

**Early life physiological and energetic responses of Atlantic silversides (*Menidia menidia*) to
ocean acidification, warming, and hypoxia**

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Teresa Schwemmer

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Teresa Schwemmer

We, the dissertation committee for the above candidate for the
Doctor of Philosophy degree, hereby recommend
acceptance of this dissertation.

Janet Nye – Dissertation Advisor
Adjunct Associate Professor, School of Marine and Atmospheric Sciences

Robert Cerrato – Chairperson of Defense
Professor, School of Marine and Atmospheric Sciences

Michael Frisk – Committee Member
Professor, School of Marine and Atmospheric Sciences

Amy Maas – Committee Member
Associate Scientist, Bermuda Institute of Ocean Sciences (BIOS)

Roger Nisbet – Committee Member
Research Professor, University of California, Santa Barbara

This dissertation is accepted by the Graduate School

Celia Marshik

Interim Dean of the Graduate School

Abstract of the Dissertation

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Global environmental change caused by human actions is making the oceans warmer, deoxygenating coastal waters, and causing acidification through dissolution of atmospheric carbon dioxide (CO₂). Understanding physiological mechanisms of fish responses to multiple co-occurring stressors is critical to conservation of marine ecosystems and the fish populations they support. In this dissertation I quantified physiological impacts of near-future levels of multiple stressors in the early life stages of the Atlantic silverside, *Menidia menidia*. In Chapter 1, I measured routine metabolic rates of embryos and larvae reared in combinations of temperature, CO₂, and oxygen levels. An interactive effect of acidification and hypoxia in embryos prompted closer examination in Chapter 2, in which I characterized the relationship between metabolism and acute hypoxia in *M. menidia* offspring reared in different CO₂ levels. In Chapter 3 I examined the density of skin surface ionocytes, cells used for acid-base balance, as an early life mechanism of high CO₂ tolerance. The first three chapters highlighted how different CO₂ effects could be depending on temperature, oxygen levels, and life stage. They also showed variable, but often high, tolerance of CO₂ with stronger effects of temperature and hypoxia on physiology. Finally, in Chapter 4 I used a Dynamic Energy Budget model to identify the processes of energetic allocation responsible for previously observed experimental hypoxia effects on *M. menidia* hatching, growth, and survival. Energy budget modeling can enhance knowledge about stressor responses by providing the information to link organismal traits to life history and populations, making it more readily applicable to conservation and management. The findings presented here provide a foundation for a more comprehensive understanding of the highly variable effects of global change on *M. menidia* and should be applied to quantifying impacts on fitness and population growth in this ecologically important species.

Dedication Page

This dissertation is dedicated to my partner and best friend, Max Grabinski. Thank you for being by my side and supporting me in more ways than I could have imagined throughout my time working on this degree. I am so unbelievably fortunate and grateful to have you in my life, and I can't wait to see where life takes us next.

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Vita, Publications and/or Fields of Study

This dissertation includes one original manuscript that has been published. All chapters will be submitted for peer review and publication in a scientific journal.

Chapter 1

Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., Nye, J. A. 2020. Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish *Menidia menidia*. *J. Exp. Biol.*, 223: jeb228015. doi: 10.1242/jeb.228015

0. Introduction

0.1. 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 (Deigweier 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.

0.2. 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.

0.3. References

- Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97(5): 399-408. <https://doi.org/10.1139/cjz-2018-0198>
- Baumann, H., Talmage, S. C., and Gobler, C. J. 2011. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nat. Clim. Change*, 2: 38-41.
- Baumann, H., Wallace, R. B., Tagliaferri, T., and Gobler, C. J. 2015. Large Natural pH, CO₂ and O₂ Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time Scales. *Estuaries Coasts*, 38: 220-231. doi: 10.1007/s12237-014-9800-y
- Baumann, H., Cross, E. L., and Murray, C. S. 2018. Robust quantification of fish early life CO₂ sensitivities via serial experimentation. *Biol. Lett.* 14: 20180408. doi:10.1098/rsbl.2018.0408
- Bengtson, D. A., Barkman, R. C., and Berry, W. J., 1987. Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*. *J. Fish. Biol.*, 31: 697-704.
- Boyd, P. W., Collins, S., Dupont, S., Fabricios, K., Gattuso, J.-P., et al. 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change. *Glob. Change Biol.*, 24: 2239-2261.
- Brauner, C. J., Shartau, R. B., Damsgaard, C., Esbaugh, A. J., Wilson, R. W., and Grosell, M. 2019. Acid-base physiology and CO₂ homeostasis: Regulation and compensation in response to elevated environmental CO₂. In: *Fish Physiology, Vol. 37: Carbon Dioxide* (ed. A. P. Farrell and C. J. Brauner), pp. 69-132. San Diego: Academic Press.
- Breitbart, D., Levin, L. A., Oschlies, A., et al. 2018. Declining oxygen in the global ocean and coastal waters. *Science*, 359(6371): eaam7240.
- Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., et al. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nat. Geosci.*, 4: 766-770.
- Cai, W.-J., Feely, R. A., Testa, J. M., Li, M., Evans, W., et al. 2021. Natural and Anthropogenic Drivers of Acidification in Large Estuaries. *Annu. Rev. Mar. Sci.*, 13: 23-55.
- Cattano, C., Claudet, J., Domenici, P., and Milazzo, M. 2018. Living in a high CO₂ world: a global meta-analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecol. Monogr.*, 88(3): 320-335.
- Chabot, D. and Claireaux, G. 2008. Environmental hypoxia as a metabolic constraint on fish: The case of Atlantic cod, *Gadus morhua*. *Mar. Pollut. Bull.*, 57: 6-12.

- Chapman, L. J. and McKenzie, D. J. 2009. Behavioral responses and ecological consequences. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. Anthony P. Farrell and Colin J. Brauner), pp. 25-77. San Diego: Academic Press.
- Claireaux, G. and Lagardère, J.-P. 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *J. Sea Res.*, 42: 157-168.
- Claireaux, G. and Chabot, D. 2016. Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. *J. Fish Biol.*, 88: 232-251.
- Clements, J. C., Sundin, J., Clark, T. D., and Jutfelt, F. 2022. Meta-analysis reveals an extreme “decline effect” in the impacts of ocean acidification on fish behavior. *PLoS Biol.*, 20(2): e3001511. <https://doi.org/10.1371/journal.pbio.3001511>
- Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., and Baumann, H. 2021. Temperature-dependent effects on fecundity in a serial broadcast spawning fish after whole-life high CO₂ exposure. *ICES J. Mar. Sci.*, 78(10): 3724-3734.
- Crain, C. M., Kroeker, K., and Halpern, B. S. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol. Lett.*, 11: 1304-1315.
- Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal pCO₂ x O₂ fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9: 18146.
- Deigweiher, K., N. Koschnick, H.-O. Pörtner, M. Lucassen. 2008. Acclimation of ion regulatory capacities in gills of marine fish under environmental hypercapnia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295: R1660-R1670.
- Diaz, R. J. and Rosenberg, R. 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. *Science*, 321: 926-929.
- Diaz, R. J. and Breitburg, D. L. 2009. The Hypoxic Environment. In: *Fish Physiology, Vol. 27: Hypoxia*. (ed. Jeffrey G. Richards, Anthony P. Farrell, and Colin J. Brauner), pp. 1-23. San Diego: Academic Press.
- Dixon, R. L., Greco, P. A., and Targett, T. E. 2017. Responses of juvenile Atlantic silverside, striped killifish, mummichog, and striped bass to acute hypoxia and acidification: Aquatic surface respiration and survival. *J. Exp. Mar. Biol. Ecol.*, 493: 20-30.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. 2009. Ocean Acidification: The Other CO₂ Problem. *Annu. Rev. Mar. Sci.*, 1: 169-192.
- Esbaugh, A. J. 2018. Physiological implications of ocean acidification for marine fish: emerging patterns and new insights. *J. Comp. Physiol. B*, 188: 1-13. <https://doi.org/10.1007/s00360-017-1105-6>

- Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.*, 65: 414-432.
- Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J. 2004. Impact of Anthropogenic CO₂ on the CaCO₃ System in the Oceans. *Science*, 305: 362-366.
- Foo, S. A. and Byrne, M. 2016. Chapter Two – Acclimatization and Adaptive Capacity of Marine Species in a Changing Ocean. In: *Advances in Marine Biology*, Vol. 74. [Barbara E. Curry, ed.]. Academic Press, Cambridge, MA, USA, pp. 69-116.
- Fry, F. E. J. 1947. *Effects of the Environment on Animal Activity*. Publications of the Ontario Fisheries Research Laboratory, No. 68. Toronto: The University of Toronto Press.
- Gamperl, A. K. and Driedzic, W. R. 2009. Cardiovascular Function and Cardiac Metabolism. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. Jeffrey G. Richards, Anthony P. Farrell, and Colin J. Brauner), pp. 25-77. San Diego: Academic Press.
- Gledhill, D. K., White, M. M., Salisbury, J., Thomas, H., Misna, I., et al. 2015. Ocean and Coastal Acidification off New England and Nova Scotia. *Oceanogr.*, 28(2), Special Issue on Emerging Themes in Ocean Acidification Science: 182-197.
- Heuer, R. M and Grosell, M. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 307: R1061-R1084. doi: 10.1152/apjregu.00064.2014
- Houde, E. D. 1989. Subtleties and episodes in the early life of fishes. *J. Fish Biol.*, 35: 29-38.
- Intergovernmental Panel on Climate Change. 2022. Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama, eds.]. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.
- Ishimatsu, A., Hayashi, M., and Kikkawa, T. 2008. Fishes in high-CO₂, acidified oceans. *Mar. Ecol. Prog. Ser.*, 373: 295-302.
- Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18: 81-92.
- Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.*, 13: 1419-1434.

- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J.-P. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Change Biol.*, 19: 1884-1896.
- Melzner, F., Gutkowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Pörtner, H.-O. 2009. Physiological basis for high CO₂ tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosci.*, 6: 2313-2331.
- Middaugh, D. P., Hemmer, M. J., and Goodman, L. R. 1987. Methods for Spawning, Cultureing and Conducting Toxicity-Tests with Early Life Stages of Four Atherinid Fishes: The Inland Silverside, *Menidia beryllina*, Atlantic silverside, *M. menidia*, Tidewater silverside, *M. peninsulae* and California grunion, *Leuresthes tenuis*. Gulf Breeze, FL: United States Environmental Protection Agency.
- Morrell, B. K. and Gobler, C. J. 2020. Negative Effects of Diurnal Changes in Acidification and Hypoxia on Early-Life Stage Estuarine Fishes. *Diversity*, 12: 25. doi: 10.3390/d12010025
- Munday, P. L., Cheal, A. J., Dixon, D. L., Rummer, J. L., and Fabricius, K. E. 2014. Behavioural impairment in reef fishes caused by ocean acidification at CO₂ seeps. *Nat. Clim. Change*, 4: 487-492.
- Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*, 10: 69. doi: 10.3390/d10030069
- Murray, C. S., Malvezzi, A., Gobler, C. J., and Baumann, H. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.*, 504: 1-11. doi: 10.3354/meps10791
- Murray, C. S., Fuiman, L. A., and Baumann, H. 2017. Consequences of elevated CO₂ exposure across multiple life stages in a coastal forage fish. *ICES J. Mar. Sci.*, 74(4): 1051-1061. doi: 10.1093/icesjms/fsw179
- National Research Council (2005). *Marine Mammal Populations and Ocean Noise: Determining When Noise Causes Biologically Significant Effects*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/11147>
- Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to ecosystems through dynamic energy budget models. *J. Anim. Ecol.*, 69: 913-926.
- Orr, J. A., Vinebrooke, R. D., Jackson, M. C., Kroeker, K. J., Kordas, R. L., et al. 2020. Towards a unified study of multiple stressors: divisions and common goals across research disciplines. *Proc. R. Soc. B*, 287: 20200421. <http://dx.doi.org/10.1098/rspb.2020.0421>

- Porteus, C. S., Roggatz, C. C., Velez, Z., Hardege, J. D., and Hubbard, P. C. 2021. Acidification can directly affect olfaction in marine organisms. *J. Exp. Biol.*, 224: jeb237941. doi: 10.1242/jeb.237941
- Pörtner, H.-O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.*, 213: 881-893.
- Pörtner, H.-O. and Knust, R. 2007. Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance. *Science*, 315: 95-97.
- Richards, J. G., Farrell, A. P., and Brauner, C. J. 2009. *Fish Physiology, Vol. 27: Hypoxia*. San Diego: Academic Press.
- Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: *Fish Physiology, Vol. 11: The Physiology of Developing Fish, Part A: Eggs and Larvae*. (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego: Academic Press.
- Saba, V. S., Griffies, S. M., Anderson, W. G., Winton, M., Alexander, M. A., et al. 2016. Enhanced warming of the Northwest Atlantic Ocean under climate change. *J. Geophys. Res. Oceans*, 121: 118-132.
- Scannell, J. A., Pershing, A. J., Alexander, M. A., Thomas, A. C., and Mills, K. E. 2016. Frequency of marine heatwaves in the North Atlantic and North Pacific since 1950. *Geophys. Res. Lett.*, 43: 2069-2076.
- Schultz, E. T., Conover, D. O., and Ehtisham, A. 1998. The dead of winter: size-dependent variation and genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia menidia*) from different latitudes. *Can. J. Fish. Aquat. Sci.*, 55: 1149-1157.
- Stiasny, M. H., Mittermayer, F. H., Sswat, M., Voss, R., Jutfelt, F., Chierici, M., Puvanendran, V., Mortensen, A., Reusch, T. B. H., and Clemmesen, C. 2016. Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fishes Population. *PLoS ONE*, 11(8): e0155448. doi: 10.1371/journal.pone.0155448
- Stierhoff, K. L., Tyler, R. M., and Targett, T. E. 2009. Hypoxia tolerance of juvenile weakfish (*Cynoscion regalis*): Laboratory assessment of growth and behavioral avoidance responses. *J. Exp. Mar. Biol. Ecol.*, 381: S173-S179.
- Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., and Reusch, T. B. H. 2014. Evolution in an acidifying ocean. *Trends Ecol. Evol.*, 29(2): 117-125.

- Taylor, J. C. and Miller, J. M. 2001. Physiological performance of juvenile southern flounder, *Paralichthys lethostigma* (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. *J. Exp. Mar. Biol. Ecol.*, 258: 195-214.
- Wallace, R. B., Baumann, H., Gear, J. S., Aller, R. C., and Gobler, C. J. 2014. Coastal ocean acidification: The other eutrophication problem. *Estuar. Coast. Shelf Sci.*, 148: 1-13.
- Watson, J. W., Hyder, K., Boyd, R., Thorpe, R., Weltersbach, M. S., Ferter, K., Cooke, S. J., Roy, S., and Sibly, R. M. 2020. Assessing the sublethal impacts of anthropogenic stressors on fish: An energy-budget approach. *Fish Fish.*, 21: 1034-1045. doi: 10.1111/faf.12487
- Welch, M. J. and Munday, P. L. 2016. Contrasting effects of ocean acidification on reproduction in reef fishes. *Coral Reefs*, 35: 485-493.
- Wells, R. M. G. 2009. Blood-gas transport and hemoglobin function: adaptations for functional and environmental hypoxia. In *Fish Physiology*, Vol. 27, Hypoxia (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 255-299. San Diego: Academic Press.
- Whitney, M. M. and Vlahos, P. 2021. Reducing Hypoxia in an Urban Estuary Despite Climate Warming. *Environ. Sci. Technol.*, 55: 941-951.
- Wittmann, A. C. and Pörtner, H.-O. 2013. Sensitivities of extant animal taxa to ocean acidification. *Nat. Clim. Change*, 3: 995-1001.

1. Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish *Menidia menidia*

1.1. Introduction

Anthropogenic ocean warming and acidification are emerging potential stressors to marine fishes (Hollowed et al., 2013; Heuer and Grosell, 2014), many of which are already threatened by overexploitation (Dulvy et al., 2003). Ocean acidification is the result of carbon dioxide (CO₂) dissolving into seawater and changing the equilibrium of carbonate species, resulting in more hydrogen ions and thus reduced pH. In shallow coastal and estuarine waters, this process is amplified by natural and anthropogenic fluctuations in pH related to ecosystem metabolism (Cai et al., 2011; Wallace et al., 2014; Baumann and Smith, 2018). The fluctuating and often extreme conditions in estuarine habitats require that studies account for the multiple stressors that coastal organisms simultaneously experience, including extreme temperature, pH, and oxygen (Gunderson et al., 2016). In the past twenty years, studies on the biological effects of ocean acidification have rapidly expanded, particularly those focusing on calcifying organisms (Riebesell et al., 2000; Orr et al., 2005; Kroeker et al., 2013; Browman, 2016). More recently, ocean acidification research has expanded to marine fishes (Munday et al., 2009a; Munday et al., 2010; Frommel et al., 2012; Baumann et al., 2012; Chambers et al., 2014; Baumann et al., 2018), and incorporated additional environmental factors such as warming (Munday et al., 2009b; Rosa et al., 2014; Murray and Baumann, 2018; Dahlke et al., 2017) and hypoxia (DePasquale et al., 2015; Gobler and Baumann, 2016; Miller et al., 2016). While adult fishes are expected to be largely resilient to elevated CO₂ levels predicted for the average surface ocean over the next centuries (Ishimatsu et al., 2008), exposure of fish early life stages to acidified water can elicit significant reductions in growth and survival (Baumann et al., 2012; Murray et al., 2014; Chambers et al., 2014; Dahlke et al., 2017). The underlying metabolic changes that contribute to observed growth and survival effects in fish are less well documented, and relatively few studies have quantified effects on oxygen consumption, mitochondrial respiration, and acid-base regulation (Heuer and Grosell, 2014; Dahlke et al., 2017). Studies on fish responses to seawater acidification have often revealed large variation among species, populations, and even subsequent experiments (Couturier et al., 2013; Munday et al., 2009b; Rummer et al., 2013;

Murray and Baumann, 2018; Clark et al., 2020), making mechanistic understanding of long-term CO₂ tolerance challenging.

The Atlantic silverside, *Menidia menidia*, and the closely related inland silverside, *Menidia beryllina*, are widely used fish models for quantifying biological effects of anthropogenic stressors (Conover and Munch, 2002; Baumann et al., 2012; DePasquale et al., 2015). Although silversides are not commercially exploited, these abundant estuarine forage fish represent an ecologically important link between planktonic and higher piscivorous trophic levels in coastal ecosystems (Bayliff, 1950; Cadigan and Fell, 1985). Offspring growth and survival in *M. menidia* and *M. beryllina* have been shown to decrease under elevated CO₂ levels in some (Baumann et al., 2012; Murray et al., 2017), but not all studies (Baumann et al., 2018; Murray and Baumann, 2018).

Acidification has also been applied simultaneously with low partial pressure of oxygen (P_{O_2}) in several *M. menidia* experiments because CO₂-acidified conditions co-occur with hypoxia in eutrophied estuaries (Melzner et al., 2012; Wallace et al., 2014; Gobler and Baumann, 2016; Baumann and Smith, 2018) and both stressors are intensifying globally (Doney et al., 2009; Keeling et al., 2010; Gruber, 2011). Hypoxia alone can cause fish to either expend energy escaping patches of low oxygen (Pihl et al., 1991; Weltzien et al., 1999) or engage mechanisms such as metabolic suppression (Richards, 2009), adjustment of oxygen-binding and transport properties of the blood (Silkin and Silkina, 2005; Wells, 2009), and altered behavior (Pollock et al., 2007). In early life stages, when compensatory mechanisms are still developing, hypoxia can delay hatching, slow growth, and reduce pre- and post-hatch survival (Rombough, 1988; DePasquale et al. 2015). Combined high CO₂ and low oxygen can have synergistic effects on hatching success and larval survival, for example in *M. menidia* (DePasquale et al., 2015; Cross et al., 2019; Morrell and Gobler, 2020). In *M. menidia* adults, exposure to acidified water increased the oxygen levels at which surface respiration, loss of equilibrium, and death occurred (Miller et al., 2016). These studies highlight variable sensitivity to acidification and the likelihood of *M. menidia* to respond to multiple interacting stressors differently than they do to the sum of individual stressors. Responses to CO₂ and P_{O_2} treatments have been documented without interacting effects for other fish species and response variables, including cellular metabolic enzyme activity of juvenile rockfish (Davis et al., 2018), survival of juvenile weakfish (Lifavi et al., 2017), and growth and survival of inland silversides (DePasquale et al., 2015). One

mechanism by which acidification could interact with hypoxia is by modifying the critical oxygen partial pressure (P_{crit}), or the P_{O_2} threshold below which routine metabolic rate (RMR) becomes oxygen-dependent (Richards, 2009). In the case of the intertidal sculpin *Clinocottus analis*, CO_2 -acidification not only increased RMR, but also raised P_{crit} , making the fish less hypoxia-tolerant.

In order to evaluate which species of fish are most vulnerable to ocean acidification and how fishes may be able to acclimate or adapt to it, it is important to have a mechanistic understanding of how acidification affects fishes. Although many trait responses to high CO_2 have been examined for many species, the mechanistic responses and bioenergetic consequences of elevated CO_2 combined with other stressors are poorly understood. Because most physiological processes, including metabolic rates (Peck and Moyano, 2016), directly depend on temperature, acidification effects are likely temperature-dependent, although there is both supporting and contradicting empirical evidence for this in fishes (Pörtner et al., 2017; Jutfelt et al., 2018; Murray and Baumann, 2018). For example, while elevated CO_2 increased survival and development speed in Antarctic dragonfish (*Gymnodraco acuticeps*) embryos, rearing embryos in water 3°C warmer caused CO_2 to have the opposite effect (Flynn et al., 2015). Additive and synergistic interactions between CO_2 and temperature were detected in ventilation and metabolic rates of juvenile emerald rockcod, in which elevated rates under high temperature at 14 days of exposure were compensated in the ambient but not elevated CO_2 treatments by 28 days (Davis et al., 2017).

RMR has been measured in fishes exposed to elevated CO_2 and temperature, and results depend heavily on the species and the methods used (Heuer and Grosell, 2014). For different species, acclimation to combined temperature and CO_2 treatments may show temperature but not CO_2 effects on RMR (Strobel et al., 2012), separate effects of both variables, or synergistic interactions (Munday et al., 2009b). Negative effects of high CO_2 may manifest mostly at the upper and lower ends of a species' thermal tolerance window because aerobic scope and thus the energetic capacity for acid-base regulation may become limited near thermal extremes (Fry, 1971; Pörtner, 2010; Lefevre, 2016). Responses to stressors at thermal extremes are also useful in understanding how climate change will affect distributions, because thermal extremes are a strong determinant of geographic ranges (Calosi et al., 2010; Lynch et al., 2014). It is therefore

critical to quantify responses of multiple endpoints across a range of temperatures (Pörtner, 2012; Twiname et al., 2019).

Over the course of two spawning seasons, we conducted six independent experiments to quantify routine metabolic rates of *M. menidia* embryos and newly hatched larvae reared in elevated CO₂ partial pressure (P_{CO_2}) and across a range of oxygen and thermal conditions that already occur in the species' habitat (Middaugh et al., 1987). We focused on the early life stages because these stages tend to be more sensitive to stressful conditions than adults (Pörtner et al., 2005; Baumann et al., 2012; Harvey et al., 2013; Dahlke et al., 2020), and growth and survival of early life stages influence recruitment and population dynamics (Chambers and Trippel, 1997; Houde, 1987; Pörtner and Peck, 2010). Both synergistic (Munday et al., 2009b) and antagonistic (Lefevre, 2016) interactions between CO₂ and temperature treatments on resting metabolic rate have been quantified in fishes, and the nature of the interaction may depend on whether the species in question increases or decreases metabolism under elevated CO₂. Our previous results on *M. menidia* show reduced or unchanged growth and survival under high CO₂ (Murray and Baumann, 2018; Baumann et al., 2018), which suggests additional energetic demands of pH regulation and thus greater oxygen demand. Therefore, we hypothesized that embryonic and larval metabolic rates would increase with both P_{CO_2} and temperature, and that P_{CO_2} effects would synergistically interact with temperature. Low P_{O_2} conditions were expected to induce metabolic suppression, thereby potentially counteracting or concealing any elevation in metabolism due to high P_{CO_2} . An interaction between P_{O_2} and P_{CO_2} was also expected because reduced pH from elevated CO₂ can reduce the oxygen-binding capacity of the blood (Brauner and Randall, 1996), so acidified seawater may differentially affect oxygen consumption depending on the P_{O_2} level. Overall, we expected larvae to be less sensitive to the treatments than embryos based on results of previous studies (Chambers et al., 2014; Murray and Baumann, 2018; Cross et al., 2019) and the more advanced regulatory mechanisms of fish larvae relative to embryos (Rombough, 1988).

1.2. Materials and Methods

1.2.1. Animals and Experimental Design

Detailed descriptions of animal collection and husbandry methods can be found in Murray and Baumann (2018). Briefly, wild, spawning-ripe adult *Menidia menidia* (Linnaeus, 1766) were collected from Mumford Cove (41°19'25" N, 72°1'7" W), Groton, CT, in late spring and early summer of 2016 and 2017 and transported to the Rankin seawater laboratory at University of Connecticut's Avery Point Campus. Adults were strip-spawned, and fertilized eggs were allowed to attach to 1-mm mesh screens, which were randomly distributed into 20-L rearing containers with mesh-covered (100 μ m) holes to allow overflow without loss of embryos or larvae. One container with 100 embryos was placed into each treatment tank within 2h post-fertilization, so initial exposure was acute and exposure time (in days) is equivalent to age at sampling. All experimental methods were approved and conducted according to University of Connecticut Institutional Animal Care and Use Committee protocol #A14-032.

Of six factorial experiments conducted in 2016 and 2017, experiments 1-4 quantified $\text{CO}_2 \times$ temperature effects and experiments 5-6 quantified $\text{CO}_2 \times$ oxygen effects (Table 1.1). Experiment 1 used 400 and 2200 μatm as target P_{CO_2} levels, crossed with two temperatures: 17°C and 24°C. Experiments 2 and 3 factorially crossed three P_{CO_2} levels (400, 2200, and 4200 μatm) with three temperatures (17°C, 20°C, and 24°C). Experiment 4 used the same three target P_{CO_2} levels crossed with 24°C and 28°C. The target temperature levels represent the range of temperatures typically encountered by *M. menidia* in Long Island Sound during the spawning season of late April to early July, except for 28°C which is closer to the species' upper thermal extreme (Middaugh et al., 1987). The target P_{CO_2} levels represent the current average oceanic P_{CO_2} (400 μatm), the approximate level predicted as the average oceanic P_{CO_2} for the next 300 years under a rising CO_2 emissions scenario (RCP8.5; IPCC, 2013) and often experienced by estuarine fishes such as *M. menidia* in the summer (2200 μatm), and a higher level (4200 μatm) that will occur more often in the future as average P_{CO_2} rises in conjunction with coastal eutrophication and hypoxia (Wallace et al., 2014). Experiments 5 and 6 exposed *M. menidia* early life stages to three levels of P_{CO_2} (400, 2200, and 4200 μatm) crossed factorially with three target levels of oxygen partial pressure (P_{O_2}): normoxic (23 kPa), suboxic (12 kPa) and hypoxic (7.5 or 9 kPa). The hypoxic P_{O_2} condition was increased to 9 kPa in experiment 6 to avoid the complete larval mortality observed at 7.5 kPa in experiment 5. The different treatments used across all experiments, each with $n=1$ rearing container per treatment, result in one to four

treatment replicates for Experiment 1-4, and one or two treatment replicates for Experiments 5 and 6. The lack of consistent replication is dealt with by using measured treatment means as quantitative rather than categorical variables in the analysis (see *Data Analysis*). We sampled at least 10 individuals per treatment for each respirometry trial, so after outlier removal and pooling of experiments the final dataset contained 8 to 55 embryos and 9 to 68 larvae from each treatment (Table 1.2). The target treatment levels of all six experiments are summarized in Table 1.1, and measured water chemistry, temperature, and oxygen values are summarized in Tables S1.1 and S1.2.

Gas and temperature manipulations and measurements are described in detail by Murray and Baumann (2018) and Cross et al. (2019). The actual P_{CO_2} levels were calculated based on measured pH, temperature, salinity, and total alkalinity (A_T). A_T samples were collected three times per experiment and measured using an endpoint titration (G20 Potentiometric Titrator, Mettler Toledo®, Columbus, OH, USA). Based on these measurements, the P_{CO_2} (μatm), fugacity of CO_2 (f_{CO_2} ; μatm), dissolved inorganic carbon (DIC; $\mu\text{mol kg}^{-1}$), and carbonate ion concentration (CO_3^{2-} ; $\mu\text{mol kg}^{-1}$) were calculated in CO2SYS (V2.1). In the $\text{CO}_2 \times \text{temperature}$ experiments, oxygen was maintained at ~100% air saturation ($>20 \text{ kPa}$). For experiments 1 and 4 this was achieved with continuous bubbling and validated daily for each tank with a handheld probe (Intellical LDO101 Laboratory Luminescent Dissolved Oxygen Sensor, Hach®, Loveland, CO, USA). For experiments 2, 3, 5, and 6, dissolved oxygen (DO, mg L^{-1}) measurements were automatically taken twice hourly in each tank by a DO probe (LDO Model 2, Hach®, Loveland, CO, USA) connected to a LabView (National Instruments®, Austin, TX, USA) program, which adjusted bubbling of CO_2 -stripped air or nitrogen gas to maintain target oxygen levels. DO measurements were converted to P_{O_2} using the oxygen solubility at the measured temperature and salinity (García and Gordon, 1992). Mean temperature, P_{O_2} , pH and carbon chemistry parameters measured and calculated over the duration of each experiment can be found in Tables S1.1 ($\text{CO}_2 \times \text{temperature}$ experiments) and S1.2 ($\text{CO}_2 \times \text{oxygen}$ experiments).

1.2.2. Microrespirometry

Closed respirometry measurements were conducted on embryos (901-1227 μm diameter) randomly sampled from each treatment 1-3 days prior to hatch and larvae (4075-6330 μm total

length) sampled on the day of hatching. Because temperature and P_{O_2} influenced the development speed, the sampling days correspond to different numbers of days-post-fertilization, but similar developmental stages (see Table 1.2 for exact ages). The P_{CO_2} level did not affect time to hatching, but embryos from higher temperatures and higher P_{O_2} levels hatched up to seven days earlier than the other treatments (Table 1.2; Murray and Baumann, 2018; Cross et al., 2019). Individual embryos were gently removed from screening and placed into microrespirometry wells. Newly hatched larvae were removed from the rearing containers with pipettes with at least a 5-mm wide tip to avoid injury and minimize handling stress. All respirometry trials were conducted during daylight hours between 09:00 and 18:00, during which time newly hatched larvae exhibited consistent levels of routine activity.

Oxygen consumption rates of embryos and larvae were measured by two 24-channel SensorDish readers (SDR; PreSens Precision Sensing, GmbH, Regensburg, Germany) and glass well plates equipped with an optical oxygen sensor spot in each well (Loligo Systems®, Viborg, Denmark). Each 0.5-mL well received a single embryo or larva, and at least one well contained only treatment water to measure background (control) respiration. We used both 24-well sensor plates and loaded individuals from each P_{CO_2} treatment within a given temperature or P_{O_2} level into the plates simultaneously. The wells were filled completely with water from the treatment tank that each individual came from, cleared of air bubbles, and sealed airtight with parafilm, silicone, and acrylic sheets. Well plates were placed in temperature-controlled water baths during the respirometry measurement period, and the system was covered to prevent light from interfering with the sensors and to minimize larval activity. Individuals were allowed to recover from any handling stress and sensors were allowed to equilibrate with the water bath temperature for 10 minutes before measurements started. Dissolved oxygen (DO, mg L^{-1}) was recorded every fifteen seconds by the PreSens SDR software until DO had decreased by 3 mg L^{-1} in at least one of the wells. Trial duration ranged from 15 to 60 minutes, depending on the temperature. In the case of the suboxic and hypoxic treatments from experiments 5 and 6, however, the trials lasted five minutes regardless of the DO differential, given the already low oxygen in the treatment water. Stirring is recommended to maintain homogeneous oxygen concentrations throughout closed respirometry chambers (Peck and Moyano, 2016), but magnetic stir bars cannot be used with this system. The system can be used atop a shaker, but shaking induces premature hatching of embryos and could disturb the larvae. However, this type of system has been used in the past

without shaking or stirring (e.g. Flynn and Todgham, 2018; Zimmer et al., 2020), and calculations of oxygen diffusion rates for our conditions showed that diffusion should maintain homogeneity of oxygen as the fish consumes oxygen. At the end of each measurement period, embryos and larvae were checked for injury or death, and any other factors that might have affected oxygen consumption rates were noted. Between trials the wells were rinsed with DI water but not dried in order to keep the sensors hydrated, and between experiments they were rinsed and gently swabbed with a 35% ethanol solution.

1.2.3. Data Analysis

Calculations of metabolic rates and all statistical tests were conducted in R statistical software (v4.0.0; R Core Team, 2020) using the stats package and olsrr package (Hebbali, 2020). Because temperature influences oxygen solubility and metabolic rates of fish, we measured temperature simultaneously with DO throughout the measurement period and only used data for periods of time in which the temperature changed by less than $\sim 0.03^{\circ}\text{C min}^{-1}$. The SDRs measure phase from each optical sensor spot, and the SDR software uses phase and temperature to calculate DO (mg L^{-1}) at each time point. An approximately linear section of data was chosen via visual inspection, and a linear model was fit to the DO values with respect to time for each well. The slope ($\text{mg O}_2 \text{ L}^{-1} \text{ s}^{-1}$) of the linear model was used to calculate oxygen consumption rate (R_{O_2} ; $\mu\text{mol O}_2 \text{ h}^{-1}$) with the following formula: $R_{\text{O}_2} = \frac{\text{slope}}{0.032} \times 3600 \times 0.0005$, where 0.032 is the molar mass of O_2 (mg mol^{-1}), 3600 converts seconds to hours, and 0.0005 L is the well volume. The mean R_{O_2} from control wells was subtracted from each fish-containing well of the same treatment to account for microbial respiration and obtain fish R_{O_2} . Size differences in embryos were negligible and quantifying embryo mass was impractical, so R_{O_2} was not normalized to mass and is reported as whole-embryo routine metabolic rate (RMR). Larval total length (TL, mm) was measured in images (Image J) taken by digital camera (TrueChrome Metrics, Tucson Photonics Co., Fuzhou, Fujian, China) connected to a stereo microscope (Nikon Eclipse E200). TL was then converted to dry weight (DW, mg) using the relationship $\ln(\text{DW}) = 2.997 \times \ln(\text{TL}) - 6.703$ (H. Baumann, personal communication, June 23, 2017). DW was then used to calculate the larval mass-specific RMR ($\mu\text{mol mg}^{-1} \text{ h}^{-1}$) as $\text{RMR} = \frac{R_{\text{O}_2}}{\text{DW}}$ (Peck and Moyano, 2016).

As a measure of sensitivity of metabolic rates to rearing temperature, the temperature coefficient (Q_{10}), the change in metabolic rate associated with a 10°C rearing temperature increase, was calculated at each P_{CO_2} . The formula used for this calculation is $Q_{10} =$

$\left(\frac{M_{O_2(b)}}{M_{O_2(a)}}\right)^{\left(\frac{10}{(T_b - T_a)}\right)}$, where T is the mean temperature in 17°C treatments (a) and 28°C treatments (b). Within P_{CO_2} treatments and life stages, Q_{10} was calculated for every possible pairing of individuals from the 17°C and 28°C treatments. Bootstrapping was used to sample with replacement from the pool of Q_{10} values 100,000 times, in order to estimate a mean Q_{10} and 95% confidence intervals.

Multiple linear regression (MLR) was used to determine if metabolic rate changed as a linear function of P_{CO_2} , temperature, P_{O_2} , or the interaction between P_{CO_2} and either of these secondary variables. Our experiments had pseudoreplication, in that individuals were sampled from only one experimental unit (rearing container) per treatment in each experiment. Using the measured predictor variables and treating them as quantitative (continuous) values rather than categorical groups allows the use of linear regression and does not require more than one experimental unit (Hurlbert, 2004). This analysis gave us more statistical power and more informative results than an analysis with categorical predictor variables would have (Cottingham et al., 2005; Havenhand et al., 2010). MLR also has the benefits of accounting for the variability in, order of, and distance between experimental treatments. This approach allows us to define quantitative relationships between the predictor and response variables that have predictive capacity beyond the treatment levels used and can be incorporated into ecological models such as dynamic energy budgets (Cottingham et al., 2005; Kooijman, 2009).

Outliers were identified using Cook's distance and standardized residuals and removed from the dataset under the assumption that extreme values represented active or stressed fish, rather than routine metabolism. Data were square-root transformed in the regression in order to satisfy the assumptions of normality and homoscedasticity, as tested using the `ols_test_normality` and `ols_test_breusch_pagan` functions, respectively, in the `olsrr` package (v0.5.3; Hebbali, 2020). All statistical tests were interpreted with a significance threshold of $\alpha = 0.05$.

1.3. Results

1.3.1. $CO_2 \times Temperature$

In experiments 1-4, embryonic metabolic rates increased significantly (MLR, $F_{3,248} = 11.94$, $p < 0.001$) with temperature, with a 79% increase in mean metabolic rate from the lowest (17°C) to the highest (28°C) temperature across all P_{CO_2} treatments. However, metabolic rates were unaffected by P_{CO_2} treatment (MLR, $F_{3,248} = 11.94$, $p = 0.07$; Figure 1.1A). In larvae, metabolic rates increased significantly (MLR, $F_{3,364} = 69.87$, $p < 0.001$) with temperature but were also statistically unaffected by P_{CO_2} treatment (MLR, $F_{3,364} = 69.87$, $p = 0.189$; Figure 1.1B). Mean metabolism increased by 173% from the lowest (17°C) to the highest (28°C) temperature across all P_{CO_2} treatments. Mean metabolic rates by treatment and sampling age are reported in Table 1.2, and multiple regression model results are summarized in Table S1.3. Bootstrapped mean Q_{10} values for *M. menidia* embryos decreased as P_{CO_2} increased (Figure 1.2). The bootstrapped 95% confidence intervals for embryonic Q_{10} did not overlap between the three P_{CO_2} levels (Table S1.4). In larvae, however, Q_{10} increased from 400 μatm to 2200 and 4200 μatm P_{CO_2} (Figure 1.2). The bootstrapped 95% confidence intervals for the elevated P_{CO_2} levels overlapped with each other, but not with that of the 400 μatm P_{CO_2} level (Table S1.4). Overall, the Q_{10} was higher in larvae than embryos under elevated P_{CO_2} but similar for both life stages under ambient P_{CO_2} .

1.3.2. $CO_2 \times \text{Oxygen}$

For experiments 5-6, we detected a significant interaction of $P_{CO_2} \times P_{O_2}$ on embryonic metabolic rates (MLR, $F_{3,255} = 8.74$, $p = 0.004$; Figure 1.3A). For embryos reared under normoxic (23.0 kPa) conditions, exposure to elevated (4200 μatm) P_{CO_2} increased metabolic rates by 55% compared to ambient P_{CO_2} . However, this effect of elevated P_{CO_2} diminished with decreasing P_{O_2} . Additionally, metabolic rates of embryos in ambient P_{CO_2} treatments were similar across all P_{O_2} levels, but they increased with P_{O_2} under elevated P_{CO_2} (Table 1.2). Metabolic rates of newly hatched larvae were statistically unaffected by P_{CO_2} or P_{O_2} (MLR, $F_{3,142} = 0.325$, $p > 0.05$; Figure 1.3B).

1.4. Discussion

Rearing *M. menidia* offspring and measuring their routine metabolic rates in combinations of P_{CO_2} and temperature expectedly revealed that temperature is the dominant determinant of embryonic and larval metabolism at these treatment levels. This is consistent with other studies in which temperature had stronger effects than CO_2 on physiological endpoints in fishes, including larval growth and survival (Murray and Baumann, 2018), time to hatch (Gobler et al., 2018), metabolic rates and aerobic scope (Gräns et al., 2014), and hatching success and ionocyte abundance (Dahlke et al., 2017). In other cases, sensitivity of fishes to CO_2 has depended on temperature through interacting effects (Munday et al., 2009b; Pimentel et al., 2014; Murray et al., 2019). Seawater acidification is thought to affect fish by limiting functional capacity of tissues, particularly at thermal extremes (Pörtner, 2012). If such a CO_2 response exists in *M. menidia*, perhaps our temperature treatments were not extreme enough to elicit a limitation of functional capacity that is detectable through routine metabolism.

Thermal sensitivity (Q_{10}) of RMR was similar for embryos and larvae at ambient (400 μatm) P_{CO_2} but declined with elevated P_{CO_2} in embryos and increased with P_{CO_2} in larvae (Figure 1.2; Table 1.3). A study on larval flatfish (*Solea senegalensis*) exposed to elevated CO_2 and temperature found a similar trend to our embryos, that increasing CO_2 reduced Q_{10} , but also found that Q_{10} increased with fish age (Pimentel et al., 2014). Fish embryos and larvae are generally more stenothermal than juveniles and young adults, and in some species these early life stages are incapable of thermal acclimation (Rombough, 1988; Rijnsdorp et al., 2009). However, *M. menidia* nursery habitats have rapidly fluctuating conditions (Conover and Ross, 1982), hence some capacity for acclimation to temperature and CO_2 is expected (Baumann, 2019). Reduced thermal sensitivity of embryonic RMR under elevated P_{CO_2} may be a beneficial adaptation that helps embryos with limited mobility survive periods of high temperature and acidity. In contrast, larvae are free swimming and active, with a higher metabolic rate overall and more well-developed mechanisms for gas and ion exchange (Nilsson and Östlund-Nilsson, 2008). The increase in larval Q_{10} with elevated P_{CO_2} indicates that larvae are slightly more sensitive to combined high P_{CO_2} and temperature, but their more advanced ability to regulate their activity and physiology may make this sensitivity less detrimental than it would be for embryos.

The combined P_{CO_2} and P_{O_2} treatments had a significant interactive effect on embryonic, but not larval, metabolic rates. In normoxia (23.0 kPa), RMR increased with P_{CO_2} , consistent with our hypothesis that seawater acidification increases metabolic costs of acid-base balance. However, the RMR of high P_{CO_2} embryos decreased with declining P_{O_2} , suggesting RMR became oxygen-dependent and the embryos from these treatments had reached their critical oxygen partial pressure (P_{crit}), the P_{O_2} level below which aerobic metabolism depends directly on available oxygen (Richards, 2009). This oxygen-dependence was not detected in the ambient P_{CO_2} treatments, indicating that P_{crit} was higher in acidified water. Increased P_{crit} can be expected as a result of elevated P_{CO_2} because the Bohr effect of reduced blood pH leads to lower hemoglobin- O_2 binding efficiency, and thus inhibits oxygen uptake (Brauner and Randall, 1996; Wells, 2009; Gobler and Baumann, 2016). Severely reduced oxygen uptake inhibits mitochondrial production of ATP and forces the fish to activate anaerobic pathways, which are much less efficient than aerobic metabolism and thus unsustainable as a long-term response (Richards, 2009). Although we did not quantify anaerobic metabolism in this study, doing so in future work would improve understanding of energetic costs of chronic acidification and hypoxia. Nevertheless, the synergistic effect we detected on embryos suggests that P_{CO_2} influences both P_{crit} and the oxygen-independent RMR (Figure 1.4). This further highlights the importance of multistressor studies, as hypoxia sensitivity could be underestimated when co-occurring effects of acidification are not considered. Such a principle has important implications for informing policy initiatives aimed at maintaining ecologically sound oxygen levels in coastal marine systems (Paerl, 2006).

Embryos were more sensitive than larvae to the P_{CO_2} and P_{O_2} treatments, with larvae having no significant response to either variable. However, survival to hatching was so low in the 7.5 kPa P_{O_2} treatments that we did not have enough larvae left to measure larval metabolic rates for that treatment. This lowest P_{O_2} level yielded the lowest growth and survival at both stages (Cross et al. 2019), so exclusion of this level from larval metabolic measurements likely precluded detection of any P_{O_2} effect, such as oxygen-dependence indicative of P_{crit} , in larvae. Future work should investigate whether acidification increases P_{crit} of *M. menidia* larvae as might be expected due to reduced oxygen affinity of hemoglobin under ambient P_{CO_2} (Brauner

and Randall, 1996; Wells, 2009). Surprisingly, recent work on juvenile European sea bass found a decrease in P_{crit} accompanied by increased hemoglobin-oxygen affinity under acutely increasing P_{CO_2} (Montgomery et al., 2019), so it is also possible that *M. menidia* are capable of reducing P_{crit} under short-term acidification. In contrast, woolly sculpin exposed to long-term elevated P_{CO_2} had higher RMR and P_{crit} relative to ambient treated fish (Hancock and Place, 2016), which suggests a similar response to our embryos may occur in later stage fishes under long enough acidified duration. Nonetheless, high P_{CO_2} exposed larvae may be more hypoxia-resistant than embryos due to increased metabolic control upon hatching, given that embryos often become less hypoxia-tolerant closer to hatching as oxygen demand increases but egg surface area for diffusion remains constant (Rombough, 1988; Kamler, 1992).

The embryos' metabolic response to elevated P_{CO_2} in the $CO_2 \times$ oxygen experiments is consistent with previous work that has shown highest sensitivity to seawater acidification in the early life stages of marine animals (Pörtner et al., 2005; Baumann et al., 2012; Harvey et al., 2013; Baumann et al., 2018; Dahlke et al., 2020). Early-life mortality has the potential to impact fish population sizes through reduced recruitment (Chambers and Trippel, 1997; Houde, 1987; Hurst and Conover, 1998), and our results indicate a metabolic mechanism for increased *M. menidia* embryo mortality measured in combined high P_{CO_2} and hypoxia (Cross et al., 2019). With these experiments we have shown that it is critical to conduct experiments that expose animals to treatment conditions directly after fertilization to imitate natural exposure and allow detection of stage-specific, early-life effects.

Aside from the interactive effect of P_{CO_2} and P_{O_2} on embryos, our experiments showed no other metabolic responses of *M. menidia* early life stages to seawater acidification. A review of fish responses to CO_2 indicated that routine metabolism, the response that we measured, might not be as sensitive to CO_2 as other measures, such as standard or active metabolism (Heuer and Grosell, 2014). Additionally, previous experiments exposing *M. menidia* to elevated P_{CO_2} have yielded highly variable results for growth and survival of embryos and larvae, potentially due to genetic variability or maternal egg provisioning (Baumann et al., 2018; Snyder et al., 2018). The high inherent variability in *M. menidia* responses to high P_{CO_2} suggests that populations will be resilient and able to adapt to acidified conditions. Based on our experiments, this species is metabolically tolerant of isolated high P_{CO_2} , but the negative synergistic response of embryos to

high P_{CO_2} and low P_{O_2} , conditions that co-occur in estuaries, demonstrates the potential for stage-specific responses and importance of considering multiple co-occurring stressors.

1.5. References

- Baumann, H. (2019). Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.* 97, 399-408.
- Baumann, H., and Smith, E. M. (2018). Quantifying Metabolically Driven pH and Oxygen Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuar. Coasts.* 41, 1102-1117.
- Baumann, H., Talmage, S. C. and Gobler, C. J. (2012). Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nat. Clim. Change.* 2, 38-41.
- Baumann, H., Cross, E. L. and Murray, C. S. (2018). Robust quantification of fish early life CO₂ sensitivities via serial experimentation. *Biol. Lett.* 14, 20180408.
- Bayliff, W. H. (1950). *The life history of the silverside Menidia menidia (Linnaeus)*. Solomons Island, Maryland: State of Maryland Board of Natural Resources, Department of Research and Education.
- Brauner, C. J., and Randall, D. J. (1996). The Interaction Between Oxygen and Carbon Dioxide Movements in Fishes. *Comp. Biochem. Physiol.* 113A, 83-90.
- Browman, H. I. (2016). Applying organized scepticism to ocean acidification research. *ICES J. Mar. Sci.* 73(3), 529-536.
- Cadigan, K. M. and Fell, P. E. (1985). Reproduction, Growth and Feeding Habits of *Menidia menidia* (Atherinidae) in a Tidal Marsh-estuarine System in Southern New England. *Copeia.* 1985(1), 21-26.
- Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., Chou, W.-C., Zhai, W., Hollibaugh, J. T., Wang, Y., et al. (2011). Acidification of subsurface coastal waters enhanced by eutrophication. *Nat. Geosci.* 4, 766-770.
- Calosi, P., Bilton, D. T., Spicer, J. I., Votier, S. C. and Atfield, A. (2010). What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *J. Anim. Ecol.* 79, 194-204.
- Chambers, R. C. and Trippel, E. A. (1997). *Early Life History and Recruitment in Fish Populations*. New York: Chapman & Hall.
- Chambers, R. C., Candelmo, A. C., Habeck, E. A., Poach, M. E., Wieczorek, D., Cooper, K. R., Greenfield, C. E. and Phelan, B. A. (2014). Effects of elevated CO₂ in the early life stages of summer flounder, *Paralichthys dentatus*, and potential consequences of ocean acidification. *Biogeosciences.* 11, 1613-1626.

- Clark, T. D., Raby, G. D., Roche, D. G., Binning, S. A., Speers-Roesch, B., Jutfelt, F. and Sundin, J. (2020). Ocean acidification does not impair the behavior of coral reef fishes. *Nature*. 577, 370-375.
- Conover, D. O. and Ross, M. R. (1982). Patterns in Seasonal Abundance, Growth and Biomass of the Atlantic Silverside, *Menidia menidia*, in a New England Estuary. *Estuaries*. 5(4), 275-286.
- Conover, D. O. and Munch, S. B. (2002). Sustaining Fisheries Yields Over Evolutionary Time Scales. *Science*. 297, 94-96.
- Cottingham, K. L., Lennon, J. T. and Brown, B. L. (2005). Knowing when to draw the line: designing more informative ecological experiments. *Front. Ecol. Environ.* 3(3), 145-152.
- Couturier, C. S., Stecyk, J. A. W., Rummer, J. L., Munday, P. L. and Nilsson, G. E. (2013). Species-specific effects of near-future CO₂ on the respiratory performance of two tropical prey fish and their predator. *Comp. Biochem. Physiol. A*. 166, 482-489.
- Cross, E. L., Murray, C. S. and Baumann, H. (2019). Diel and tidal pCO₂ × O₂ fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.* 9, 18146. doi:10.1038/s41598-019-53930-8.
- Dahlke, F. T., Leo, E. A., Mark, F. C., Pörtner, H.-O., Bickmeyer, U., Frickenhaus, S. and Storch, D. (2017). Effects of ocean acidification increase embryonic sensitivity to thermal extremes in Atlantic cod, *Gadus morhua*. *Global Change Biol.* 23, 1499-1510.
- Dahlke, F. T., Wohlrab, S., Butzin, M., and Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*. 369, 65-70.
- Davis, B. E., Flynn, E. E., Miller, N. A., Nelson, F. A., Fangue, N. A., and Todgham, A. E. (2017). Antarctic emerald rockcod have the capacity to compensate for warming when uncoupled from CO₂-acidification. *Glob. Change Biol.* 24, e655-e670.
- Davis, B. E., Komoroske, L. M., Hansen, M. J., Poletto, J. B., Perry, E. N., Miller, N. A., Ehlman, S. M., Wheeler, S. G., Sih, A., Todgham, A. E. and Fangue, N. A. (2018). Juvenile rockfish show resilience to CO₂-acidification and hypoxia across multiple biological scales. *Conserv. Physiol.* 6, coy038. doi:10.1093/conphys/coy038.
- DePasquale, E., Baumann, H. and Gobler, C. J. (2015). Vulnerability of early life stage Northwest Atlantic forage fish to ocean acidification and low oxygen. *Mar. Ecol. Prog. Ser.* 523, 145-156.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. (2009). Ocean Acidification: The Other CO₂ Problem. *Annu. Rev. Mar. Sci.* 1, 169-192

- Dulvy, N. K., Sadovy, Y. and Reynolds, J. D. (2003). Extinction vulnerability in marine populations. *Fish Fish.* 4, 25-64.
- Flynn, E. E. and Todgham, A. E. (2018). Thermal windows and metabolic performance curves in a developing Antarctic fish. *J. Comp. Physiol. B.* 188, 271-282.
- Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., Folkvord, A., Piatkowski, U., Reusch, T. B. H. and Clemmesen, C. (2012). Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nat. Clim. Change.* 2, 42-46.
- Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In *Fish Physiology, Vol. 6, Environmental Relations and Behavior* (ed. W. S. Hoar and D. J. Randall), pp. 1-98. New York: Academic Press.
- García, H. E. and Gordon, L. I. (1992). Oxygen solubility in seawater: Better fitting equations. *Limnol. Oceanogr.* 37, 1307-1312.
- Gobler, C. J. and Baumann, H. (2016). Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. *Biol. Lett.* 12, 20150976. <http://dx.doi.org/10.1098/rsbl.2015.0976>.
- Gobler, C. J., Merlo, L. R., Morrell, B. K. and Griffith, A. W. (2018). Temperature, Acidification, and Food Supply Interact to Negatively Affect the Growth and Survival of the Forage Fish, *Menidia beryllina* (Inland Silverside), and *Cyprinodon variegatus* (Sheepshead Minnow). *Front. Mar. Sci.* 5, 86. doi: 10.3389/fmars.2018.0086.
- Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I., et al. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO₂ in Atlantic halibut. *J. Exp. Biol.* 217, 711-717.
- Gruber, N. (2011). Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Phil. Trans. R. Soc. A.* 369, 1980-1996.
- Gunderson, A. R., Armstrong, E. J. and Stillman, J. H. (2016). Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment. *Annu. Rev. Mar. Sci.* 8, 357-78.
- Hancock, J. R., and Place, S. P. (2016). Impact of ocean acidification on the hypoxia tolerance of the woolly sculpin, *Clinocottus analis*. *Conserv. Physiol.* 4, cow040. doi: 10.1093/conphys/cow040.
- Harvey, B. P., Gwynn-Jones, D. and Moore, P. J. (2013). Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecol. Evol.* 3(4), 1016-1030.

- Havenhand, J., Dupont, S. and Quinn, G. P. (2010). Designing ocean acidification experiments to maximize inference. In *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (ed. U. Riebesell, V. J. Fabry, L. Hansson and J.-P. Gattuso), pp. 67-136. Luxembourg: Publications Office of the European Union Luxembourg.
- Hebbali, A. (2020). Olsrr: Tools for Building OLS Regression Models. R package version 0.5.3. <https://CRAN.R-project.org/package=olsrr>.
- Heuer, R. M. and Grosell, M. (2014). Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 307, R1061-R1084.
- Hollowed, A. B., Barange, M., Beamish, R. J., Brander, K., Cochrane, K., Drinkwater, K., Foreman, M. G. G., Hare, J. A., Holt, J. Ito, S. et al. (2013). Projected impacts of climate change on marine fish and fisheries. *ICES J. Mar. Sci.* 70(5), 1023-1037.
- Houde, E. D. (1987). Fish Early Life Dynamics and Recruitment Variability. *Am. Fish. Soc. Symp.* 2, 17-29.
- Hurlbert, S. H. (2004). On misinterpretations of pseudoreplication and related matters: a reply to Oksanen. *Oikos*, 104, 591-597.
- Hurst, T. P. and Conover, D. O. (1998). Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): size-dependent patterns and effects on recruitment. *Can. J. Fish. Aquat. Sci.* 55, 1122-1130.
- Intergovernmental Panel on Climate Change. (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley). Cambridge, UK, and New York, NY, USA: Cambridge University Press.
- Ishimatsu, A., Hayashi, M. and Kikkawa, T. (2008). Fishes in high-CO₂, acidified oceans. *Mar. Ecol. Prog. Ser.* 373, 295-302.
- Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G. E., Metcalfe, N. B., Hickey, A. J. R., et al. (2018). Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *J. Exp. Biol.* 221, jeb169615. doi: 10.1242/jeb.162693.
- Kamler, E. (1992). *Early Life History of Fish: An Energetics Approach*. New York: Chapman & Hall.
- Keeling, R. F., Körtzinger, A., and Gruber, N. (2010). Ocean Deoxygenation in a Warming World. *Annu. Rev. Mar. Sci.* 2, 463-493.

- Kooijman, S. A. L. M. (2009). *Dynamic Energy Budget theory for metabolic organisation*. Cambridge: Cambridge University Press.
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M. and Gattuso, J.-P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biol.* 19, 1884-1896.
- Lefevre, S. (2016). Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO₂ and their interaction. *Conserv. Physiol.* 4(1), cow009. doi: 10.1093/conphys/cow009.
- Lifavi, D. M., Targett, T. E., and Greco, P. A. (2017). Effects of diel-cycling hypoxia and acidification on juvenile weakfish *Cynoscion regalis* growth, survival, and activity. *Mar. Ecol. Prog. Ser.* 564, 163-174.
- Lynch, H. J., Rhainds, M., Calabrese, J. M., Cantrell, S., Cosner, C. and Fagan, W. F. (2014). How climate extremes – not means – define a species’ geographic range boundary via a demographic tipping point. *Ecol. Monogr.* 84(1), 131-149.
- Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A. (2013). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Mar. Biol.* 160, 1875-1888.
- Middaugh, D. P., Hemmer, M. J. and Goodman, L. (1987). *Methods for Spawning, Culturing and Conducting Toxicity-Tests with Early Life Stages of four Atherinid Fishes: The Inland Silverside, Menidia beryllina, Atlantic silverside, M. menidia, Tidewater silverside, M. peninsulae and California grunion, Leuresthes tenuis*. Gulf Breeze, FL: United States Environmental Protection Agency.
- Miller, S. H., Breitburg, D. L., Burrell, R. B., and Keppel, A. G. (2016). Acidification increases sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.* 549, 1-8.
- Montgomery, D. W., Simpson, S. D., Engelhard, G. H., Birchenough, S. N. R., and Wilson, R. W. (2019). Rising CO₂ enhances hypoxia tolerance in a marine fish. *Sci. Rep.* 9, 15152.
- Morrell, B. K. and Gobler, C. J. (2020). Negative Effects of Diurnal Changes in Acidification and Hypoxia on Early-Life Stage Estuarine Fishes. *Diversity*. 12, 25. doi: 10.3390/d12010025.
- Munday, P. L., Dixson, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V. and Doving, K. B. (2009a). Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl. Acad. Sci. U S A.* 106(6), 1848-1852.

- Munday, P. L., Crawley, N. E. and Nilsson, G. E. (2009b). Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Mar. Ecol. Prog. Ser.* 388, 235-242.
- Munday, P. L., Dixon, D. L., McCormick, M. I., Meekan, M., Ferrari, M. C. O. and Chivers, D. P. (2010). Replenishment of fish populations is threatened by ocean acidification. *Proc. Natl. Acad. Sci.* 107(29), 12930-12934.
- Murray, C. S. and Baumann, H. (2018). You Better Repeat It: Complex CO₂ × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*. 10, 69. doi: 10.3390/d10030069.
- Murray, C. S., Malvezzi, A., Gobler, C. J. and Baumann, H. (2014). Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.* 504, 1-11.
- Murray, C. S., Fuiman, L. A. and Baumann, H. (2017). Consequences of elevated CO₂ exposure across multiple life stages in a coastal forage fish. *ICES J. Mar. Sci.* 74(4), 1051-1061.
- Murray, C. S., Wiley, D. and Baumann, H. (2019). High sensitivity of a keystone forage fish to elevated CO₂ and temperature. *Conserv. Physiol.* 7(1), coz084. doi: 10.1093/conphys/coz084.
- Nilsson, G. E. and Östlund-Nilsson, S. (2008). Does size matter for hypoxia tolerance in fish? *Biol. Rev.* 83, 173-189.
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., et al. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*. 437, 681-686.
- Paerl, H. W. (2006). Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: Interactive effects of human and climatic perturbations. *Ecol. Eng.* 26(1), 40-54.
- Peck, M. A., and Moyano, M. (2016). Measuring respiration rates in marine fish larvae: challenges and advances. *J. Fish Biol.* 88, 173-205.
- Pihl, L., Baden, S. P., and Diaz, R. J. (1991). Effects of periodic hypoxia on distribution of demersal fish and crustaceans. *Mar. Biol.* 108, 349-360.
- Pimentel, M. S., Faleiro, F., Dionísio, G., Repolho, T., Pousão-Ferreira, P., Machado, J. and Rosa, R. (2014). Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. *J. Exp. Biol.* 217, 2062-2070.
- Pollock, M. S., Clarke, L. M. J., and Dubé, M. G. (2007). The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environ. Rev.* 15, 1-14.

- Pörtner, H.-O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213, 881-893.
- Pörtner, H.-O. (2012). Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Mar. Ecol. Prog. Ser.* 470, 273-290.
- Pörtner, H.-O. and Peck, M. A. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J. Fish Biol.* 77(8), 1745-1779.
- Pörtner, H.-O., Langenbuch, M. and Michaelidis, B. (2005). Synergistic effects of temperature extremes, hypoxia, and increases in CO₂ on marine animals: From Earth history to global change. *J. Geophys. Res.* 110, C9S10.
- Pörtner, H.-O., Bock, C. and Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *J. Exp. Biol.* 220, 2685-2696.
- R Core Team (2020). R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Richards, J. G. (2009). Metabolic and molecular responses of fish to hypoxia. In *Fish Physiology, Vol. 27, Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 443-485. San Diego: Academic Press.
- Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E. and Morel, F. M. M. (2000). Reduced calcification of marine plankton in response to increased atmospheric CO₂. *Nature*. 407, 364-367.
- Rijnsdorp, A. D., Peck, M. A., Engelhard, G. H., Möllmann, C., and Pinnegar, J. K. (2009). Resolving the effect of climate change on fish populations. *ICES J. Mar. Sci.* 66, 1570-1583.
- Rombough, P. J. (1988). Respiratory Gas Exchange, Aerobic Metabolism, and Effects of Hypoxia during Early Life. In *Fish Physiology, Vol. 11, The Physiology of Developing Fish, Part A: Eggs and Larvae* (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego: Academic Press.
- Rosa, R., Baptista, M., Lopes, V. M., Pegado, M. R., Paula, J. R., Trübenbach, K., Leal, M. C., Calado, R. and Repolho, T. (2014). Early-life exposure to climate change impairs tropical shark survival. *Proc. R. Soc. B.* 281, 20141738. <http://doi.org/10.1098/rspb.2014.1738>.
- Rummer, J. L., Stecyk, J. A. W., Couturier, C. S., Watson, S.-A., Nilsson, G. E. and Munday, P. L. (2013). Elevated CO₂ enhances aerobic scope of a coral reef fish. *Conserv. Physiol.* 1(1), 1-7. doi: 10.1093/conphys/cot023.

- Silkin, Y. A. and Silkina, E. N. (2005). Effect of Hypoxia on Physiological-Biochemical Blood Parameters in Some Marine Fish. *J. Evol. Biochem. Physiol.* 41, 527-532.
- Snyder, J. T., Murray, C. S. and Baumann, H. (2018). Potential for maternal effects on offspring CO₂ sensitivities in the Atlantic silverside (*Menidia menidia*). *J. Exp. Mar. Biol. Ecol.* 449, 1-8.
- Twinaime, S., Fitzgibbon, Q. P., Hobday, A. J., Carter, C. G. and Pecl, G. T. (2019). Multiple measures of thermal performance of early stage eastern rock lobster in a fast-warming ocean region. *Mar. Ecol. Prog. Ser.* 624, 1-11.
- Wallace, R. B., Baumann, H., Gear, J. S., Aller, R. C. and Gobler, C. J. (2014). Coastal ocean acidification: The other eutrophication problem. *Estuar. Coast. Shelf Sci.* 148, 1-13.
- Wells, R. M. G. (2009). Blood-gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. In *Fish Physiology, Vol. 27, Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 255-299. San Diego: Academic Press.
- Weltzien, F.-A., Døving, K. B., and Carr, W. E. S. (1999). Avoidance reaction of yolk-sac larvae of the inland silverside *Menidia beryllina* (Atherinidae) to hypoxia. *J. Exp. Biol.* 202, 2869-2876.
- Zimmer, A. M., Mandic, M., Rourke, K. M. and Perry, S. F. (2020). Breathing with fins: do the pectoral fins of larval fishes play a respiratory role? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 318, R89-R97.

1.6. Figures

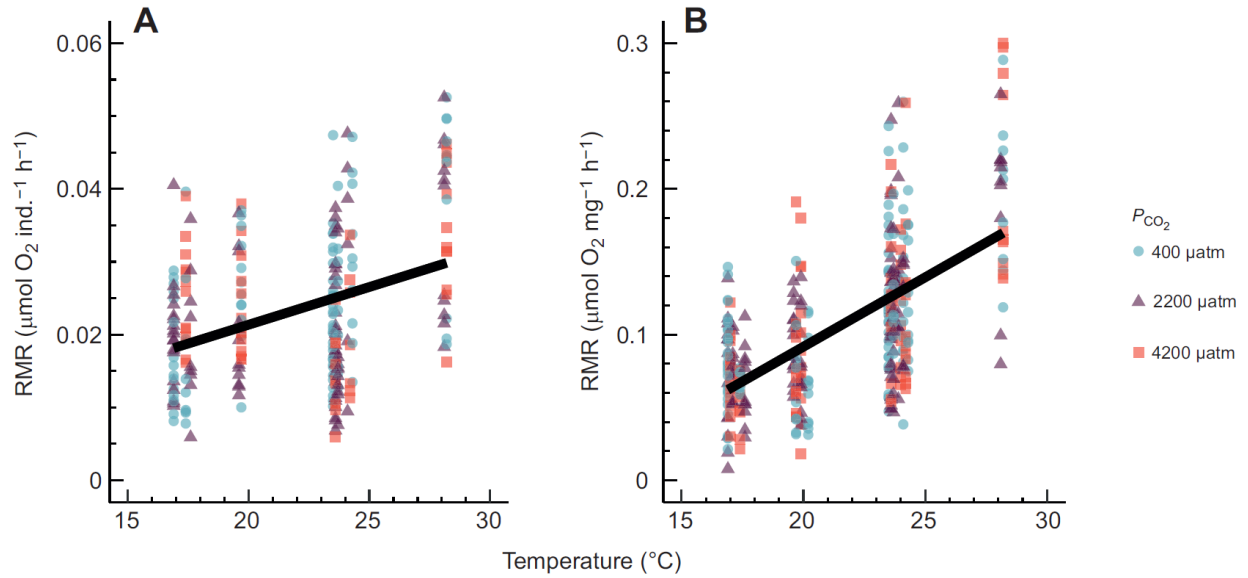


Figure 1.1. Temperature- and P_{CO_2} -dependent routine metabolic rates (RMR) of *M. menidia* embryos and larvae. (A) Whole-body RMR ($\mu\text{mol O}_2 \text{ individual}^{-1} \text{ h}^{-1}$) of embryos and (B) mass-specific RMR ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ h}^{-1}$) of newly hatched larvae from experiments 1-4. Regression lines are fitted to the metabolic rates as a function of temperature to illustrate the significant effect of temperature (MLR, embryos: $F_{3,247} = 10.96$, $p < 0.001$; larvae: $F_{3,364} = 69.87$, $p < 0.001$). Sample sizes for each temperature and P_{CO_2} treatment combination are $n=10-46$ for embryos and $n=9-68$ for larvae.

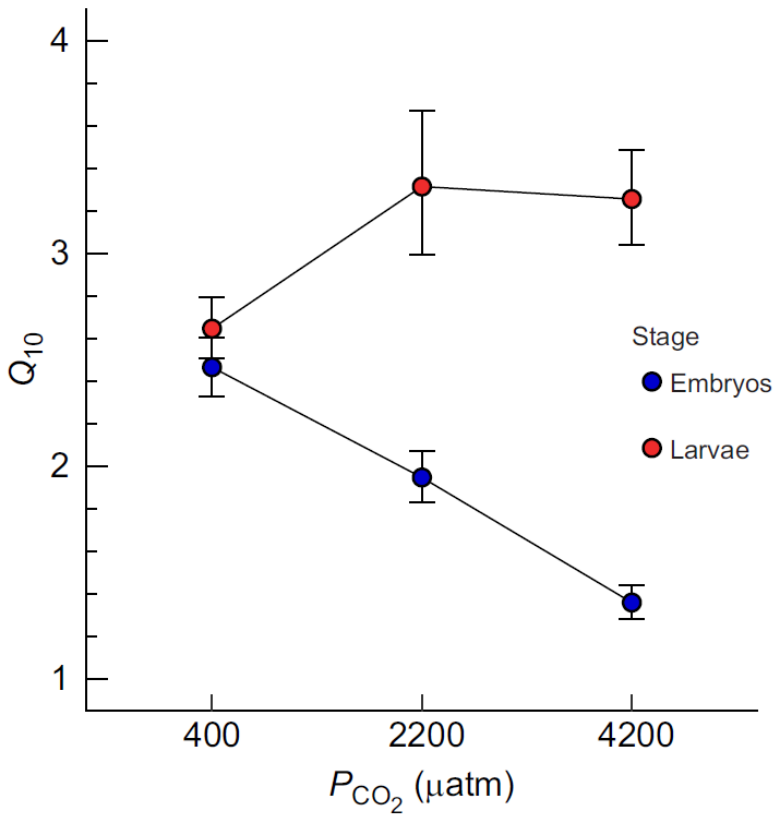


Figure 1.2. The effect of P_{CO_2} on Q_{10} of routine metabolic rates in *M. menidia* embryos and larvae. Bootstrapped mean Q_{10} values of *M. menidia* embryos and larvae calculated from routine metabolic rates at 17°C and 28°C, from experiments 1-4. Error bars indicate bootstrapped 95% confidence intervals, and sample sizes for each P_{CO_2} level are $n=10-27$ for embryos and $n=9-41$ for larvae.

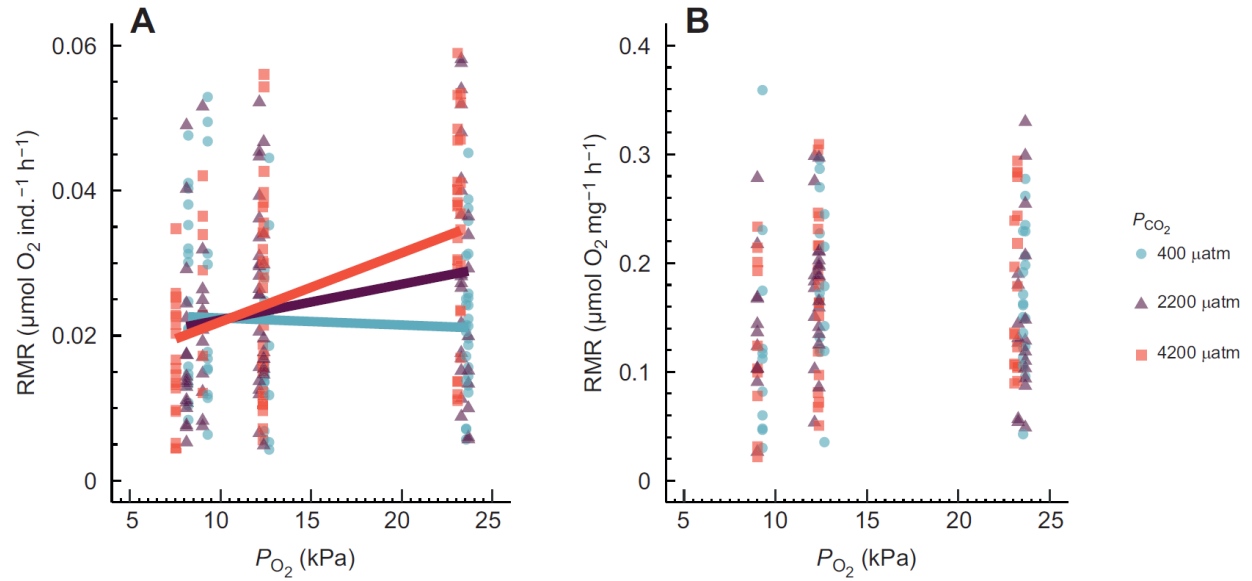


Figure 1.3. P_{O_2} - and P_{CO_2} -dependent routine metabolic rates of *M. menidia* embryos and larvae. (A) Whole-body RMR ($\mu\text{mol O}_2 \text{ individual}^{-1} \text{ h}^{-1}$) of embryos and (B) mass-specific RMR ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ h}^{-1}$) of newly hatched larvae from experiments 5-6. Regression lines are fitted to embryonic metabolic rates (A) as a function of P_{O_2} within each P_{CO_2} treatment to illustrate the significant P_{CO_2} and P_{O_2} interaction (MLR, $F_{3,258} = 7.96$, $p = 0.005$). No regression lines are shown for larvae (B) because there were no significant effects (MLR, $F_{3,142} = 0.325$, $p > 0.05$). Sample sizes in each P_{O_2} and P_{CO_2} combination are $n=8-31$ for embryos and $n=10-22$ for larvae.

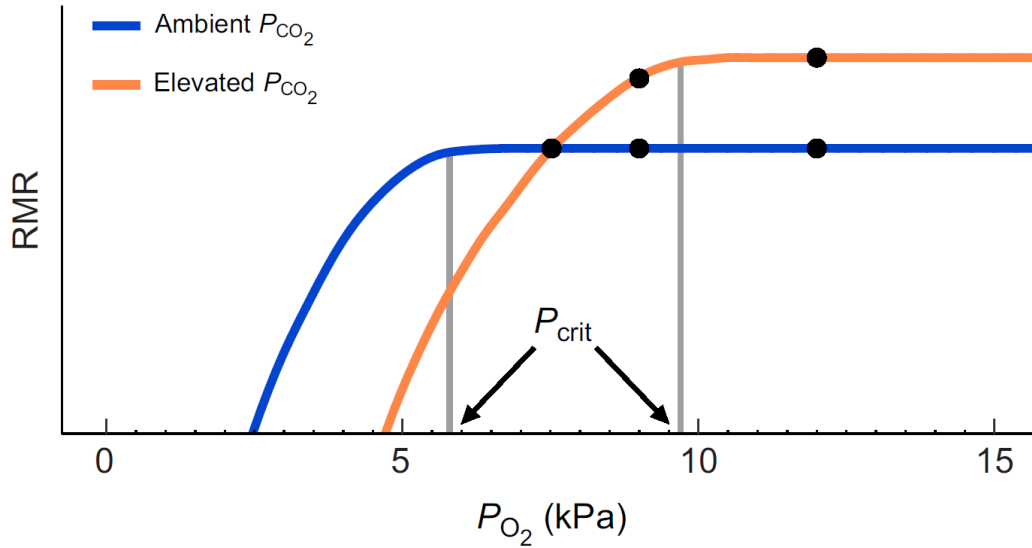


Figure 1.4. Conceptual diagram of the relationship between P_{O_2} and routine metabolic rate of *M. menidia* embryos in ambient and elevated P_{CO_2} . Hypothesized shifts in the relationship between embryonic RMR and P_{O_2} are shown for elevated (orange) versus ambient (blue) P_{CO_2} . Our results (measured at the P_{O_2} levels marked by black dots) suggest that P_{CO_2} can influence both the critical oxygen partial pressure (P_{crit} , gray lines) and the oxygen-independent RMR. At higher P_{O_2} levels, RMR increases with P_{CO_2} , potentially due to increased metabolic demand. As P_{O_2} decreases, embryonic RMR reaches P_{crit} and becomes oxygen-dependent at a higher P_{O_2} level in acidified than in ambient P_{CO_2} conditions. Low intracellular red blood cell pH caused by high P_{CO_2} can be expected to reduce hemoglobin- O_2 affinity (Bohr effect) and make embryonic RMR less hypoxia-resistant, which could manifest as an increase in P_{crit} for embryos in elevated P_{CO_2} . See text for more information.

1.7. Tables

Table 1.1. Overview of target levels for P_{CO_2} , temperature, and oxygen partial pressure (P_{O_2}) for six experiments for which respirometry was conducted on embryos (E) and newly hatched larvae (L) of *Menidia menidia*.

Experiment	Fertilization Date	P_{CO_2} (μ atm)	Temperature ($^{\circ}$ C)	P_{O_2} (kPa)	Stage
1	4/22/2016	400, 2200	17, 24	21-23	E, L
2	5/3/2016	400, 2200, 4200	17, 20, 24	21-23	L
3	5/19/2016	400, 2200, 4200	17, 20, 24	21-23	E, L
4	5/26/2017	400, 2200, 4200	24, 28	21-23	E, L
5	5/9/2017	400, 2200, 4200	24	7.5, 12.0, 23.0	E, L*
6	6/9/2017	400, 2200, 4200	24	9.0, 12.0, 23.0	E, L

*In Experiment 5, respirometry was only done on larvae from the 23.0 and 12.0 kPa P_{O_2} treatments due to low hypoxic hatch survival.

Table 1.2. Age at sampling (equivalent to exposure time) in days-post-fertilization (dpf) and mean routine metabolic rates (\pm s.e.m.) of *M. menidia* embryos and larvae across P_{CO_2} , temperature, and P_{O_2} treatments.

Temp (°C)	P_{O_2} (kPa)	Age at Embryonic Sampling (dpf)	Age at Larval Sampling (dpf)	P_{CO_2} (μ atm)	Embryonic Sample Size	Mean Embryonic RMR (\pm s.e.m.) (μ mol O_2 h ⁻¹)	Larval Sample Size	Mean Larval RMR (\pm s.e.m.) (μ mol O_2 mg ⁻¹ h ⁻¹)
17	>20	11-12	14-15	400	26	0.016(\pm 0.002)	41	0.078(\pm 0.004)
				2200	27	0.020(\pm 0.001)	35	0.071(\pm 0.005)
				4200	12	0.026(\pm 0.002)	24	0.066(\pm 0.005)
20	>20	8	10-11	400	12	0.027(\pm 0.002)	23	0.071(\pm 0.007)
				2200	12	0.020(\pm 0.002)	26	0.088(\pm 0.006)
				4200	13	0.024(\pm 0.002)	24	0.087(\pm 0.009)
24	>20	5	6-7	400	55	0.024(\pm 0.001)	65	0.126(\pm 0.006)
				2200	46	0.021(\pm 0.002)	68	0.118(\pm 0.006)
				4200	16	0.017(\pm 0.002)	33	0.118(\pm 0.009)
28	>20	4	5	400	11	0.039(\pm 0.004)	9	0.196(\pm 0.018)
				2200	11	0.035(\pm 0.004)	10	0.191(\pm 0.018)
				4200	11	0.034(\pm 0.002)	10	0.207(\pm 0.022)
24	23.0	5	6-7	400	26	0.022(\pm 0.002)	21	0.165(\pm 0.013)
				2200	28	0.028(\pm 0.003)	19	0.148(\pm 0.018)
				4200	29	0.034(\pm 0.003)	19	0.174(\pm 0.017)
24	12.0	5-6	7-8	400	26	0.018(\pm 0.002)	15	0.182(\pm 0.019)
				2200	31	0.026(\pm 0.002)	22	0.179(\pm 0.013)
				4200	30	0.025(\pm 0.002)	18	0.173(\pm 0.019)
24	9.0	7	9	400	13	0.025(\pm 0.004)	11	0.126(\pm 0.030)
				2200	14	0.022(\pm 0.003)	11	0.142(\pm 0.020)
				4200	8	0.027(\pm 0.004)	10	0.130(\pm 0.024)
24	7.5	7	-	400	18	0.025(\pm 0.003)	-	-
				2200	17	0.018(\pm 0.003)	-	-
				4200	19	0.017(\pm 0.002)	-	-

Dashes (-) indicates treatments for which too few embryos survived to hatching for larval respirometry to be done.

2. Metabolic response of the coastal fish *Menidia menidia* reared in elevated CO₂ to progressive hypoxia

2.1. Introduction

Acidification of coastal waters and estuaries is both gradually increasing as the oceans absorb atmospheric carbon dioxide (CO₂) emissions (IPCC, 2022; Doney et al., 2009) and eutrophication intensifies globally (Diaz and Rosenberg, 2008) and fluctuating on diel and seasonal time scales due to anthropogenic and oceanographic factors that increase community respiration (Cai et al., 2011). The latter also depletes oxygen, and consequentially hypoxia and acidification often co-occur (Wallace and Gobler, 2021). In Long Island Sound and the smaller estuaries connected to it, for example, nutrient loading due to dense human populations on land combines with restricted circulation and high temperatures to create extreme temperature, oxygen, and pH fluctuations in the summer (Baumann et al., 2015) as well as persistent acidification and hypoxia due to stratification and accumulation of organic matter (Wallace and Gobler, 2021). This makes environmental conditions stressful to marine organisms, many of which use estuaries, as critical nursery habitat (Beck et al., 2001). For the species and life stages that do or will experience such conditions, it is critical to understand the physiological responses to current and future combinations of acidification and hypoxia, which are intensifying over time (Keeling et al., 2010; Gruber, 2011).

The responses of fishes to seawater acidification by carbon dioxide (CO₂) are typically subtle and variable, particularly in species adapted to estuarine waters that have fluctuating CO₂ and pH levels (Kroeker et al., 2010; Kroeker et al., 2013; Heuer and Grosell, 2014; Cattano et al., 2018; Baumann, 2019). Unlike the relatively strong and straightforward mode by which moderately elevated CO₂ can impact growth and survival through calcification in shellfish (Orr et al., 2005), fish responses tend to be low in magnitude (Murray et al., 2017; Cattano et al., 2018), life stage-dependent (Harvey et al., 2013; Ishimatsu et al., 2008), and facilitated by uncertain and variable mechanisms (Kroeker et al., 2010; Esbaugh, 2018). Often the presence of an additional stressor, such as temperature or hypoxia, can elicit a response when there otherwise was none or interactively enhance an existing response (Pörtner et al., 2005; Crain et al., 2008; Kroeker et al., 2013; Melzner et al., 2013). In such cases the underlying physiological mechanisms can shed light on the meaning of whole-organism responses, or lack thereof, and

explain what makes some species more tolerant of global change than others (Melzner et al., 2009). Quantifying physiological processes by which fish respond to multiple stressors can also aid understanding of whether the responses will facilitate tolerance and adaptation over time or lead to population decline (Wittmann and Pörtner, 2013). Estimates of the energetic costs of responding to stressors, such as metabolic rates and enzyme activity, can be useful for predicting or explaining changes to other aspects of the energy budget that have population-level implications, such as reproduction, growth, and survival to recruitment stage (National Research Council, 2005; Watson et al., 2020).

In recent years, increasing discussions and developments have gone into quantitative descriptors of hypoxia tolerance in ectotherms, particularly in the context of metabolic responses to environmental change (Wood, 2018; Regan et al., 2019; Seibel and Deutsch, 2020; Zhang et al., 2022). A commonly used measure of hypoxia sensitivity is the critical oxygen partial pressure (P_{crit}) below which a given measure of metabolic rate becomes oxygen-dependent, or oxyregulation switches to oxyconformity (Richards, 2011). Above P_{crit} , it is typically assumed that most oxygen demands are being met with the ambient environmental O_2 . Although P_{crit} does not strictly mark the onset of anaerobic metabolism (Wood, 2018), below P_{crit} there are demands that must be met with anaerobic metabolism or simply go unmet (Pörtner and Grieshaber, 1993). P_{crit} can be a useful way to examine the relationship between hypoxia and ocean acidification and understand the underlying mechanisms of their interactive effects. Ocean acidification may affect the ability to tolerate another stressor such as hypoxia, despite having small or no effects on biological responses when all other conditions are optimal, through physiological processes that go undetected when stressors are not combined. Measurement of embryonic routine metabolism (MO_2) in the Atlantic silverside, *Menidia menidia*, demonstrated that while pCO_2 alone has little effect, MO_2 becomes increasingly oxygen-dependent in elevated pCO_2 levels (Schwemmer et al., 2020). This suggests a shift from oxyregulation to oxyconformity indicative of P_{crit} being reached at higher oxygen levels than in ambient pCO_2 . Effects of acidification on hypoxia response may be stage- and species-dependent, as larvae from Schwemmer et al. (2020) did not exhibit the response embryos did, and studies on juvenile European sea bass (Montgomery et al., 2019) and adult woolly sculpin (Hancock and Place, 2016) showed reduced and increased P_{crit} , respectively, under elevated pCO_2 . Impacts of high pCO_2 on P_{crit} and other measures of hypoxia response may result from a reduction in blood pH, which reduces affinity and capacity of oxygen to bind to

hemoglobin via the Bohr and Root effects, respectively (Brauner and Randall, 1996; Wells, 2009). P_{crit} can also be useful in setting hypoxia thresholds or indicators used by marine resource managers. In a management and policy context, hypoxia is often somewhat arbitrarily set as 2 or 3 mg L⁻¹ dissolved oxygen but a more ecologically and biologically relevant threshold could be set using knowledge about thresholds of hypoxia response in local organisms, and further enhanced with information about how coinciding stressors affect this threshold.

M. menidia is an extensively studied estuarine species that has shown variable, but broadly tolerant, responses to ocean acidification (Baumann et al., 2018). The early life stages, which reside in estuaries during summer, have had reduced growth and survival in elevated pCO₂ in some (Murray et al., 2014; Murray et al., 2017) but not all experiments (Baumann et al., 2018). Multistressor experiments have shown interactive effects of pCO₂ and hypoxia on hatching and metabolism (Cross et al., 2019; Schwemmer et al., 2020) and of pCO₂ and temperature on growth and survival (Murray and Baumann, 2018). *M. menidia* lives in estuarine habitats with great fluctuations in CO₂, oxygen, and temperature, and because of their spring and summer spawning season, the early life stages experience the most severe of these conditions while having less mobility than adults (Cadigan and Fell, 1985). The mechanistic responses behind these interactive effects can explain their tolerance and help establish its limits.

Here we use respirometry to quantify the metabolic response of *M. menidia* early life stages reared in elevated CO₂ to acute progressive hypoxia. Over the course of two experiments, we reared *M. menidia* offspring of wild-caught adults in various pCO₂ levels and measured the metabolic rates of embryos, newly hatched larvae, and 5-day post-hatch (dph) larvae in closed respirometers as hypoxia intensified due to oxygen consumption. The objective was to quantify the relationship between routine metabolic rate (MO₂) and ambient pO₂, then calculate P_{crit} and analyze how the MO₂-oxygen relationship changes under elevated pCO₂. We hypothesized that at high oxygen levels the metabolic rates would be elevated under high pCO₂, but only in embryos. We also hypothesized that high pCO₂ would increase P_{crit} based on the previously recorded increase in oxygen-dependence at high pCO₂ (Schwemmer et al., 2020), work on other species that exhibit this response (Cruz-Neto and Steffensen, 1997; Hancock and Place, 2016), and the changes to hemoglobin-oxygen binding affinity when blood pH is low (Wells, 2009).

2.2. Methods

2.2.1. *Experimental Design and Treatments*

Two experiments were conducted simultaneously in June 2021. In each experiment, three pCO₂ levels were used and replicated across three rearing tanks each (n=3). Target pCO₂ levels were ambient (450 µatm), moderately elevated (2000 µatm), and high (4000 µatm; Figure 2.1) to easily compare with previous work on this species using similar treatment levels. To accommodate logistical constraints on respirometry equipment availability, Experiments 1 and 2 were maintained at slightly different temperatures (24 and 22°C, respectively) which allowed us to stagger developmental timepoints for respirometry and measure treatment effects at two different temperatures common in local estuaries in June. Measured mean treatment and water quality parameters for all tanks are reported in Tables 2.1 and S2.1.

Both experiments were conducted in closed, recirculating systems at two separate Stony Brook University laboratory facilities: Experiment 1 in Dana Hall, Stony Brook University, Stony Brook, NY, and Experiment 2 in Flax Pond Marine Laboratory, Old Field, NY. Water equilibration baths were filled with seawater that was pumped from a nearby estuary and filtered to 5 µm. All equilibration baths were bubbled with laboratory air using a 50W pump (Air Pump 1110 GPH, Vivosun, Ontario, CA) and air stones. In each elevated CO₂ water bath CO₂ was added using a gas proportioner in Experiment 1 (Masterflex® Variable-Area Flowmeter, Cole-Parmer, Vernon Hills, IL, USA) and solenoid valve controlled by an Apex AquaController unit in Experiment 2 (Neptune Systems, Morgan Hill, CA, USA). The pH in each water bath was monitored continuously through the Apex Fusion online interface with pH probes (Double Junction pH Probe, Neptune Systems, Morgan Hill, CA, USA). CO₂ input was adjusted based on pH readings, manually in Experiment 1 and automatically using Apex Fusion web interface in Experiment 2. Water was kept at target temperatures using various aquarium heaters with integrated thermostats for Experiment 1 or heaters and chillers controlled by the Apex units and the Apex Fusion software in Experiment 2. Water was pumped slowly into rearing containers then allowed to drain back into water baths through 120-µm mesh-covered holes, to mix the water and keep conditions in the rearing containers the same as those in the water equilibration baths.

We manually measured and recorded temperature, pH, salinity, dissolved oxygen, ammonia, nitrate, and nitrite to validate treatment levels and ensure consistent, optimal rearing

conditions. One partial water change was done 2 days after larvae hatched to keep ammonia levels down after feeding began. In both laboratories, lights were set on timers for a 14h:10h light to dark cycle to simulate the approximate daylight hours of June in New York. To quantify the carbonate system in each treatment tank, water samples were taken at the beginning and end of each experiment, with an additional sample collected in the middle of Experiment 2, in 250 mL borosilicate bottles, sealed with vacuum grease, and stored at 4°C. The water samples were analyzed for dissolved inorganic carbon (DIC) and total alkalinity (TA) using a VINDTA 3C system (Marianda, Kiel, Germany). DIC and TA were used to calculate pCO₂ (Figure 2.1), fugacity of CO₂ (*f*CO₂), bicarbonate (HCO₃⁻), and carbonate (CO₃²⁻) for each treatment. The samples from the third sampling date for Experiment 2 were not sealed well and gas exchange affected DIC but not TA, so the carbonate chemistry parameters were calculated using TA and pH from a handheld probe for this date. Water quality measurements for both experiments can be found in Tables 2.1 and S2.1.

2.2.2. *Animal Acquisition and Rearing*

Wild *M. menidia* adults were caught on June 8, 2021, with a 30-meter beach seine in Stony Brook Harbor, New York, USA. Individuals were separated by sex and acclimated to 20°C for 48 hours in two tanks in a dry lab at Stony Brook University. On June 10 gonads were ripe and females' ovaries released hydrated eggs under gentle pressure. Eggs of 5 females were stripped into trays of seawater containing pieces of 1-mm mesh for chorionic filaments to adhere to. Milt of 8 males was stripped into beakers of seawater, which were then poured over the eggs. Trays were gently stirred and allowed to rest for 15 minutes. Then pieces of mesh were cut to make several small pieces containing ~15 viable eggs, and two pieces of mesh were hung with fishing line in each rearing container so that at least 30 viable eggs were randomly distributed into each container in both experimental systems within 3 hours of spawning. Upon hatching, larvae were fed *ad libitum* with newly hatched brine shrimp nauplii, and excess or dead nauplii were siphoned from rearing containers daily. Brine shrimp cysts were chemically decapsulated prior to hatching to enhance hatch success and ensure no hard shells would be consumed by larvae.

2.2.3. *Respirometry and Analysis*

On the day before hatching, the day after hatching (2dph), and 5 days after hatching (5dph), *M. menidia* offspring were sampled for respirometry. Microrespirometry protocols and equipment are described in detail in Schwemmer et al. (2020). Briefly, several fish were removed from each rearing container and sealed in individual microrespirometers, or well plates equipped with optical oxygen sensors (Loligo Systems). These plates were sealed with parafilm and a silicone membrane and compressed with several acrylic sheets in a temperature control chamber to create an air- and water-proof seal. Oxygen sensors were read with a PreSens sensor dish reader and SDR software v4.0.0. The respirometers were shaded with a box to help minimize stress and activity and because the oxygen sensors require darkness. Respirometry trials were conducted until oxygen fell below 0.1 mg L^{-1} so that metabolic rates could be calculated for a range of ambient oxygen levels including hypoxic ones.

All calculations and statistical analyses were conducted with R software (v4.2.1, R Core Team, 2022), including the packages ‘respirometry’, ‘car’, ‘segmented’, and ‘zoo’ (Birk, 2021; Fox and Weisberg, 2019; Muggeo, 2008; Zeileis and Grothendieck, 2005). Although room temperature was fairly constant, formulas provided by Loligo Systems were used to convert phase data from the microrespirometers into oxygen concentration with data from the integrated temperature sensor, rather than assuming a constant temperature. Oxygen data were subset to exclude changes in temperature at rates greater than $0.5^{\circ}\text{C h}^{-1}$ and extreme changes in oxygen that were likely due to temperature change or air bubbles dissolving or degassing in the wells. Any individuals that died, were injured, escaped, or for which the well was not fully sealed were excluded from analysis so that the final sample size for each treatment and time point combination ranged from 8 to 15. For each treatment there were at least two control (animal free) wells used to estimate microbial respiration.

For each well, the `make_bins()` function from the ‘respirometry’ package was used to set variable bin widths that were widest at high O_2 to produce low-noise routine MO_2 estimates and narrowest at low O_2 to produce high-resolution P_{crit} estimates (Birk, 2021). The maximum bin width was 1/10 of the total trial duration and the minimum bin width was 1/30 of the total trial duration. Then `calc_MO2()` from ‘respirometry’ was used, along with the well volume, to calculate the metabolic rate as the slope of $\text{O}_2 \sim \text{Time}$ for each bin within each individual’s dataset, in $\mu\text{mol O}_2 \text{ h}^{-1}$). Then the average oxygen depletion rate from the control wells were

subtracted from the oxygen consumption rates for each individual from the corresponding rearing tank to account for microbial respiration. For larvae, individuals were preserved then measured so that total length could be converted to dry weight using a formula based on previous *M. menidia* data (H. Baumann, personal communication). Each larval oxygen consumption rate was divided by the calculated dry weight of the individual to obtain mass-specific metabolic rates for each bin ($\mu\text{mol O}_2 \text{ mg dw}^{-1} \text{ h}^{-1}$).

First, P_{crit} was determined using segmented regression with the function `selgmented()` in the ‘segmented’ package in R (Muggeo, 2008). This function allows for multiple breakpoints, which was necessary due to some fluctuations in metabolic rates in high O_2 from random activity and in low O_2 from temporary sharp increases in MO_2 likely related to anaerobic byproducts (Pörtner and Grieshaber, 1993). The segmented regression found the best fitting model and when multiple breakpoints were identified, we visually inspected the model fit with the observed data to identify which breakpoint corresponded to a marked shift from oxygen-independence to relative oxygen-dependence. We also recorded presence or absence of a sharp temporary increase in MO_2 below P_{crit} . If no P_{crit} was identified, either because it was outside of the range of oxygen levels measured or didn’t exist, this was also recorded. For embryos, many individuals did not have a P_{crit} but rather displayed oxyconformity throughout the respirometry trial. Percentages of individuals within each experiment and treatment exhibiting such oxyconformity or a sharp temporary increase in MO_2 below P_{crit} were calculated. To test for significant differences in proportion of individuals displaying either of these patterns across pCO_2 treatments, a test of equal proportions was conducted. Routine metabolism was calculated as the mean of all MO_2 values above P_{crit} or, when no P_{crit} was found, the mean of all MO_2 values above $3 \text{ mg l}^{-1} \text{ O}_2$. This means that the routine metabolism reported here encompassed all levels of non-hypoxia-influenced oxygen consumption for the individual, including variable routine swimming activity. Observing the fish during respirometry to identify periods of rest and activity was not possible with this equipment.

Statistical analyses were performed separately for each experiment and statistical comparisons were not made between temperatures because of the methodological differences in implementing treatment conditions, as described above. Routine MO_2 and P_{crit} data were analyzed for significant differences across treatments and tank effects using a nested ANOVA with the `aov()` function in R, with the formula: $\text{Response} \sim \text{pCO}_2 / \text{factor}(\text{Tank})$. Outliers were

identified using residuals and Cook's distance with a threshold of $4/(N - K - 1)$ where N is the sample size and K is the number of parameters in the model, and these outliers were removed. The assumptions of ANOVA, normality of data and homogeneity of variances, were tested with the Shapiro-Wilk test and Levene test, respectively. A square root transformation was applied to embryonic MO₂ data, and a reciprocal transformation was applied to 2dph larval MO₂ data to correct for significant heterogeneity of variances. In cases where the ANOVA detected significant pCO₂ effects, a Tukey test was used to test for pairwise significant differences between treatment levels. All statistical tests were interpreted with a significance level of $\alpha=0.05$.

2.3. Results

2.3.1. Routine Metabolism

M. menidia routine MO₂ was generally higher in Experiment 1, which was performed at a higher temperature than Experiment 2. Metabolic rates of *M. menidia* embryos in Experiment 2, but not Experiment 1, were significantly affected by pCO₂ (Figure 2.2A; ANOVA, $p<0.0001$, $F_{2,26} = 23.2$). In Experiment 2, which was conducted at 22°C, embryonic MO₂ was significantly greater in the highest pCO₂ treatment relative to both the ambient and moderately elevated pCO₂ levels (Tukey test, $p<0.0001$ and $p=0.0003$, respectively). The moderately elevated pCO₂ embryos did not have significantly higher routine MO₂ than those in ambient pCO₂ (Tukey test, $p=0.47$; Table 2.2).

At 2 dph there was no significant effect of pCO₂ on routine metabolic rates in either experiment (Figure 2.2B; ANOVA, $p>0.05$). Mass-specific metabolic rates of larvae generally decreased slightly between 2 and 5 dph. Increasing pCO₂ significantly affected the mass-specific metabolic rates of 5 dph larvae in both the 24°C (ANOVA, $F_{2,14}=3.78$, $p=0.049$) and 22°C experiment (ANOVA, $p<0.0001$, $F_{2,22} = 14.8$). At 24°C no significant pairwise differences were found (Tukey test, $p>0.05$) but the mean MO₂ at moderate pCO₂ was lower than those at ambient and high pCO₂ (Figure 2.2C). The 5 dph larvae reared under high pCO₂ in Experiment 2 had significantly lower MO₂ than those at both ambient pCO₂ (Tukey test, $p=0.003$) and moderate pCO₂ (Tukey test, $p<0.0001$; Table 2.2). Experiment 2 also had a significant tank effect for MO₂ (ANOVA, $p=0.009$, $F_{4,22}=4.4$), but there were no other significant tank effects found in nested ANOVAs for any life stage or experiment (ANOVA, $p>0.05$).

2.3.2. Relationship Between MO_2 and O_2

A slight decrease in P_{crit} of *M. menidia* offspring was observed over time across experiments and treatments. A substantial proportion of embryos displayed a steady decline in MO_2 as oxygen decreased such that no breakpoint could be identified at which a relatively oxygen-independent portion of the data shifted to become oxygen-dependent (Figure 2.3). In these cases, P_{crit} could not be estimated and embryos appeared to be oxyconformers regardless of ambient O_2 levels. In Experiment 1 the frequency of this phenomenon was not significantly different across CO_2 treatments (Proportion Test, $X^2=0.695$, $p=0.7$), with 40% of ambient embryos, 22.2% of moderate pCO_2 embryos, and 33.3% of high pCO_2 embryos displaying oxyconformity (Table 2.3). In contrast, embryos from Experiment 2 had significant differences in proportion with oxyconformity (Proportion Test, $X^2=18.936$, $p<0.001$), as the highest pCO_2 level had 71.4% oxyconformers compared to 0% in ambient and 8.3% of moderate pCO_2 and 71.4% of high pCO_2 embryos showing the pattern and no embryos showing this in ambient pCO_2 (Table 2.3).

There were no significant effects of pCO_2 on P_{crit} at the embryo stage in either experiment (Figure 2.4A; ANOVA, $p>0.05$). The pCO_2 significantly affected larval P_{crit} at 2dph in Experiment 1 (Figure 2.4B; ANOVA, $p=0.049$, $F_{2,20}=3.53$). The P_{crit} in high pCO_2 treated larvae was significantly lower than that in ambient pCO_2 (Tukey test, $p=0.04$), while the moderate pCO_2 level was not significantly different from either of the other levels (Tukey test, $p>0.05$; Table 2.2). However, no similar effects were detected in Experiment 2 (ANOVA, $p>0.05$). At 5 dph, P_{crit} declined significantly with increasing pCO_2 in both Experiment 1 (Figure 2.4C, ANOVA, $F_{2,14}=4.25$, $p=0.036$) and Experiment 2 (ANOVA, $F_{2,20}=4.97$, $p=0.018$). Pairwise comparisons showed that high pCO_2 was significantly lower than at ambient pCO_2 in the 24°C experiment (Figure 2.4C, ANOVA, $p>0.05$) and the 22°C experiment (Tukey test, $p=0.015$) but that moderate pCO_2 groups were not significantly different from the other treatments. There was a significant decrease in P_{crit} with elevated pCO_2 in 5dph larvae from Experiment 2 (Figure 2.4C, ANOVA, $p=0.018$, $F_{2,20}=4.97$). There were no significant tank effects on P_{crit} (nested ANOVA, $p>0.05$).

The percentages of offspring with a low-oxygen increase in MO_2 below P_{crit} (Figure 2.2) are reported in Table 2.3. The prevalence of this pattern was lowest among embryos and highest at 2dph. In Experiment 1 larvae (2 and 5 dph) the phenomenon occurs slightly less frequently in

elevated pCO₂ levels. There were no statistically significant differences in proportion of individuals across treatment groups displaying this pattern (Proportion Test, $p > 0.05$).

2.4. Discussion

By rearing *M. menidia* offspring in different pCO₂ treatments and quantitatively analyzing their MO₂-oxygen relationship we showed that high pCO₂ effects on MO₂ depend on rearing temperature and occur primarily at pCO₂ levels above 4000 μ atm, yet high temperatures and high pCO₂ are expected as anthropogenic effects intensify. Routine MO₂ of embryos and 5 dph larvae at 22°C were significantly affected by pCO₂, while their counterparts at 24°C were unaffected. Because of differences in equipment used to control experimental conditions, the 22°C experiment had higher mean moderate and high pCO₂ than the 24°C one had. The highest pCO₂ treatment in the 24°C experiment was 3609.8 μ atm, while the highest treatment at 22°C, and the only treatment that elicited changes in routine MO₂, was 4324.5 μ atm. Past studies have pointed out that responses to acidification are not always linear but may instead have a bell-curve or threshold-type response (Ries et al., 2009; Bednaršek et al., 2019). The difference we observed between experiments may be indicative of a threshold existing between ~3600-4300 μ atm pCO₂ for early life stages of this species. This parallels the upper limits of recorded diel pCO₂ fluctuations in a Long Island estuary near where the adults were collected for this experiment (Baumann et al., 2015). It is possible that the metabolic impacts of high CO₂ are negligible until levels approach the limits of what naturally occurs in the range of the populations these individuals are from.

Because fertilized eggs were gathered from the same batch spawned by several parents and randomly divided between Experiments 1 and 2, genetic variability likely did not cause the different responses between experiments. However, the 2°C temperature difference between experiments may have influenced responses. Temperature-dependent effects of pCO₂ have been found in the past for *M. menidia*, with temperature altering how embryo survival and hatch length were impacted by pCO₂ but not consistently increasing CO₂-sensitivity (Murray and Baumann, 2018). Additionally, past experiments in which significant effects of CO₂ were detected mainly took place early or late in the spawning season (Baumann et al., 2018), and the present study utilized one of the last spawning events of the season. In contrast, a past study in which pCO₂ did not affect routine metabolism used experiments on offspring from early- and

mid-season spawning events (Schwemmer et al., 2020). The sensitivity of *M. menidia* to pCO₂ is thought to vary based on the timing of spawning, potentially due to the environment adults are experiencing and their condition or energetic contribution to eggs (Murray et al., 2014; Snyder et al., 2017). Temperature also influences developmental rate, yolk consumption rate, and hatch timing (Blaxter, 1988). This means that even though embryos and larvae from each experiment were sampled at the same time points relative to hatching, they may have been at slightly different points in their development of homeostatic and metabolic mechanisms.

The response shown in the 22°C experiment of embryonic MO₂ increasing with pCO₂ is similar to the results of Schwemmer et al. (2020) in which mean MO₂ increased by over 30% from the lowest to highest pCO₂ treatment under normoxia. Increased oxygen consumption suggests that additional ATP is being expended on acid-base balance at the cellular level, as embryos do not yet have the ability to remove CO₂ from the blood by increasing ventilation (Rombough, 1988). By 5 dph the pCO₂ effect was reversed and the highest pCO₂ treatment led to a significantly lower MO₂ than the lower pCO₂ levels, again only at 22°C. Reduced routine MO₂ has been observed in other marine fish species exposed to high CO₂, including embryos (Rosa et al., 2014), larvae (Pimentel et al., 2014; Pimentel et al., 2015) and juveniles (Stiller et al., 2015). Reduced MO₂ indicates that high pCO₂ is not causing additional energy to be expended on homeostasis at this stage (Stiller et al., 2015), which could help explain why *M. menidia* persists in regularly elevated pCO₂ levels. This evidence that little, if any, additional energy is being expended on pumping ions for acid-base balance leaves the possibility that routine ventilation is enough to keep blood pH at acceptable levels. The shift in response from embryos to 5 dph could also be a result of differential mortality with increasing pCO₂ of only the most sensitive embryos, leaving tolerant larvae with lower MO₂ by 5 dph. Turtle cells incubated in an experiment used the majority of their ATP on the ion exchanger Na⁺/K⁺-ATPase and protein synthesis, so if this also holds for fish and metabolism is suppressed under high CO₂ both of these functions may be reduced to the detriment of the fish under longer term exposure (Storey and Storey, 2004). Currently, such high pCO₂ levels only occur for a few hours at a time certain times of year but as they become more common due to anthropogenic CO₂ emissions (Gledhill et al., 2015; IPCC, 2022) chronic exposure to these conditions could slow down growth and development (Hochachka and Somero, 2002). Even if natural variability results in only some fish being

sensitive, this could have population-level consequences through recruitment, reproductive output, and predation rates (Houde, 1997).

Contrary to our hypothesis, P_{crit} did not significantly increase with seawater acidification, but only in embryos in the 22°C experiment. These results differ from previous findings that routine MO_2 became more oxygen-dependent in *M. menidia* reared in CO_2 and O_2 treatments (Schwemmer et al., 2020). We expanded upon this past work by quantifying the MO_2 - O_2 relationship directly in individuals as oxygen decreases acutely, rather than inferring it from separate individuals kept in separate static DO levels long-term. Oxygen-sensitivity may be resistant to pCO_2 effects because of the capability for cutaneous oxygen uptake and high surface area to volume ratio of embryos allowing sufficient oxygen uptake capability (Rombough, 1988). This lack of sensitivity is surprising, as low blood pH can reduce the oxygen that fish can take up into the blood through the Root effect, reduced hemoglobin- O_2 binding capacity, and Bohr effect, reduced hemoglobin- O_2 binding affinity (Brauner and Randall, 1996; Wells, 2009). Embryonic P_{crit} was unaffected by pCO_2 despite increased routine MO_2 under high pCO_2 in the same individuals. When metabolism increases and approaches maximum metabolic rate due to activity and other metabolic demands, P_{crit} generally increases as well (Pörtner, 2010). Therefore, elevated P_{crit} would be an expected consequence of increased metabolic demand under high pCO_2 . A study on intertidal sculpin found increased P_{crit} after acclimation to high pCO_2 coincided with not only elevated MO_2 but also greater activity rates of Na^+/K^+ -ATPase, an ion exchange enzyme indirectly involved in acid-base regulation (Hancock and Place, 2016; Claiborne et al., 2002; Catches et al., 2006). Further measurements, such as ionoregulatory enzyme activity and gene expression, could elucidate whether *M. menidia* in high pCO_2 are spending additional energy on acid-base regulation, which may prevent the Bohr and Root effects from impairing oxygen uptake ability.

Hypoxia-sensitivity under acidification in embryos from the 22°C experiment may be hidden by the prevalence of individuals displaying full oxyconformity. In these embryos, MO_2 declined steadily as oxygen decreased such that a breakpoint indicative of P_{crit} could not be detected. Although this phenomenon occurred similarly across pCO_2 levels in the 24°C experiment, it significantly increased with high pCO_2 and was absent from the ambient treatment in the 22°C experiment (Table 2.3). The starting DO concentration of a respirometry trial was around 5 mg L^{-1} after a sensor equilibration and warmup period, so this pattern may indicate a

P_{crit} above that level. If we had been able to start at a higher DO level we may have detected such a high P_{crit} , which would have drawn the high pCO_2 effect on P_{crit} up, as these embryos were excluded from our analysis due to inability to detect P_{crit} . Embryos of most fish species oxyregulate to some extent, although exceptions exist, but the P_{crit} typically increases as embryos near the time of hatching (Rombough, 1988). Therefore, it is likely a higher P_{crit} exists in these individuals rather than high pCO_2 inducing full oxyconformity. Regardless of this distinction, this pattern suggests that oxygen demand is high but the ability to meet it across depends greatly on ambient DO, but only in a subset of individuals. This could contribute to immediate mortality or later reductions in growth and survival, as seen in Cross et al. (2019), from reduced ability to use energy and damage from anaerobic byproducts (Richards, 2011; Thomas et al., 2019).

In contrast to 22°C embryos, P_{crit} decreased under high pCO_2 in 2 dph larvae in the 24°C, as did 5 dph P_{crit} in both temperatures. Other studies have measured P_{crit} in fish exposed to seawater acidification and found no effects, as we did in embryos at 24°C and 2 dph larvae at 22°C (McKenzie et al., 2003; Couturier et al., 2013; Heinrich et al., 2014). Multiple studies link species' tolerance of future global change conditions to the degree of fluctuating severe conditions they currently experience (Pörtner and Farrell, 2008; Baumann, 2019). A study on two damselfish species found that P_{crit} and resting metabolism were both unaffected by four days of high- CO_2 exposure, potentially owing to the periodic hypoxia these species encounter on coral reefs (Couturier et al., 2013). Much like *M. menidia*'s estuarine habitat, the naturally fluctuating conditions on the reef appear to have resulted in fish that tolerate a wide range of conditions without disruption to metabolic processes. Similarly, an elasmobranch that is regularly exposed to diurnal CO_2 and O_2 fluctuations on shallow coral reef habitats was also displayed no effects of long term high CO_2 exposure on its resting metabolism or P_{crit} (Heinrich et al., 2014). Although low blood pH is known to reduce the oxygen-binding capacity and affinity of hemoglobin (Brauner and Randall, 1996) the effect may not be strong enough at our pCO_2 levels to detectably affect oxygen uptake rates or the fish may have the ability to prevent CO_2 from affecting blood pH.

A recent study that took measurements of European sea bass under constant low pCO_2 to acutely increasing pCO_2 found that P_{crit} decreased and hemoglobin affinity for oxygen increased, both of which indicate an increase in hypoxia tolerance with acidification (Montgomery et al., 2019). Furthermore, the authors found no difference in European sea bass blood pH during the

high-CO₂ exposure, which supports the idea that environmentally typical pCO₂ levels may not alter blood pH to the extent necessary for the Bohr and Root effects to inhibit hypoxia tolerance. Montgomery et al. (2019) propose several mechanisms to account for the increase in hemoglobin-O₂ affinity at high pCO₂, such as the possibility that increasing pCO₂ increases the rate of β -adrenergic stimulated increase in intracellular erythrocyte pH that occurs in acute hypoxia or that erythrocyte chloride levels decrease following increases in plasma bicarbonate resulting from respiratory acidosis compensation (Montgomery et al., 2019). The present study did not include the measurements needed to confirm such mechanisms, and future work doing so would help determine if such processes aid tolerance in *M. menidia*.

Interestingly, the impacts most suggestive of sensitivity to high pCO₂ only occurred in the 22°C experiment: stage specific changes in MO₂ and increased prevalence of oxyconformity in embryos. Meanwhile, the only high pCO₂ response in the 24°C experiment was reduced P_{crit} in larvae. This suggests the offspring reared at the lower temperature (22°C, Experiment 2) were more sensitive to acidification. The higher temperature, which is not considered stressfully high for this species (Middaugh et al., 1987), may lead to not only earlier hatching but also faster development of the metabolic and ionoregulatory mechanisms that help *M. menidia* withstand fluctuating conditions, which intensify as summer goes on and temperatures rise. The lower prevalence of embryo oxyconformity in the 24°C experiment is further evidence that developmental rate may have played a role, as P_{crit} is highly dependent on developmental stage before hatching and some species have reduced ability to oxyregulate as embryos (Rombough, 1988). *M. menidia* also has a distribution extending south to Florida where summer temperatures are even higher, so it makes sense that the species may thrive under warmer temperatures.

Differences in prevalence of a temporary, sharp increase in oxygen consumption at low oxygen were not significant among experiments and pCO₂ treatments. However, there was a large difference between embryos and larvae, with the percentage of embryos with this pattern ranging from 10-35.71% while in larvae it ranged from 33.33-100%. This is consistent with the explanation that stress and accumulation of anaerobic byproducts stimulates activity (Pörtner and Grieshaber, 1993) because embryos are less capable of activity than larvae (although at the stage sampled their bodies are developed enough to move around in the chorion). Older *M. menidia* are known to exhibit aquatic surface respiration during hypoxia (Miller et al., 2016) and larvae have also been observed selectively occupying surface water in hypoxic conditions (Cross et al.,

2019), showing that the instinct to swim to escape hypoxia starts early. This activity is unconfirmed in our fish, however, because we were not able to observe the respirometry wells during trials. The highest occurrence of this pattern was at the 2dph larval stage in the 24°C experiment, the same group in which P_{crit} was reduced by high pCO_2 . If this low-oxygen sharp increase in MO_2 is indeed indicative of greater rates of anaerobiosis, this suggests that the fish with increased hypoxia-tolerance under high pCO_2 may have also had a greater capacity for anaerobic metabolism to meet their metabolic needs despite the lack of oxygen.

It is recommended that the MO_2 -oxygen relationship be assessed in combination with another measure that can provide more physiological information to explain the relationship (Ultsch and Regan, 2019). Future work can uncover more about *M. menidia* hypoxia tolerance under multiple stressors by measuring anaerobic metabolism, behavioral responses (activity, aquatic surface respiration, loss of equilibrium), and enzyme activity as oxygen is depleted. Measuring and observing activity of larvae during respiration was not possible in this experiment because the inside of respirometers was not visible when they are sealed, but future experiments that record video of larvae during respirometry would allow experimenters to separate out periods of rest and activity and better characterize what happens to the fish as oxygen approaches zero, physiological needs are no longer met, and anaerobic metabolites build up that may stimulate activity (Pörtner and Grieshaber, 1993; Pörtner, 2010). Multiple replication studies should be done to encompass the range of natural genetic variation that likely confers population persistence amid extreme environmental fluctuations (Baumann et al., 2018). This study describes two experiments that, for logistical reasons, were done simultaneously at different temperatures and with different methods of acidifying the water. Despite the methodological differences that prevent statistical comparison of the different temperatures, the resulting dataset captures responses across two typical temperatures and a range of slightly different pCO_2 treatments to give a more complete picture of how the metabolic response to hypoxia can vary in these conditions.

We quantified life-stage dependent responses of routine metabolism and P_{crit} to seawater acidification. As we hypothesized, acidification increased routine metabolism in embryos, which suggests increased metabolic demands associated with acid-base balance, but the lack of accompanying increase in embryonic P_{crit} indicates the higher metabolism enables adequate blood pH regulation. This could have important implications for the energy budget influencing

when offspring hatch and at what size. Our results conflict with the proposed mechanism of previous findings that acidification interactions with DO stem from high pCO₂ increasing hypoxia sensitivity (Schwemmer et al., 2020). Evidence of metabolic suppression under high pCO₂ at 5 dph shows important changes in the energy budget after hatching and potentially explains previously observed reductions in larval growth under high pCO₂ (Murray et al., 2017; Baumann et al., 2018). Surprisingly, decreased P_{crit} in the highest pCO₂ level of 2 dph larvae in one experiment indicates acidification may increase hypoxia tolerance. However, the lack of significant effects in some life stages within each experiment highlights natural variability in sensitivity and the effects slight differences in temperature, and thus developmental rate, can have. Hypoxia and acidification are concurrently increasing gradually around the globe and already co-occur in coastal waters due to periodic fluctuations in community respiration (Cai et al., 2011; Gruber, 2011). Understanding the combined effects of these stressors on fish metabolism will aid in predicting and managing population-level impacts of global change and provide insight into the optimal mitigation actions. It is important to understand the responses of tolerant species, which *M. menidia* appears to be, as well as sensitive species, because the mechanisms that lead to tolerance can shed light on potential for future adaptation in a variety of species.

2.5. References

- Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97(5): 399-408. <https://doi.org/10.1139/cjz-2018-0198>
- Baumann, H., Wallace, R. B., Tagliaferri, T., and Gobler, C. J. 2015. Large Natural pH, CO₂ and O₂ Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time Scales. *Estuaries Coasts*, 38: 220-231. doi: 10.1007/s12237-014-9800-y
- Baumann, H., Cross, E. L., and Murray, C. S. 2018. Robust quantification of fish early life CO₂ sensitivities via serial experimentation. *Biol. Lett.*, 14: 20180408. <http://dx.doi.org/10.1098/rsbl.2018.0408>
- Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., et al. 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates: A better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. *BioScience*, 51(8): 633-641.
- Bednaršek, N., Feely, R. A., Howes, E. L., Hunt, B. P. B., Kessouri, Faycal., León, P., Lischka, S., Maas, A. E., McLaughlin, K., Nezlin, N. P., Sutula, M., and Weisberg, S. B. 2019. Systematic Review and Meta-Analysis Toward Synthesis of Thresholds of Ocean Acidification Impacts on Calcifying Pteropods and Interactions With Warming. *Front. Mar. Sci.*, 6: 227. doi: 10.3389/fmars.2019.00227
- Birk, M. A. 2021. respirometry: Tools for Conducting and Analyzing Respirometry Experiments. R package version 1.3.0, <<https://CRAN.R-project.org/package=respirometry>>.
- Blaxter, J. H. S. 1988. Pattern and Variety in Development. In: *Fish Physiology, Vol. 11A: The Physiology of Developing Fish: Eggs and Larvae* (ed. W. S. Hoar and D. J. Randall), pp. 1-58. San Diego: Academic Press.
- Brauner, C. J. and Randall, D. J. 1996. The interaction between oxygen and carbon dioxide movements in fishes. *Comp. Biochem. Physiol.*, 113A: 83-90. doi: 10.1016/0300-9629(95)02062-4
- Cadigan, K. M. and Fell, P. E. 1985. Reproduction, growth and feeding habits of *Menidia menidia* (Atherinidae) in a tidal marsh-estuarine system in southern New England. *Copeia*, 1985: 21-26. doi:10.2307/1444786
- Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., et al. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geosci.*, 4: 766-770. <https://doi.org/10.1038/ngeo1297>

- Catches, J. S., Burns, J. M., Edwards, S. L., and Claiborne, J. B. 2006. Na⁺/H⁺ antiporter, V-H⁺-ATPase and Na⁺/K⁺-ATPase immunolocalization in a marine teleost (*Myoxocephalus octodecemspinosus*). *J. Exp. Biol.*, 209: 3440-3447.
- Cattano, C., Claudet, J., Domenici, P., and Milazzo, M. 2018. Living in a high CO₂ world: a global meta-analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecol. Monogr.*, 88(3): 320-335.
- Claiborne, J. B., Edwards, S. L., and Morrison-Shetlar, A. I. 2002. Acid-Base Regulation in Fishes: Cellular and Molecular Mechanisms. *J. Exp. Zool.*, 293: 302-319.
- Couturier, C. S., Stecyk, J. A. W., Rummer, J. L., Munday, P. L. and Nilsson, G. E. 2013. Species-specific effects of near-future CO₂ on the respiratory performance of two tropical prey fish and their predator. *Comp. Biochem. Physiol. A*, 166: 482-489. doi:10.1016/j.cbpa.2013.07.025
- Crain, C. M., Kroeker, K., and Halpern, B. S. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol. Lett.*, 11: 1304-1315.
- Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal pCO₂ × O₂ fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9: 18146. <https://doi.org/10.138/s41598-019-53930-8>
- Cruz-Neto, A. P. and Steffensen, J. F. 1997. The effects of acute hypoxia and hypercapnia on oxygen consumption of the freshwater European eel. *J. Fish. Biol.*, 50: 759-769.
- Diaz, R. J. and Rosenberg, R. 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. *Science*, 321: 926-929.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. 2009. Ocean Acidification: The Other CO₂ Problem. *Annu. Rev. Mar. Sci.*, 1: 169-192. <https://doi.org/10.1146/annurev.marine.010908.163834>
- Esbaugh, A. J. 2018. Physiological implications of ocean acidification for marine fish: emerging patterns and new insights. *J. Comp. Physiol. B*, 188: 1-13. <https://doi.org/10.1007/s00360-017-1105-6>
- Fox, J. and Weisberg, S. 2019. An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL: <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Gledhill, D. K., White, M. M., Salisbury, J., Thomas, H., Misna, I., et al. 2015. Ocean and Coastal Acidification off New England and Nova Scotia. *Oceanogr.*, 28(2), Special Issue on Emerging Themes in Ocean Acidification Science: 182-197.

- Gruber, N. 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Phil. Trans. R. Soc. A.*, 369: 1980-1996. <http://doi.org/10.1098/rsta.2011.0003>
- Hancock, J. R. and Place, S. P. 2016. Impact of ocean acidification on the hypoxia tolerance of the woolly sculpin, *Clinocottus analis*. *Conserv. Physiol.* 4, cow040. doi:10.1093/conphys/cow040
- Harvey, B. P., Gwynn-Jones, D., and Moore, P. J. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecol. Evol.*, 3(4): 1016-1030. doi: 10.1002/ece3.516
- Heinrich, D. D. U., Rummer, J. L., Morash, A. J., Watson, S.-A., Simpfendorfer, S. A., Heupel, M. R., and Munday, P. L. 2014. A product of its environment: the epaulette shark (*Hemiscyllium ocellatum*) exhibits physiological tolerance to elevated environmental CO₂. *Conserv. Physiol.*, 2(1): cou047. <https://doi.org/10.1093/conphys/cou047>
- Heuer, R. M and Grosell, M. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 307: R1061-R1084. doi: 10.1152/apjregu.00064.2014
- Hochachka, P. W., and Somero, G. N. 2002. Biochemical adaptation: mechanisms and process in physiological evolution. Oxford University Press, Oxford.
- Houde, E. D. 1997. Patterns and consequences of selective processes in teleost early life histories. In: *Early Life History and Recruitment in Fish Populations*. (ed. R. Christopher Chambers and Edward A Trippel), pp. 173-196. London: Chapman & Hall.
- Intergovernmental Panel on Climate Change. 2022. Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama, eds.]. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.
- Ishimatsu, A., Hayashi, M., and Kikkawa, T. 2008. Fishes in high-CO₂, acidified oceans. *Mar. Ecol. Prog. Ser.*, 373: 295-302. doi: 10.3354/meps07823
- Keeling, R. F., Körtzinger, A., and Gruber, N. 2010. Ocean Deoxygenation in a Warming World. *Annu. Rev. Mar. Sci.*, 2: 463-493. doi: 10.1146/annurev.marine.010908.163855
- Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.*, 13: 1419-1434. doi: 10.1111/j.1461-0248.2010.01518.x
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J.-P. 2013. Impacts of ocean acidification on marine organisms: quantifying

- sensitivities and interaction with warming. *Glob. Change Biol.*, 19: 1884-1896. doi: 10.1111/gcb.12179
- Marshall, D. J., Burgess, S. C., and Connallon, T. 2016. Global change, life-history complexity and the potential for evolutionary rescue. *Evol. Appl.*, 9: 1189-1201.
- McKenzie, D. J., Piccolella, M., Dalla Valle, A. Z, Taylor, E. W., Bolis, C. L., and Steffensen, J. F. 2003. Tolerance of chronic hypercapnia by the European eel *Anguilla anguilla*. *J. Exp. Biol.*, 206(10): 1717-1726. <https://doi.org/10.1242/jeb.00352>
- Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Pörtner, H.-O. 2009. Physiological basis for high CO₂ tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosci.*, 6: 2313-2331. www.biogeosciences.net/6/2313/2009/
- Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A. 2013. Future ocean acidification will be amplified by hypoxia in coastal habitats. *Mar. Biol.*, 160: 1875-1888. doi: 10.1007/s00227-012-1954-1
- Middaugh, D. P., Hemmer, M. J., and Goodman, L. 1987. Methods for Spawning, Culturing and Conducting Toxicity-Tests with Early Life Stages of four Atherinid Fishes: The Inland Silverside, *Menidia beryllina*, Atlantic silverside, *M. Menidia*, Tidewater silverside, *M. peninsula*, and California grunion, *Leuresthes tenuis*. Gulf Breeze, FL: United States Environmental Protection Agency.
- Montgomery, D. W., Simpson, S. D., Engelhard, G. H., Birchenough, S. N. R. and Wilson, R. W. 2019. Rising CO₂ enhances hypoxia tolerance in a marine fish. *Sci. Rep.* 9, 15152. doi:10.1038/s41598-019-51572-4
- Muggeo, V. M. R. 2008. Segmented: an R Package to Fit Regression Models with Broken-Line Relationships. *R News*, 8/1, 20-25. URL <https://cran.r-project.org/doc/Rnews/>.
- Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*, 10: 69. doi: 10.3390/d10030069
- Murray, C. S., Malvezzi, A., Gobler, C. J., and Baumann, H. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.*, 504: 1-11. doi: 10.3354/meps10791
- Murray, C. S., Fuiman, L. A., and Baumann, H. 2017. Consequences of elevated CO₂ exposure across multiple life stages in a coastal forage fish. *ICES J. Mar. Sci.*, 74(4): 1051-1061. doi: 10.1093/icesjms/fsw179

- National Research Council (2005). *Marine Mammal Populations and Ocean Noise: Determining When Noise Causes Biologically Significant Effects*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/11147>
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., et al. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437: 681-686. <https://doi.org/10.1038/nature04095>
- Pimentel, M., Pegado, M., Repolho, T., and Rosa, R. 2014. Impact of ocean acidification in the metabolism and swimming behavior of the dolphinfish (*Coryphaena hippurus*) early larvae. *Mar. Biol.*, 161: 725-729.
- Pimentel, M. S., Faleiro, F., Diniz, M., Machado, J., Pousão-Ferreira, P., Peck, M. A., Pörtner, H.-O., and Rosa, R. 2015. Oxidative Stress and Digestive Enzyme Activity of Flatfish Larvae in a Changing Ocean. *PLoS ONE*, 10(7): e0134082. doi: 10.1371/journal.pone.0134082
- Pörtner, H.-O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.*, 213: 881-893. doi: 10.1242/jeb.037523
- Pörtner, H.-O. and Farrell, A. P. 2008. Physiology and Climate Change. *Science*. 322(5902): 690-692.
- Pörtner, H.-O. and Grieshaber, M. K. 1993. Critical PO₂(s) in oxyconforming and oxyregulating animals: gas exchange, metabolic rate and the mode of energy production. In *The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life* (J. E. P. W. Bicudo, ed), pp. 330-357. Boca Raton, FL, USA: CRC Press.
- Pörtner, H.-O., Langenbuch, M., and Michaelidis, B. 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO₂ on marine animals: From Earth history to global change. *J. Geophys. Res. Oceans*, 110: C09S10. doi: 10.1029/2004JC002561
- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.
- Regan, M. D., Mandic, M., Dhillon, R. S., Lau, G. Y., Farrell, A. P., Schulte, P. M., Seibel, B. A., Speers-Roesch, B., Ultsch, G. R., and Richards, J. G. 2019. Don't throw the fish out with the respirometry water. *J. Exp. Biol.*, 222(6): jeb200253.
- Richards, J. G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.*, 214: 191-199.
- Ries, J. B., Cohen, A. L., and McCorkle, D. C. 2009. Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology*, 37(12): 1131-1134.

- Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: *Fish Physiology, Vol. 11: The Physiology of Developing Fish, Part A: Eggs and Larvae*. (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego: Academic Press.
- Rosa, R., Baptista, M., Lopes, V. M., Pegado, M. R., Paula, J. R., Trübenbach, K., Leal, M. C., Calado, R., and Repolho, T. 2014. Early-life exposure to climate change impairs tropical shark survival. *Proc. R. Soc. B*, 281: 20141738. <http://dx.doi.org/10.1098/rspb.2014.1738>
- Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., and Nye, J. A. 2020. Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish *Menidia menidia*. *J. Exp. Biol.*, 223: jeb228015. doi: 10.1242/jeb.228015
- Seibel, B. A. and Deutsch, C. 2020. Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature. *J. Exp. Biol.*, 223(12): jeb210492. <https://doi.org/10.1242/jeb.210492>
- Snyder, J. T., Murray, C. S., and Baumann, H. 2018. Potential for maternal effects on offspring CO₂ sensitivities in the Atlantic silverside (*Menidia menidia*). *J. Exp. Mar. Biol. Ecol.*, 499: 1-8.
- Stiller, K. T., Vanselow, K. H., Moran, D., Bojens, G., Voigt, W., Meyer, S., and Schulz, C. 2015. The effect of carbon dioxide on growth and metabolism in juvenile turbot *cophthalmus maximus* L. *Aquaculture*, 444: 143-150.
- Storey, K. B., and Storey, J. M. 2002. Oxygen limitation and metabolic rate depression. In: Storey, K. B. (ed), *Functional Metabolism: Regulation and Adaptation*. Wiley, Hoboken, NJ, pp-415-442.
- Thomas, Yoann., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G. M., and Pecquerie, Laure. 2019. Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB) theory. *J. Sea Res.*, 143: 231-242.
- Ultsch, G. R. and Regan, M. D. 2019. The utility and determination of P_{crit} in fishes. *J. Exp. Biol.*, 222: jeb203646. doi: 10.1242/jeb.203646
- Wallace, R. B. and Gobler, C. J. 2021. The role of algal blooms and community respiration in controlling the temporal and spatial dynamics of hypoxia and acidification in eutrophic estuaries. *Mar. Pollut. Bul.*, 172: 112908. <https://doi.org/10.1016/j.marpolbul.2021.112908>
- Watson, J. W., Hyder, K., Boyd, R., Thorpe, R., Weltersbach, M. S., Ferter, K., Cooke, S. J., Roy, S., and Sibly, R. M. 2020. Assessing the sublethal impacts of anthropogenic

- stressors on fish: An energy-budget approach. *Fish Fish.*, 21: 1034-1045. doi: 10.1111/faf.12487
- Wells, R. M. G. 2009. Blood-gas transport and hemoglobin function: adaptations for functional and environmental hypoxia. In: *Fish Physiology, Vol. 27, Hypoxia* (Ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 255-299. San Diego: Academic Press.
- Wittmann, A. C. and Pörtner, H.-O. 2013. Sensitivities of extant animal taxa to ocean acidification. *Nat. Clim. Change*, 3: 995-1001. <https://doi.org/10.1038/nclimate1982>
- Wood, C. M. 2018. The fallacy of the P_{crit} – are there more useful alternatives? *J. Exp. Biol.*, 221: jeb163717. doi: 10.1242/jeb.163717
- Zeileis, A. and Grothendieck, G. 2005. zoo: S3 Infrastructure for Regular and Irregular Time Series. *Journal of Statistical Software*, 14(6): 1-27. doi: 10.18637/jss.v014.i06
- Zhang, Y., Montgomery, D. W., White, C. F., Richards, J. G., Brauner, C. J., and Farrell, A. P. 2022. Characterizing the hypoxic performance of a fish using a new metric: P_{AAS-50} . *J. Exp. Biol.*, 225: jeb244239. doi: 10.1242/jeb.244239

2.6. Figures

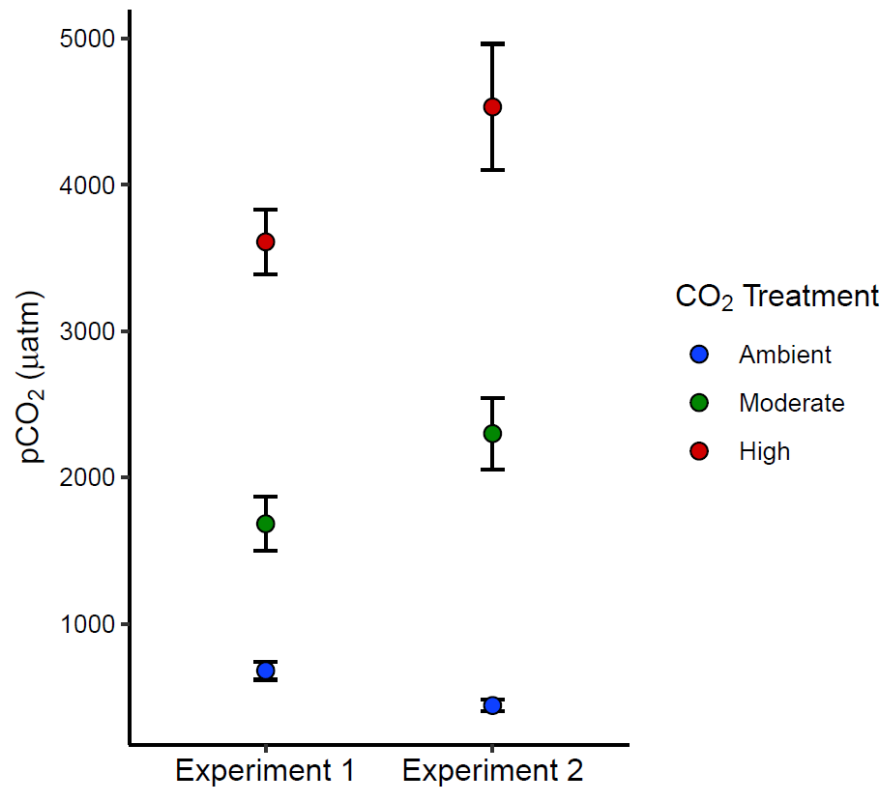


Figure 2.1. Experiment 1 and 2 mean pCO₂ levels for each treatment with error bars showing standard error. In Experiment 1, each rearing tank was sampled twice. In Experiment 2, each equilibration tank was sampled three times.

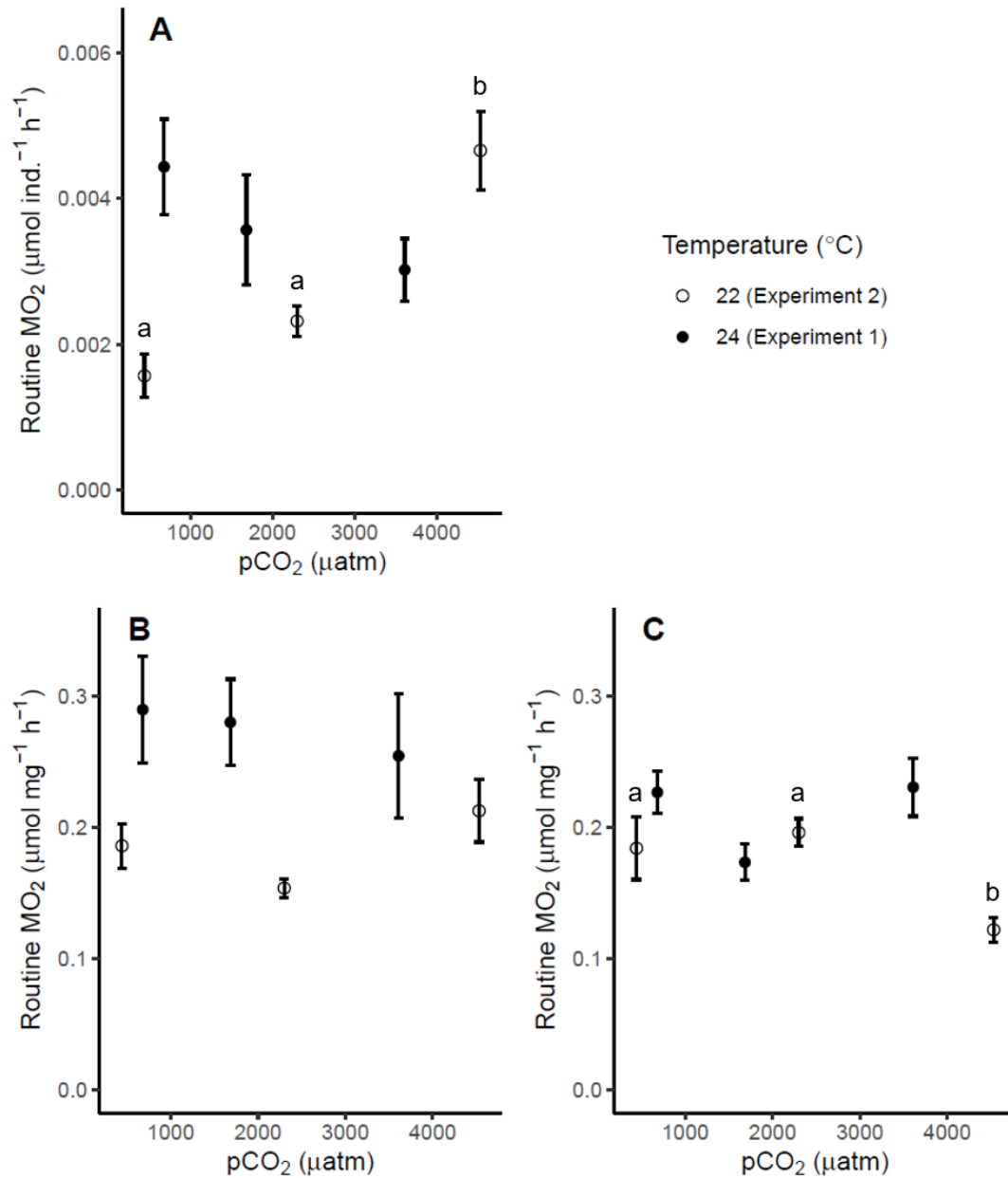


Figure 2.2. Routine metabolic rates of whole *M. menidia* embryos (A, $\mu\text{mol O}_2 \text{ individual}^{-1} \text{ h}^{-1}$) and mass-specific routine metabolic rates ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ h}^{-1}$) of 2-dph larvae (B) and 5-dph larvae (C) as a function of pCO_2 (μatm). Error bars display standard error about the mean. Closed circles represent means from Experiment 1 (24°C) and open circles represent means from Experiment 2 (22°C). Letters denote significant differences between pCO_2 levels within an experiment (temperature level), as detected with a Tukey test, with points that do not share a letter being significantly different. Points that lack significance letters had no significant pCO_2 effect according to ANOVA.

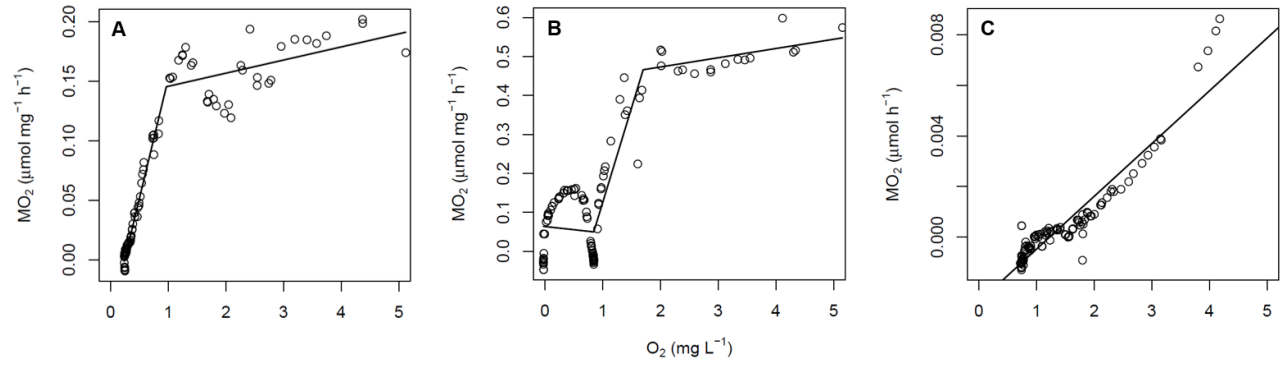


Figure 2.3. Examples of a typical MO_2 - O_2 curve with a fitted breakpoint regression (A), an MO_2 - O_2 curve with a sudden transient increase in MO_2 at low oxygen with a fitted breakpoint regression (B), and an oxyconforming MO_2 - O_2 curve that frequently occurred in embryos with a fitted linear regression (C). In (A) and (B) the dashed line shows P_{crit} , the breakpoint identified by the regression and the point at which relatively oxygen-independent metabolism shifts to highly oxygen-dependent. In (C) no such breakpoint exists because metabolism is consistently oxygen-dependent throughout the trial. The proportions of individuals exhibiting the low-oxygen increase in metabolism in (B) and the oxyconformity shown in (C) for each treatment group are listed in Table 2.3.

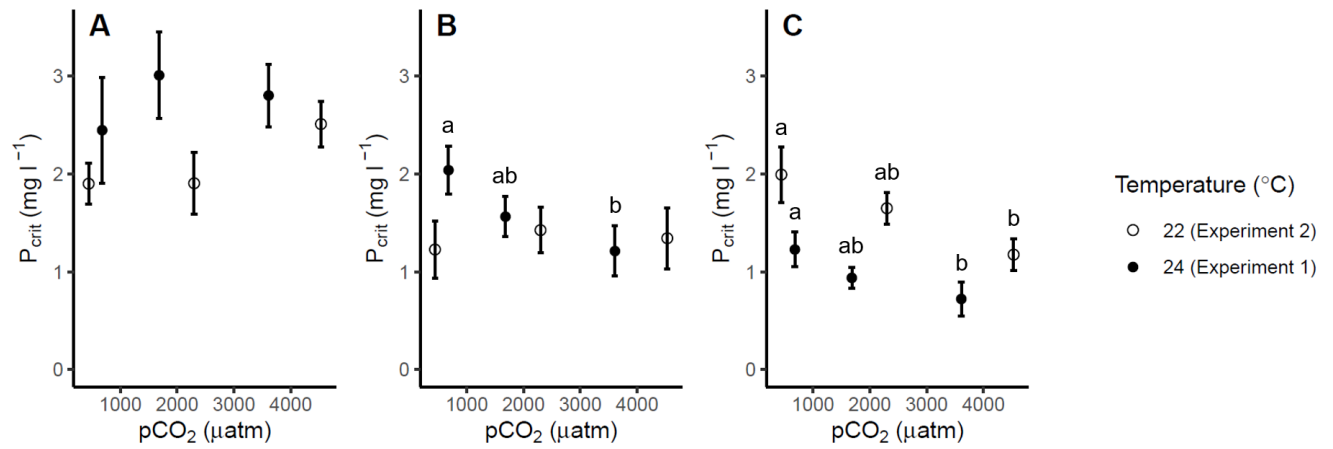


Figure 2.4. Critical oxygen levels of *M. menidia* offspring in response to pCO_2 treatments at the embryo (A), 2dph larval (B), and 5dph larval (C) stages. Error bars display standard error about the mean. Closed circles represent means from Experiment 1 (24°C) and open circles represent means from Experiment 2 (22°C). Letters denote significant differences between pCO_2 levels within an experiment (temperature level), as detected with a Tukey test, with points that do not share a letter being significantly different. Points that lack significance letters had no significant pCO_2 effect according to ANOVA.

2.7. Tables

Table 2.1. Target and measured mean pH, pCO₂, and temperature levels in Experiments 1 and 2.

	pH			pCO₂ (μatm)			Temperature (°C)
	Amb	Mod	High	Amb	Mod	High	
Target levels	8.1	7.5	7.2	450	2000	4000	24 (Exp. 1), 22 (Exp. 2)
Exp. 1	7.94	7.41	7.13	680.0	1683.0	3609.1	23.8
Exp. 2	8.08	7.39	7.09	441.7	2299.2	4530.9	22.3

Table 2.2. Mean routine MO₂ and P_{crit} (±standard error) for *M. menidia* embryos and larvae reared in three pCO₂ treatments in two experiments (Experiment 1 at 24°C and Experiment 2 at 22°C). Routine MO₂ for embryos is µmol O₂ individual⁻¹ h⁻¹ and for larvae is µmol O₂ mg dw⁻¹ h⁻¹. P_{crit} is in mg l⁻¹.

		Routine MO ₂			P _{crit}		
		Ambient pCO ₂	Moderate pCO ₂	High pCO ₂	Ambient pCO ₂	Moderate pCO ₂	High pCO ₂
Embryos	Exp. 1	0.0044(±0.00066)	0.0036(±0.00075)	0.0030(±0.00043)	2.44(±0.54)	3.01(±0.44)	2.80(±0.32)
	Exp. 2	0.0016(±0.00030)	0.0023(±0.00020)	0.0047(±0.00054)	1.90(±0.21)	1.90(±0.32)	2.51(±0.23)
2dph	Exp. 1	0.29(±0.041)	0.28(±0.033)	0.25(±0.047)	2.04(±0.25)	1.56(±0.21)	1.21(±0.26)
Larvae	Exp. 2	0.19(±0.017)	0.15(±0.0071)	0.21(±0.024)	1.23(±0.29)	1.42(±0.23)	1.34(±0.31)
5dph	Exp. 1	0.23(±0.016)	0.17(±0.014)	0.23(±0.022)	1.23(±0.18)	0.94(±0.11)	0.72(±0.17)
Larvae	Exp. 2	0.18(±0.024)	0.20(±0.011)	0.12(±0.0092)	1.99(±0.29)	1.65(±0.16)	1.17(±0.16)

Table 2.3. The percentages of *M. menidia* offspring in which full oxyconformity or a low-oxygen increase in MO₂ following a decline in MO₂ below P_{crit}, for each Experiment, pCO₂ treatment, and life stage.

			Ambient pCO₂	Moderate pCO₂	High pCO₂	<i>p</i>-value
Oxyconformity	Embryos	Exp. 1 (24°C)	40.0%	22.2%	33.3%	0.7065
		Exp. 2 (22°C)	0.00%	8.33%	71.4%	7.7e-5
Low-DO increase	Embryos	Exp. 1 (24°C)	10.0%	22.2%	11.1%	0.71
		Exp. 2 (22°C)	18.2%	16.7%	35.7%	0.45
	2dph Larvae	Exp. 1 (24°C)	100%	90.0%	77.8%	0.28
		Exp. 2 (22°C)	66.7%	83.3%	53.8%	0.29
	5dph Larvae	Exp. 1 (24°C)	55.6%	37.5%	33.3%	0.60
		Exp. 2 (22°C)	85.7%	75.0%	40.0%	0.10

3. Ionoregulatory responses of *Menidia menidia* to seawater acidification and temperature

3.1. Introduction

Ocean acidification, the gradual decrease in global ocean pH due to dissolution of atmospheric carbon dioxide (CO₂), has highly variable effects on fishes, both within (Frommel et al., 2012; Dahlke et al., 2017; Baumann et al., 2018) and among species (Kroeker et al., 2010; Couturier et al., 2013; Heuer and Grosell, 2014), and this only becomes more complex when multiple co-occurring stressors are involved, such as high temperature and hypoxia (Gunderson et al., 2016). Ocean acidification affects responses such as growth, mortality, metabolism, and behavior in many studies (Cattano et al., 2018; Esbaugh, 2018; Espinel-Velasco et al., 2018) while in other studies fish are unaffected (Jarrold and Munday, 2018; Sundin et al., 2019; Clark et al., 2020). The earliest life stages tend to have greater sensitivity to high CO₂ (Ishimatsu et al., 2005; Baumann et al., 2012; Rodriguez-Dominguez et al., 2018), owing to their lower mobility as well as incomplete development of regulatory organ systems and internal buffering capacity (Melzner et al., 2009; Marshall et al., 2016). In some cases, effects of elevated CO₂ are only detected in combination with another stressor (Cross et al., 2019; Schwemmer et al., 2020). Although the growing number of studies on fish responses to acidification seem to paint an increasingly complicated picture, this information will be critical to anticipating how ecologically and economically valuable species will be impacted as pH changes alongside warming, deoxygenation, pollution, and overfishing (Baumann, 2019).

One way to increase understanding of such variable, subtle, and complex responses to stressors and, importantly, their implications for fitness, is to find evidence of the mechanisms behind observed sensitivity or tolerance. Knowledge of physiological mechanisms can enhance predictive models that link organismal processes to scales relevant for management, such as population models and energy budgets (Le Quesne and Pinnegar, 2012; Lavaud et al., 2021). This knowledge will help answer the question of whether the underlying processes are subject to phenotypic plasticity that could mitigate negative responses under persistent acidification (Esbaugh, 2018). Like many of the invertebrates most sensitive to ocean acidification, formation of calcium carbonate in some teleosts is impacted by increased acidity (Grosell, 2019). Unlike in invertebrates, however, the primary threat posed to fish by ocean acidification is thought to come

directly from hypercapnia rather than the changes to ocean pH and calcium carbonate formation (Esbaugh et al., 2012). Fish have effective mechanisms of acid-base homeostasis and CO₂ removal, enabling them to survive higher CO₂ levels than predicted with global ocean acidification in the coming centuries (e.g. Randall et al., 1976; Ishimatsu et al., 2004). When CO₂ enters the blood it reacts with water to form bicarbonate (HCO₃⁻) and hydrogen (H⁺) ions, which reduces the blood pH. This is rapidly compensated by exchanging bicarbonate ions (HCO₃⁻) for chloride ions and by hemoglobin (Hb) and other compounds that buffer H⁺ (Brauner et al., 2019). A variety of enzymes in the gills and kidneys transport ions to support proton removal for pH regulation, often requiring ATP for active transport (Deigweiher et al., 2008; Brauner et al., 2019). While these processes may confer some tolerance, studies documenting changes in growth, percent survival, behavior, and more suggest that acid-base regulation has costs that could impact populations and ecology. For example, additional energy required for H⁺ transport may require additional food or yolk consumption and may result in slower somatic growth (Deigweiher et al., 2008; Lefevre, 2019). Even effective compensation may entail at least brief disturbances to extra- and intracellular pH that could disrupt physiological processes, such as Hb-oxygen binding (Root and Bohr effect; Brauner and Randall, 1996), and cause malformations or tissue damage (Frommel et al., 2012; Pimentel et al., 2016). There is great interspecies variability in the efficacies of these regulatory mechanisms so it cannot be assumed that what is observed in one species applies to all (Brauner et al., 2019), although there may be some commonalities in tolerances and mechanisms in species with similar habitat conditions (Cattano et al., 2018; Baumann, 2019).

The Atlantic silverside, *Menidia menidia*, is a useful model species with numerous studies on its responses to acidification and other environmental stressors (Bengtson et al., 1987; Schultz et al., 1998; Dixon et al., 2017; Baumann et al., 2018). Abundant throughout the estuaries of the North American east coast, *M. menidia* is easy to collect in the wild, spawn, and maintain for laboratory experiments (Middaugh et al., 1987). It is adapted to great environmental fluctuations, so using wild *M. menidia* can provide unique information that is representative of how fish in the wild would respond to stressors compared to using captive fish from genetically homogeneous populations/broodstocks. A pressing question in ocean acidification research is whether CO₂ fluctuations experienced in the wild mediate species' sensitivities to long term exposure to the higher levels that will occur as the global oceans acidify (Baumann, 2019). One

study showed that diel cycling CO₂ and dissolved oxygen (DO) had a less severe effect on *M. menidia* early life growth and survival metrics when compared to the detrimental effects of static treatments (Cross et al., 2019). Earlier work suggested a parental effect in which offspring of silversides that were collected at more acidified times of the spawning season were more tolerant of static high CO₂ (Murray et al., 2014). A study quantifying metabolic rates found that high CO₂ only impacted embryonic metabolism through an interaction effect with hypoxia (Schwemmer et al., 2020). Although this species was unaffected by high CO₂ in some experiments (Murray and Baumann, 2018), a meta-analysis of 20 acidification experiments on *M. menidia* showed reductions in early life survival and growth that tended to be strongest near the beginning and end of the spawning season (Baumann et al., 2018).

Observed declines in early life growth and increases in metabolism under elevated CO₂ suggest tradeoffs between maintenance costs and growth may be occurring in *M. menidia*. Fish eggs and larvae are highly subject to diffusion relative to adults, lack gills, and have little control over ventilation or mobility to remove CO₂. Prior to cell differentiation, pH disturbances may be passively buffered by respiratory proteins and ions in the perivitelline fluid (Alderdice, 1988; Melzner et al., 2009). Embryos later form epithelial ionocytes, specialized mitochondria-rich cells with enzymes that transport ions, thus allowing more active control over both acid-base balance and osmoregulation (Alderdice, 1988). The use of ATP to actively transport H⁺ and other ions involved in acid-base balance may be one mechanism behind energetic tradeoffs leading to observed changes in metabolism and growth. Buffering by Hb can preserve intracellular pH under moderate CO₂ elevations, and this is enhanced in teleosts through the Bohr effect, in which low pH reduces Hb-oxygen affinity (Brauner et al., 2019). Low pH can also lead to low internal oxygen levels due to reduced oxygen carrying capacity (Root effect; Berenbrink et al., 2005), so another tradeoff associated with acid-base regulation may be reduced oxygen uptake. In *M. menidia* embryos, oxygen consumption increased with CO₂ unless hypoxia was simultaneously imposed (Schwemmer et al., 2020), suggesting that maintenance demands associated with ion exchange outweigh any reduction in oxygen uptake ability due to Hb buffering.

Ionocytes typically form on the yolk sac epithelium of the embryo but may also be found in other areas of the body, especially in late embryonic and larval stages (Alderdice, 1988). In some species embryonic ionocytes are particularly abundant near the blood vessels of the yolk

sac (Guggino, 1980; Alderdice, 1988). A number of ion transporters can be found in ionocytes, and their composition varies between species and habitat salinity, as many of these transporters are also involved in osmoregulation (Christensen et al., 2012). Marine teleosts are thought to use Na^+/K^+ -ATPase (NKA), carbonic anhydrase (CAc), apical Na^+/H^+ exchangers (NHE2/3), vacuolar H^+ -ATPase (VHA) and $\text{Na}^+/\text{HCO}_3^-$ cotransporter (NBC) to hydrate CO_2 and remove the resulting ions, H^+ and HCO_3^- (Esbaugh, 2018). Basolateral NKA is involved in acid-base balance through secondary active transport, moving Na^+ from the ionocyte cytoplasm into the blood to create a gradient that allows apical NHE to remove H^+ to the external seawater by bringing in Na^+ from seawater (Silva et al., 1977; Claiborne et al., 2002; Catches et al., 2006). Because of their role in acid-base regulation and use of ATP to do so, ionocytes and their ion transporting proteins have become a mechanism of interest for high- CO_2 tolerance (Esbaugh, 2018). NKA-positive ionocyte abundance was found to decrease with increasing temperature but not respond to CO_2 in the yolk sac of newly hatched Atlantic cod (*Gadus morhua*) larvae reared in combinations of temperature and CO_2 treatments (Dahlke et al., 2017). Another study on *G. morhua* found similar levels of ion pump enzyme activity in larvae to those of adult gill tissue, demonstrating great capacity for ion regulation despite their lack of fully developed organs (Dahlke et al., 2020). This study also highlighted the importance of measuring multiple metrics of ion regulation, such as ionocyte abundance, enzyme activity, and gene expression of ion transporting enzymes; enzyme activity of NKA, VHA, and ATP-synthase increased significantly with CO_2 in cod embryos and larvae (Dahlke et al., 2020), even though ionocyte abundance was not affected in the previous study (Dahlke et al., 2017). A study on white seabass (*Atractoscion nobilis*) eggs and larvae from a broodstock that had previously been exposed to high CO_2 found that rearing offspring in elevated CO_2 increased ionocyte abundance and density, but that accounting for ionocyte size to calculate total ionocyte area negated this effect (Kwan et al., 2021). This suggests that populations historically experiencing periods of high CO_2 , including estuarine fishes like *M. menidia*, may already have sufficient mechanisms in place to cope with predicted global CO_2 increases without making substantial changes to their physiology.

With this study, we aimed to mechanistically explain previously recorded growth and survival responses of *M. menidia* to elevated CO_2 by quantifying the temperature-dependent effects of seawater acidification on ionic regulation in the early life stages. We sampled embryos and larvae that were reared in four experiments combining factorial combinations of temperature

and partial pressure of CO₂ (pCO₂) and measured ionocyte density on the skin. A separate study also measured gene expression of seven ionoregulatory enzymes in fish from these experiments (Schwemmer et al., in prep). We hypothesized that early life *M. menidia* reared in elevated pCO₂ would have increased density of ionocytes as a mechanism for maintaining internal acid-base balance. We expected to see greater ionocyte densities on the skin surface to increase capacity for ion exchange in the absence of gills. Based on previous results showing reduced growth and survival and increased embryonic routine metabolic rates under high pCO₂ (Murray et al., 2014; Murray and Baumann 2018; Schwemmer et al., 2020), we hypothesized that more energy was redirected from growth to maintenance at the embryo stage, and therefore there would be fewer ionocytes in embryos exposed to high CO₂.

3.2. Methods

3.2.1. Animals, Sampling, and Treatments

Collection, rearing, and harvesting of live *M. menidia* were conducted as previously described in Murray and Baumann (2018) and Schwemmer et al. (2020). For the experiments in this study, wild, spawning ripe *M. menidia* adults were collected in 2016 from Mumford Cove, a salt marsh adjacent to Long Island Sound, New York, USA. Adults were transported to laboratory facilities at the Rankin Seawater Facility at University of Connecticut's Avery Point campus in Groton, Connecticut, USA. Females were strip-spawned at room temperature by gently squeezing their eggs into shallow spawning dishes containing pieces of plastic window screen (1 mm mesh). Milt from several males was collected, pooled, mixed in seawater, and poured into the spawning dishes. After 15 minutes, fertilized eggs attached to the screen via uncoiled chorionic filaments while unfertilized eggs could be gently rinsed off later. Screens were cut into pieces which were randomly divided and hung in 20-L rearing containers with mesh-covered holes for water to overflow without fish escaping. One rearing container containing 100 viable embryos was placed in each tank of treatment seawater within 2 hours of fertilization (filtered to 1 µm and UV sterilized).

Larvae hatched 4-11 days postfertilization and were immediately fed *ad libitum* with newly hatched brine shrimp nauplii (*Artemia* spp.). Embryos were collected one day before hatching for ionocyte staining (Figure 3.1). Larvae were collected for ionocyte staining at 1-day post hatch (dph) and once the larvae within a temperature treatment reached ~10 mm standard

length (SL; Figure 3.1). The final sampling point was determined by SL rather than time to allow assessment of treatment effects at a similar developmental stage (Tables 3.2 and 3.3).

Temperature strongly affects rates of growth and development, and this allowed us to correct for that so that differences in development would not be confounding treatment effects.

Consequently, age at final sampling ranged from 10 to 23 dph. Embryos and larvae used for ionocyte analysis first underwent respirometry (Schwemmer et al., 2020), then were fixed and preserved for staining.

Experimental treatment conditions are described in detail in Murray and Baumann (2018) and Schwemmer et al. (2020). Briefly, four factorial experiments were conducted in 2016 and 2017 combining environmentally relevant CO₂ and temperature levels as currently experienced in Long Island Sound estuaries and predicted worldwide by 2100 (IPCC, 2022). Target treatment levels and spawning dates for each experiment and life stages sampled for each response variable are summarized in Table 3.1. CO₂ levels were manipulated by continuously bubbling 100% CO₂ (bone dry grade, AirGas) mixed with air via gas proportioners (Cole Parmer, Vernon Hills, IL, USA) into rearing containers (Experiments 1 and 4) or by controlling bubbling 100% CO₂ into sumps of treatment tanks with an automated program designed in LabView software (National Instruments, Austin, TX, USA; Experiments 2 and 3). In Experiments 1 and 4, pH was monitored daily with a handheld probe and in Experiments 2 and 3 pH was measured once per hour automatically by pumping water from each tank to a central pH electrode, which was then used to control CO₂ input to maintain static treatments. Laboratory air was also bubbled continuously to maintain oxygen at 100% air saturation. Thermostats controlled aquarium heaters or chillers (DeltaStar, Lynchburg, VA, USA) to maintain temperature treatments. Carbonate chemistry conditions were characterized by measuring pH, temperature, salinity, and total alkalinity (TA). TA samples were collected three times per experiment and measured using an endpoint titration (G20 Potentiometric Titrator, Mettler Toledo, Columbus, OH, USA). CO₂SYN (V2.1) was used to calculate pCO₂ (μatm), fugacity of CO₂ (fCO₂, μatm), dissolved inorganic carbon (DIC, μmol kg⁻¹), and carbonate ion concentration (CO₃²⁻, μmol kg⁻¹). Measured seawater conditions are reported in Table S1.1.

3.2.2. *Ionocyte Density*

At the time of sampling, embryos and larvae were fixed in 4% paraformaldehyde (buffered, pH 7.4) and stored at 4°C for 4 hours, then transferred to 70% ethanol and stored at

4°C. Embryos were manually dechorionated before staining. Samples were equilibrated in phosphate buffered saline (PBS) for 5 minutes, blocked in normal horse serum for 20 minutes to minimize background staining, blotted dry, then incubated for 30 minutes in $\alpha 5$ primary antibody, a mouse monoclonal antibody raised against the alpha subunit of chicken NKA (D. M. Fambrough, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). The primary antibody was diluted to 1 $\mu\text{g/mL}$ for embryos and 2 $\mu\text{g/mL}$ for larvae, in PBS with 1% bovine serum albumin. Primary antibody binding was detected using the avidin-biotin complex (ABC) method (Hsu et al., 1981). Samples were incubated for 30 minutes each in a secondary antibody and ABC reagent (Vectastain[®] ABC HRP Kit, Peroxidase, Mouse IgG, Vector Laboratories, Burlingame, CA) and rinsed for 5 minutes in PBS between each step. The specific binding was then visualized with a peroxidase substrate kit (Vector[®] VIP HRP Substrate Kit, Vector Laboratories, Burlingame, CA). Conducting this protocol in the absence of primary antibody produced no staining, confirming that the staining indicated presence of the antigen NKA.

All samples were photographed through a compound microscope (Nikon Eclipse E200) at several depths of focus to capture the entirety of the three-dimensional structure. Images were analyzed using the software ImageJ. For each individual fish, the images from all focus depths were loaded as a stack and projected into a single image with all ionocytes in focus. We selected and measured the area (mm^2) of a section of upward-facing skin surface in the resulting photo and counted the ionocytes within that area. For larvae, the ionocytes on the head, abdomen, and trunk skin surface, excluding the eyes and mouth, were enumerated. For embryos, yolk sac epithelium was analyzed separately from the rest of the skin. Ionocyte density was calculated as ionocytes mm^{-2} of skin surface area. Ionocyte density was quantified by two different people or twice by the same person with at least one month in between to account for subjectivity and uncertainty in ionocyte identification. Standard deviation between the two densities was calculated and if it was greater than three standard deviations above the mean, the sample was analyzed again by the same methods to rectify differences in analysis.

3.2.3. *Data Analysis*

Statistical analysis of the ionocyte density data was conducted using linear regression models with the formula: Ionocyte Density $\sim \text{pCO}_2 \times \text{Temperature}$. The independent variables

were input as quantitative variables using the mean measured pCO₂ and temperatures for each treatment. In each experiment we sampled one experimental unit (n=1) per treatment, and not all treatment levels were repeated in multiple experiments (e.g. 28°C) so we had pseudoreplication. When tests that use categorical independent variables, such as analysis of variance, cannot be used due to pseudoreplication, a model can instead be fit with quantitative (continuous) independent variables (Hurlbert, 2004), which in fact provides greater statistical power and more informative results than an analysis with categorical independent variables would (Cottingham et al., 2005; Havenhand et al., 2010). Assumptions of normally distributed residuals and homoskedasticity were confirmed using the Kolmogorov-Smirnov test and Breusch-Pagan test, respectively. Linear model residuals and Cook's distance were used to identify extreme and highly influential datapoints, which resulted in one outlier being removed from the data for each stage. A significance threshold of $\alpha=0.05$ was used for all statistical tests. All statistical tests were performed in R statistical software (v4.2.2, R Core Team, 2022) using the 'stats' and 'olsrr' (Hebbali, 2020) packages.

3.3. Results

Embryos and hatchlings had higher maximum skin ionocyte densities, 722.6 and 611.4 ionocytes mm⁻², respectively, while 10-mm larvae had lower ionocyte densities, with a maximum of 418.8 ionocyte mm⁻². However, embryo data were distributed around a lower mean with a strong positive skew while both stages of larvae had normally distributed ionocyte densities (Figure 3.2). Yolk data were more skewed than skin data. Therefore, to meet the assumptions of normality and homoskedasticity the embryo yolk data were log-transformed and embryo skin data were square-root transformed in the linear model. Additionally, across all treatments combined the non-yolk portion of the embryos' bodies had significantly greater ionocyte density than the yolks (t-test, $p<0.001$, $t_{(558)}=-3.7$), and progression of ionocyte development commonly differs between the yolk sac and rest of the body (Alderdice, 1988) so we analyzed these regions separately.

Temperature and pCO₂ had a significant interactive effect on embryo yolk sac (linear regression, $F_{(3,280)}=23.94$, $p < 0.01$) and skin (linear regression, $F_{(3,280)}=16.3$, $p < 0.001$) surface ionocyte density (Figure 3.3). Ionocyte density decreased with increasing temperature, with 28°C embryos having the lowest ionocyte densities across all temperature and pCO₂ levels. The

greatest mean yolk sac ionocyte density occurred in the highest pCO₂ and 17°C treatment combination, while skin ionocytes exhibited a weaker pCO₂ effect at this temperature. Within the 17°C treatments, the mean yolk ionocyte density at 4200 µatm pCO₂ is 118.4% greater than that at 400 µatm, while the skin ionocyte density at 4200 µatm is only 61.9% greater than the density at 400 µatm. In the yolk and body skin it appears that high CO₂ increases the temperature-dependence of ionocyte density, making it decrease more sharply as temperature increases. Within the 4200 µatm pCO₂ level, yolk sac ionocyte density decreases more dramatically with temperature than skin ionocyte density does, illustrating the greater response of yolk sac ionocytes to elevated pCO₂.

Temperature and pCO₂ had a significant interactive effect on ionocyte densities of newly hatched larvae (linear regression, $F_{(3,393)}=9.461$, $p = 0.001$). Hatchlings show the opposite temperature effect of embryos, with ionocyte density increasing with temperature, particularly in the 28°C treatment (Figure 3.4A). The 400 µatm pCO₂ treatment groups have a slight decrease in ionocytes as temperature decreases up to 24°C before increasing sharply at 28°C. In contrast, the 4200 µatm larvae have a more steady increase in ionocytes with temperature across all temperature levels. Within the 24°C treatments, ionocyte density at 4200 µatm pCO₂ is 16.7% higher than at 400 µatm. At the 10-mm larval sampling point the ionocyte densities are no longer affected by temperature or pCO₂ (linear regression, $F_{(3,337)}=7.946$, $p>0.05$). The most substantial difference is that at 17°C ionocyte density at 4200 µatm pCO₂ is 12.7% and 17.4% greater than at 400 µatm and 2200 µatm, respectively (Figure 3.4B). At this stage, three gill arches with filaments and lamellae had developed on each side and skin surface ionocyte densities decreased to just over half the density of newly hatched larvae, regardless of treatments.

Embryos and hatchlings in this study were also used for respirometry measurements (full results reported in Chapter 1). We used Pearson's correlation test to test whether ionocyte density is significantly correlated with mass-specific routine metabolic rates (RMR) in individuals for which both quantities were measured. In embryos, RMR significantly decreased with increasing ionocyte density (Pearson's correlation coefficient = -0.21, $t_{(241)}=-3.83$, $p<0.001$) while in larvae RMR significantly increased with ionocyte density (Pearson's correlation coefficient = 0.13, $t_{(353)}=2.55$, $p=0.01$). Both response variables are strongly affected by temperature, as RMR increases with temperature while ionocyte density's relationship with temperature is stage-dependent. Before hatching ionocyte density is lower at the highest

temperatures, leading to a negative correlation of RMR with ionocyte density (Figure 3.5A). After hatching ionocyte density is highest at high temperature and the RMR-ionocyte correlation becomes positive (Figure 3.5B).

3.4. Discussion

Across four experiments exposing *M. menidia* embryos and larvae to pCO₂ and temperature treatments, ionocyte density was primarily influenced by temperature with some interacting, low magnitude pCO₂ effects. In embryos sampled about one day before hatching, high pCO₂ increased overall temperature-sensitivity of ionocyte density. Embryos reared at the lowest temperature had greater ionocyte density at the highest pCO₂ level, particularly on the yolk sac epithelium. Embryonic ionocytes have been noted to occur on the yolk sac in proximity to vitelline blood vessels (Guggino, 1980; Alderdice, 1988), likely to facilitate ion transport for blood pH regulation. Low temperature slows growth, with offspring in our 17°C taking the longest to hatch, so the additional incubation time and delay in gill development may have led to increased ionocyte formation in response to high pCO₂ to promote H⁺ removal and HCO₃⁻ accumulation, particularly in the yolk sac area. Previous studies support the idea that substantial ion transport is possible in early life stages exposed to high pCO₂, despite incomplete development of organ systems. Acidified conditions have been shown to elicit both an increase in ionocyte density (Kwan et al., 2021) and accumulation of HCO₃⁻ (Kwan and Tresguerres, 2022). While fish in the earliest stages of embryogenesis and gastrulation may rely on passive defenses such as non-HCO₃⁻ buffers (Alderdice, 1988; Melzner et al., 2009), differentiation of cells like ionocytes and plasticity in homeostatic mechanisms seem to confer sufficient tolerance for high pCO₂ (Dahlke et al., 2020). The pCO₂ effect observed in *M. menidia* embryos from 17°C treatments, as well as the overall abundance of NKA-rich ionocytes on the skin of near-hatching embryos and newly hatched larvae, indicate a substantial capacity for removing H⁺ ions by creating a Na⁺ gradient to drive NHE2/3.

Although ionocyte density was low among most embryos, at 17°C embryos were able to produce more ionocytes in response to high pCO₂, which could aid acid-base balance and improve chances of hatch survival. Ionocyte density is subject to phenotypic plasticity, as demonstrated by past studies that have shown changes in ionocyte density, distribution, and functioning in response to environmental conditions such as salinity in euryhaline species

(Schwerdtfeger and Bereiter-Hahn, 1978; Zydlewski et al., 2003; Hiroi and McCormick, 2012) and air exposure in amphibious species (Tunnah et al., 2022). Our results support our hypothesis that chronic, environmentally relevant high CO₂ can similarly induce a plastic response in skin ionocytes of *M. menidia*, although this response was only detected at the lowest temperature. Without measurements of enzyme activity levels, energetic costs of additional ionocytes cannot be ascertained. Nonetheless, this finding implies that additional energy may be directed to pumping ions under these conditions, which may boost chances of survival but could also be expected to divert energy away from growth, which could have implications for post hatch survival, predation vulnerability, and ultimately recruitment (Houde, 1997).

Temperature evidently affects ionocyte density either directly, such as by increasing enzyme activity rates and reducing the need for greater quantities of ionocytes, or indirectly through impacts on development rate. We observed a decrease in embryonic ionocytes with increasing temperature and an increase in larval ionocyte density at the highest temperature treatment (28°C). However, it is uncertain if these differences in ionocyte density are a direct result of temperature, and thus further evidence of phenotypic plasticity, or instead an indirect effect of temperature through its influence on developmental rate. Although we set our sampling timepoints to compensate for effects of temperature on development rates – sampling based on hatching and total length rather than equal times from fertilization – we have still observed temperature effects on body size at each time point, for example reduced length at hatching for 28°C relative to those reared at 24°C (Murray and Baumann, 2018).

The differences in distribution of ionocyte abundances directly before and after hatching are potentially an important mechanism by which sensitivity improves once larvae have hatched. Before hatching, the frequency distribution has a strong positive skew, particularly on the yolk sac, showing that while the majority of embryos have a relatively low ionocyte density, a smaller subset have great ionocyte abundances that are similar to those of hatchlings. By the day of hatching, which in our experiments was only one or two days after sampling of embryos, the skew is gone and ionocyte densities are symmetrically distributed around a much higher mean (Figure 3.2). There are two possible explanations for this rapid shift, one being that ionocytes proliferate around the time of hatching and only low numbers of the embryos we sampled had such high abundances. The second explanation is that there is broad natural variability in ionocyte production and embryos with lower ionocytes are less likely to survive to hatching

because of their reduced capacity for homeostasis, leaving the remaining hatchlings with greater ionocyte abundances. Such selective mortality would not be restricted to fish in acidified conditions, as ionocytes are also central to salt secretion for osmoregulation (Hwang and Lin, 2013; Liu et al., 2016), but it still could favorably produce survivors that are better prepared to regulate acid-base balance. The reality may be a combination of these two explanations, and both help explain why post-hatch larvae were less sensitive to both pCO₂ and temperature. This pattern also may be responsible for the reversal in temperature effect on ionocyte density. Mean hatch survival in these experiments was lower at 28°C than the other temperatures (Table 3.5; Murray and Baumann, 2018) and these embryos also had the lowest ionocyte density. The rapid shift to 28°C fish having the greatest ionocyte densities makes sense if the individuals with less ionocytes were less likely to survive hatching.

The large impact of 28°C compared to differences among the other temperature treatments aligns with results for other response variables from the same set of experiments: quick hatching time, small larval size, and high metabolism (Table 3.5; Figure 3.5; Murray and Baumann, 2018; Schwemmer et al., 2020). Combined, these results indicate that the temperature of 28°C – rarely encountered in the spawning season and regional habitat of this species – requires high maintenance rates that quickly deplete the yolk while reserving less energy for growth and development. The rapid increase we observed in ionocyte density after hatching at 28°C may serve as evidence of delayed ionocyte formation, either due to temperature-related energetic limitations or growth and hatching exceeding the limits of ionocyte formation speed. At the same time, evidence for *G. morhua* show increased enzyme activity and reduced ionocyte density with high temperature (Dahlke et al., 2017; Dahlke et al., 2020), suggesting that fish reared at high temperature have fewer ionocytes that are more active to keep up with ionoregulatory needs. While we did not measure enzyme activity in this study, metabolic rates of hatchlings slightly increased with ionocyte density, suggesting a low magnitude increase in energy used for ionic regulation in fish with more ionocytes. More likely, however, is that this correlation is related to the high ionocyte densities we found at high temperature. As shown in Figure 3.5, RMR increases with temperature (Schwemmer et al., 2020), and the correlations of RMR with ionocyte density are likely an indirect result of how temperature affects both; ionocyte density is negatively correlated with temperature before hatching and positively correlated with it after hatching. Even if we cannot be certain that the additional ionocytes at

28°C are accompanied by additional energy spent on pH regulation at high CO₂, the lack of CO₂ effects on survival in these experiments (Murray and Baumann, 2018) provides further evidence that the ionocytes were sufficient for acid-base regulation, or at least a bare minimum for survival.

When larvae reached 10-mm standard length, skin surface ionocyte densities had decreased to just over half of the densities quantified in hatchlings. These mature larvae consistently had three gill arches on each side of the body, with several gill filaments and secondary lamellae usually visible and ionocytes present. These trends are consistent with the transition from reliance on cutaneous to branchial ionocytes as fish progress through the larval stage (Alderdice, 1988; Hiroi et al., 1998). There were no CO₂ or temperature effects on skin ionocyte density of 10-mm larvae. This is not surprising, as surface area is increasing in the gills and the number of ionocytes may no longer be a limiting factor in ion exchange or plasticity may become centralized in the gills, which are more efficient at ion regulation due to their proximity to both the blood and outside environment. The oldest individuals sampled at this stage were 23 dph, so the latest most silversides would be reaching this stage is around the end of July. In Long Island Sound, this is approaching the hottest time of year with greatest incidences of eutrophication, stratification, hypoxia, and coastal acidification. It bodes well for larvae that the gills are already developing, mobility is increasing, and reliance on skin surface ionocytes appears to be declining by this stage. We did not sample mature larvae in Experiments 3 or 4, the ones that included 28°C treatments, so it is possible this prevented us from detecting a high temperature effect as was observed in the earlier stages. Future work should continue to include such high temperatures to aid understanding of the interactions between temperature stress and acid-base regulation and further establish the environmental limits of this species with physiological mechanisms.

This study presents a mechanism for the generally high tolerance *M. menidia* have for high CO₂ while showing some evidence for temperature-dependent plasticity in ionocyte density, potentially exchanging energy that would have gone towards growth for maintenance costs under high CO₂. Further support for this mechanism could be found by measuring activity of ion transporting enzymes, which could confirm whether the ionocyte differences we did observe can be considered a proxy for energy expended on ion exchange. This could also improve understanding of how responsive *M. menidia* ionocytes are to changes in CO₂ when density is

unaffected, as it is possible the ionocytes simply increase activity without the fish needing to produce more cells. There are two types of ionocytes that marine fish use to transport ions for pH and osmoregulation. We stained for Type-I ionocytes, in which NKA play a role in pH compensation by creating a Na^+ gradient that allows NHE2/3 to passively exchange H^+ in the cell for Na^+ from the seawater (Silva et al., 1977; Claiborne et al., 2002; Kwan et al., 2020). Type-II ionocytes are high in carbonic anhydrase and VHA, which allows them to contribute to both transport of HCO_3^- into the endolymph and H^+ out of the body (Kwan et al., 2020). Although logistical constraints prevented us from staining for Type-II ionocytes, it would be useful to know the extent to which the cellular mechanisms of *M. menidia* early life CO_2 responses include HCO_3^- transport and whether Type-II ionocytes exhibit similar patterns of sensitivity and resistance to acidification and high temperature to those of Type-I ionocytes. Quantifying VHA-rich cells would also contribute to our knowledge of the energy expended on ion exchange early in life. Consistent with what is expected for an estuarine species that has historically experienced periods of high CO_2 (Baumann, 2019; Kwan et al., 2021), *M. menidia* develop ionocytes before hatching that likely confer the ability to tolerate a wide range of acidity levels. While CO_2 effects on ionocyte density were minimal in the early life stages of *M. menidia*, extreme high temperatures and the accompanying high rates of development and yolk depletion may compromise the ability of embryos and larvae to develop cells for active ionic regulation. Knowledge of the mechanisms behind interacting stressor impacts such as these has important implications for accurately predicting energetically determined responses, such as reduced body size or survival, that may have population-level consequences, such as predation mortality or reproductive output.

3.5. References

- Alderdice, D. F. 1988. Osmotic and ionic regulation in teleost eggs and larvae. *In: Fish Physiology, Vol. 11A: The Physiology of Developing Fish, Part A: Eggs and Larvae.* (ed. W. S. Hoar and D. J. Randall), pp. 163-251. San Diego: Academic Press.
- Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97(5): 399-408. <https://doi.org/10.1139/cjz-2018-0198>
- Baumann, H., Talmage, S. C., and Gobler, C. J. 2012. Reduced early life growth and survival in a fish in direct response to increase carbon dioxide. *Nat. Clim. Change*, 2: 38-41. doi: 10.1038/nclimate1291
- Baumann, H., Cross, E. L., and Murray, C. S. 2018. Robust quantification of fish early life CO₂ sensitivities via serial experimentation. *Biol. Lett.* 14: 20180408. doi:10.1098/rsbl.2018.0408
- Bengtson, D. A., Barkman, R. C., and Berry, W. J., 1987. Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*. *J. Fish. Biol.*, 31: 697-704.
- Berenbrink, M., Koldkjær, P., Keep, O. and Cossins, A. R. 2005. Evolution of Oxygen Secretion in Fishes and the Emergence of a Complex Physiological System. *Science*, 307(5716): 1752-1757.
- Brauner, C. J. and Randall, D. J. 1996. The interaction between oxygen and carbon dioxide movements in fishes. *Comp. Biochem. Physiol.*, 113A: 83-90. doi: 10.1016/0300-9629(95)02062-4
- Brauner, C. J., Shartau, R. B., Damsgaard, C., Esbaugh, A. J., Wilson, R. W., and Grosell, M. 2019. Acid-base physiology and CO₂ homeostasis: Regulation and compensation in response to elevated environmental CO₂. *In: Fish Physiology, Vol. 37: Carbon Dioxide* (ed. A. P. Farrell and C. J. Brauner), pp. 69-132. San Diego: Academic Press.
- Catches, J. S., Burns, J. M., Edwards, S. L., and Claiborne, J. B. 2006. Na⁺/H⁺ antiporter, V-H⁺-ATPase and Na⁺/K⁺-ATPase immunolocalization in a marine teleost (*Myoxocephalus octodecemspinosus*). *J. Exp. Biol.*, 209: 3440-3447.
- Cattano, C., Claudet, J., Domenici, P., and Milazzo, M. 2018. Living in a high CO₂ world: a global meta-analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecol. Monogr.*, 88(3): 320-335.

- Christensen, A. K., Hiroi, J., Schultz, E. T., and McCormick, S. D. 2012. Branchial ionocyte organization and ion-transport protein expression in juvenile alewives acclimated to freshwater or seawater. *J. Exp. Biol.*, 215: 642-652.
- Claiborne, J. B., Edwards, S. L., and Morrison-Shetlar, A. I. 2002. Acid-Base Regulation in Fishes: Cellular and Molecular Mechanisms. *J. Exp. Zool.*, 293: 302-319.
- Clark, T. D., Raby, G. D., Roche, D. G., Binning, S. A., Speers-Roesch, B., Jutfelt, F., and Sundin, J. 2020. Ocean acidification does not impair the behavior of coral reef fishes. *Nature*, 577: 370-375. doi:10.1038/s41586-019-1903-y
- Cottingham, K. L., Lennon, J. T., and Brown, B. L. 2005. Knowing when to draw the line: designing more informative ecological experiments. *Front. Ecol. Environ.*, 3: 145-152. doi:10.1890/1540-9295(2005)003[0145:KWTDTL]2.0.CO;2
- Couturier, C. S., Stecyk, J. A. W., Rummer, J. L., Munday, P. L., and Nilsson, G. E. 2013. Species-specific effects of near-future CO₂ on the respiratory performance of two tropical prey fish and their predator. *Comp. Biochem. Physiol. A*, 166: 482-489. doi:10/1016/j.cbpa.2013.07.025
- Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal pCO₂ x O₂ fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9: 18146. doi:10.1038/s41598-019-53930-8
- Dahlke, F. T., Leo, E., Mark, F. C., Pörtner, H.-O., Bickmeyer, U., Frickenhaus, S., and Storch, D. 2017. Effects of ocean acidification increase embryonic sensitivity to thermal extremes in Atlantic cod, *Gadus morhua*. *Glob. Change Biol.*, 23: 1499-1510. doi: 10.1111/gcb.13527
- Dahlke, F., Lucassen, M., Bickmeyer, U., Wohlrab, S., Puvanendran, V., Mortensen, A., Chierici, M., Pörtner, H.-O., and Storch, D. 2020. Fish embryo vulnerability to combined acidification and warming coincides with a low capacity for homeostatic regulation. *J. Exp. Biol.*, 223: jeb212589. doi: 10.1242/jeb.212589
- Deigweiher, K., N. Koschnick, H.-O. Pörtner, M. Lucassen. 2008. Acclimation of ion regulatory capacities in gills of marine fish under environmental hypercapnia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295: R1660-R1670.
- Dixon, R. L., Greco, P. A., and Targett, T. E. 2017. Responses of juvenile Atlantic silverside, striped killifish, mummichog, and striped bass to acute hypoxia and acidification: Aquatic surface respiration and survival. *J. Exp. Mar. Biol. Ecol.*, 493: 20-30.
- Esbaugh, A. J. 2018. Physiological implications of ocean acidification for marine fish: emerging patterns and new insights. *J. Comp. Physiol. B*, 188: 1-13. <https://doi.org/10/1007/s00360-017-1105-6>

- Esbaugh, A. J., R. Heuer, M. Grosell. 2012. Impacts of ocean acidification on respiratory gas exchange and acid-base balance in a marine teleost, *Opsanus beta*. *J. Comp. Physiol. B*. 182: 921-934.
- Espinel-Velasco, N., Hoffmann, L., Agüera, A., Byrne, M., Dupont, S., Uthicke, S., Webster, N. S., and Lamare, M. 2018. Effects of ocean acidification on the settlement and metamorphosis of marine invertebrate and fish larvae: a review. *Mar. Ecol. Prog. Ser.*, 606: 237-257. <https://doi.org/10.3354/meps12754>
- Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., et al. 2012. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nat. Clim. Change*, 2: 42-46. doi: 10.1038/nclimate1324
- Grosell, M. 2019. CO₂ and calcification processes in fish. In: *Fish Physiology, Vol. 37: Carbon Dioxide* (ed. A. P. Farrell and C. J. Brauner), pp. 133-159. San Diego: Academic Press.
- Guggino, W. B. 1980. Salt balance in embryos of *Fundulus heteroclitus* and *F. bermudae* adapted to seawater. *Am. J. Physiol.*, 238: R36-R41.
- Gunderson, A. R., Armstrong, E. J., and Stillman, J. H. 2016. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Annu. Rev. Mar. Sci.*, 8: 357-378. doi:10.1146/annurev-marine-122414-033953
- Havenhand, J., Dupont, S., and Quinn, G. P. 2010. Designing ocean acidification experiments to maximize inference. In *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (ed. U. Riebesell, V. J. Fabry, L. Hansson and J.-P. Gattuso), pp. 67-136. Luxembourg: Publications Office of the European Union Luxembourg.
- Hebbali, A. 2020. olsrr: Tools for Building OLS Regression Models. R package version 0.5.3, <<https://CRAN.R-project.org/package=olsrr>>.
- Heuer, R. M and Grosell, M. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 307: R1061-R1084. doi: 10.1152/apjregu.00064.2014
- Hiroi, J. and McCormick, S. D. 2012. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. *Resp. Physiol. Neurobiol.*, 184: 257-268.
- Hiroi, J., Kaneko, T., Seikai, T., and Tanaka, M. 1998. Developmental Sequence of Chloride Cells in the Body Skin and Gills of Japanese Flounder (*Paralichthys olivaceus*) Larvae. *Zool. Sci.*, 15: 455-460.
- Houde, E. D. 1997. Patterns and consequences of selective processes in teleost early life histories. In: *Early Life History and Recruitment in Fish Populations*. (ed. R. Christopher Chambers and Edward A Trippel), pp. 173-196. London: Chapman & Hall.

- Hsu, S.-M., Raine, L., and Fanger, H. 1981. Use of Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques: A Comparison between ABC and Unlabeled Antibody (PAP) Procedures. *J. Histochem. Cytochem.*, 29(4): 577-580.
- Hurlbert, S. H. 2004. On misinterpretations of pseudoreplication and related matters: a reply to Oksanen. *Oikos*, 104: 591-597. doi:10.1111/j.0030-1299.2004.12752.x
- Hwang, P. P., and Lin, L. Y. 2013. Gill ionic transport, acid-base regulation and nitrogen excretion. In: *The Physiology of Fishes*. (Eds. D. Evans, J. B. Claiborne, and S. Currie), pp. 205-233, Boca Raton: CRC Press.
- Intergovernmental Panel on Climate Change. 2022. Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama, eds.]. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.
- Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K.-S., and Kita, J. 2004. Effects of CO₂ on Marine Fish: Larvae and Adults. *J. Oceanogr.*, 60: 731-741.
- Ishimatsu, A., Hayashi, M., and Lee, K.-S. 2005. Physiological effects on fishes in a high-CO₂ world. *J. Geophys. Res.*, 110: C09S09. doi: 10.1029/2004JC002564
- Jarrold, M. D. and Munday, P. L. 2018. Diel CO₂ cycles do not modify juvenile growth, survival and otolith development in two coral reef fish under ocean acidification. *Mar. Biol.*, 165: 49. <https://doi.org/10.1007/s00227-018-3311-5>
- Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.*, 13: 1419-1434. doi: 10.1111/j.1461-0248.2010.01518.x
- Kwan, G. T., and Tresguerres, M. 2022. Elucidating the acid-base mechanisms underlying otolith overgrowth in fish exposed to ocean acidification. *Sci. Total Environ.*, 832: 153690. <https://doi.org/10.1016/j.scitotenv.2022.153690>
- Kwan, G. T., Smith, T. R., and Tresguerres, M. 2020. Immunological characterization of two types of ionocytes in the inner ear epithelium of Pacific Chub Mackerel (*Scomber japonicus*). *J. Comp. Physiol. B*, 190: 419-431.
- Kwan, G. T., Shen, S. G., Drawbridge, M., Checkley Jr., D. M., and Tresguerres, M. 2021. Ion-transporting capacity and aerobic respiration of larval white seabass (*Atractoscion nobilis*) may be resilient to ocean acidification conditions. *Sci. Total Environ.*, 791: 148285. <https://doi.org/10.1016/j.scitotenv.2021.148285>

- Lavaud, R., Filgueira, R., and Augustine, S. 2021. The role of Dynamic Energy Budgets in conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi: 10.1093/conphys/coab083
- Le Quesne, W. J. F. and Pinnegar, J. K. 2012. The potential impacts of ocean acidification: scaling from physiology to fisheries. *Fish Fish.*, 13: 333-344.
- Lefevre, S. 2019. Effects of high CO₂ on oxygen consumption rates, aerobic scope and swimming performance. In: *Fish Physiology, Vol. 37: Carbon Dioxide* (ed. A. P. Farrell and C. J. Brauner), pp. 195-244. San Diego: Academic Press.
- Liu, S.-T., Horng, J.-L., Chen, P.-Y., Hwang, P.-P., and Lin, L.-Y. 2016. Salt secretion is linked to acid-base regulation of ionocytes in seawater acclimated medaka: new insights into the salt-secreting mechanism. *Sci. Rep.*, 6: 31433. doi:10.1038/srep31433
- Marshall, D. J., Burgess, S. C., and Connallon, T. 2016. Global change, life-history complexity and the potential for evolutionary rescue. *Evol. Appl.*, 9: 1189-1201.
- Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Pörtner, H.-O. 2009. Physiological basis for high CO₂ tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosci.*, 6: 2313-2331. www.biogeosciences.net/6/2313/2009/
- Middaugh, D. P., Hemmer, M. J., and Goodman, L. 1987. Methods for Spawning, Culturing and Conducting Toxicity-Tests with Early Life Stages of four Atherinid Fishes: The Inland Silverside, *Menidia beryllina*, Atlantic silverside, *M. Menidia*, Tidewater silverside, *M. peninsula*, and California grunion, *Leuresthes tenuis*. Gulf Breeze, FL: United States Environmental Protection Agency.
- Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ x Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*, 10: 69. doi:10.3390/d10030069.
- Murray, C. S., Malvezzi, A., Gobbler, C. J., and Baumann, H. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.*, 504: 1-11. doi:10.3354/meps10791
- Pimentel, M. S., Faleiro, F., Marques, T., Bispo, R., Dionísio, G., Faria, A. M., Machado, J., Peck, M. A., Pörtner, H., Pousão-Ferreira, P., Gonçalves, E. J., and Rosa, R. 2016. Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming. *Clim. Change*, 137: 495-509.
- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.

- Randall, D. J., Heisler, N., and Drees, F. 1976. Ventilatory response to hypercapnia in larger spotted dogfish *Scyliorhinus stellaris*. *Am. J. Physiol.*, 230(3): 590-594.
- Rodriguez-Dominguez, A., Connell, S. D., Baziret, C., and Nagelkerken, I. 2018. Irreversible behavioural impairment of fish starts early: Embryonic exposure to ocean acidification. *Mar. Poll. Bull.*, 133: 562-567.
- Schultz, E. T., Conover, D. O., and Ehtisham, A. 1998. The dead of winter: size-dependent variation and genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia menidia*) from different latitudes. *Can. J. Fish. Aquat. Sci.*, 55: 1149-1157.
- Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., and Nye, J. A. 2020. Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish *Menidia menidia*. *J. Exp. Biol.* 223: jeb228015. doi:10.1242/jeb.228015
- Schwerdtfeger, W. K., and Bereiter-Hahn, J. 1978. Transient occurrence of chloride cells in the abdominal epidermis of the guppy, *Poecilia reticulata* Peters, adapted to sea water. *Cell Tissue Res.*, 191: 463-471.
- Silva, P., Solomon, R., Spokes, K., and Epstein, F. H. 1977. Ouabain inhibition of gill Na-K-ATPase: Relationship to active chloride transport. *J. Exp. Zool.*, 199(3): 419-426.
- Sundin, J., Amcoff, M., Mateos-González, F., Raby, G. D., and Clark, T. D. 2019. Long-term acclimation to near-future ocean acidification has negligible effects on energetic attributes in a juvenile coral reef fish. *Oecologia*, 190: 689-702. <https://doi.org/10.1007/s00442-019-04430-z>
- Tunnah, L., Turko, A. J., and Wright, P. A. 2022. Skin ionocyte density of amphibious killifishes is shaped by phenotypic plasticity and constitutive interspecific differences. *J. Comp. Physiol. B*, 192: 701-711.
- Zydlewski, J., McCormick, S. D., and Kunkel, J. G. 2003. Late migration and seawater entry is physiologically disadvantageous for American shad juveniles. *J. Fish. Biol.*, 63: 1521-1537.

3.6. Figures

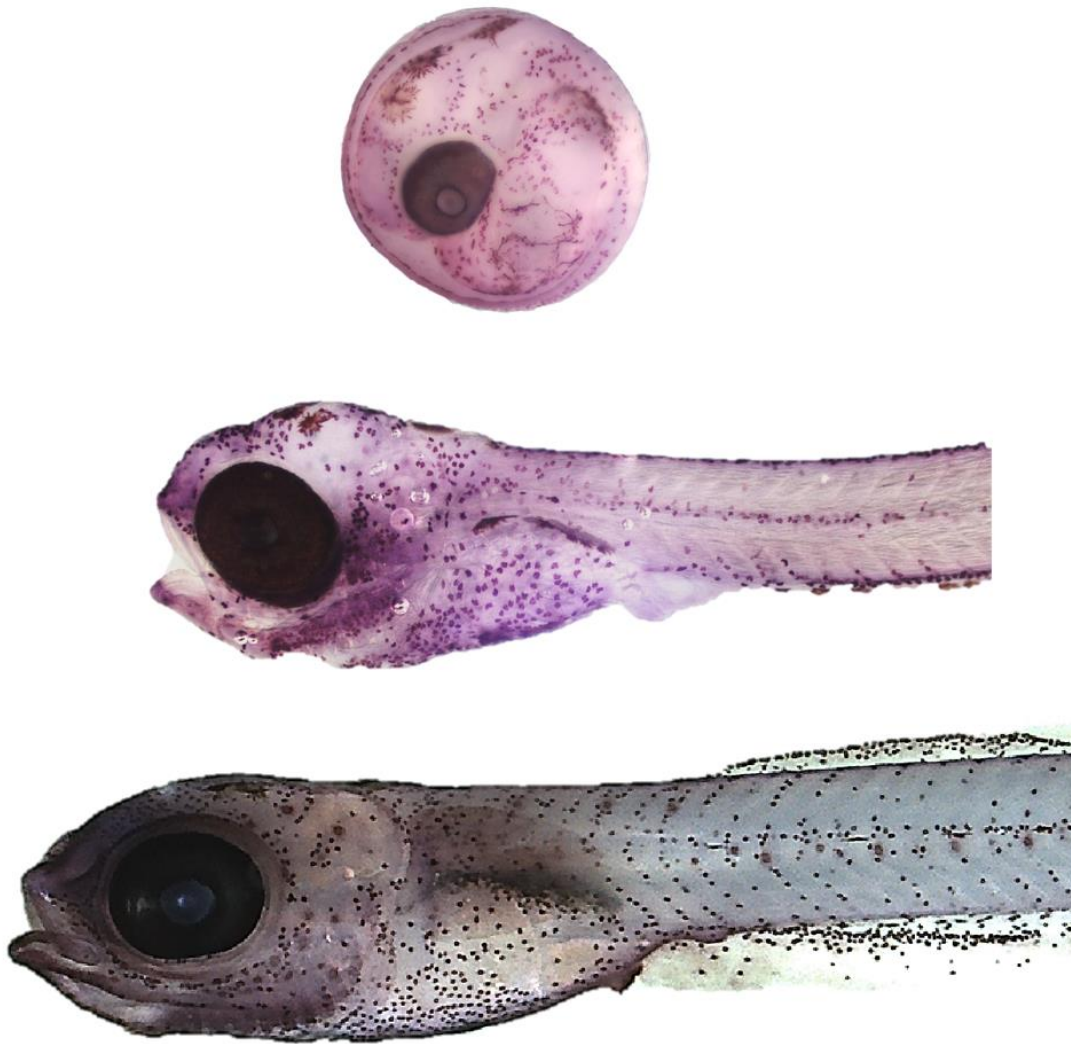


Figure 3.1. Microscope images of an *M. menidia* embryo, hatchling, and 10-mm larva (from top to bottom) with ionocytes stained dark purple.

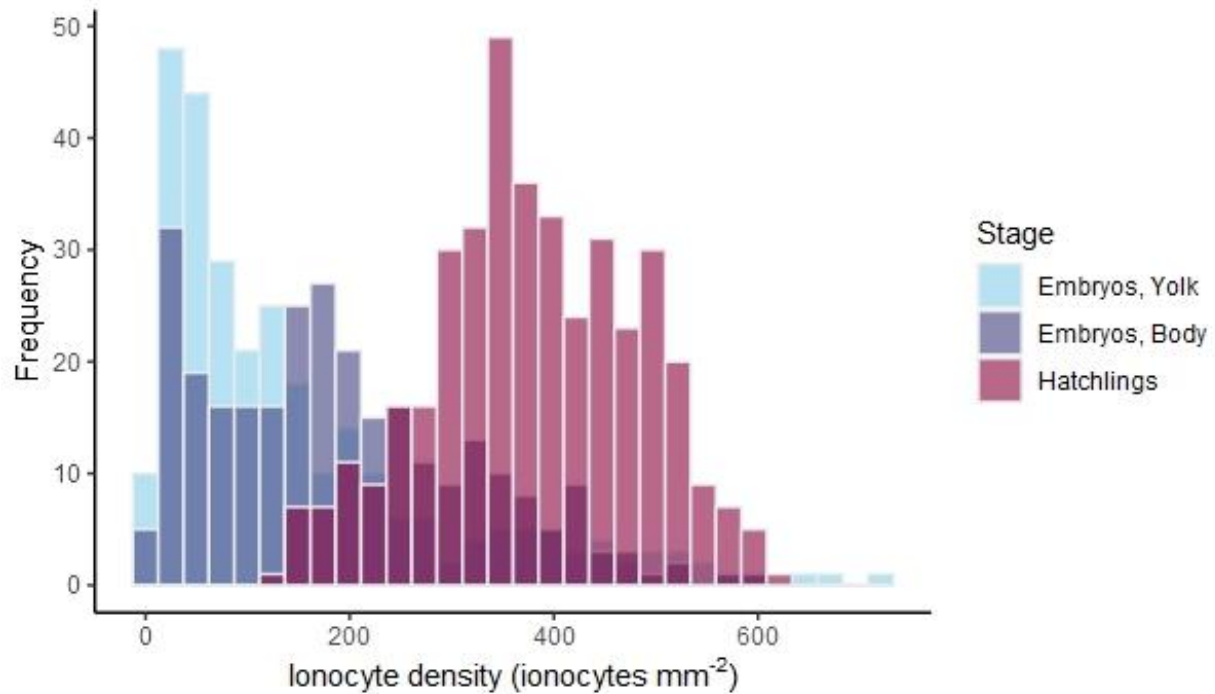


Figure 3.2. Frequency distribution of ionocyte densities at the embryo stage (yolk and skin) and hatchlings. In embryos the data are positively skewed, particularly for the yolk, but immediately after hatching the ionocyte densities are more normally distributed. The 10-mm larvae also have normally distributed ionocyte densities, although around a lower mean, but we do not display them here so the difference in distribution before and after hatching can be emphasized.

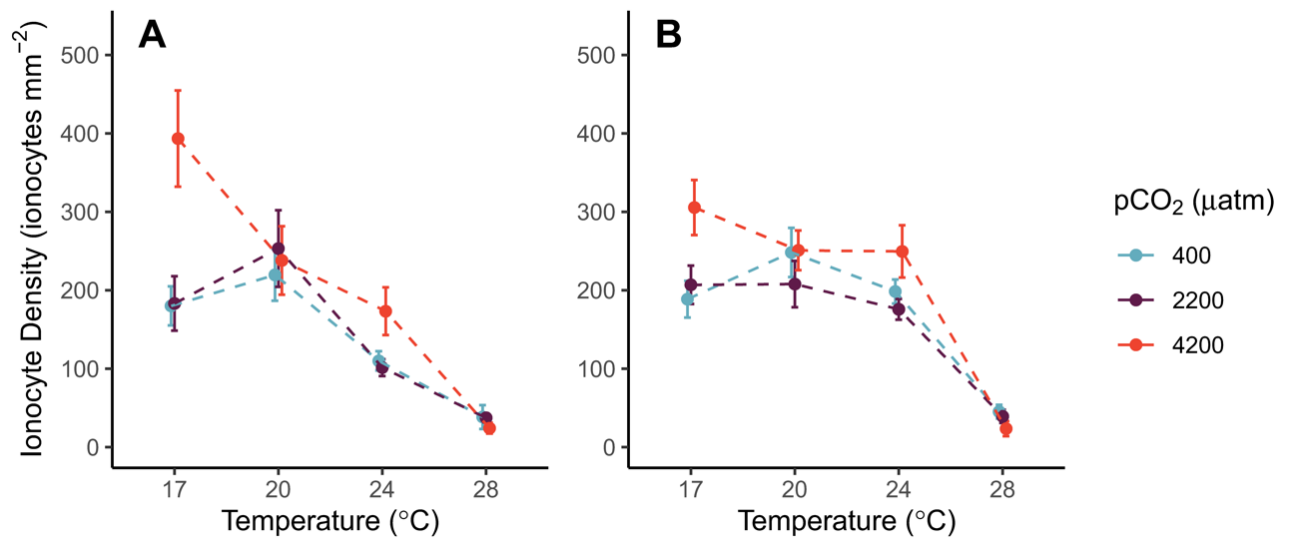


Figure 3.3. Embryo yolk sac (A) and skin (B) ionocyte density means plotted with respect to temperature and pCO₂. Error bars show standard error.

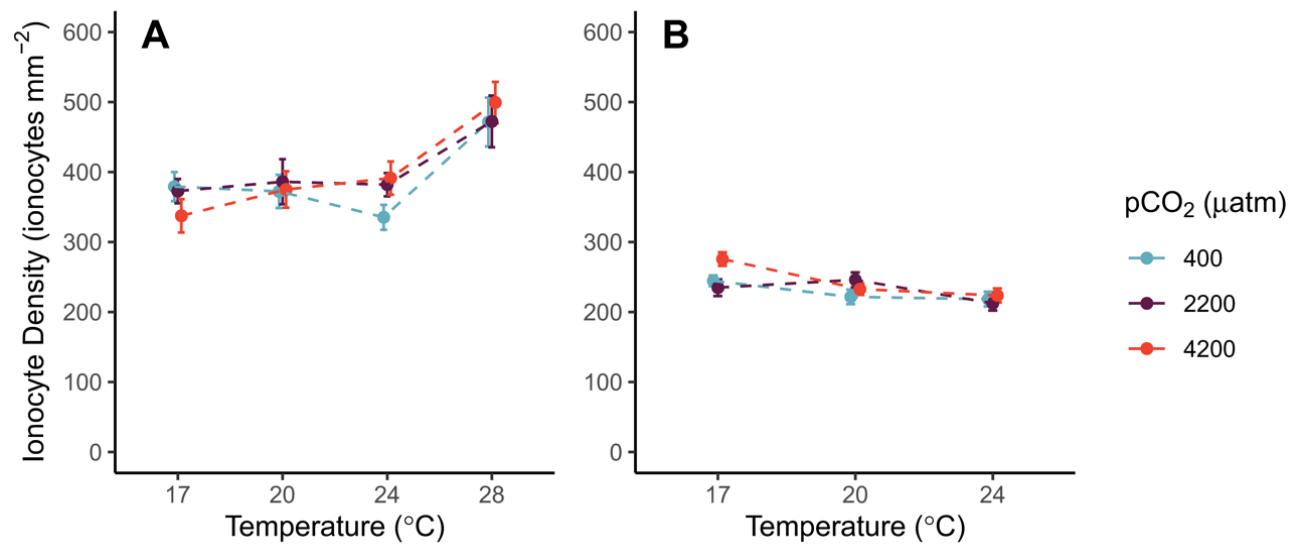


Figure 3.4. Hatchling (A) and 10-mm larvae (B) ionocyte density means plotted with respect to temperature and pCO₂. Error bars show standard error.

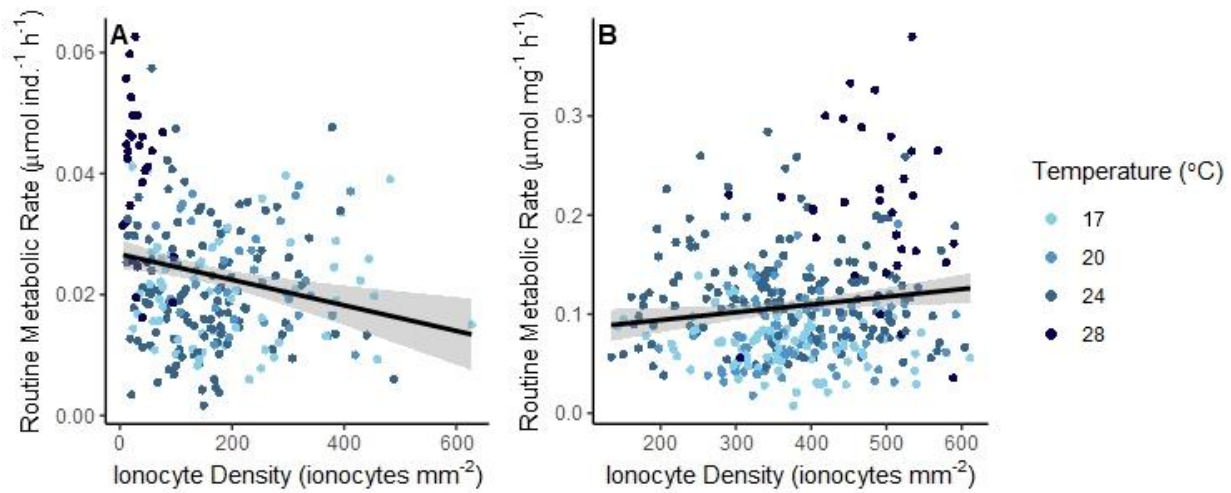


Figure 3.5. Metabolic rates of embryos (A) and mass-specific metabolic rates of hatchlings (B) plotted with respect to ionocyte density, with shades of blue indicating the treatment temperature ranging from 17°C (lightest shade) to 28°C (darkest shade). Each point represents an individual fish for which both RMR and ionocyte density were quantified. The black line and gray shading are a linear regression of RMR ~ Ionocyte Density with 95% confidence intervals.

3.7. Tables

Table 3.1. Spawning dates, target temperature, target pCO₂ levels, and stages sampled in each experiment. E stands for embryos, H stands for hatchlings, and L stands for mature (10-mm) larvae.

	400 μ atm	2200 μ atm	4200 μ atm
Experiment 1 – April 22, 2016			
17	E, H	E, H	-
20	E, H	E, H	-
24	E, H	E, H	-
Experiment 2 – May 3, 2016			
17	E, H, L	E, H, L	E, H, L
20	E, H, L	E, H, L	E, H, L
24	E, H, L	E, H, L	E, H, L
Experiment 3 – May 19, 2016			
17	E, H, L	E, H, L	E, H, L
20	E, H, L	E, H, L	E, H, L
24	E, H, L	E, H, L	E, H, L
Experiment 4 – May 26, 2017			
24	E, H	E, H	E, H
28	E, H	E, H	E, H

Table 3.2. Mean, standard error, sample size, and age at sampling (range in days post fertilization) for ionocyte density of embryo yolk sac and body skin surface in each treatment.

	Temp (°C)	pCO₂ (µatm)	N	Age (dpf)	Mean	SE
Embryo (yolk sac)	17	400	29	14-15	180.1	24.9
		2200	30	14-15	183.2	34.7
		4200	10	14-15	393.4	61.3
	20	400	13	10-11	219.6	33.0
		2200	13	10-11	253.3	48.9
		4200	13	10-11	238.1	43.7
	24	400	60	6-7	110.0	12.2
		2200	64	6-7	101.4	10.7
		4200	24	6-7	173.3	30.5
	28	400	9	5	38.4	15.2
		2200	9	5	37.4	5.9
		4200	10	5	24.2	6.8
Embryo (skin)	17	400	29	14-15	188.7	23.4
		2200	30	14-15	207.0	24.4
		4200	10	14-15	305.5	35.2
	20	400	13	10-11	248.3	31.3
		2200	13	10-11	208.0	29.6
		4200	13	10-11	250.9	25.3
	24	400	60	6-7	198.5	15.3
		2200	65	6-7	184.3	15.6
		4200	24	6-7	249.6	33.4
	28	400	9	5	45.2	8.7
		2200	9	5	39.2	8.4
		4200	10	5	23.6	9.6

Table 3.3. Mean, standard error, sample size, and age at sampling (range in days post hatching) for ionocyte density of newly hatched and 10-mm larvae in each treatment.

	Temp (°C)	pCO₂ (µatm)	N	Age (dph)	Mean	SE
Hatchlings	17	400	42	1	379.2	14.5
		2200	42	1	372.8	11.8
		4200	27	1	337.5	16.8
	20	400	27	1	372.3	11.8
		2200	27	1	386.1	17.4
		4200	25	1	375.1	16.9
	24	400	66	1	335.4	15.1
		2200	69	1	382.0	12.2
		4200	37	1	391.4	18.1
	28	400	12	1	471.5	22.4
		2200	12	1	472.3	23.6
		4200	11	1	499.3	14.7
10-mm larvae	17	400	40	15-23	244.6	8.5
		2200	37	15-23	234.8	9.0
		4200	33	15-23	275.7	8.9
	20	400	40	12-14	221.7	9.2
		2200	39	12-14	246.0	10.0
		4200	38	12-14	232.7	7.7
	24	400	38	10	218.5	10.2
		2200	37	10	212.4	8.4
		4200	39	10	223.7	8.4

Table 3.4. Linear model coefficients and p-values for ionocyte density

		Coefficient	St. Err.	t-value	p-value
Embryo (yolk sac) ¹	pCO ₂	7.8e-4	2.5e-4	3.1	0.0020
	Temp	-7.2e-2	2.7e-2	-2.7	0.0082
	pCO ₂ x Temp	-3.2e-5	1.1e-5	-2.9	0.0039
Embryo (skin) ²	pCO ₂	4.6e-3	1.2e-3	3.8	0.0002
	Temp	-0.11	0.13	-0.82	0.415
	pCO ₂ x Temp	-1.9e-4	5.3e-5	-3.7	0.0003
Hatchlings	pCO ₂	-5.9e-2	2.0e-2	-3.0	0.0031
	Temp	-0.88	2.3	-0.38	0.706
	pCO ₂ x Temp	3.0e-3	9.0e-4	3.3	0.0010
10-mm larvae	pCO ₂	2.5e-2	1.4e-2	1.7	0.0818
	Temp	-2.6	1.9	-1.4	0.169
	pCO ₂ x Temp	-1.0e-3	7.0e-4	-1.5	0.145

¹Square-root transformed²Natural log-transformed

Table 3.5. Comparison of ionocyte density results with previously reported effects of pCO₂ and temperature on growth and survival of fish from the same experiments. Experiment 2 is omitted because growth and survival were not quantified. Blue symbol is sole pCO₂ effect and red symbol is sole temperature effect, ‘↑’=positive effect, ‘↓’=negative effect, ‘↑↓’=both positive and negative, ‘×’=interaction, and ‘–’=no effect. The reference numbering is the experiment number used in the publication where the growth and survival data are reported, Murray and Baumann (2018), because the numbering for the same experiments differs in this study.

	Embryo ionocytes	Hatchling ionocytes	Hatch survival	Hatch length	Larval survival	Larval growth	Reference numbering
Exp. 1	–	×	–	–	↑	↑	‘Exp. 2’
Exp. 3	↓	↓	–	×	↑↓	↑	‘Exp. 3’
Exp. 4	↓	–	–	↑, ↓, ×	↓	↑	‘Exp. 5’

4. Attributing hypoxia responses of early life *Menidia menidia* to energetic mechanisms with Dynamic Energy Budget theory

4.1. Introduction

Hypoxia is common in coastal and estuarine waters and is expected to intensify with global warming (Diaz and Rosenberg, 2008; Breitburg et al., 2018). Between anthropogenic influence on nearshore waters and the natural dynamics of shallow, partially enclosed water bodies, hypoxia often co-occurs with other stressors such as high temperature, ocean acidification, and pollutants (Gruber, 2011). Along the Northeast United States coast, stratification and productivity associated with high temperatures in spring and summer cause hypoxic and eutrophic zones to form and great fluctuations in dissolved oxygen (DO) on diel to monthly time scales (O'Donnell et al., 2004; Baumann and Smith, 2018; Testa et al., 2018). While fish species that currently live in such areas tend to have mechanisms to cope with periods of hypoxia (Farrell and Brauner, 2009; Zhu et al., 2013; Baumann, 2019), these do not necessarily confer tolerance of longer durations. Fishes that spawn in the spring and summer face the additional threat of experiencing hypoxia during the particularly sensitive early life stages. Embryos and young larvae rely largely on diffusion for oxygen uptake and lack well-developed mechanisms, such as high surface area gills, to meet oxygen demands in low DO water (Rombough, 1988). While later stage fishes and even some early larvae can swim to avoid hypoxic habitats (Niklitschek and Secor, 2005; Chapman and McKenzie, 2009), embryos cannot utilize this response. Mortality can result directly from severe hypoxia or indirectly from reduced growth increasing susceptibility to predation. Even fish that survive may incur sublethal effects with lasting, lifelong consequences for growth, development, and reproduction (Stierhoff et al., 2006; Vanderplancke et al., 2015; Zambonino-Infante et al., 2017). Modeling the energetic mechanisms of responses to hypoxia in using unified principles on model species can help connect physiology and life history to population-level changes and serve as a valuable alternative to time- and labor-intensive laboratory experiments on other species, particularly with very small embryos and larvae.

Hypoxia is known to inhibit growth and survival in early life fishes (Rombough, 1988; Cross et al., 2019; Del Rio et al., 2019), as oxygen is required for the processes that maintain homeostasis and convert food for growth and activity. Anaerobic energy production fuels these

processes with about 1/15th the ATP yield of aerobic respiration. Hypoxic exposure leads to physiological responses such as depressed metabolism (Schwemmer, Chapter 2), limited growth, increased ventilation, and changes to hematocrit, hemoglobin, and erythrocyte quantities and characteristics (Taylor and Miller, 2001; Stierhoff et al., 2009a; Bianchini and Wright, 2013). Metabolism has also been shown to increase after temporary hypoxia as fish remove lactate accumulated from anaerobic respiration (Heath and Pritchard, 1965).

Hypoxia often has interactive effects with other stressors such as temperature (Brandt et al., 2009; McBryan et al., 2013; Earhart et al., 2022) and high carbon dioxide (CO₂; Hancock and Place, 2016; Miller et al., 2016; Morrell and Gobler, 2020). Interactive effects of high CO₂ and hypoxia were documented in an estuarine model species, Atlantic silverside (*Menidia menidia*), after offspring were reared in static or diel fluctuating combinations of oxygen and CO₂ treatments (Cross et al., 2019). Although diel fluctuations in both of these properties provided temporary relief that reduced the overall effects of hypoxia and acidification, static low DO significantly delayed hatching, reduced survival to hatching and larval survival, and reduced embryo and larval growth (Cross et al., 2019). While diel fluctuations are a realistic representation of changes in community photosynthesis and respiration between day and night, environmental change in coming years could extend hypoxic duration to reduce periods of relief. Warming reduces oxygen solubility while increasing metabolic rates of organisms that draw down oxygen when densely aggregated. At the same time, higher summer temperatures and freshwater input in some regions will intensify stratification that separates low-oxygen water from surface oxygen diffusion (Rabalais et al., 2009; Howarth et al., 2011). Currently *M. menidia* is tolerant enough that population declines are not a concern, but without knowledge of the mechanisms of early life impacts it is hard to anticipate whether this will change under intensifying deoxygenation or with additional stressors (Baumann, 2019).

Risks associated with stressors are important to quantify at the population level because targeted conservation actions operate at this level, but scaling experimental studies to population-level processes remains a challenge (but see Nisbet et al., 1989; Gear et al., 2020; Tai et al., 2021). While many laboratory experiments have measured physiological responses at the individual-level, additional steps must be taken to translate them to demographic rates like recruitment and reproductive investment in the next generation. Care must be taken as individual-level impacts do not necessarily scale linearly to the population level (Chambers and

Trippel, 1997; Galic et al., 2018). Models that use knowledge of life history and energetic allocation to connect physiological mechanisms of stressor effects to higher levels of biological organization create widely applicable tools that can be used to make population-level predictions.

Dynamic Energy Budget (DEB) modeling is a bioenergetic framework designed to bridge multiple levels of biological organization in assessing stressor effects in a vast variety of species (Kooijman, 2010; AmP, 2023). This approach follows energy allocation, in the form of suborganismal metabolic fluxes, and how it leads to life history outcomes such as growth rate, reproductive output, and survival, using physical and biological concepts that are generalizable to most species (Jusup et al., 2017). It accounts for differences in the energy budget at each stage to allow modeling of life stage transition timing and stage-specific responses to stressors (Kooijman, 2010). DEB theory is often used to connect experimental observations of multiple stressor effects to both the underlying energetic mechanisms (Kooijman, 2018) and life history outcomes that feed into population dynamics (Nisbet et al., 2000; Martin et al., 2013; Smallegange et al., 2017). These capabilities make DEB theory an excellent tool for enhancing the utility of experimental hypoxia data in conservation and management (Lavaud et al., 2021).

Depending on the application and types of data available, simplified versions of the standard DEB model can be used (e.g. Kooijman and Metz, 1984; Jager, 2018; Martin et al., 2017). Although complexity can be beneficial (Evans et al., 2013), simpler models with fewer parameters are often preferable for their predictive power and ability to be applied, tested, and interpreted widely (Holling, 1966; May, 2001; Jusup et al., 2017). The DEBkiss framework (Figure 4.1) is a moderately simplified variation on the standard DEB model for animals that eliminates the concept of reserve, a pool of assimilates that are allocated to structure, maintenance, and reproduction in the standard DEB model (Jager et al., 2013). This framework reduces the data requirements, the role of compound parameters, and, depending on the data, the total number of parameters to be estimated (Jager et al., 2013). The simplicity of DEBkiss and its easily understandable equations make it ideal for adaptation to many species of ecological or commercial value, even when the existing studies were not originally intended for this use, because it uses commonly measured data, such as growth and survival rates.

We used a DEBkiss model to test the hypothesis that changes in animal performance can be explained by one or more of the rate processes in the model, and to identify the bioenergetic

mechanisms underlying experimental hatching, growth, and survival effects of hypoxia in early life stages of *M. menidia* observed in Cross et al. (2019). First, we fit a DEBkiss model to full-life data on total length, reproductive output, hatch timing, and survival and estimated or calculated parameters under fully oxygenated conditions. Second, we modified a subset of parameters with one of two assumed oxygen-dependent correction factors to control the relationship between the correction factor and DO to fit the model to early-life data for three low DO treatments. We evaluated which parameter or combination of parameters, when adjusted with the correction factors, was able to best account for the full set of hypoxia responses observed in experiments.

We hypothesized that the following parameters would account for some or all of the hypoxia effects: maximum assimilation rate, conversion efficiency of assimilates into structure (growth), maximum somatic maintenance rate, embryo mortality rate, and post-hatch mortality rate. Maintenance in DEBkiss is the energy allocated to any processes that support the integrity and functioning of the structural body (Jager, 2018), including homeostasis, damage repair, and activity. The maintenance rate could be elevated by the activity required for some of the behavioral responses fish exhibit under hypoxia (Thomas et al., 2019). *M. menidia* exposed to hypoxia swim to the surface to use aquatic surface respiration, taking advantage of the diffusion of oxygen from the air (Miller et al., 2016). This behavior is impossible in embryos but has been observed in larvae (Cross et al., 2019). Fishes also expend energy on faster ventilation and heartbeat to increase oxygen uptake when ambient DO is low (Kramer, 1987; Maxime et al., 2000), but these capabilities may be limited until development has progressed further. We therefore hypothesize that maintenance does not account for a substantial portion of the early life changes in growth, hatch timing, and survival.

The conversion efficiency of assimilates to structure controls growth and hatch timing because it is the fraction of assimilates that are converted into structure rather than burned on overhead costs of growth (Jager, 2018). When oxygen is low enough that anaerobic metabolism must be used, this reduces conversion efficiency so that less growth results from the same amount of yolk or food (Thomas et al., 2019). We hypothesize that this contributed to a smaller hatch size and slower growth post-hatch.

Assimilation is the transformation of food and oxygen into compounds that will go to structure, maintenance, or reproduction. Reduced food consumption is a primary mechanism by

which the fish energy budget is thought to be impacted by hypoxia (Chabot and Dutil, 1999; Thomas et al., 2019). However, feeding effects cannot explain the observed hypoxia impacts on *M. menidia* hatch survival, timing, and size (Cross et al., 2019) because embryos do not yet ingest food. But because oxygen is also used in assimilation, low oxygen could reduce the assimilation rate of yolk resulting in slower depletion of the egg buffer and smaller size at hatching. Changes to assimilation efficiency under hypoxia have been recorded in other species, but the direction of that effect is species-dependent (reviewed in Thomas et al., 2019). Without hypoxia effects, our fitted survival parameter for embryo mortality is greater than that of larvae. If assimilation rate or conversion efficiency of *M. menidia* decreases under hypoxia, the resulting slower egg buffer depletion would delay hatching, extending individuals' time in the stage with greater mortality and thus accounting for reduced hatch survival under hypoxia. We therefore hypothesize that either maximum assimilation rate or conversion efficiency for growth will be the best parameter to explain the bioenergetic mechanism of early life hypoxia effects, and that modifying the embryo mortality parameter will consequently not be necessary. However, we hypothesize that this will not be the case for the post-hatch mortality parameter because none of the processes in the DEBkiss model indirectly affect mortality after hatching, so changing either the assimilation or conversion efficiency parameter in combination with the post-hatch mortality parameter may be necessary to fully replicate the observed changes to growth, hatch timing, and survival under hypoxia.

4.2. Methods

4.2.1. DEBkiss Model Description

To model the stage-specific energy budget of *M. menidia* in a way that would allow us to explain early-life hypoxia effects with bioenergetic processes, we used DEBkiss, a simplified and widely applicable DEB model (Jager et al., 2013; Jager, 2018). DEBkiss uses fewer parameters than the standard DEB model, which reduces data requirements and the risk of overfitting. It also has no reserve compartment between food assimilation and allocation, and for embryos this means that the egg buffer is assimilated into body structure and for maintenance, with hatching occurring when the egg buffer is fully depleted, instead of following reserve dynamics of the standard DEB model (Jager et al., 2013). The lack of reserve makes DEBkiss well-suited for animals with a small ultimate body size because reserve plays a smaller role in

such species under DEB theory (Nisbet et al., 2000), but DEBkiss has been successfully applied to larger animals as well (e.g. Desforbes et al., 2017).

The DEBkiss assumptions and equations we used are from Jager (2018). The parameters are defined in Table 4.1 and the variables, differential equations, and conversions are defined in Table 4.2. The flux of food or, for embryos, the egg buffer (W_B) is immediately converted to assimilates which are allocated to a somatic fraction (κ) and a reproductive fraction ($1-\kappa$; Figure 4.1); these fractions are constant throughout the life cycle. The assimilation flux (J_A) is the product of the scaled measure of resource availability (f), the volumetric surface area (L^2), and the parameter maximum area-specific assimilation rate (J_{Am}^a) where $f = 1$ for embryos and for post-hatching fish fed *ad libitum*. Egg buffer mass decreases at a rate inversely proportional to assimilation flux (Table 4.2). Within the somatic branch, which does not change with life stage, a flux to maintenance (J_M) is prioritized while the remainder goes to the flux for structure (J_V) with a conversion efficiency y_{VA} . The maintenance flux is the product of volume and the parameter for the volume-specific cost for maintenance (J_M^v ; Table 4.2).

For juveniles, the non-somatic fraction of assimilates is spent on maturation, or increasing complexity through gonad development. While the standard DEB formulation uses a state variable for maturity that triggers changes between life stages, DEBkiss instead uses a constant size at puberty to specify when reproduction is initiated (Kooijman, 2010; Jager et al., 2013). Once the mass at puberty is reached (W_{vp}), reproductive flux (J_R) toward egg production begins in adults with a conversion efficiency y_{BA} . Although *M. menidia* have a distinct larval and juvenile stage, both are treated as the juvenile stage because the relevant aspects of their energy budget for DEBkiss are assumed to be identical. DEBkiss also uses an optional flux to maturity maintenance (J_J) that comes from the $1-\kappa$ fraction of assimilates (Jager, 2018), which we chose to use in our model.

Because the model equations use dry weight for body size and our growth data is in total length, we calculated a shape correction coefficient (δ_M) and dry weight density (d_V) to allow the model to convert between the two (Table 4.2). We calculated these constants using data on *M. menidia* length and egg volume (Klahre, 1997) and a total length to dry weight conversion (H. Baumann, personal communication):

$$W_V = e^{(2.997 * \ln(L^M) - 6.7)} \quad (4.1)$$

To address the assumption of DEBkiss that eggs hatch when buffer is depleted, regardless of body size or developmental progress (Jager et al., 2013), we added a survival state variable (S). In addition to allowing an alternative outcome to hatching, this allowed us to examine survival as a consequence of hypoxia effects on the energy budget. We fit mortality parameters for embryos and post-hatch fish (μ_{emb} and μ_{lar}) to data for survival to hatching and larval/juvenile survival (Figure 4.1; Table 4.2). In our implementation of survival, the only DEB process influencing survival is egg buffer depletion, which determines the time to hatch and thus when the embryo mortality rate switches to the post-hatch mortality rate. This means survival is indirectly affected by the assimilation rate and conversion efficiency of assimilates into structure.

4.2.2. Data and Fitting

For the DEBkiss model we calculated and estimated parameters based on four types of data (state variables): total length over time, egg buffer mass over time (and through this, time to hatching), cumulative egg production over time, and proportion surviving since fertilization over time. Data for the total length were sourced from three studies. Length at hatching and 15 days post-hatching (dph) came from a study that reared *M. menidia* offspring in different static oxygen levels across two experiments (Cross et al., 2019). This provided data for control oxygen levels used in fitting the DEBkiss model and three reduced oxygen treatments (Table 4.2). The study featured two additional experiments that exposed offspring to fluctuating oxygen and CO₂ levels but the control conditions were static, so we used total length data from these treatments to fit the model as well (Cross et al., 2019). We sourced additional length data from control levels of experiments that exposed *M. menidia* offspring to ambient and elevated CO₂ levels (Murray and Baumann, 2018; Murray and Baumann, 2020; Concannon et al., 2021). All total length data were obtained from fish maintained in static laboratory conditions at 24°C. Data for the state variables on egg buffer mass (i.e. time to hatching when egg buffer mass is zero) and survival to hatching and 15 dph under different oxygen levels were obtained from Cross et al. (2019). We also used survival data from the 24°C and control CO₂ groups of a study on the effects of temperature and CO₂ on *M. menidia* early life survival (Murray and Baumann, 2018). Four additional data points for long-term survival in laboratory conditions at 17°C were obtained from a study that exposed *M. menidia* offspring until 122 dph to two CO₂ levels, of which we only used data from the control level (Murray et al., 2017). Lastly, the data for cumulative egg

production over time was also obtained from control groups in Concannon et al. (2021), a study in which wild-caught juveniles were held in the laboratory at 20°C in different CO₂ treatments and strip-spawned once they reached reproductive maturity.

We estimated three parameters by fitting them to data (y_{VA} , μ_{emb} , and μ_{lar}) and fixed at suggested values parameters for which we had insufficient data to calculate or estimate. The primary parameters and their calculated or estimated values are found in Table 4.1. Fitting was done in Matlab with the packages BYOM v.6.4 (Jager, 2022) and DEBkiss v.2.3a (Jager, 2021). BYOM uses a Nelder-Mead simplex search to optimize the parameters for a set of ordinary differential equations by minimizing negative log-likelihood (NLL). The DEBkiss package works under BYOM to bring in the DEBkiss model parameters, variables, and equations so that the parameters can be estimated based on their effect on the DEBkiss equations and the equations derived from them. The differential equations give the predicted data for each type of observed data (length, egg production, egg buffer mass, and survival over time) the difference between which is used to calculate NLL.

BYOM allows users to turn fitting on and off for each parameter, and with fitting turned off for all parameters it runs a simulation that calculates predicted values over time for each state variable using the initial parameter values. Before estimating any parameters with the optimization described above, we ran simulations with fitting turned off using a set of recommended parameters (Jager, 2018) and parameters obtained from existing data on *M. menidia*. We visually assessed fit and checked NLL as we adjusted parameters to obtain a reasonable set of initial parameters before estimating any. Testing a range of parameters and obtaining realistic initial parameters helps avoid detecting local minima with the optimization. This also helped us reduce the number of parameters being estimated to avoid overfitting and so that there were not multiple correlated parameters free at once, because we were able to obtain a reasonable fit using suggested default values for y_{AV} , y_{BA} , and κ . The default value for y_{VA} of 0.8 from the literature (Jager, 2018) did not allow a realistic fit to the length data, but the length, reproduction, and egg buffer depletion data allowed it to be estimated with the BYOM optimization. Ultimate length was used to fix J^a_{Am} to a reasonable value before estimating y_{VA} because both parameters affect growth and egg buffer depletion in the model and therefore can not be estimated simultaneously. Finally, we fixed all parameters except μ_{emb} and μ_{lar} to estimate

these parameters, again using the visually best-fitting parameters from the simulations as initial values. The full-life predicted and observed data are shown in Figure 4.2.

The length and reproductive data allowed us to calculate length at puberty (L_{vp}), which in the DEB literature is defined as the length at which egg production begins. We obtained W_{B0} from *M. menidia* egg dry weight data (Klahre, 1997) and calculated δ_M and d_V from total length, egg diameter, and egg mass data (Cross et al., 2019; Klahre, 1997; Concannon et al., 2021). To calculate volume-specific maintenance costs (J'_M), we used data on the rate of decrease in larval dry weight over a period of starvation in the congeneric species *M. beryllina* (Letcher and Bengtson, 1993). Borrowing from closely related species is a common practice in bioenergetic modeling when the species has similar habitat, life history, and physiology (Sibly et al., 2013). *M. menidia* and *M. beryllina* have overlapping habitats and similar life history, egg sizes, and body sizes, although *M. beryllina* reaches a smaller ultimate length (Middaugh, 1981; Bengtson, 1984; Middaugh and Hemmer, 1992). All *M. menidia* experiments used in this study fed fish *ad libitum* in all treatment levels, so f was set to 1. For experiments that exposed fish to different CO₂ levels, we only used data from control groups to avoid potential stressor effects in the data.

4.2.3. Hypoxia Stress

We tested the hypothesis that changes in *M. menidia* early life growth, hatch timing, and survival under reduced oxygen (Cross et al., 2019) can be explained by one or more DEBkiss processes (Figure 4.1). To summarize the experimental data on static hypoxia effects we are attempting to explain by altering these parameters, the mean values of data for each oxygen treatment are listed in Table 4.3. We used the parameter values from the model fit to full life data, and altered one or more parameters at a time with oxygen-dependent correction factors, then fit the model to data for only the first 136 days by estimating a parameter that controls the correction factor's relationship with DO. We only used early life data to fit the hypoxia-altered parameters because we did not have late-life data for multiple oxygen treatments later in life to validate observed changes against and did not have any reproduction data for oxygen treatments. It did not make sense to include later life data in the calculations of NLL that influence the parameter estimates or to speculate about how well the predicted data match what we might expect to happen later in life if we not only lack late-life hypoxia data but also do not expect full life hypoxia to occur in nature.

Building on the physiological rationale for hypotheses described in Section 4.1, we used two initial criteria to identify the candidate processes to explain changes in animal performance. The initial criteria for a given DEBkiss parameter were 1) that altering the parameter must lead to a change in at least one state variable within the range of life history for which we have hypoxia data, and in the same direction as the observed effect of hypoxia, and 2) that the final best model must include parameter(s) that account for the changes in all three state variables for which low oxygen data exist (growth, egg buffer mass, and survival). For example, y_{BA} does not meet Criterion 1 because changing it has no effect on the state variables for length, egg buffer mass, or survival. μ_{emb} meets Criterion 1 because adjusting it changes the survival state variable, but it does not change total length or egg buffer mass over time so a model in which hypoxia changes μ_{emb} alone does not meet Criterion 2. Once we narrowed down the list of candidate parameters that met Criterion 1 (summarized in Table 4.3 with examples in Figures 4.4-4.6) we applied one of two correction factors to them based on the assumption that hypoxia would either reduce or increase them. We did this for each individual parameter and every combination of two, three, or four parameters.

The first correction factor, c , is calculated with an assumed plausible functional form for parameters that are hypothesized to exponentially decrease as DO decreases (Figure 4.3A):

$$c = \begin{cases} 1 - e^{-Z(DO - DO_c)} & \text{if } DO > DO_c \\ 0 & \text{if } DO \leq DO_c \end{cases} \quad (4.2)$$

where Z is the exponential coefficient that affects the strength of the DO effect on predicted values of the state variables. DO is the treatment level of oxygen, and DO_c is the critical oxygen level below which the $c = 0$. The value of c cannot exceed 1. A larger Z value keeps c higher as oxygen decreases before a more abrupt drop, while a smaller Z gives a more constant decline in c with hypoxia (Figure 4.3). Attempts to estimate DO_c and Z simultaneously showed that leaving DO_c free did not improve the ability of the correction factor to fit the hypoxia data. Instead, DO_c was fixed at a biologically relevant level of 2.044 mg L^{-1} , which is the critical oxygen level below which embryonic routine metabolism becomes highly oxygen-dependent (Schwemmer, Chapter 2). This correction factor was multiplied by J^a_{Am} and y_{VA} because these parameters were hypothesized to decrease under hypoxia. To alter the parameters hypothesized to increase under hypoxia (J^v_M , μ_{emb} , and μ_{lar}) a secondary correction factor, c_I , was calculated from c with the assumed functional form:

$$c_1 = \min\left(\frac{1}{c}, c_{1(max)}\right) \quad (4.3)$$

where $c_{1(max)}$ is the upper limit to the correction factor, or a maximum factor by which we are willing to multiply the parameters. We set $c_{1(max)} = 10$ because the value does not affect the shape of the curve below the limit and only very low Z values would lead c_1 to reach this level at the DO treatments of the data. The correction factor c_1 was multiplied by J'_M , μ_{emb} , and μ_{lar} to increase them with decreasing DO.

To find the best value of Z for each DEBkiss parameter or combination of parameters, we added Z as a model parameter and estimated it using the BYOM optimization to minimize NLL. We did not apply the correction factor to J^a_{Am} and y_{VA} simultaneously because they are multiplied together to obtain J_V and their individual contributions to the growth and egg buffer depletion can not be fully separated. To identify the most likely version of the model (which parameter or combination of parameters best explain the hypoxia effects on the state variables), we estimated Z for each of these scenarios and calculated Akaike's Information Criterion for small sample sizes (AICc). We compared the AICc between each model using the difference between AICc values ($\Delta AICc$) and the relative likelihood of each model using Akaike weights:

$$w_i(AICc) = e^{-0.5 \cdot \Delta_i AICc} / \sum_{k=1}^K e^{-0.5 \cdot \Delta_k AICc}, \quad (4.4)$$

where $w_i(AICc)$ is the Akaike weight of each model i , $\Delta_i AICc$ is the difference between each model i and the model with the lowest AICc ($AICc_{min}$), and the denominator calculates the sum of relative likelihoods for every model starting at the first model k (Wagenmakers and Farrell, 2004). We used $\Delta AICc$ and ratios of Akaike weights to determine which combination of parameters that also met Criterion 2 best fit the data and, therefore, which DEB processes best explain the hypoxia effects observed in experiments (Table 4.4).

4.3. Results

4.3.1. DEBkiss Model

We obtained realistic fits to all datasets (Figure 4.2). The only exception is late-life survival, for which the mortality was too high beyond the larval stage but could not be better fit due to lack of full-life survival data. However, this did not impair our ability to model the effects of hypoxia on early life survival. Estimating y_{VA} returned a lower than typical value for conversion efficiency of assimilates to growth, but this gave a realistic fit to the length data and

allowed a detailed and very close fit to egg buffer mass over time (hatch timing). The observed and predicted data for full life span and early life are plotted in Figure 4.2.

4.3.2. Hypoxia Stress: Results of Criterion 1

Preliminary testing ruled out five of the parameters as having no effect on the three state variables for which we have hypoxia data when increased or decreased based on hypothesized hypoxia effects (Table 4.4). The parameters that did affect the state variables were able to be changed so as to reproduce the direction of experimentally observed hypoxia effects, e.g. increasing J^a_{Am} reduced total length, increased time until egg buffer mass reaches 0, and reduced survival (Figure 4.4). Although κ met Criterion 1, we did not include it as a candidate because we lacked the reproductive data needed to model any potential changes in relative energy allocation under hypoxia. We also did not include f despite it meeting Criterion 1 because feeding was *ad libitum* across all experiments, and f_B – the food level for embryos – was excluded because *M. menidia* embryos do not feed. The remaining parameters (Table 4.5) underwent the model selection process of multiplying each parameter and combination of two or three parameters by the oxygen-based correction factors.

4.3.3. Hypoxia Stress: Results of Criterion 2 and Model Selection

The best model of experimental hypoxia effects on *M. menidia* early life stages had correction factors applied to y_{VA} , μ_{emb} , and μ_{lar} . The correction factor c was used to reduce y_{VA} and c_I was used to increase both μ_{emb} and μ_{lar} . This model met Criterion 1 of affecting all three state variables (total length, egg buffer mass, and survival) in the same direction as hypoxia affected them in experimental data (Figure 4.7, Figure S4.1). Although adjusting y_{VA} alone met Criterion 2 by affecting all three state variables, also increasing both mortality parameters improved the fit to the data (Table 4.5). It also had a lower AICc than all but one of the other models that met the initial criteria, with an AICc of 584.78. Adding a correction factor to J^v_M in addition to these three parameters reduced AICc slightly to 584.65 (AICc_{min}). The ratio of Akaike weights shows that relative to the model with correction factors for y_{VA} , μ_{emb} , and μ_{lar} , adding an additional correction factor to J^v_M gives a model that is only 1.06 times more likely to be the best model (Table 4.5). It was therefore not considered to have improved the fit, and in the interest of parsimony is not beneficial enough to justify the added complexity of applying the

correction factor to a fourth parameter. After estimating Z we calculated the values of y_{VA} , μ_{emb} , and μ_{lar} when their respective correction factors are applied for each DO level (Table 4.6).

Reducing J^a_{Am} with hypoxia using correction factor c also resulted in a good fit to the data across oxygen levels and fulfilled the initial criteria. Combining the adjusted J^a_{Am} with correction factors to increase both mortality rates improved the fit as well, but this model fit slightly less well than the version that corrected y_{VA} , μ_{emb} , and μ_{lar} , with an AIC value of 586.72 in the former model compared to 584.72 in the latter. The ΔAIC for this pair of models is 2, indicating that the model with c multiplied by J^a_{Am} performs similarly to the model with c multiplied by y_{VA} , when correction factor c_I is also included for both mortality parameters. However, ratio of Akaike weights for the model applying hypoxia correction factors to $y_{VA} + \mu_{emb} + \mu_{lar}$ and the one for $J^a_{Am} + \mu_{emb} + \mu_{lar}$ is 2.68, which makes the former 2.68 times more plausible than the latter model. The ΔAIC values relative to the AIC_{min} for the models applying correction factors to $J^a_{Am} + \mu_{emb}$ and $y_{VA} + \mu_{emb}$ are 5.95 and 4.64, respectively (ΔAIC for all models listed in Table 4.5). This suggests that although these are not the best fitting models, there is a moderate level of support for them, contrary to our hypothesis that adjusting μ_{lar} with oxygen would be required to get a good fit.

4.4. Discussion

By combining experimental data with unified principles for energetic allocation that are broadly applicable across species, we identified the conversion efficiency of assimilates into structure and the maximum area-specific assimilation rate as the most likely processes by which low oxygen levels affect early life stages of *M. menidia*. After we eliminated the parameters in DEBkiss that had no effect on the ecological endpoints (size, hatch timing, and survival), we discovered that applying correction factors to reduce the conversion efficiency for growth (y_{VA}) and increase pre- and post-hatching mortality rates (μ_{emb} and μ_{lar}) best predicted the experimental effects of hypoxia on larval length, time to hatching, and early life survival. Through this model we have found evidence that the mechanism largely responsible for the observed hypoxia impacts on growth, hatch timing, and survival is the efficiency by which assimilated food or egg yolk is converted into structure. The estimated best value of Z , the exponential coefficient in the correction factor c , enables us to calculate that y_{VA} at the lowest oxygen level is 58% of its value with no hypoxia stress. Reducing conversion efficiency alone produced small differences in

survival at hatching because it prolongs the time spent in the embryo stage, which has a greater mortality rate than post-hatching in our model. Multiplying both the pre- and post-hatching mortality rates by the correction factor c_I more closely predicted the reduced survival rates in the low DO treatments, resulting in a best fitting model that explained observed hypoxia effects well by altering conversion efficiency, embryo mortality, and post-hatch mortality. Our best fitting model, according to AICc and parsimony, underestimated time to hatching and overestimated size at age, which suggests there either may be a different correction factor function that better fits the nonlinear relationship between DO and the DEBkiss parameters or that there were additional factors contributing to these differences that the model does not account for. For example, we do not have data on gonad development or reproductive output later in life after rearing *M. menidia* in hypoxia, which would allow us investigate if κ is an affected parameter. Hypoxia can reduce gonadosomatic index and gonad development in fish, suggesting that the reproductive branch of the energy budget might require additional energy to be redirected from the somatic branch (Wu et al., 2002; Thomas et al., 2006; Landry et al., 2007). Despite the underestimation of some hypoxia effects, the model was able to replicate the direction of effects and even account for some hypoxia effects in all three state variables simultaneously by changing only one parameter, either conversion efficiency or assimilation.

Replacing conversion efficiency with assimilation as the hypoxia-reduced parameter yielded a similar fit, likely because both parameters are used to calculate predicted growth and egg buffer depletion. However, applying correction factor c to conversion efficiency explained the data slightly better than assimilation based on AICc. Hypoxia could influence either the assimilation rate or the efficiency with which assimilates turn into structure could be affected by hypoxia. Under *ad libitum* feeding, differences in assimilation of hatched larvae could indicate reduced ingestion with low oxygen, a common hypoxia response in fishes (Chabot and Dutil, 1999; Thomas et al., 2019). For embryos, on the other hand, reduced assimilation rates indicate slower absorption of the yolk. Hypoxia has been shown to delay development in Atlantic salmon by reducing yolk absorption rates (Polymeropoulos et al., 2017). If assimilation rate were the only difference between hypoxia treatments, one would expect the offspring to reach the same size at hatching regardless of the timing. However, *M. menidia* larvae had significant differences in hatch lengths between DO treatments (Cross et al., 2019), indicating that y_{VA} played a role in the hypoxia response as well. When oxygen is low, conversion efficiencies of assimilates can be

reduced by the far less efficient production of ATP through anaerobic respiration combined with slower rates of tissue differentiation. Extending developmental time while continuing to pay maintenance costs can further increase the energy expended to produce each unit of structure (Kamler, 2008). After hatching, these mechanisms would continue to reduce y_{VA} but it may also be reduced by increased ventilation required during digestion (Chabot and Claireaux, 2008). The experimental DO levels are greater than the critical oxygen levels for oxygen-independent routine metabolism (P_{crit}) of 2.04 mg L^{-1} and 1.56 mg L^{-1} for embryos and 5dph larvae, respectively (Schwemmer, Chapter 2). P_{crit} has been assumed by some to be the oxygen level at which anaerobic metabolism is triggered, but there is abundant evidence that some level of anaerobic metabolism can occur well above P_{crit} (Nonnotte et al., 1993; Maxime et al., 2000; Wood et al., 2018). Additional activity such as swimming bursts can drive up the need for anaerobiosis (Di Santo et al., 2017). Our identification of conversion efficiency as a primary component of the energy budget that is reduced by hypoxia suggests that anaerobic metabolism is a mechanism of hypoxia effects in *M. menidia* early life stages even at oxygen levels above P_{crit} . A limitation of this study is the inability to fully separate the relative influences of conversion efficiency and assimilation from each other because flux for growth is calculated from the product of conversion efficiency and the somatic fraction of assimilation; we can adjust one or the other and get similar effects on the flux for growth with no way of determining which is correct.

Adding a correction factor to the volume-specific maintenance rate in addition to this model did not substantially improve the fit according to AICc, suggesting that increasing maintenance costs is not a bioenergetic mechanism underlying hypoxia response in early life stages. In this model, egg buffer depletion is insensitive to changes in volume-specific maintenance costs, requiring a quadrupling to see a noticeable delay in hatching (Figure 4.4). Changing maintenance has much greater effects on length later in life while failing to explain differences in length at the time of hatching (Figure 4.4). One way that maintenance costs could increase under hypoxia is through additional activity related to ventilation and mobility (Thomas et al., 2019), but at the embryo stage very little activity is possible so it makes sense that the correction factor for maintenance doesn't model the hypoxia effects well. A common response to hypoxia in fish embryos is premature hatching (Kamler, 2008) which could allow swimming escape responses that increase maintenance costs, but studies on chorion removal have shown

that the increased mobility can improve growth despite hypoxia exposure (Ciuhandu et al., 2005; Ninness et al., 2006). In contrast, *M. menidia* embryos' delayed growth and hatching do not appear to be related to elevated maintenance costs, and rearing them in hypoxia did not significantly change their oxygen consumption rates as may be expected if maintenance was elevated (Cross et al., 2019; Schwemmer, Chapter 1). Some studies on fish responses to hypoxia suggest maintenance may drop temporarily due to the reduced capacity for aerobic metabolism at low DO levels, then subsequently be temporarily elevated after oxygen is restored because of recovery demands such as paying oxygen debt and removing or repairing damage from anaerobic byproducts (Heath and Pritchard, 1965; Claireaux and Chabot, 2016; Thomas et al., 2019). If such fluctuations were occurring in the *M. menidia* offspring from this dataset, the net effect on maintenance was not discernible by our model.

Although both conversion efficiency and assimilation can explain hypoxia effects on total length and egg buffer mass over time, reducing them only produced a small decrease in survival relative to the data. Applying correction factor c_I to both mortality rates better captured the great reductions in survival at both hatching 15 dph with hypoxia. In the experiments, the lowest oxygen level (2.7 mg L⁻¹) had a mean hatch survival of 30.2% while the mean survival in the other three treatments was over 70% (Cross et al., 2019). By 15 dph fish from all three low oxygen treatments had lower survival than those from the normoxic treatment (Cross et al., 2019; Table 4.3). Including hypoxia effects for both pre- and post-hatching mortality rates allowed the model to more closely predict these differences in hypoxia effects in both stages and improve the fit based on $\Delta AICc$ (Table 4.6). However, an intrinsic mortality rate isn't as explicitly indicative of underlying energetic processes as the other DEB parameters are. The additional mortality that was not accounted for by y_{VA} may have been related to tissue damage from buildup of toxic compounds during anaerobic metabolism (Richards, 2011). The mortality could also have resulted from failing to meet energetic demands with either aerobic or anaerobic metabolism (Richards, 2009) and, specifically in embryos, failure to reach a viable level of complexity before the yolk is depleted (Jager et al., 2013). The latter could be an indirect effect of reduced y_{VA} that the model does not account for, as mortality rates are not influenced by the other model parameters in our formulation. Measurement of anaerobic byproducts such as lactate and morphological evaluation of dead embryos and larvae could help to identify the mechanisms underlying the mortality rates in future work. Although survival does not approach 0% during

the larval stage in our best fitting model (Figure 4.7), all experimental replicates of the 2.7 mg L⁻¹ DO treatment had 0% survival by 15 dph, making larvae apparently more sensitive than embryos (Cross et al., 2019). The authors of the study attribute this to a possibly lower ability to suppress metabolism in larvae compared to embryos. While the increased mobility of larvae may allow escape from hypoxia in a patchy and stratified estuarine environment, activity comes with elevated maintenance costs and, regardless of escape behavior, some level of swimming is required for *M. menidia* to begin feeding almost immediately after hatching (Middaugh and Lempeis, 1976). Furthermore, swimming upward for aquatic surface respiration may inhibit feeding, thus creating a positive feedback of additional energetic costs with decreasing assimilates to meet them (Miller et al., 2016; Cross et al., 2019). Though beyond the scope of this work, a model that captures stage-specific differences in maintenance costs and links them explicitly to survival may better capture the high mortality in larvae and their reduced ability to suppress metabolism.

Understanding the mechanisms of reduced growth and survival under hypoxia through DEB theory can be useful for predicting life history effects, and although not within the scope of this study, the predictions can be used to model population growth rates, which are useful for resource management (Kooijman et al., 2020; Lavaud et al., 2021). An important assumption of our model is that several of the parameters have the same value across life stages (e.g. J^a_{Am} , J^v_M , y_{VA}) and similarly that values of the hypoxia correction factors are the same regardless of life stage. Future work could evaluate full-life sensitivity with higher resolution data for the later life stages. We lacked reproductive data to look at hypoxia effects on the proportion of total energy allocated to reproduction ($1-\kappa$), which is an additional component of DEB useful in connecting organismal effects to populations, but future experimentation could provide the needed information. Nonetheless, our model fit to early life data with a hypoxia-based correction factor predicts reductions in long-term growth and survival that would certainly be detrimental to population growth under extended periods of low oxygen. Under this model, even restoring normoxia after 15 days would result in smaller size at age and survival rates than the groups exposed to 7.7 mg L⁻¹, although compensation of growth may be possible after exposure to hypoxia (Wei et al., 2008) and other stressors (Russell and Wootton, 1992; Nicieza and Metcalfe, 1997; Ali et al., 2003). Delayed hatching and slower growth can both lead to enhanced

vulnerability to predation, which could further reduce survival rates beyond those observed in controlled laboratory conditions.

With this simple and widely applicable DEBkiss model we were able to attribute much of the hypoxia-related variability in total length, egg buffer mass, and survival over time to changes in DEB processes. The evidence for the mechanisms is inferred from a combination of experimentally observed responses and unified principles that apply to virtually all animal species (Jager et al., 2013). Similar approaches have applied correction factors to DEB parameters to model other species' responses to hypoxia (Lavaud et al., 2019; Aguirre-Velarde et al., 2019) and other stressors such as seawater acidification (Jager et al., 2016; Pousse et al., 2022) and pollutants (Muller et al., 2010; Desforges et al., 2017). The success of this approach with a wide variety of stressors makes it an ideal supplement to multistressor experiments, which are limited by logistical constraints. Modeling stressor effects with DEBkiss parameters can yield additional information about energetic mechanisms of responses and, with careful attention to the assumptions being made, may be useful in extrapolating stressor effects to additional levels or combinations of stressors that would have been impractical to test experimentally or to species with certain shared physiology or life history traits (Goussen et al., 2020; Boulton and Evans, 2021). The patterns modeled in this study should not be interpreted as a direct prediction of what will happen to wild *M. menidia* populations as coastal hypoxia intensifies. Lifelong constant oxygen levels do not occur and are not expected to occur in the future, but rather fluctuating oxygen levels will provide opportunities for recovery and may confer tolerance of temporary stress (Cross et al., 2019; Baumann, 2019). Instead, this approach demonstrates the value of identifying DEB parameters responsible for whole-organism effects of hypoxia to understand underlying energetic processes that are often time, labor, and cost-intensive to measure empirically, particularly in the early life stages, when biomass available for sampling is small and developmental changes are rapid. Through doing so we were able to highlight the conversion of assimilates to structure as a primary, but not sole, mechanism by which hypoxia reduces size, delays hatching, and increases mortality in an ecologically important forage fish.

4.5. References

- Aguirre-Velarde, A., Pecquerie, L., Frederic, J., Gerard, T., and Flye-Sainte-Marie, J. 2019. Predicting the energy budget of the scallop *Argopecten purpuratus* in an oxygen-limiting environment. *J. Sea Res.*, 143: 254-261.
- Ali, M., Nicieza, A., and Wootton, R. J. 2003. Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries*, 4: 147-190.
- AmP. 2021. Online database of DEB parameters, implied properties and referenced underlying data. www.bio.vu.nl/thb/deb/deblab/add_my_pet/ (data accessed: March 3, 2023).
- Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97: 399-408.
- Baumann, H. and Smith, E. M. 2018. Quantifying Metabolically Driven pH and Oxygen Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuaries and Coasts*, 41: 1102-1117.
- Bengtson, D. A. 1984. Resource partitioning by *Menidia menidia* and *Menidia beryllina* (Osteichthyes: Atherinidae). *Mar. Ecol. Prog. Ser.*, 18: 21-30.
- Bianchini, K. and Wright, P. A. 2013. Hypoxia delays hematopoiesis: retention of embryonic hemoglobin and erythrocytes in larval rainbow trout, *Oncorhynchus mykiss*, during chronic hypoxia exposure. *J. Exp. Biol.*, 216(23): 4415-4425.
- Boult, V. L. and Evans, L. C. 2021. Mechanisms matter: Predicting the ecological impacts of global change. *Glob. Change Biol.*, 27(9): 1689-1691.
- Brandt, S. B., Gerken, M., Hartman, K. J., and Demers, E. 2009. Effects of hypoxia on food consumption and growth of juvenile striped bass (*Morone saxatilis*). *J. Exp. Mar. Biol. Ecol.*, 381: S143-S149. doi: 10.1016/j.jembe.2009.07.028
- Breitburg, D., Levin, L. A., Oschlies, A., et al. 2018. Declining oxygen in the global ocean and coastal waters. *Science*, 359(6371): eaam7240.
- Chabot, D. and Claireaux, G. 2008. Environmental hypoxia as a metabolic constraint on fish: The case of Atlantic cod, *Gadus morhua*. *Mar. Pollut. Bull.*, 57: 6-12.
- Chabot, D. and Dutil, J.-D. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *J. Fish. Biol.*, 55: 472-491.
- Chambers, R. C. and Trippel, E. A. 1997. *Early Life History and Recruitment in Fish Populations*. Fish and Fisheries Series, No. 21. New York: Chapman & Hall.

- Chapman, L. J. and McKenzie, D. J. 2009. Behavioral responses and ecological consequences. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. Jeffrey G. Richards, Anthony P. Farrell, and Colin J. Brauner), pp. 25-77. San Diego: Academic Press.
- Ciuhandu, C. S., Stevens, E. D., and Wright, P. A. 2005. The effect of oxygen on the growth of *Oncorhynchus mykiss* embryos with and without a chorion. *J. Fish. Biol.*, 67: 1544-1551.
- Claireaux, G. and Chabot, D. 2016. Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. *J. Fish Biol.*, 88: 232-251.
- Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., and Baumann, H. 2021. Temperature-dependent effects on fecundity in a serial broadcast spawning fish after whole-life high CO₂ exposure. *ICES J. Mar. Sci.*, 78(10): 3724-3734.
- Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal pCO₂ x O₂ fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9: 18146.
- Del Rio, A. M., Davis, B. E., Fangue, N. A., and Todgham, A. E. 2019. Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conserv. Physiol.*, 7(1): coy078. doi: 10.1093/conphys/coy078
- Desforges, J.-P. W., Sonne, C., and Dietz, R. 2017. Using energy budgets to combine ecology and toxicology in a mammalian sentinel species. *Sci. Rep.*, 7: 46267. doi: 10.1038/srep46267
- Di Santo, V., Kenaley, C. P., and Lauder, G. V. 2017. High postural costs and anaerobic metabolism during swimming support the hypothesis of a U-shaped metabolism–speed curve in fishes. *Proc. Nat. Acad. Sci.*, 114(49): 13048-13053.
- Diaz, R. J. and Rosenberg, R. 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. *Science*, 321: 926-929.
- Earhart, M. L., Blanchard, T. S., Harman, A. A., and Schulte, P. M. 2022. Hypoxia and High Temperature as Interacting Stressors: Will Plasticity Promote Resilience of Fishes in a Changing World? *Biol. Bull.*, 243: 149-170.
- Evans, M. R., Grimm, V., Johst, K., et al. 2013. Do simple models lead to generality in ecology? *Trends in Ecology & Evolution*, 28(10): 578-583.
- Farrell, A. P. and Brauner, C. J. 2009. *Fish Physiology, Vol. 27: Hypoxia*. Academic Press, London.
- Galic, N., Sullivan, L. L., Grimm, V., and Forbes, V. E. 2018. When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. *Ecol. Lett.*, 21(4): 568-577.

- Goussen, B., Rendal, C., Sheffield, D., Butler, E., Price, O. R., and Ashauer, R. 2020. Bioenergetics modelling to analyze and predict the joint effects of multiple stressors: Meta-analysis and model corroboration. *Sci. Total. Environ.*, 749: 141509.
- Grear, J. S., O’Leary, C. A., Nye, J. A., Tettelbach, S. T., and Gobler, C. J. 2020. Effects of coastal acidification on North Atlantic bivalves: interpreting laboratory responses in the context of *in situ* populations. *Mar. Ecol. Prog. Ser.*, 633: 89-104.
- Gruber, J. 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Phil. Trans. R. Soc. A*, 369: 1980-1996.
- Hancock, J. R. and Place, S. P. 2016. Impact of ocean acidification on the hypoxia tolerance of the woolly sculpin, *Clinocottus analis*. *Conserv. Physiol.* 4, cow040. doi:10.1093/conphys/cow040
- Heath, A. G. and Pritchard, A. W. 1965. Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of fresh-water fish. *Physiol. Zool.*, 38(4): 325-334.
- Holling, C. S. 1966. The strategy of building models of complex ecological systems. In: *Systems Analysis in Ecology*. (K. E. F. Watt, Ed.) Academic Press. Pp. 195-214.
- Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., and Billen, G. 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Front. Ecol. Environ.*, 9(1): 18-26. doi: 10.1890/100008
- Jager, T. 2018. DEBkiss: A Simple Framework for Animal Energy Budgets. Version 2.0. Leanpub: https://leanpub.com/debkiss_book.
- Jager, T., Martin, B. T., and Zimmer, E. I. 2013. DEBkiss or the quest for the simplest generic model of animal life history. *J. Theor. Biol.*, 328: 9-18.
- Jager, T., Ravagnan, E., and Dupont, S. 2016. Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points using a DEB modelling approach. *J. Exp. Mar. Biol. Ecol.*, 474: 11-17.
- Jusup, M., Sousa, T., Domingos, T., Labinac, V., Marn, N., Wang, Z., and Klanjšček, T. 2017. Physics of metabolic organization. *Physics of Life Reviews*, 20: 1-39.
- Kamler, E. 2008. Resource allocation in yolk-feeding fish. *Rev. Fish. Biol. Fisheries*, 18: 143-200.
- Klahre, L. E. 1997. Countergradient Variation in Egg Production Rate of the Atlantic Silverside *Menidia menidia*. [Master’s thesis]. Stony Brook University.

- Kooijman, S. A. L. M. 2010. Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press, Cambridge.
- Kooijman, S. A. L. M. 2018. Models in stress research. *Ecol. Complex.*, 34: 161-177.
- Kooijman, S. A. L. M., and Metz, J. A. J. 1984. On the dynamics of chemically stressed populations: The deduction of population consequences from effects on individuals. *Ecotoxicology and Environmental Safety*, 8(3): 254-274.
- Kooijman, S. A. L. M., Lika, K., Augustine, S., Marn, N., and Kooi, B. W. 2020. The energetic basis of population growth in animal kingdom. *Ecol. Model.*, 428: 109055.
- Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18: 81-92.
- Landry, C. A., Steele, S. L., Manning, S., and Cheek, A. O. 2007. Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.*, 148(2): 317-323.
- Lavaud, R., Filgueira, R., and Augustine, S. 2019. The role of Dynamic Energy Budgets in conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi: 10.1093/conphys/coab083
- Lavaud, R., Filgueira, R., and Augustine, S. 2021. The role of Dynamic Energy Budgets in conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi: 10.1093/conphys/coab083.
- Letcher, B. H. and Bengtson, D. A. 1993. Effects of food density and temperature on feeding and growth of young inland silversides (*Menidia beryllina*). *J. Fish Biol.*, 43: 671-686.
- Martin, B. T., Jager, T., Nisbet, R. M., Preuss, T. G., and Grimm, V. 2013. Predicting Population Dynamics from the Properties of Individuals: A Cross-Level Test of Dynamic Energy Budget Theory. *The American Naturalist*, 181(4): 506-519.
- Martin, B. T., Heintz, R., Danner, E. M., and Nisbet, R. M. 2017. Integrating lipid storage into general representations of fish energetics. *Journal of Animal Ecology*, 86: 812-825.
- Maxime, V., Pichavant, K., Boeuf, G., and Nonnotte, G. 2000. Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. *Fish Physiology and Biochemistry*, 22: 51-59.
- May, R. M. 2001. Stability and Complexity in Model Ecosystems. 2nd Edition. Princeton University Press.

- McBryan, T. L., Anttila, K., Healy, T. M., and Schulte, P. M. 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integr. Comp. Biol.*, 53: 648-659. doi: 10.1093/icb/ict066
- Middaugh, D. P. 1981. Reproductive Ecology and Spawning Periodicity of the Atlantic Silverside, *Menidia menidia* (Pisces: Atherinidae). *Copeia*, 1981(4): 766-776.
- Middaugh, D. P. and Hemmer, M. J. 1992. Reproductive Ecology of the Inland Silverside, *Menidia beryllina*, (Pisces: Atherinidae) from Blackwater Bay, Florida. *Copeia*, 1992(1): 53-61.
- Middaugh, D. P. and Lempesis, P. W. 1976. Laboratory spawning and rearing of a marine fish, the silverside *Menidia menidia menidia*. *Mar. Biol.*, 35: 295-300.
- Miller, S. H., Breitburg, D. L., Burrell, R. B., Keppel, A. G. 2016. Acidification increases sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.*, 549: 1-8.
- Morrell, B. K. and Gobler, C. J. 2020. Negative Effects of Diurnal Changes in Acidification and Hypoxia on Early-Life Stage Estuarine Fishes. *Diversity*, 12: 25. doi: 10.3390/d12010025
- Muller, E. B., Nisbet, R. M., and Berkley, H. A. 2010. Sublethal toxicant effects with dynamic energy budget theory: model formulation. *Ecotoxicology*, 19: 48-60.
- Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*, 10: 69. doi: 10.3390/d10030069
- Murray, C. S. and Baumann, H. 2020. Are long-term growth responses to elevated pCO₂ sex-specific in fish? *PLoS ONE*, 15(7): e0235817.
- Murray, C. S., Fuiman, L. A., and Baumann, H. 2017. Consequences of elevated CO₂ exposure across multiple life stages in a coastal forage fish. *ICES J. Mar. Sci.*, 74(4): 1051-1061. doi: 10.1093/icesjms/fsw179
- Nicieza, A. G. and Metcalfe, N. B. 1997. Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. *Ecology*, 78(8): 2385-2400.
- Niklitschek, E. J. and Secor, D. H. 2005. Modeling spatial and temporal variation of suitable nursery habitats for Atlantic sturgeon in the Chesapeake Bay. *Estuar. Coast. Shelf Sci.*, 64: 135-148.
- Ninness, M. M., Stevens, E. D., and Wright, P. A. 2006. Removal of the chorion before hatching results in increased movement and accelerated growth in rainbow trout (*Oncorhynchus mykiss*) embryos. *J. Exp. Biol.*, 209: 1874-1882.

- Nisbet, R. M., Gurney, W. S. C., Murdoch, W. W., and McCauley, E. 1989. Structured population models: a tool for linking effects at individual and population level. *Biol. J. Linn. Soc.*, 37: 79-99.
- Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to ecosystems through dynamic energy budget models. *Journal of Animal Ecology*, 69: 913-926.
- Nonnotte, G., Maxime, V., Truchot, J. P., Williot, P., and Peyraud, C. 1993. Respiratory responses to progressive ambient hypoxia in the sturgeon, *Acipenser baeri*. *Respir. Physiol.*, 91: 71-82.
- O'Donnell, J., Dam, H. G., Bohlen, W. F., Fitzgerald, W., Gay, P. S., Houk, A. E., Cohen, D. C., and Howard-Strobel, M. M. 2008. Intermittent ventilation in the hypoxic zone of western Long Island Sound during the summer of 2004. *J. Geophys. Res.*, 113: C09025.
- Polymeropoulos, E. T., Elliott, N. G., and Frappell, P. B. 2017. Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comp. Biochem. Physiol. A*, 213: 28-35.
- Pousse, É., Munroe, D., Hart, D., Hennen, D., Cameron, L. P., Rheuban, J. E., Wang, Z. A., Wikfors, G. H., and Meseck, S. L. 2022. Dynamic energy budget modeling of Atlantic surfclam, *Spisula solidissima*, under future ocean acidification and warming. *Mar. Environ. Res.*, 177: 105602. <https://doi.org/10.1016/j.marenvres.2022.105602>
- Rabalais, N. N., Turner, R. E., Díaz, R. J., and Justić, D. 2009. Global change and eutrophication of coastal waters. *ICES J. Mar. Sci.*, 66(7): 1528-1537.
- Richards, J. G. 2009. Metabolic and Molecular Responses of Fish to Hypoxia. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 443-485. San Diego: Academic Press.
- Richards, J. G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.*, 214: 191-199.
- Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: *Fish Physiology, Vol. 11: The Physiology of Developing Fish, Part A: Eggs and Larvae*. (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego: Academic Press.
- Russell, N. R., and Wootton, R. J. 1992. Appetite and growth compensation in the European minnow, *Phoxinus phoxinus* (Cyprinidae), following short periods of food restriction. *Environ. Biol. Fishes*, 34: 277-285.

- Sibly, R. M., Grimm, V., Martin, B. T., Johnston, A. S. A., et al. 2013. Representing the acquisition and use of energy by individuals in agent-based models of animal populations. *Methods in Ecology and Evolution*, 4: 151-161.
- Smallegange, I. M., Caswell, H., Toorians, M. E. M., and de Roos, A. M. 2017. Mechanistic description of population dynamics using dynamic energy budget theory incorporated into integral projection models. *Methods in Ecology and Evolution*, 8: 146-154.
- Stierhoff, K. L., Targett, T. E., and Miller, K. 2006. Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. *Mar. Ecol. Prog. Ser.*, 325: 255-266.
- Stierhoff, K. L., Targett, T. E., and Power, J. H. 2009a. Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA. *Can. J. Fish. Aquat. Sci.*, 66(7): 1033-1047.
- Tai, T. C., Sumaila, U. R., and Cheung, W. W. L. 2021. Ocean Acidification Amplifies Multi-Stressor Impacts on Global Marine Invertebrate Fisheries. *Front. Mar. Sci.*, 8: 596644. doi: 10.3389/fmars.2021.596644
- Taylor, J. C. and Miller, J. M. 2001. Physiological performance of juvenile southern flounder, *Paralichthys lethostigma* (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. *J. Exp. Mar. Biol. Ecol.*, 258: 195-214.
- Testa, J. M., Murphy, R. R., Brady, D. C., and Kemp, W. M. 2018. Nutrient- and Climate-Induced Shifts in the Phenology of Linked Biogeochemical Cycles in a Temperate Estuary. *Front. Mar. Sci.*, 5: 114.
- Thomas, P., Rahman, M. S., Kummer, J. A., and Lawson, S. 2006. Reproductive endocrine dysfunction in Atlantic croaker exposed to hypoxia. *Mar. Environ. Res.*, 62: S249-S252.
- Thomas, Yoann., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G. M., and Pecquerie, Laure. 2019. Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB) theory. *J. Sea Res.*, 143: 231-242.
- Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferraresso, S., Sévère, A., Zambonino-Infante, J.-L., and Mazurais, D. 2014. Hypoxic episode during the larval period has long-term effects on European sea bass juveniles (*Dicentrarchus labrax*). *Mar. Biol.*, 162: 367-376.
- Wagenmakers, E.-J. and Farrell, S. 2004. AIC model selection using Akaike weights. *Psychon. Bull. Rev.*, 11(1): 192-196.

- Wei, L.-Z., Zhang, X.-M., Li, J., and Huang, G.-Q. 2008. Compensatory growth of Chinese shrimp, *Fenneropenaeus chinensis* following hypoxic exposure. *Aquacult. Int.*, 16: 455-470.
- Wood, C. M. 2018. The fallacy of the P_{crit} – are there more useful alternatives? *J. Exp. Biol.*, 221: jeb163717. doi: 10.1242/jeb.163717
- Wu, R. S. S., Zhou, B. S., Randall, D. J., Woo, N. Y. S., and Lam, P. K. S. 2003. Aquatic Hypoxia Is an Endocrine Disruptor and Impairs Fish Reproduction. *Environ. Sci. Technol.*, 37(6): 1137-1141.
- Zambonino-Infante, J. L., Mazurais, D., Dubuc, A., Quéau, P., Vanderplancke, G., Servili, A., Cahu, C., Le Bayon, N., Huelvan, C., and Claireaux, G. 2017. An early life hypoxia event has a long-term impact on protein digestion and growth in juvenile European sea bass. *J. Exp. Biol.*, 220(10): 1846-1851.
- Zhu, C.-D., Wang, Z.-H., and Yan, B. 2013. Strategies for hypoxia adaptation in fish species: a review. *J. Comp. Physiol. B*, 183: 1005-1013.

4.6. Figures

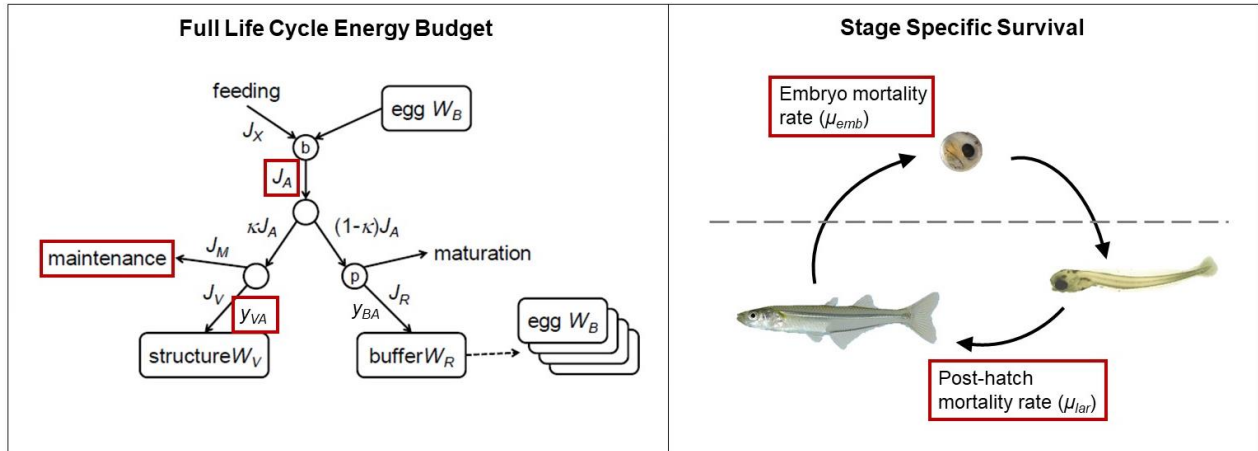


Figure 4.1. The DEBkiss model (diagram adapted from Jager et al., 2013) with stage-specific survival parameters. The candidate parameters for hypoxia stress mechanisms are highlighted in red boxes. The left panel shows the energy budget for the full life cycle and the right panel shows the stage-specific survival modification.

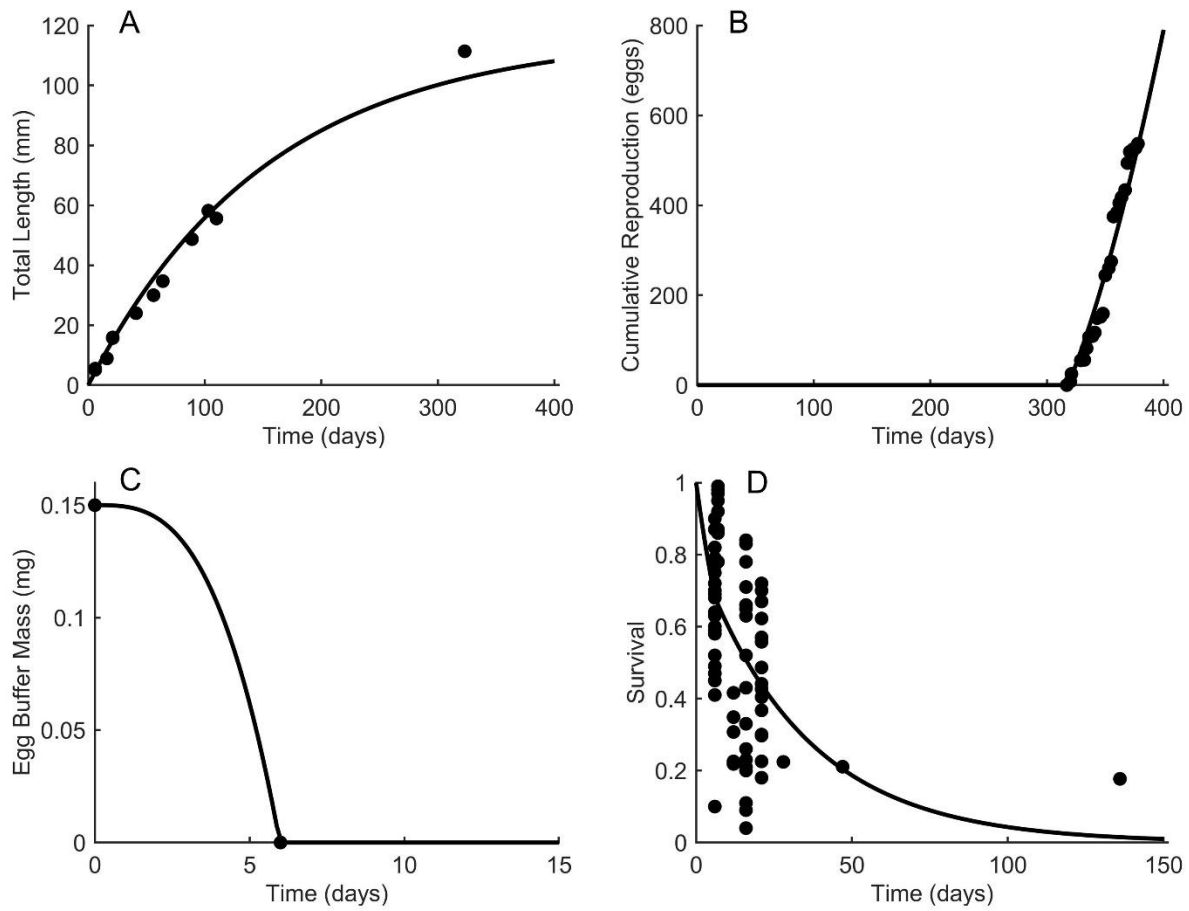


Figure 4.2. Predicted (lines) and observed data (dots) for the DEBkiss model of *M. menidia*. The state variables are (A) total length (mm) over time (days), (B) cumulative reproduction (eggs) over time (days), (C) egg buffer mass (mg) over time (days), and (D) survival over time (days). Predicted data lines are calculated with the parameter values listed in Table 4.1.

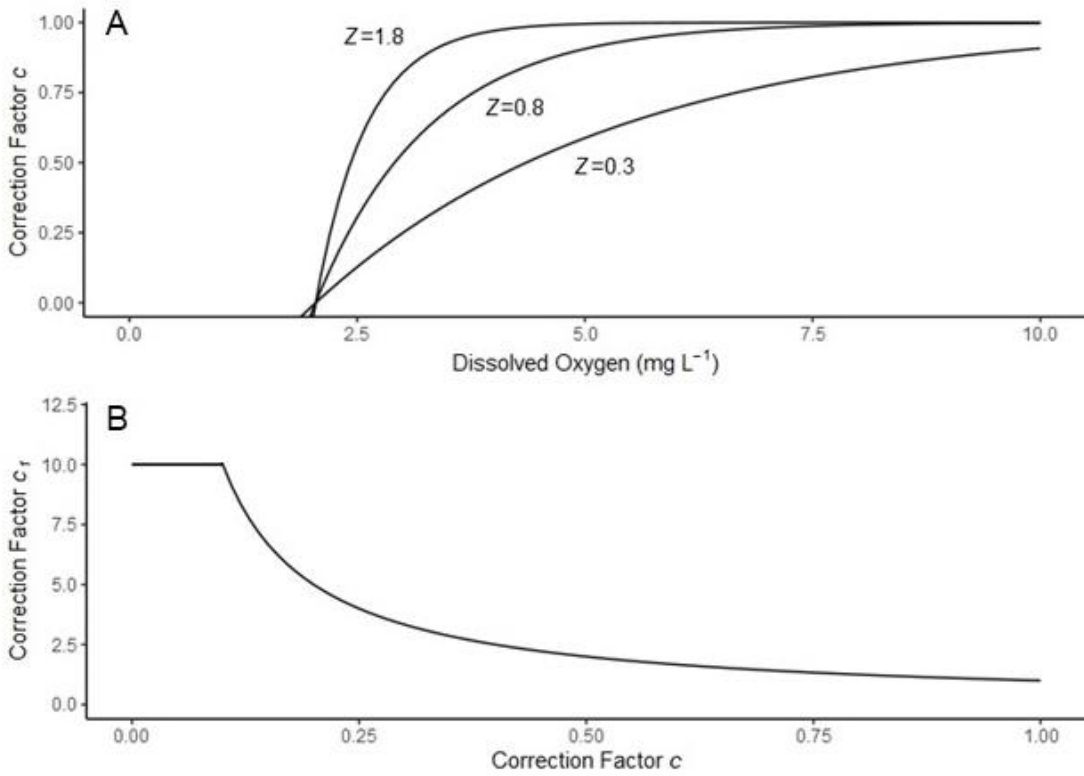


Figure 4.3. The effect of DO on correction factor c (A) at three different values of the exponential coefficient Z , and correction factor c_l (B) as a function of c . Actual estimated Z values are listed in Table 4.5, and the three Z values used in (A) are sample values to show how Z affects the relationship between DO and c . Correction factor c_l is secondarily impacted by DO through c , so that c_l increases as DO and c decrease.

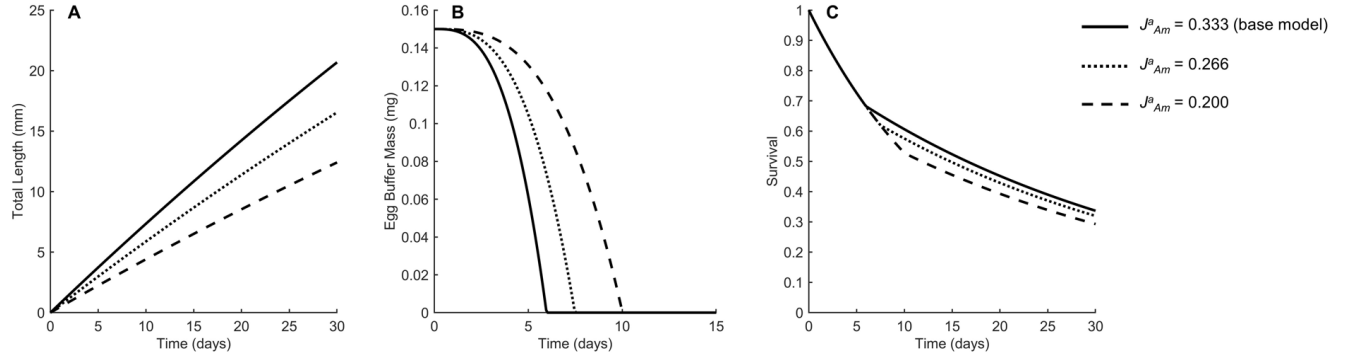


Figure 4.4. Simulated effect of hypoxia on the state variables total length (A), egg buffer mass (B), and survival (C), brought about by reducing J^a_{Am} . These sample plots use J^a_{Am} as an example to show how we simulated the hypothesized effect of hypoxia on each parameter to test whether a given parameter met Criterion 1. The solid line is the predicted data for the control value of J^a_{Am} while the dotted lines show how the predicted data changes as J^a_{Am} is reduced (to arbitrary example values), as it would be by correction factor c . Table 4.4 shows the full results of running these simulations on all parameters.

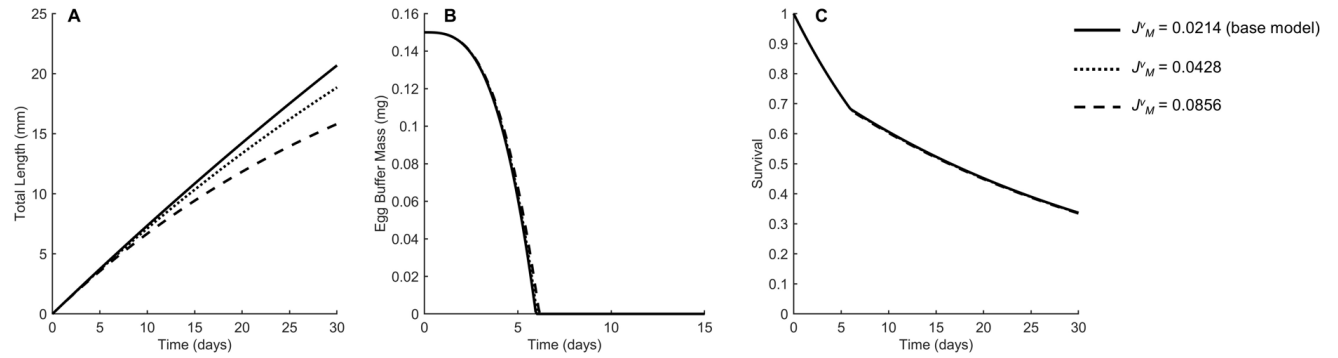


Figure 4.5. Simulated effect of hypoxia on the state variables total length (A), egg buffer mass (B), and survival (C), brought about by increasing J_M^v . These sample plots use J_M^v as an example to show how we simulated the hypothesized effect of hypoxia on each parameter to test whether a given parameter met Criterion 1. The solid line is the predicted data for the control value of J_M^v while the dotted lines show how the predicted data changes as J_M^v is increased (to arbitrary example values), as it would be by correction factor c_I . Table 4.4 shows the full results of running these simulations on all parameters.

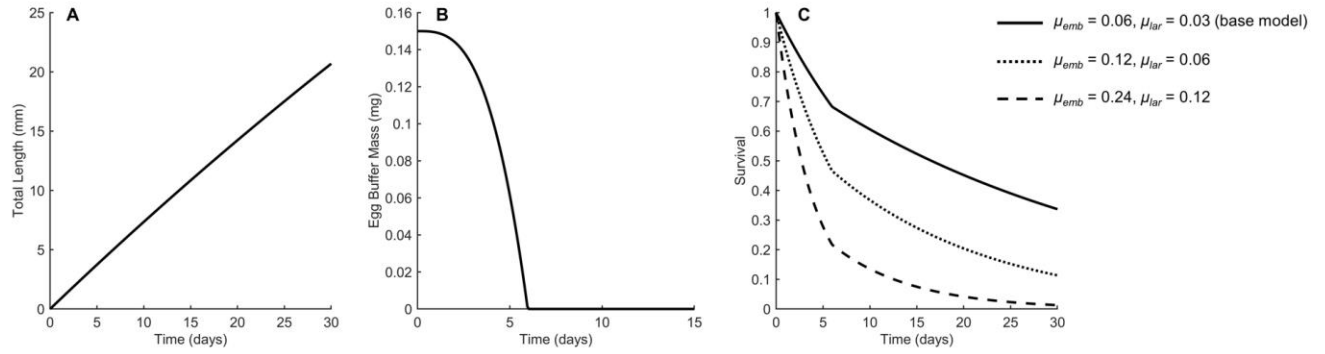


Figure 4.6. Simulated effect of hypoxia on the state variables total length (A), egg buffer mass (B), and survival (C), brought about by increasing μ_{emb} and μ_{lar} simultaneously. These sample plots use μ_{emb} and μ_{lar} as an example to show how we simulated the hypothesized effect of hypoxia on each parameter to test whether a given parameter met Criterion 1. The solid line is the predicted data for the control values of μ_{emb} and μ_{lar} while the dotted lines show how the predicted data changes as mortality is increased (to arbitrary example values), as it would be by correction factor c_I . Table 4.4 shows the full results of running these simulations on all parameters.

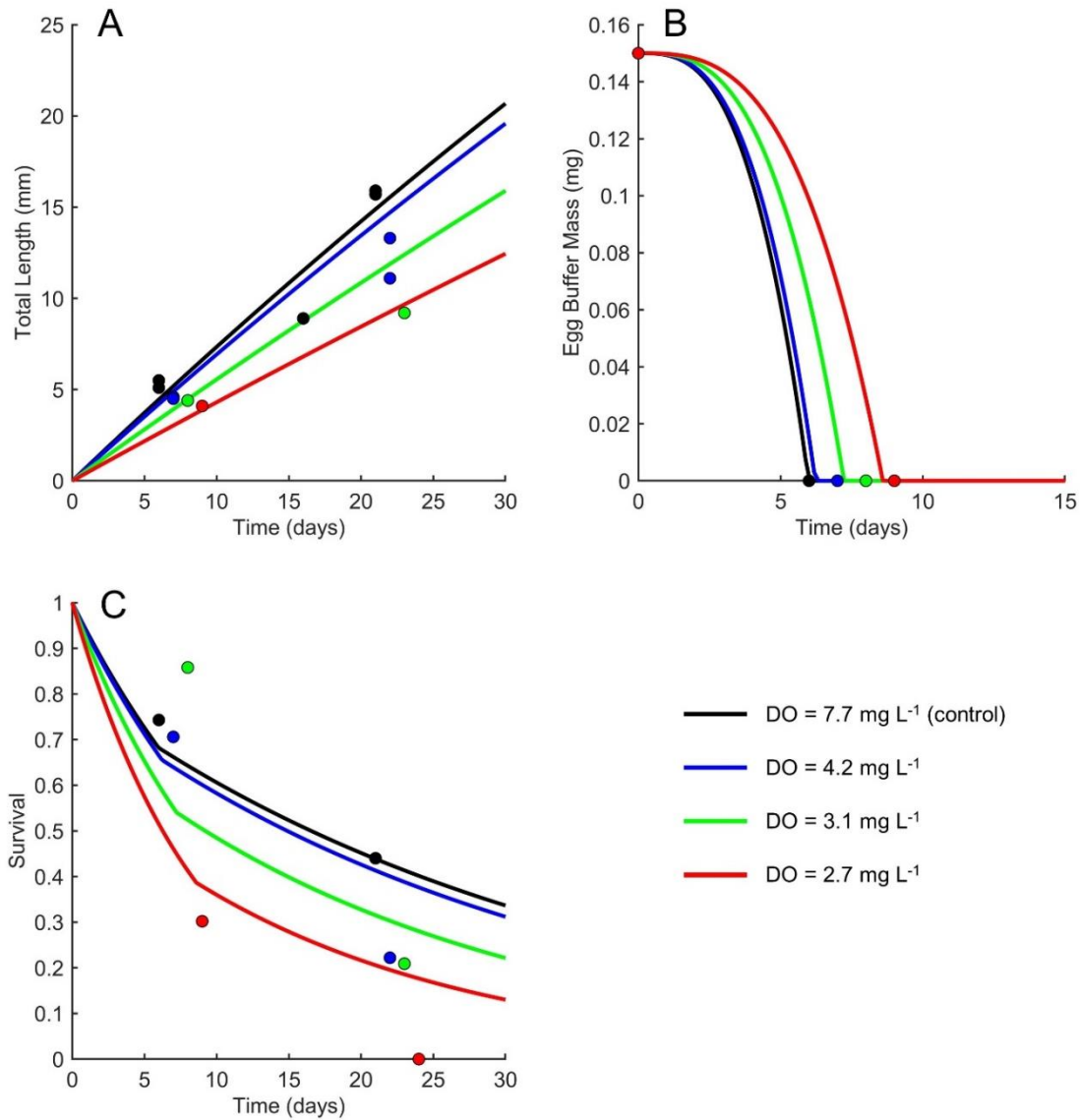


Figure 4.7. Best fit of DEBkiss model to experimental data from four DO levels, showing fit to early life data only. The best fitting model was selected based on a combination of initial criteria that all three response variables' predicted values are affected by the hypoxia correction factor and $\Delta AICc$. (A) is total length (mm) over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time (days), with means rather than all data plotted for survival for ease of viewing patterns.

4.7. Tables

Table 4.1. DEBkiss parameters, their abbreviations, and their fixed or estimated values from fitting to full life data. Units are given with the value unless the parameter is a unitless ratio.

Parameter	Symbol	Fixed or estimated	Value
Max. area-specific assimilation rate	J_{Am}^a	Estimated	0.333 mg mm ⁻² d ⁻¹
Max. volume-specific maintenance rate	J_M^v	Fixed	0.0214 mg mm ⁻³ d ⁻¹
Initial egg buffer mass	W_{B0}	Fixed	0.15 mg
Total length at puberty	L_{Vp}	Fixed	102 mm
Yield of assimilates on volume	y_{AV}	Fixed	0.8
Yield of egg buffer on assimilates	y_{BA}	Fixed	0.95
Yield of structure on assimilates	y_{VA}	Estimated	0.365
Fraction of assimilates allocated to soma	κ	Fixed	0.8
Scaled food level	f	Fixed	1
Scaled food level for embryo	f_B	Fixed	1
Half-saturation total length	L_{Vf}	Fixed	0
Mortality rate for embryos	μ_{emb}	Estimated	0.0639
Mortality rate for larvae	μ_{lar}	Estimated	0.0294

Table 4.2. Fluxes, state variables, and differential equations in the DEBkiss model.

Flux	Symbol	Equation	Units
Assimilation flux	J_A	$J_A = f J_{Am}^a L^2$	mg day ⁻¹
Maintenance flux	J_M	$J_M = J_M^v L^3$	mg day ⁻¹
Flux to structural growth	J_V	$J_V = y_{VA} (\kappa J_A - J_M)$	mg day ⁻¹
Flux to reproduction buffer	J_R	$J_R = (1 - \kappa) J_A - J_J$ when $W_V \geq W_{Vp}$ $J_R = 0$ when $W_V < W_{Vp}$	mg day ⁻¹
Flux to maturity	J_J	$J_J = J_J^v L^3$ when $W_V < W_{Vp}$ $J_J = J_J^v L_{Vp}^3$ when $W_V \geq W_{Vp}$	mg day ⁻¹
State Variable	Symbol	Equation	Units
Structural dry mass over time	W_V	$\frac{dW_V}{dt} = J_V$	mg day ⁻¹
Continuous reproduction rate	R	$\frac{dR}{dt} = \frac{y_{BA} J_R}{W_{B0}}$	eggs day ⁻¹
Egg buffer (yolk) mass	W_B	$\frac{dW_B}{dt} = -J_A$	mg day ⁻¹
Survival	S	$\frac{dS}{dt} = -\mu_{emb} S$ when $W_B > 0$ $\frac{dS}{dt} = -\mu_{lar} S$ when $W_B = 0$	unitless (range 0-1)
Other variables and conversions	Symbol	Equation	Units
Total physical length	L^M	$L^M = \frac{L}{\delta_M}$	mm
Volumetric length	L	$L = \sqrt[3]{L^3}$	mm (cubic root of volume)
Shape coefficient	δ_M	$\delta_M = \frac{L}{L^M}$	unitless
Dry weight density of structure	d_V	$d_V = \frac{W_V}{L^3}$	mg mm ⁻³
Dry mass at puberty	W_{Vp}	$W_{Vp} = d_V * (L_{Vp} * \delta_M)^3$	mg
Volume-specific maturity maintenance costs	J_J^v	-	mg mm ⁻³ day ⁻¹
Structural volume at puberty	L_{Vp}^3	-	mm ⁻³
Scaled measure of resource availability	f	-	unitless (range 0-1)

Table 4.3. The mean survival to hatching, hatch time (at which egg buffer is zero), length at hatching, length at 15 dph, and survival to 15 dph from the different oxygen treatments in Cross et al. (2019). The control DO level means (7.7 mg l^{-1}) also include data from Murray and Baumann (2018).

	7.7 mg L^{-1}	4.2 mg L^{-1}	3.1 mg L^{-1}	2.7 mg L^{-1}
Survival to hatching	74.3%	70.6%	85.8%	30.2%
Hatch time (egg buffer mass = 0)	6 days	7 days	8 days	9 days
Length at hatching	5.3 mm	4.6 mm	4.4 mm	4.1 mm
Larval length at 15 dph	15.8 mm	12.2 mm	9.2 mm	-
Larval survival to 15 dph	44.0%	22.2%	20.9%	0%

Table 4.4. Summary of impacts of altering each DEBkiss parameter on predicted data for total length, time to hatching (egg buffer mass = 0), and survival over time. We used this information to choose which parameters to which to apply hypoxia-based correction factors by identifying those that best meet our requirement of allowing hypoxia effects on at least one of three state variables, within the range of our data. The last column indicates whether the effect of changing the parameter matches the overall patterns observed in the data (i.e. an increase or decrease in at least one state variable).

Parameter	Correction factor	Impact on predicted values of:			Criterion 1 met?
		Total length (mm)	Time to hatching (d)	Survival proportion	
J_{Am}^a	c	✓	✓	✓	yes
J_M^v	c_l	✓	✓ (weak)	✓ (weak)	yes
W_{B0}	c				no
L_{Vp}	c, c_l				no
y_{AV}	c				no
y_{BA}	c				no
y_{VA}	c	✓	✓	✓	yes
κ	c	✓	✓	✓	yes
f	c	✓	✓	✓	yes
f_B	c	✓ (prehatch only)	✓	✓	yes
L_{Vf}	c_l				no
μ_{emb}	c_l			✓	yes
μ_{lar}	c_l			✓	yes

Table 4.5. The estimated Z value and AICc when the correction factors were applied to each parameter or combination of parameters. ΔAICc and Akaike weights are listed only for models that satisfied Criterion 2 as the ones that do not fit the criteria are not eligible to be selected as the best model, and was calculated with $\text{AICc}_{\min} = 584.65$ for the $y_{VA} + J^v_M + \mu_{emb} + \mu_{lar}$ model.

Parameter(s) affected by hypoxia	Correction factor(s)	Estimated Z [95% CI]	AICc	ΔAICc	Akaike weight
J^a_{Am}	c	1.70 [1.69-2.70]	600.73	16.08	1.16e-4
y_{VA}	c	1.48 [1.20-3.21]	602.38	17.73	5.07e-5
J^v_M	c_I	0.365 [0.302-0.5178]	599.42	-	-
μ_{emb}	c_I	0.626 [0.435-0.992]	585.76	-	-
μ_{lar}	c_I	0.303 [0.201-0.492]	575.06	-	-
$J^a_{Am} + J^v_M$	$c + c_I$	1.72 [1.72-2.69]	600.65	16.00	1.20e-4
$y_{VA} + J^v_M$	$c + c_I$	1.47 [1.22-3.08]	602.23	17.58	5.46e-5
$J^v_M + \mu_{emb}$	$c_I + c_I$	0.520 [0.374-0.851]	582.83	-	-
$J^a_{Am} + \mu_{emb}$	$c + c_I$	1.70 [1.69-2.04]	590.30	5.95	0.0213
$y_{VA} + \mu_{emb}$	$c + c_I$	1.31 [1.20-1.78]	589.29	4.64	0.0353
$J^v_M + \mu_{lar}$	$c_I + c_I$	0.354 [0.299-0.448]	568.13	-	-
$J^a_{Am} + \mu_{lar}$	$c + c_I$	1.70 [1.69-2.25]	595.45	10.80	0.00162
$y_{VA} + \mu_{lar}$	$c + c_I$	1.34 [1.20-1.98]	594.67	10.02	0.00239
$\mu_{emb} + \mu_{lar}$	$c_I + c_I$	0.766 [0.543-1.15]	580.11	-	-
$J^a_{Am} + \mu_{emb} + \mu_{lar}$	$c + c_I + c_I$	1.70 [1.69-2.02]	586.75	2.10	0.126
$y_{VA} + \mu_{emb} + \mu_{lar}$	$c + c_I + c_I$	1.32 [1.20-1.76]	584.78	0.13	0.336
$J^v_M + \mu_{emb} + \mu_{lar}$	$c_I + c_I + c_I$	0.712 [0.482-1.09]	578.82	-	-
$J^a_{Am} + J^v_M + \mu_{emb} + \mu_{lar}$	$c + c_I + c_I + c_I$	1.72 [1.72-2.04]	586.86	2.21	0.119
$y_{VA} + J^v_M + \mu_{emb} + \mu_{lar}$	$c + c_I + c_I + c_I$	1.31 [1.22-1.75]	584.65	0	0.359

Table 4.6. The value of the DEBkiss parameters that best reproduce the hypoxia effects observed experimentally, calculated (along with 95% confidence intervals in brackets) for each DO treatment level using the correction factors c and c_I and the estimated value of $Z = 1.315$.

	Product of correction factor and initial parameter value			
	7.7 mg L⁻¹	4.2 mg L⁻¹	3.1 mg L⁻¹	2.7 mg L⁻¹
y_{VA}	0.364 [0.364, 0.365]	0.343 [0.337, 0.356]	0.274 [0.261, 0.308]	0.211 [0.198, 0.249]
μ_{emb}	0.175 [0.175, 0.175]	0.186 [0.179, 0.190]	0.234 [0.207, 0.244]	0.303 [0.256, 0.322]
μ_{lar}	0.0807 [0.0806, 0.0807]	0.0856 [0.0825, 0.0872]	0.107 [0.0956, 0.112]	0.139 [0.118, 0.148]

5. Discussion

5.1. 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).

5.2. 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.

5.3. References

- Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97(5): 399-408. <https://doi.org/10.1139/cjz-2018-0198>
- Baumann, H., Wallace, R. B., Tagliaferri, T., and Gobler, C. J. 2015. Large Natural pH, CO₂ and O₂ Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time Scales. *Estuaries and Coasts*, 38: 220-231.
- Baumann, H., Cross, E. L., and Murray, C. S. 2018. Robust quantification of fish early life CO₂ sensitivities via serial experimentation. *Biol. Lett.*, 14: 20180408. <http://dx.doi.org/10.1098/rsbl.2018.0408>
- Cheung, W. W. L., Sarmiento, J. L., Dunne, J., Fröhlicher, T. L., Lam, V. W. Y., Palomares, M. L. D., Watson, R., and Pauly, D. 2013. Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. *Nat. Clim. Change*, 3: 254-258.
- Chevin, L.-M., Lande, R., and Mace, G. M. 2010. Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biol.*, 8(4): e1000357. doi: 10.1371/journal.pbio.1000357
- Cooley, S., Schoeman, D., Bopp, L., Boyd, P., Donner, S., Ghebrehewet, D. Y., Ito, S.-I., Kiessling, W., Martinetto, P., Ojea, E., Racault, M.-F., Rost, B., and Skern-Mauritzen, M. 2022. Oceans and Coastal Ecosystems and Their Services. In: *Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 379–550, doi:10.1017/9781009325844.005.
- Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal pCO₂ x O₂ fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9: 18146.
- DePasquale, E., Baumann, H., and Gobler, C. J. 2015. Vulnerability of early life stage Northwest Atlantic forage fish to ocean acidification and low oxygen. *Mar. Ecol. Prog. Ser.*, 523: 145-156.
- Foo, S. A. and Byrne, M. 2016. Chapter Two – Acclimatization and Adaptive Capacity of Marine Species in a Changing Ocean. In: *Advances in Marine Biology*, Vol. 74. [Barbara E. Curry, ed.]. Academic Press, Cambridge, MA, USA, pp. 69-116.
- Gobler, C. J., Merlo, L. R., Morrell, B. K., and Griffith, A. W. 2018. Temperature, Acidification, and Food Supply Interact to Negatively Affect the Growth and Survival of the Forage

- Fish, *Menidia beryllina* (Inland Silverside), and *Cyprinodon variegatus* (Sheepshead Minnow). *Front. Mar. Sci.*, 5: 86. doi: 10.3389/fmars.2018.00086
- Harvey, B. P., Gwynn-Jones, D., and Moore, P. J. 2013. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecol. Evol.*, 3(4): 1016-1030.
- Jager, T., Ravagnan, E., and Dupont, S. 2016. Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points using a DEB modelling approach. *J. Exp. Mar. Biol. Ecol.*, 474: 11-17.
- Leslie, H. M. and McLeod, K. L. 2007. Confronting the challenges of implementing marine ecosystem-based management. *Front. Ecol. Environ.*, 5(10): 540-548. doi: 10.1890/060093
- Miller, S. H., Breitburg, D. L., Burrell, R. B., and Keppel, A. G. 2016. Acidification increases sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.*, 549: 1-8.
- Montgomery, D. W., Simpson, S. D., Engelhard, G. H., Birchenough, S. N. R., and Wilson, R. W. 2019. Rising CO₂ enhances hypoxia tolerance in a marine fish. *Sci. Rep.*, 9: 15152. <https://doi.org/10.1038/s41598-019-51572-4>
- Moreira, J. M., Mendes, A. C., Maulvault, A. L., Marques, A., Rosa, R., Pousão-Ferreira, P., Sousa, T., Anacleto, P., and Marques, G. M. 2022. Impacts of ocean warming and acidification on the energy budget of three commercially important fish species. *Conserv. Physiol.*, 10(1): coac048. doi: 10.1093/conphys/coac048
- Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*, 10: 69. doi: 10.3390/d10030069
- Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to ecosystems through dynamic energy budget models. *J. Anim. Ecol.*, 69: 913-926.
- Pousse, É., Munroe, D., Hart, D., Hennen, D., Cameron, L. P., Rheuban, J. E., Wang, Z. A., Wikfors, G. H., and Meseck, S. L. 2022. Dynamic energy budget modeling of Atlantic surfclam, *Spisula solidissima*, under future ocean acidification and warming. *Mar. Environ. Res.*, 177: 105602. <https://doi.org/10.1016/j.marenvres.2022.105602>
- Saba, G. K., Goldsmith, K. A., Cooley, S. R., Grosse, D., Meseck, S. L., et al. 2019. Recommended priorities for research on ecological impacts of ocean and coastal acidification in the U.S. Mid-Atlantic. *Estuar. Coast. Shelf Sci.*, 225: 106188. <https://doi.org/10.1016/j.ecss.2019.04.022>

- Sampaio, E., Santos, C., Rosa, I. C., Ferreira, V., Pörtner, H.-O., Duarte, C. M., Levin, L. A., and Rosa, R. 2021. Impacts of hypoxic events surpass those of future ocean warming and acidification. *Nat. Ecol. Evol.*, 5: 311-321.
- Stiasny, M. H., Sswat, M., Mittermayer, F. H., Falk-Peterson, I.-B., Schnell, N. K., Puvanendran, V., Mortensen, A., Reusch, T. B. H., and Clemmesen, C. 2019. Divergent responses of Atlantic cod to ocean acidification and food limitation. *Glob. Change Biol.*, 25: 839-849.
- Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., and Reusch, T. B. H. 2014. Evolution in an acidifying ocean. *Trends Ecol. Evol.*, 29(2): 117-125.
- Targett, T. E., Greco, P. A., and Dixon, R. L. 2019. Growth of the estuarine fish *Fundulus heteroclitus* in response to diel-cycling hypoxia and acidification: interaction with temperature. *Can. J. Fish. Aquat. Sci.*, 76: 1295-1304.
- Wallace, R. B., Baumann, H., Gear, J. S., Aller, R. C., and Gobler, C. J. 2014. Coastal ocean acidification: The other eutrophication problem. *Estuar. Coast. Shelf Sci.*, 148: 1-13.
- Watson, J. W., Hyder, K., Boyd, R., Thorpe, R., Weltersbach, M. S., Ferter, K., Cooke, S. J., Roy, S., and Sibly, R. M. 2020. Assessing the sublethal impacts of anthropogenic stressors on fish: An energy-budget approach. *Fish Fish.*, 21: 1034-1045. doi: 10.1111/faf.12487

Master Reference List

- Aguirre-Velarde, A., Pecquerie, L., Frederic, J., Gerard, T., and Flye-Sainte-Marie, J. 2019. Predicting the energy budget of the scallop *Argopecten purpuratus* in an oxygen-limiting environment. *J. Sea Res.*, 143: 254-261.
- Alderdice, D. F. 1988. Osmotic and ionic regulation in teleost eggs and larvae. *In: Fish Physiology, Vol. 11A: The Physiology of Developing Fish, Part A: Eggs and Larvae.* (ed. W. S. Hoar and D. J. Randall), pp. 163-251. San Diego: Academic Press.
- Ali, M., Nicieza, A., and Wootton, R. J. 2003. Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries*, 4: 147-190.
- AmP. 2021. Online database of DEB parameters, implied properties and referenced underlying data. www.bio.vu.nl/thb/deb/deblab/add_my_pet/ (data accessed: March 3, 2023).
- Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97(5): 399-408. <https://doi.org/10.1139/cjz-2018-0198>
- Baumann, H. and Smith, E. M. 2018. Quantifying Metabolically Driven pH and Oxygen Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuaries and Coasts*, 41: 1102-1117.
- Baumann, H., Cross, E. L., and Murray, C. S. 2018. Robust quantification of fish early life CO₂ sensitivities via serial experimentation. *Biol. Lett.* 14: 20180408. doi:10.1098/rsbl.2018.0408
- Baumann, H., Talmage, S. C., and Gobler, C. J. 2012. Reduced early life growth and survival in a fish in direct response to increase carbon dioxide. *Nat. Clim. Change*, 2: 38-41. doi: 10.1038/nclimate1291
- Baumann, H., Wallace, R. B., Tagliaferri, T., and Gobler, C. J. 2015. Large Natural pH, CO₂ and O₂ Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time Scales. *Estuaries Coasts*, 38: 220-231. doi: 10.1007/s12237-014-9800-y
- Bayliff, W. H. (1950). *The life history of the silverside Menidia menidia (Linnaeus)*. Solomons Island, Maryland: State of Maryland Board of Natural Resources, Department of Research and Education.
- Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., et al. 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates: A better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. *BioScience*, 51(8): 633-641.

- Bednaršek, N., Feely, R. A., Howes, E. L., Hunt, B. P. B., Kessouri, Faycal., León, P., Lischka, S., Maas, A. E., McLaughlin, K., Nezlin, N. P., Sutula, M., and Weisberg, S. B. 2019. Systematic Review and Meta-Analysis Toward Synthesis of Thresholds of Ocean Acidification Impacts on Calcifying Pteropods and Interactions With Warming. *Front. Mar. Sci.*, 6: 227. doi: 10.3389/fmars.2019.00227
- Bengtson, D. A. 1984. Resource partitioning by *Menidia menidia* and *Menidia beryllina* (Osteichthyes: Atherinidae). *Mar. Ecol. Prog. Ser.*, 18: 21-30.
- Bengtson, D. A., Barkman, R. C., and Berry, W. J., 1987. Relationships between maternal size, egg diameter, time of spawning season, temperature, and length at hatch of Atlantic silverside, *Menidia menidia*. *J. Fish. Biol.*, 31: 697-704.
- Berenbrink, M., Koldkjær, P., Keep, O. and Cossins, A. R. 2005. Evolution of Oxygen Secretion in Fishes and the Emergence of a Complex Physiological System. *Science*, 307(5716): 1752-1757.
- Bianchini, K. and Wright, P. A. 2013. Hypoxia delays hematopoiesis: retention of embryonic hemoglobin and erythrocytes in larval rainbow trout, *Oncorhynchus mykiss*, during chronic hypoxia exposure. *J. Exp. Biol.*, 216(23): 4415-4425.
- Birk, M. A. 2021. respirometry: Tools for Conducting and Analyzing Respirometry Experiments. R package version 1.3.0, <<https://CRAN.R-project.org/package=respirometry>>.
- Blaxter, J. H. S. 1988. Pattern and Variety in Development. In: *Fish Physiology, Vol. 11A: The Physiology of Developing Fish: Eggs and Larvae* (ed. W. S. Hoar and D. J. Randall), pp. 1-58. San Diego: Academic Press.
- Boult, V. L. and Evans, L. C. 2021. Mechanisms matter: Predicting the ecological impacts of global change. *Glob. Change Biol.*, 27(9): 1689-1691.
- Boyd, P. W., Collins, S., Dupont, S., Fabricios, K., Gattuso, J.-P., et al. 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change. *Glob. Change Biol.*, 24: 2239-2261.
- Brandt, S. B., Gerken, M., Hartman, K. J., and Demers, E. 2009. Effects of hypoxia on food consumption and growth of juvenile striped bass (*Morone saxatilis*). *J. Exp. Mar. Biol. Ecol.*, 381: S143-S149. doi: 10.1016/j.jembe.2009.07.028
- Brauner, C. J. and Randall, D. J. 1996. The interaction between oxygen and carbon dioxide movements in fishes. *Comp. Biochem. Physiol.*, 113A: 83-90. doi: 10.1016/0300-9629(95)02062-4
- Brauner, C. J., Shartau, R. B., Damsgaard, C., Esbaugh, A. J., Wilson, R. W., and Grosell, M. 2019. Acid-base physiology and CO₂ homeostasis: Regulation and compensation in response to elevated environmental CO₂. In: *Fish Physiology, Vol. 37: Carbon Dioxide* (ed. A. P. Farrell and C. J. Brauner), pp. 69-132. San Diego: Academic Press.

- Breitburg, D., Levin, L. A., Oschlies, A., et al. 2018. Declining oxygen in the global ocean and coastal waters. *Science*, 359(6371): eaam7240.
- Browman, H. I. (2016). Applying organized scepticism to ocean acidification research. *ICES J. Mar. Sci.* 73(3), 529-536.
- Cadigan, K. M. and Fell, P. E. 1985. Reproduction, growth and feeding habits of *Menidia menidia* (Atherinidae) in a tidal marsh-estuarine system in southern New England. *Copeia*, 1985: 21-26. doi:10.2307/1444786
- Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C, et al. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geosci.*, 4: 766-770. <https://doi.org/10.1038/ngeo1297>
- Calosi, P., Bilton, D. T., Spicer, J. I., Votier, S. C. and Atfield, A. (2010). What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *J. Anim. Ecol.* 79, 194-204.
- Catches, J. S., Burns, J. M., Edwards, S. L., and Claiborne, J. B. 2006. Na⁺/H⁺ antiporter, V-H⁺-ATPase and Na⁺/K⁺-ATPase immunolocalization in a marine teleost (*Myoxocephalus octodecemspinosus*). *J. Exp. Biol.*, 209: 3440-3447.
- Cattano, C., Claudet, J., Domenici, P., and Milazzo, M. 2018. Living in a high CO₂ world: a global meta-analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecol. Monogr.*, 88(3): 320-335.
- Chabot, D. and Claireaux, G. 2008. Environmental hypoxia as a metabolic constraint on fish: The case of Atlantic cod, *Gadus morhua*. *Mar. Pollut. Bull.*, 57: 6-12.
- Chabot, D. and Dutil, J.-D. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *J. Fish. Biol.*, 55: 472-491.
- Chambers, R. C. and Trippel, E. A. (1997). *Early Life History and Recruitment in Fish Populations*. New York: Chapman & Hall.
- Chambers, R. C., Candelmo, A. C., Habeck, E. A., Poach, M. E., Wiczorek, D., Cooper, K. R., Greenfield, C. E. and Phelan, B. A. (2014). Effects of elevated CO₂ in the early life stages of summer flounder, *Paralichthys dentatus*, and potential consequences of ocean acidification. *Biogeosciences*. 11, 1613-1626.
- Chapman, L. J. and McKenzie, D. J. 2009. Behavioral responses and ecological consequences. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. Jeffrey G. Richards, Anthony P. Farrell, and Colin J. Brauner), pp. 25-77. San Diego: Academic Press.

- Cheung, W. W. L., Sarmiento, J. L., Dunne, J., Fröhlicher, T. L., Lam, V. W. Y., Palomares, M. L. D., Watson, R., and Pauly, D. 2013. Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. *Nat. Clim. Change*, 3: 254-258.
- Chevin, L.-M., Lande, R., and Mace, G. M. 2010. Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biol.*, 8(4): e1000357. doi: 10.1371/journal.pbio.1000357
- Christensen, A. K., Hiroi, J., Schultz, E. T., and McCormick, S. D. 2012. Branchial ionocyte organization and ion-transport protein expression in juvenile alewives acclimated to freshwater or seawater. *J. Exp. Biol.*, 215: 642-652.
- Ciuhandu, C. S., Stevens, E. D., and Wright, P. A. 2005. The effect of oxygen on the growth of *Oncorhynchus mykiss* embryos with and without a chorion. *J. Fish. Biol.*, 67: 1544-1551.
- Claiborne, J. B., Edwards, S. L., and Morrison-Shetlar, A. I. 2002. Acid-Base Regulation in Fishes: Cellular and Molecular Mechanisms. *J. Exp. Zool.*, 293: 302-319.
- Claireaux, G. and Chabot, D. 2016. Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. *J. Fish Biol.*, 88: 232-251.
- Claireaux, G. and Lagardère, J.-P. 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *J. Sea Res.*, 42: 157-168.
- Clark, T. D., Raby, G. D., Roche, D. G., Binning, S. A., Speers-Roesch, B., Jutfelt, F., and Sundin, J. 2020. Ocean acidification does not impair the behavior of coral reef fishes. *Nature*, 577: 370-375. doi:10.1038/s41586-019-1903-y
- Clements, J. C., Sundin, J., Clark, T. D., and Jutfelt, F. 2022. Meta-analysis reveals an extreme “decline effect” in the impacts of ocean acidification on fish behavior. *PLoS Biol.*, 20(2): e3001511. <https://doi.org/10.1371/journal.pbio.3001511>
- Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., and Baumann, H. 2021. Temperature-dependent effects on fecundity in a serial broadcast spawning fish after whole-life high CO₂ exposure. *ICES J. Mar. Sci.*, 78(10): 3724-3734.
- Conover, D. O. and Munch, S. B. (2002). Sustaining Fisheries Yields Over Evolutionary Time Scales. *Science*. 297, 94-96.
- Conover, D. O. and Ross, M. R. (1982). Patterns in Seasonal Abundance, Growth and Biomass of the Atlantic Silverside, *Menidia menidia*, in a New England Estuary. *Estuaries*. 5(4), 275-286.
- Cooley, S., Schoeman, D., Bopp, L., Boyd, P., Donner, S., Ghebrehiwet, D. Y., Ito, S.-I., Kiessling, W., Martinetto, P., Ojea, E., Racault, M.-F., Rost, B., and Skern-Mauritzen, M. 2022. Oceans and Coastal Ecosystems and Their Services. In: *Climate Change 2022*:

- Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 379–550, doi:10.1017/9781009325844.005.
- Cottingham, K. L., Lennon, J. T., and Brown, B. L. 2005. Knowing when to draw the line: designing more informative ecological experiments. *Front. Ecol. Environ.*, 3: 145-152. doi:10.1890/1540-9295(2005)003[0145:KWTDTL]2.0.CO;2
- Couturier, C. S., Stecyk, J. A. W., Rummer, J. L., Munday, P. L. and Nilsson, G. E. 2013. Species-specific effects of near-future CO₂ on the respiratory performance of two tropical prey fish and their predator. *Comp. Biochem. Physiol. A*, 166: 482-489. doi:10.1016/j.cbpa.2013.07.025
- Crain, C. M., Kroeker, K., and Halpern, B. S. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol. Lett.*, 11: 1304-1315.
- Cross, E. L., Murray, C. S. and Baumann, H. (2019). Diel and tidal pCO₂ × O₂ fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.* 9, 18146. doi:10.1038/s41598-019-53930-8.
- Cruz-Neto, A. P. and Steffensen, J. F. 1997. The effects of acute hypoxia and hypercapnia on oxygen consumption of the freshwater European eel. *J. Fish. Biol.*, 50: 759-769.
- Dahlke, F. T., Leo, E., Mark, F. C., Pörtner, H.-O., Bickmeyer, U., Frickenhaus, S., and Storch, D. 2017. Effects of ocean acidification increase embryonic sensitivity to thermal extremes in Atlantic cod, *Gadus morhua*. *Glob. Change Biol.*, 23: 1499-1510. doi: 10.1111/gcb.13527
- Dahlke, F. T., Wohlrab, S., Butzin, M., and Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*. 369, 65-70.
- Dahlke, F., Lucassen, M., Bickmeyer, U., Wohlrab, S., Puvanendran, V., Mortensen, A., Chierici, M., Pörtner, H.-O., and Storch, D. 2020. Fish embryo vulnerability to combined acidification and warming coincides with a low capacity for homeostatic regulation. *J. Exp. Biol.*, 223: jeb212589. doi: 10.1242/jeb.212589
- Davis, B. E., Flynn, E. E., Miller, N. A., Nelson, F. A., Fangue, N. A., and Todgham, A. E. (2017). Antarctic emerald rockcod have the capacity to compensate for warming when uncoupled from CO₂-acidification. *Glob. Change Biol.* 24, e655-e670.
- Davis, B. E., Komoroske, L. M., Hansen, M. J., Poletto, J. B., Perry, E. N., Miller, N. A., Ehلمان, S. M., Wheeler, S. G., Sih, A., Todgham, A. E. and Fangue, N. A. (2018).

- Juvenile rockfish show resilience to CO₂-acidification and hypoxia across multiple biological scales. *Conserv. Physiol.* 6, coy038. doi:10.1093/conphys/coy038.
- Deigweiher, K., N. Koschnick, H.-O. Pörtner, M. Lucassen. 2008. Acclimation of ion regulatory capacities in gills of marine fish under environmental hypercapnia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295: R1660-R1670.
- Del Rio, A. M., Davis, B. E., Fangue, N. A., and Todgham, A. E. 2019. Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conserv. Physiol.*, 7(1): coy078. doi: 10.1093/conphys/coy078
- DePasquale, E., Baumann, H., and Gobler, C. J. 2015. Vulnerability of early life stage Northwest Atlantic forage fish to ocean acidification and low oxygen. *Mar. Ecol. Prog. Ser.*, 523: 145-156.
- Desforges, J.-P. W., Sonne, C., and Dietz, R. 2017. Using energy budgets to combine ecology and toxicology in a mammalian sentinel species. *Sci. Rep.*, 7: 46267. doi: 10.1038/srep46267
- Di Santo, V., Kenaley, C. P., and Lauder, G. V. 2017. High postural costs and anaerobic metabolism during swimming support the hypothesis of a U-shaped metabolism–speed curve in fishes. *Proc. Nat. Acad. Sci.*, 114(49): 13048-13053.
- Diaz, R. J. and Breitburg, D. L. 2009. The Hypoxic Environment. *In: Fish Physiology, Vol. 27: Hypoxia.* (ed. Jeffrey G. Richards, Anthony P. Farrell, and Colin J. Brauner), pp. 1-23. San Diego: Academic Press.
- Diaz, R. J. and Rosenberg, R. 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. *Science*, 321: 926-929.
- Dixon, R. L., Greco, P. A., and Targett, T. E. 2017. Responses of juvenile Atlantic silverside, striped killifish, mummichog, and striped bass to acute hypoxia and acidification: Aquatic surface respiration and survival. *J. Exp. Mar. Biol. Ecol.*, 493: 20-30.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. 2009. Ocean Acidification: The Other CO₂ Problem. *Annu. Rev. Mar. Sci.*, 1: 169-192.
<https://doi.org/10.1146/annurev.marine.010908.163834>
- Dulvy, N. K., Sadovy, Y. and Reynolds, J. D. (2003). Extinction vulnerability in marine populations. *Fish Fish.* 4, 25-64.
- Earhart, M. L., Blanchard, T. S., Harman, A. A., and Schulte, P. M. 2022. Hypoxia and High Temperature as Interacting Stressors: Will Plasticity Promote Resilience of Fishes in a Changing World? *Biol. Bull.*, 243: 149-170.

- Esbaugh, A. J. 2018. Physiological implications of ocean acidification for marine fish: emerging patterns and new insights. *J. Comp. Physiol. B*, 188: 1-13.
<https://doi.org/10/1007/s00360-017-1105-6>
- Esbaugh, A. J., R. Heuer, M. Grosell. 2012. Impacts of ocean acidification on respiratory gas exchange and acid-base balance in a marine teleost, *Opsanus beta*. *J. Comp. Physiol. B*. 182: 921-934.
- Espinel-Velasco, N., Hoffmann, L., Agüera, A., Byrne, M., Dupont, S., Uthicke, S., Webster, N. S., and Lamare, M. 2018. Effects of ocean acidification on the settlement and metamorphosis of marine invertebrate and fish larvae: a review. *Mar. Ecol. Prog. Ser.*, 606: 237-257. <https://doi.org/10.3354/meps12754>
- Evans, M. R., Grimm, V., Johst, K., et al. 2013. Do simple models lead to generality in ecology? *Trends in Ecology & Evolution*, 28(10): 578-583.
- Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.*, 65: 414-432.
- Farrell, A. P. and Brauner, C. J. 2009. Fish Physiology, Vol. 27: Hypoxia. Academic Press, London.
- Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J. 2004. Impact of Anthropogenic CO₂ on the CaCO₃ System in the Oceans. *Science*, 305: 362-366.
- Flynn, E. E. and Todgham, A. E. (2018). Thermal windows and metabolic performance curves in a developing Antarctic fish. *J. Comp. Physiol. B*. 188, 271-282.
- Foo, S. A. and Byrne, M. 2016. Chapter Two – Acclimatization and Adaptive Capacity of Marine Species in a Changing Ocean. In: *Advances in Marine Biology*, Vol. 74. [Barbara E. Curry, ed.]. Academic Press, Cambridge, MA, USA, pp. 69-116.
- Fox, J. and Weisberg, S. 2019. An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL:
<https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., Folkvord, A., Piatkowski, U., Reusch, T. B. H. and Clemmesen, C. (2012). Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nat. Clim. Change*. 2, 42-46.
- Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., et al. 2012. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nat. Clim. Change*, 2: 42-46. doi: 10.1038/nclimate1324

- Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In *Fish Physiology, Vol. 6, Environmental Relations and Behavior* (ed. W. S. Hoar and D. J. Randall), pp. 1-98. New York: Academic Press.
- Fry, F. E. J. 1947. *Effects of the Environment on Animal Activity*. Publications of the Ontario Fisheries Research Laboratory, No. 68. Toronto: The University of Toronto Press.
- Galic, N., Sullivan, L. L., Grimm, V., and Forbes, V. E. 2018. When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. *Ecol. Lett.*, 21(4): 568-577.
- Gamperl, A. K. and Driedzic, W. R. 2009. Cardiovascular Function and Cardiac Metabolism. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. Jeffrey G. Richards, Anthony P. Farrell, and Colin J. Brauner), pp. 25-77. San Diego: Academic Press.
- García, H. E. and Gordon, L. I. (1992). Oxygen solubility in seawater: Better fitting equations. *Limnol. Oceanogr.* 37, 1307-1312.
- Gledhill, D. K., White, M. M., Salisbury, J., Thomas, H., Misna, I., et al. 2015. Ocean and Coastal Acidification off New England and Nova Scotia. *Oceanogr.*, 28(2), Special Issue on Emerging Themes in Ocean Acidification Science: 182-197.
- Gobler, C. J. and Baumann, H. (2016). Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. *Biol. Lett.* 12, 20150976.
<http://dx.doi.org/10.1098/rsbl.2015.0976>.
- Gobler, C. J., Merlo, L. R., Morrell, B. K., and Griffith, A. W. 2018. Temperature, Acidification, and Food Supply Interact to Negatively Affect the Growth and Survival of the Forage Fish, *Menidia beryllina* (Inland Silverside), and *Cyprinodon variegatus* (Sheepshead Minnow). *Front. Mar. Sci.*, 5: 86. doi: 10.3389/fmars.2018.00086
- Goussen, B., Rendal, C., Sheffield, D., Butler, E., Price, O. R., and Ashauer, R. 2020. Bioenergetics modelling to analyze and predict the joint effects of multiple stressors: Meta-analysis and model corroboration. *Sci. Total. Environ.*, 749: 141509.
- Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I., et al. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO₂ in Atlantic halibut. *J. Exp. Biol.* 217, 711-717.
- Greear, J. S., O'Leary, C. A., Nye, J. A., Tettelbach, S. T., and Gobler, C. J. 2020. Effects of coastal acidification on North Atlantic bivalves: interpreting laboratory responses in the context of *in situ* populations. *Mar. Ecol. Prog. Ser.*, 633: 89-104.
- Grosell, M. 2019. CO₂ and calcification processes in fish. In: *Fish Physiology, Vol. 37: Carbon Dioxide* (ed. A. P. Farrell and C. J. Brauner), pp. 133-159. San Diego: Academic Press.

- Gruber, N. 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Phil. Trans. R. Soc. A.*, 369: 1980-1996. <http://doi.org/10.1098/rsta.2011.0003>
- Guggino, W. B. 1980. Salt balance in embryos of *Fundulus heteroclitus* and *F. bermudae* adapted to seawater. *Am. J. Physiol.*, 238: R36-R41.
- Gunderson, A. R., Armstrong, E. J., and Stillman, J. H. 2016. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Annu. Rev. Mar. Sci.*, 8: 357-378. doi:10.1146/annurev-marine-122414-033953
- Hancock, J. R. and Place, S. P. 2016. Impact of ocean acidification on the hypoxia tolerance of the woolly sculpin, *Clinocottus analis*. *Conserv. Physiol.* 4, cow040. doi:10.1093/conphys/cow040
- Harvey, B. P., Gwynn-Jones, D., and Moore, P. J. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecol. Evol.*, 3(4): 1016-1030. doi: 10.1002/ece3.516
- Havenhand, J., Dupont, S. and Quinn, G. P. (2010). Designing ocean acidification experiments to maximize inference. In *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (ed. U. Riebesell, V. J. Fabry, L. Hansson and J.-P. Gattuso), pp. 67-136. Luxembourg: Publications Office of the European Union Luxembourg.
- Heath, A. G. and Pritchard, A. W. 1965. Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of fresh-water fish. *Physiol. Zool.*, 38(4): 325-334.
- Hebbali, A. 2020. olsrr: Tools for Building OLS Regression Models. R package version 0.5.3, <<https://CRAN.R-project.org/package=olsrr>>.
- Heinrich, D. D. U., Rummer, J. L., Morash, A. J., Watson, S.-A., Simpfendorfer, S. A., Heupel, M. R., and Munday, P. L. 2014. A product of its environment: the epaulette shark (*Hemiscylium ocellatum*) exhibits physiological tolerance to elevated environmental CO₂. *Conserv. Physiol.*, 2(1): cou047. <https://doi.org/10.1093/conphys/cou047>
- Heuer, R. M and Grosell, M. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 307: R1061-R1084. doi: 10.1152/apjregu.00064.2014
- Hiroi, J. and McCormick, S. D. 2012. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. *Resp. Physiol. Neurobiol.*, 184: 257-268.
- Hiroi, J., Kaneko, T., Seikai, T., and Tanaka, M. 1998. Developmental Sequence of Chloride Cells in the Body Skin and Gills of Japanese Flounder (*Paralichthys olivaceus*) Larvae. *Zool. Sci.*, 15: 455-460.

- Hochachka, P. W., and Somero, G. N. 2002. Biochemical adaptation: mechanisms and process in physiological evolution. Oxford University Press, Oxford.
- Holling, C. S. 1966. The strategy of building models of complex ecological systems. In: *Systems Analysis in Ecology*. (K. E. F. Watt, Ed.) Academic Press. Pp. 195-214.
- Hollowed, A. B., Barange, M., Beamish, R. J., Brander, K., Cochrane, K., Drinkwater, K., Foreman, M. G. G., Hare, J. A., Holt, J. Ito, S. et al. (2013). Projected impacts of climate change on marine fish and fisheries. *ICES J. Mar. Sci.* 70(5), 1023-1037.
- Houde, E. D. (1987). Fish Early Life Dynamics and Recruitment Variability. *Am. Fish. Soc. Symp.* 2, 17-29.
- Houde, E. D. 1989. Subtleties and episodes in the early life of fishes. *J. Fish Biol.*, 35: 29-38.
- Houde, E. D. 1997. Patterns and consequences of selective processes in teleost early life histories. In: *Early Life History and Recruitment in Fish Populations*. (ed. R. Christopher Chambers and Edward A Trippel), pp. 173-196. London: Chapman & Hall.
- Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., and Billen, G. 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Front. Ecol. Environ.*, 9(1): 18-26. doi: 10.1890/100008
- Hsu, S.-M., Raine, L., and Fanger, H. 1981. Use of Avidin-Biotin-Peroxidase Complex (ABC) in Immunoperoxidase Techniques: A Comparison between ABC and Unlabeled Antibody (PAP) Procedures. *J. Histochem. Cytochem.*, 29(4): 577-580.
- Hurlbert, S. H. 2004. On misinterpretations of pseudoreplication and related matters: a reply to Oksanen. *Oikos*, 104: 591-597. doi:10.1111/j.0030-1299.2004.12752.x
- Hurst, T. P. and Conover, D. O. (1998). Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): size-dependent patterns and effects on recruitment. *Can. J. Fish. Aquat. Sci.* 55, 1122-1130.
- Hwang, P. P., and Lin, L. Y. 2013. Gill ionic transport, acid-base regulation and nitrogen excretion. In: *The Physiology of Fishes*. (Eds. D. Evans, J. B. Claiborne, and S. Currie), pp. 205-233, Boca Raton: CRC Press.
- Intergovernmental Panel on Climate Change. (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley). Cambridge, UK, and New York, NY, USA: Cambridge University Press.

- Intergovernmental Panel on Climate Change. 2022. Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [H.-O. Pörtner, D. C. Roberts, M. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama, eds.]. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.
- Ishimatsu, A., Hayashi, M., and Kikkawa, T. 2008. Fishes in high-CO₂, acidified oceans. *Mar. Ecol. Prog. Ser.*, 373: 295-302. doi: 10.3354/meps07823
- Ishimatsu, A., Hayashi, M., and Lee, K.-S. 2005. Physiological effects on fishes in a high-CO₂ world. *J. Geophys. Res.*, 110: C09S09. doi: 10.1029/2004JC002564
- Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K.-S., and Kita, J. 2004. Effects of CO₂ on Marine Fish: Larvae and Adults. *J. Oceanogr.*, 60: 731-741.
- Jager, T. 2018. DEBkiss: A Simple Framework for Animal Energy Budgets. Version 2.0. Leanpub: https://leanpub.com/debkiss_book.
- Jager, T., Martin, B. T., and Zimmer, E. I. 2013. DEBkiss or the quest for the simplest generic model of animal life history. *J. Theor. Biol.*, 328: 9-18.
- Jager, T., Ravagnan, E., and Dupont, S. 2016. Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points using a DEB modelling approach. *J. Exp. Mar. Biol. Ecol.*, 474: 11-17.
- Jarrold, M. D. and Munday, P. L. 2018. Diel CO₂ cycles do not modify juvenile growth, survival and otolith development in two coral reef fish under ocean acidification. *Mar. Biol.*, 165: 49. <https://doi.org/10.1007/s00227-018-3311-5>
- Jusup, M., Sousa, T., Domingos, T., Labinac, V., Marn, N., Wang, Z., and Klanjšček, T. 2017. Physics of metabolic organization. *Physics of Life Reviews*, 20: 1-39.
- Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G. E., Metcalfe, N. B., Hickey, A. J. R., et al. (2018). Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *J. Exp. Biol.* 221, jeb169615. doi: 10.1242/jeb.162693.
- Kamler, E. (1992). *Early Life History of Fish: An Energetics Approach*. New York: Chapman & Hall.
- Kamler, E. 2008. Resource allocation in yolk-feeding fish. *Rev. Fish. Biol. Fisheries*, 18: 143-200.
- Keeling, R. F., Körtzinger, A., and Gruber, N. (2010). Ocean Deoxygenation in a Warming World. *Annu. Rev. Mar. Sci.* 2, 463-493.

- Keeling, R. F., Körtzinger, A., and Gruber, N. 2010. Ocean Deoxygenation in a Warming World. *Annu. Rev. Mar. Sci.*, 2: 463-493. doi: 10.1146/annurev.marine.010908.163855
- Klahre, L. E. 1997. Countergradient Variation in Egg Production Rate of the Atlantic Silverside *Menidia menidia*. [Master's thesis]. Stony Brook University.
- Kooijman, S. A. L. M. 2010. Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press, Cambridge.
- Kooijman, S. A. L. M. 2018. Models in stress research. *Ecol. Complex.*, 34: 161-177.
- Kooijman, S. A. L. M., and Metz, J. A. J. 1984. On the dynamics of chemically stressed populations: The deduction of population consequences from effects on individuals. *Ecotoxicology and Environmental Safety*, 8(3): 254-274.
- Kooijman, S. A. L. M., Lika, K., Augustine, S., Marn, N., and Kooi, B. W. 2020. The energetic basis of population growth in animal kingdom. *Ecol. Model.*, 428: 109055.
- Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18: 81-92.
- Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.*, 13: 1419-1434. doi: 10.1111/j.1461-0248.2010.01518.x
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J.-P. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Change Biol.*, 19: 1884-1896.
- Kwan, G. T., and Tresguerres, M. 2022. Elucidating the acid-base mechanisms underlying otolith overgrowth in fish exposed to ocean acidification. *Sci. Total Environ.*, 832: 153690. <https://doi.org/10.1016/j.scitotenv.2022.153690>
- Kwan, G. T., Shen, S. G., Drawbridge, M., Checkley Jr., D. M., and Tresguerres, M. 2021. Ion-transporting capacity and aerobic respiration of larval white seabass (*Atractoscion nobilis*) may be resilient to ocean acidification conditions. *Sci. Total Environ.*, 791: 148285. <https://doi.org/10.1016/j.scitotenv.2021.148285>
- Kwan, G. T., Smith, T. R., and Tresguerres, M. 2020. Immunological characterization of two types of ionocytes in the inner ear epithelium of Pacific Chub Mackerel (*Scomber japonicus*). *J. Comp. Physiol. B*, 190: 419-431.
- Landry, C. A., Steele, S. L., Manning, S., and Cheek, A. O. 2007. Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.*, 148(2): 317-323.

- Lavaud, R., Filgueira, R., and Augustine, S. 2019. The role of Dynamic Energy Budgets in conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi: 10.1093/conphys/coab083
- Lavaud, R., Filgueira, R., and Augustine, S. 2021. The role of Dynamic Energy Budgets in conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi: 10.1093/conphys/coab083
- Le Quesne, W. J. F. and Pinnegar, J. K. 2012. The potential impacts of ocean acidification: scaling from physiology to fisheries. *Fish Fish.*, 13: 333-344.
- Lefevre, S. (2016). Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO₂ and their interaction. *Conserv. Physiol.* 4(1), cow009. doi: 10.1093/conphys/cow009.
- Lefevre, S. 2019. Effects of high CO₂ on oxygen consumption rates, aerobic scope and swimming performance. In: *Fish Physiology, Vol. 37: Carbon Dioxide* (ed. A. P. Farrell and C. J. Brauner), pp. 195-244. San Diego: Academic Press.
- Leslie, H. M. and McLeod, K. L. 2007. Confronting the challenges of implementing marine ecosystem-based management. *Front. Ecol. Environ.*, 5(10): 540-548. doi: 10.1890/060093
- Letcher, B. H. and Bengtson, D. A. 1993. Effects of food density and temperature on feeding and growth of young inland silversides (*Menidia beryllina*). *J. Fish Biol.*, 43: 671-686.
- Lifavi, D. M., Targett, T. E., and Greco, P. A. (2017). Effects of diel-cycling hypoxia and acidification on juvenile weakfish *Cynoscion regalis* growth, survival, and activity. *Mar. Ecol. Prog. Ser.* 564, 163-174.
- Liu, S.-T., Horng, J.-L., Chen, P.-Y., Hwang, P.-P., and Lin, L.-Y. 2016. Salt secretion is linked to acid-base regulation of ionocytes in seawater acclimated medaka: new insights into the salt-secreting mechanism. *Sci. Rep.*, 6: 31433. doi:10.1038/srep31433
- Lynch, H. J., Rhinds, M., Calabrese, J. M., Cantrell, S., Cosner, C. and Fagan, W. F. (2014). How climate extremes – not means – define a species' geographic range boundary via a demographic tipping point. *Ecol. Monogr.* 84(1), 131-149.
- Marshall, D. J., Burgess, S. C., and Connallon, T. 2016. Global change, life-history complexity and the potential for evolutionary rescue. *Evol. Appl.*, 9: 1189-1201.
- Martin, B. T., Heintz, R., Danner, E. M., and Nisbet, R. M. 2017. Integrating lipid storage into general representations of fish energetics. *Journal of Animal Ecology*, 86: 812-825.

- Martin, B. T., Jager, T., Nisbet, R. M., Preuss, T. G., and Grimm, V. 2013. Predicting Population Dynamics from the Properties of Individuals: A Cross-Level Test of Dynamic Energy Budget Theory. *The American Naturalist*, 181(4): 506-519.
- Maxime, V., Pichavant, K., Boeuf, G., and Nonnotte, G. 2000. Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. *Fish Physiology and Biochemistry*, 22: 51-59.
- May, R. M. 2001. Stability and Complexity in Model Ecosystems. 2nd Edition. Princeton University Press.
- McBryan, T. L., Anttila, K., Healy, T. M., and Schulte, P. M. 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integr. Comp. Biol.*, 53: 648-659. doi: 10.1093/icb/ict066
- McKenzie, D. J., Piccolella, M., Dalla Valle, A. Z., Taylor, E. W., Bolis, C. L., and Steffensen, J. F. 2003. Tolerance of chronic hypercapnia by the European eel *Anguilla anguilla*. *J. Exp. Biol.*, 206(10): 1717-1726. <https://doi.org/10.1242/jeb.00352>
- Melzner, F., Gutkowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C., Bleich, M., and Pörtner, H.-O. 2009. Physiological basis for high CO₂ tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosci.*, 6: 2313-2331.
- Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A. (2013). Future ocean acidification will be amplified by hypoxia in coastal habitats. *Mar. Biol.* 160, 1875-1888.
- Middaugh, D. P. 1981. Reproductive Ecology and Spawning Periodicity of the Atlantic Silverside, *Menidia menidia* (Pisces: Atherinidae). *Copeia*, 1981(4): 766-776.
- Middaugh, D. P. and Hemmer, M. J. 1992. Reproductive Ecology of the Inland Silverside, *Menidia beryllina*, (Pisces: Atherinidae) from Blackwater Bay, Florida. *Copeia*, 1992(1): 53-61.
- Middaugh, D. P. and Lempesis, P. W. 1976. Laboratory spawning and rearing of a marine fish, the silverside *Menidia menidia menidia*. *Mar. Biol.*, 35: 295-300.
- Middaugh, D. P., Hemmer, M. J., and Goodman, L. R. 1987. Methods for Spawning, Cultureing and Conducting Toxicity-Tests with Early Life Stages of Four Atherinid Fishes: The Inland Silverside, *Menidia beryllina*, Atlantic silverside, *M. menidia*, Tidewater silverside, *M. peninsulae* and California grunion, *Leuresthes tenuis*. Gulf Breeze, FL: United States Environmental Protection Agency.
- Miller, S. H., Breitburg, D. L., Burrell, R. B., and Keppel, A. G. (2016). Acidification increases sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.* 549, 1-8.

- Montgomery, D. W., Simpson, S. D., Engelhard, G. H., Birchenough, S. N. R. and Wilson, R. W. 2019. Rising CO₂ enhances hypoxia tolerance in a marine fish. *Sci. Rep.* 9, 15152. doi:10.1038/s41598-019-51572-4
- Moreira, J. M., Mendes, A. C., Maulvault, A. L., Marques, A., Rosa, R., Pousão-Ferreira, P., Sousa, T., Anacleto, P., and Marques, G. M. 2022. Impacts of ocean warming and acidification on the energy budget of three commercially important fish species. *Conserv. Physiol.*, 10(1): coac048. doi: 10.1093/conphys/coac048
- Morrell, B. K. and Gobler, C. J. (2020). Negative Effects of Diurnal Changes in Acidification and Hypoxia on Early-Life Stage Estuarine Fishes. *Diversity*. 12, 25. doi: 10.3390/d12010025.
- Muggeo, V. M. R. 2008. Segmented: an R Package to Fit Regression Models with Broken-Line Relationships. *R News*, 8/1, 20-25. URL <https://cran.r-project.org/doc/Rnews/>.
- Muller, E. B., Nisbet, R. M., and Berkley, H. A. 2010. Sublethal toxicant effects with dynamic energy budget theory: model formulation. *Ecotoxicology*, 19: 48-60.
- Munday, P. L., Cheal, A. J., Dixon, D. L., Rummer, J. L., and Fabricius, K. E. 2014. Behavioural impairment in reef fishes caused by ocean acidification at CO₂ seeps. *Nat. Clim. Change*, 4: 487-492.
- Munday, P. L., Dixon, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V. and Doving, K. B. (2009a). Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl. Acad. Sci. U S A*. 106(6), 1848-1852.
- Munday, P. L., Crawley, N. E. and Nilsson, G. E. (2009b). Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Mar. Ecol. Prog. Ser.* 388, 235-242.
- Munday, P. L., Dixon, D. L., McCormick, M. I., Meekan, M., Ferrari, M. C. O. and Chivers, D. P. (2010). Replenishment of fish populations is threatened by ocean acidification. *Proc. Natl. Acad. Sci.* 107(29), 12930-12934.
- Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*, 10: 69. doi: 10.3390/d10030069
- Murray, C. S. and Baumann, H. 2020. Are long-term growth responses to elevated pCO₂ sex-specific in fish? *PLoS ONE*, 15(7): e0235817.
- Murray, C. S., Fuiman, L. A. and Baumann, H. (2017). Consequences of elevated CO₂ exposure across multiple life stages in a coastal forage fish. *ICES J. Mar. Sci.* 74(4), 1051-1061.

- Murray, C. S., Malvezzi, A., Gobbler, C. J., and Baumann, H. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.*, 504: 1-11. doi:10.3354/meps10791
- Murray, C. S., Wiley, D. and Baumann, H. (2019). High sensitivity of a keystone forage fish to elevated CO₂ and temperature. *Conserv. Physiol.* 7(1), cox084. doi: 10.1093/conphys/cox084.
- National Research Council (2005). *Marine Mammal Populations and Ocean Noise: Determining When Noise Causes Biologically Significant Effects*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/11147>
- Nicieza, A. G. and Metcalfe, N. B. 1997. Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. *Ecology*, 78(8): 2385-2400.
- Niklitschek, E. J. and Secor, D. H. 2005. Modeling spatial and temporal variation of suitable nursery habitats for Atlantic sturgeon in the Chesapeake Bay. *Estuar. Coast. Shelf Sci.*, 64: 135-148.
- Nilsson, G. E. and Östlund-Nilsson, S. (2008). Does size matter for hypoxia tolerance in fish? *Biol. Rev.* 83, 173-189.
- Ninness, M. M., Stevens, E. D., and Wright, P. A. 2006. Removal of the chorion before hatching results in increased movement and accelerated growth in rainbow trout (*Oncorhynchus mykiss*) embryos. *J. Exp. Biol.*, 209: 1874-1882.
- Nisbet, R. M., Gurney, W. S. C., Murdoch, W. W., and McCauley, E. 1989. Structured population models: a tool for linking effects at individual and population level. *Biol. J. Linn. Soc.*, 37: 79-99.
- Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to ecosystems through dynamic energy budget models. *J. Anim. Ecol.*, 69: 913-926.
- Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to ecosystems through dynamic energy budget models. *J. Anim. Ecol.*, 69: 913-926.
- Nonnotte, G., Maxime, V., Truchot, J. P., Williot, P., and Peyraud, C. 1993. Respiratory responses to progressive ambient hypoxia in the sturgeon, *Acipenser baeri*. *Respir. Physiol.*, 91: 71-82.
- O'Donnell, J., Dam, H. G., Bohlen, W. F., Fitzgerald, W., Gay, P. S., Houk, A. E., Cohen, D. C., and Howard-Strobel, M. M. 2008. Intermittent ventilation in the hypoxic zone of western Long Island Sound during the summer of 2004. *J. Geophys. Res.*, 113: C09025.

- Orr, J. A., Vinebrooke, R. D., Jackson, M. C., Kroeker, K. J., Kordas, R. L., et al. 2020. Towards a unified study of multiple stressors: divisions and common goals across research disciplines. *Proc. R. Soc. B*, 287: 20200421. <http://dx.doi.org/10.1098/rspb.2020.0421>
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., et al. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437: 681-686. <https://doi.org/10.1038/nature04095>
- Paerl, H. W. (2006). Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: Interactive effects of human and climatic perturbations. *Ecol. Eng.* 26(1), 40-54.
- Peck, M. A., and Moyano, M. (2016). Measuring respiration rates in marine fish larvae: challenges and advances. *J. Fish Biol.* 88, 173-205.
- Pihl, L., Baden, S. P., and Diaz, R. J. (1991). Effects of periodic hypoxia on distribution of demersal fish and crustaceans. *Mar. Biol.* 108, 349-360.
- Pimentel, M. S., Faleiro, F., Diniz, M., Machado, J., Pousão-Ferreira, P., Peck, M. A., Pörtner, H.-O., and Rosa, R. 2015. Oxidative Stress and Digestive Enzyme Activity of Flatfish Larvae in a Changing Ocean. *PLoS ONE*, 10(7): e0134082. doi: 10.1371/journal.pone.0134082
- Pimentel, M. S., Faleiro, F., Dionísio, G., Repolho, T., Pousão-Ferreira, P., Machado, J. and Rosa, R. (2014). Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. *J. Exp. Biol.* 217, 2062-2070.
- Pimentel, M. S., Faleiro, F., Marques, T., Bispo, R., Dionísio, G., Faria, A. M., Machado, J., Peck, M. A., Pörtner, H., Pousão-Ferreira, P., Gonçalves, E. J., and Rosa, R. 2016. Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming. *Clim. Change*, 137: 495-509.
- Pimentel, M., Pegado, M., Repolho, T., and Rosa, R. 2014. Impact of ocean acidification in the metabolism and swimming behavior of the dolphinfish (*Coryphaena hippurus*) early larvae. *Mar. Biol.*, 161: 725-729.
- Pollock, M. S., Clarke, L. M. J., and Dubé, M. G. (2007). The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environ. Rev.* 15, 1-14.
- Polymeropoulos, E. T., Elliott, N. G., and Frappell, P. B. 2017. Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comp. Biochem. Physiol. A*, 213: 28-35.

- Porteus, C. S., Roggatz, C. C., Velez, Z., Hardege, J. D., and Hubbard, P. C. 2021. Acidification can directly affect olfaction in marine organisms. *J. Exp. Biol.*, 224: jeb237941. doi: 10.1242/jeb.237941
- Pörtner, H.-O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213, 881-893.
- Pörtner, H.-O. (2012). Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Mar. Ecol. Prog. Ser.* 470, 273-290.
- Pörtner, H.-O. and Farrell, A. P. 2008. Physiology and Climate Change. *Science*. 322(5902): 690-692.
- Pörtner, H.-O. and Grieshaber, M. K. 1993. Critical PO₂(s) in oxyconforming and oxyregulating animals: gas exchange, metabolic rate and the mode of energy production. In *The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life* (J. E. P. W. Bicudo, ed), pp. 330-357. Boca Raton, FL, USA: CRC Press.
- Pörtner, H.-O. and Knust, R. 2007. Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance. *Science*, 315: 95-97.
- Pörtner, H.-O. and Peck, M. A. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J. Fish Biol.* 77(8), 1745-1779.
- Pörtner, H.-O., Bock, C. and Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *J. Exp. Biol.* 220, 2685-2696.
- Pörtner, H.-O., Langenbuch, M. and Michaelidis, B. (2005). Synergistic effects of temperature extremes, hypoxia, and increases in CO₂ on marine animals: From Earth history to global change. *J. Geophys. Res.* 110, C9S10.
- Pousse, É., Munroe, D., Hart, D., Hennen, D., Cameron, L. P., Rheuban, J. E., Wang, Z. A., Wikfors, G. H., and Meseck, S. L. 2022. Dynamic energy budget modeling of Atlantic surfclam, *Spisula solidissima*, under future ocean acidification and warming. *Mar. Environ. Res.*, 177: 105602. <https://doi.org/10.1016/j.marenvres.2022.105602>
- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.
- Rabalais, N. N., Turner, R. E., Díaz, R. J., and Justić, D. 2009. Global change and eutrophication of coastal waters. *ICES J. Mar. Sci.*, 66(7): 1528-1537.
- Randall, D. J., Heisler, N., and Drees, F. 1976. Ventilatory response to hypercapnia in larger spotted dogfish *Scyliorhinus stellaris*. *Am. J. Physiol.*, 230(3): 590-594.

- Regan, M. D., Mandic, M., Dhillon, R. S., Lau, G. Y., Farrell, A. P., Schulte, P. M., Seibel, B. A., Speers-Roesch, B., Ultsch, G. R., and Richards, J. G. 2019. Don't throw the fish out with the respirometry water. *J. Exp. Biol.*, 222(6): jeb200253.
- Richards, J. G. (2009). Metabolic and molecular responses of fish to hypoxia. In *Fish Physiology, Vol. 27, Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 443-485. San Diego: Academic Press.
- Richards, J. G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.*, 214: 191-199.
- Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E. and Morel, F. M. M. (2000). Reduced calcification of marine plankton in response to increased atmospheric CO₂. *Nature*. 407, 364-367.
- Ries, J. B., Cohen, A. L., and McCorkle, D. C. 2009. Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology*, 37(12): 1131-1134.
- Rijnsdorp, A. D., Peck, M. A., Engelhard, G. H., Möllmann, C., and Pinnegar, J. K. (2009). Resolving the effect of climate change on fish populations. *ICES J. Mar. Sci.* 66, 1570-1583.
- Rodriguez-Dominguez, A., Connell, S. D., Baziret, C., and Nagelkerken, I. 2018. Irreversible behavioural impairment of fish starts early: Embryonic exposure to ocean acidification. *Mar. Poll. Bull.*, 133: 562-567.
- Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In: *Fish Physiology, Vol. 11: The Physiology of Developing Fish, Part A: Eggs and Larvae*. (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego: Academic Press.
- Rosa, R., Baptista, M., Lopes, V. M., Pegado, M. R., Paula, J. R., Trübenbach, K., Leal, M. C., Calado, R. and Repolho, T. (2014). Early-life exposure to climate change impairs tropical shark survival. *Proc. R. Soc. B.* 281, 20141738. <http://doi.org/10.1098/rspb.2014.1738>.
- Rummer, J. L., Stecyk, J. A. W., Couturier, C. S., Watson, S.-A., Nilsson, G. E. and Munday, P. L. (2013). Elevated CO₂ enhances aerobic scope of a coral reef fish. *Conserv. Physiol.* 1(1), 1-7. doi: 10.1093/conphys/cot023.
- Russell, N. R., and Wootton, R. J. 1992. Appetite and growth compensation in the European minnow, *Phoxinus phoxinus* (Cyprinidae), following short periods of food restriction. *Environ. Biol. Fishes*, 34: 277-285.
- Saba, G. K., Goldsmith, K. A., Cooley, S. R., Grosse, D., Meseck, S. L., et al. 2019. Recommended priorities for research on ecological impacts of ocean and coastal

- acidification in the U.S. Mid-Atlantic. *Estuar. Coast. Shelf Sci.*, 225: 106188.
<https://doi.org/10.1016/j.ecss.2019.04.022>
- Saba, V. S., Griffies, S. M., Anderson, W. G., Winton, M., Alexander, M. A., et al. 2016. Enhanced warming of the Northwest Atlantic Ocean under climate change. *J. Geophys. Res. Oceans*, 121: 118-132.
- Sampaio, E., Santos, C., Rosa, I. C., Ferreira, V., Pörtner, H.-O., Duarte, C. M., Levin, L. A., and Rosa, R. 2021. Impacts of hypoxic events surpass those of future ocean warming and acidification. *Nat. Ecol. Evol.*, 5: 311-321.
- Scannell, J. A., Pershing, A. J., Alexander, M. A., Thomas, A. C., and Mills, K. E. 2016. Frequency of marine heatwaves in the North Atlantic and North Pacific since 1950. *Geophys. Res. Lett.*, 43: 2069-2076.
- Schultz, E. T., Conover, D. O., and Ehtisham, A. 1998. The dead of winter: size-dependent variation and genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia menidia*) from different latitudes. *Can. J. Fish. Aquat. Sci.*, 55: 1149-1157.
- Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., and Nye, J. A. 2020. Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish *Menidia menidia*. *J. Exp. Biol.*, 223: jeb228015. doi: 10.1242/jeb.228015
- Schwerdtfeger, W. K., and Bereiter-Hahn, J. 1978. Transient occurrence of chloride cells in the abdominal epidermis of the guppy, *Poecilia reticulata* Peters, adapted to sea water. *Cell Tissue Res.*, 191: 463-471.
- Seibel, B. A. and Deutsch, C. 2020. Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature. *J. Exp. Biol.*, 223(12): jeb210492. <https://doi.org/10.1242/jeb.210492>
- Sibly, R. M., Grimm, V., Martin, B. T., Johnston, A. S. A., et al. 2013. Representing the acquisition and use of energy by individuals in agent-based models of animal populations. *Methods in Ecology and Evolution*, 4: 151-161.
- Silkin, Y. A. and Silkina, E. N. (2005). Effect of Hypoxia on Physiological-Biochemical Blood Parameters in Some Marine Fish. *J. Evol. Biochem. Physiol.* 41, 527-532.
- Silva, P., Solomon, R., Spokes, K., and Epstein, F. H. 1977. Ouabain inhibition of gill Na-K-ATPase: Relationship to active chloride transport. *J. Exp. Zool.*, 199(3): 419-426.
- Smallegange, I. M., Caswell, H., Toorians, M. E. M., and de Roos, A. M. 2017. Mechanistic description of population dynamics using dynamic energy budget theory incorporated into integral projection models. *Methods in Ecology and Evolution*, 8: 146-154.

- Snyder, J. T., Murray, C. S. and Baumann, H. (2018). Potential for maternal effects on offspring CO₂ sensitivities in the Atlantic silverside (*Menidia menidia*). *J. Exp. Mar. Biol. Ecol.* 449, 1-8.
- Stiasny, M. H., Mittermayer, F. H., Sswat, M., Voss, R., Jutfelt, F., Chierici, M., Puvanendran, V., Mortensen, A., Reusch, T. B. H., and Clemmesen, C. 2016. Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fishes Population. *PLoS ONE*, 11(8): e0155448. doi: 10.1371/journal.pone.0155448
- Stiasny, M. H., Sswat, M., Mittermayer, F. H., Falk-Peterson, I.-B., Schnell, N. K., Puvanendran, V., Mortensen, A., Reusch, T. B. H., and Clemmesen, C. 2019. Divergent responses of Atlantic cod to ocean acidification and food limitation. *Glob. Change Biol.*, 25: 839-849.
- Stierhoff, K. L., Targett, T. E., and Miller, K. 2006. Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. *Mar. Ecol. Prog. Ser.*, 325: 255-266.
- Stierhoff, K. L., Targett, T. E., and Power, J. H. 2009a. Hypoxia-induced growth limitation of juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics using RNA:DNA. *Can. J. Fish. Aquat. Sci.*, 66(7): 1033-1047.
- Stierhoff, K. L., Tyler, R. M., and Targett, T. E. 2009b. Hypoxia tolerance of juvenile weakfish (*Cynoscion regalis*): Laboratory assessment of growth and behavioral avoidance responses. *J. Exp. Mar. Biol. Ecol.*, 381: S173-S179.
- Stiller, K. T., Vanselow, K. H., Moran, D., Bojens, G., Voigt, W., Meyer, S., and Schulz, C. 2015. The effect of carbon dioxide on growth and metabolism in juvenile turbot *cophthalmus maximus* L. *Aquaculture*, 444: 143-150.
- Storey, K. B., and Storey, J. M. 2002. Oxygen limitation and metabolic rate depression. In: Storey, K. B. (ed), *Functional Metabolism: Regulation and Adaptation*. Wiley, Hoboken, NJ, pp-415-442.
- Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., and Reusch, T. B. H. 2014. Evolution in an acidifying ocean. *Trends Ecol. Evol.*, 29(2): 117-125.
- Sundin, J., Amcoff, M., Mateos-González, F., Raby, G. D., and Clark, T. D. 2019. Long-term acclimation to near-future ocean acidification has negligible effects on energetic attributes in a juvenile coral reef fish. *Oecologia*, 190: 689-702.
<https://doi.org/10.1007/s00442-019-04430-z>
- Tai, T. C., Sumaila, U. R., and Cheung, W. W. L. 2021. Ocean Acidification Amplifies Multi-Stressor Impacts on Global Marine Invertebrate Fisheries. *Front. Mar. Sci.*, 8: 596644. doi: 10.3389/fmars.2021.596644

- Targett, T. E., Gre cay, P. A., and Dixon, R. L. 2019. Growth of the estuarine fish *Fundulus heteroclitus* in response to diel-cycling hypoxia and acidification: interaction with temperature. *Can. J. Fish. Aquat. Sci.*, 76: 1295-1304.
- Taylor, J. C. and Miller, J. M. 2001. Physiological performance of juvenile southern flounder, *Paralichthys lethostigma* (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. *J. Exp. Mar. Biol. Ecol.*, 258: 195-214.
- Testa, J. M., Murphy, R. R., Brady, D. C., and Kemp, W. M. 2018. Nutrient- and Climate-Induced Shifts in the Phenology of Linked Biogeochemical Cycles in a Temperate Estuary. *Front. Mar. Sci.*, 5: 114.
- Thomas, P., Rahman, M. S., Kummer, J. A., and Lawson, S. 2006. Reproductive endocrine dysfunction in Atlantic croaker exposed to hypoxia. *Mar. Environ. Res.*, 62: S249-S252.
- Thomas, Yoann., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G. M., and Pecquerie, Laure. 2019. Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB) theory. *J. Sea Res.*, 143: 231-242.
- Tunnah, L., Turko, A. J., and Wright, P. A. 2022. Skin ionocyte density of amphibious killifishes is shaped by phenotypic plasticity and constitutive interspecific differences. *J. Comp. Physiol. B*, 192: 701-711.
- Twina me, S., Fitzgibbon, Q. P., Hobday, A. J., Carter, C. G. and Pecl, G. T. (2019). Multiple measures of thermal performance of early stage eastern rock lobster in a fast-warming ocean region. *Mar. Ecol. Prog. Ser.* 624, 1-11.
- Ultsch, G. R. and Regan, M. D. 2019. The utility and determination of P_{crit} in fishes. *J. Exp. Biol.*, 222: jeb203646. doi: 10.1242/jeb.203646
- Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferraresso, S., Sévère, A., Zambonino-Infante, J.-L., and Mazurais, D. 2014. Hypoxic episode during the larval period has long-term effects on European sea bass juveniles (*Dicentrarchus labrax*). *Mar. Biol.*, 162: 367-376.
- Wagenmakers, E.-J. and Farrell, S. 2004. AIC model selection using Akaike weights. *Psychon. Bull. Rev.*, 11(1): 192-196.
- Wallace, R. B. and Gobler, C. J. 2021. The role of algal blooms and community respiration in controlling the temporal and spatial dynamics of hypoxia and acidification in eutrophic estuaries. *Mar. Pollut. Bul.*, 172: 112908.
<https://doi.org/10.1016/j.marpolbul.2021.112908>
- Wallace, R. B., Baumann, H., Gear, J. S., Aller, R. C., and Gobler, C. J. 2014. Coastal ocean acidification: The other eutrophication problem. *Estuar. Coast. Shelf Sci.*, 148: 1-13.

- Watson, J. W., Hyder, K., Boyd, R., Thorpe, R., Weltersbach, M. S., Ferter, K., Cooke, S. J., Roy, S., and Sibly, R. M. 2020. Assessing the sublethal impacts of anthropogenic stressors on fish: An energy-budget approach. *Fish Fish.*, 21: 1034-1045. doi: 10.1111/faf.12487
- Wei, L.-Z., Zhang, X.-M., Li, J., and Huang, G.-Q. 2008. Compensatory growth of Chinese shrimp, *Fenneropenaeus chinensis* following hypoxic exposure. *Aquacult. Int.*, 16: 455-470.
- Welch, M. J. and Munday, P. L. 2016. Contrasting effects of ocean acidification on reproduction in reef fishes. *Coral Reefs*, 35: 485-493.
- Wells, R. M. G. 2009. Blood-gas transport and hemoglobin function: adaptations for functional and environmental hypoxia. In *Fish Physiology*, Vol. 27, Hypoxia (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 255-299. San Diego: Academic Press.
- Weltzien, F.-A., Døving, K. B., and Carr, W. E. S. (1999). Avoidance reaction of yolk-sac larvae of the inland silverside *Menidia beryllina* (Atherinidae) to hypoxia. *J. Exp. Biol.* 202, 2869-2876.
- Whitney, M. M. and Vlahos, P. 2021. Reducing Hypoxia in an Urban Estuary Despite Climate Warming. *Environ. Sci. Technol.*, 55: 941-951.
- Wittmann, A. C. and Pörtner, H.-O. 2013. Sensitivities of extant animal taxa to ocean acidification. *Nat. Clim. Change*, 3: 995-1001. <https://doi.org/10.1038/nclimate1982>
- Wood, C. M. 2018. The fallacy of the P_{crit} – are there more useful alternatives? *J. Exp. Biol.*, 221: jeb163717. doi: 10.1242/jeb.163717
- Wu, R. S. S., Zhou, B. S., Randall, D. J., Woo, N. Y. S., and Lam, P. K. S. 2003. Aquatic Hypoxia Is an Endocrine Disruptor and Impairs Fish Reproduction. *Environ. Sci. Technol.*, 37(6): 1137-1141.
- Zambonino-Infante, J. L., Mazurais, D., Dubuc, A., Quéau, P., Vanderplancke, G., Servili, A., Cahu, C., Le Bayon, N., Huelvan, C., and Claireaux, G. 2017. An early life hypoxia event has a long-term impact on protein digestion and growth in juvenile European sea bass. *J. Exp. Biol.*, 220(10): 1846-1851.
- Zeileis, A. and Grothendieck, G. 2005. zoo: S3 Infrastructure for Regular and Irregular Time Series. *Journal of Statistical Software*, 14(6): 1-27. doi: 10.18637/jss.v014.i06
- Zhang, Y., Montgomery, D. W., White, C. F., Richards, J. G., Brauner, C. J., and Farrell, A. P. 2022. Characterizing the hypoxic performance of a fish using a new metric: P_{AAS-50} . *J. Exp. Biol.*, 225: jeb244239. doi: 10.1242/jeb.244239

- Zhu, C.-D., Wang, Z.-H., and Yan, B. 2013. Strategies for hypoxia adaptation in fish species: a review. *J. Comp. Physiol. B*, 183: 1005-1013.
- Zimmer, A. M., Mandic, M., Rourke, K. M. and Perry, S. F. (2020). Breathing with fins: do the pectoral fins of larval fishes play a respiratory role? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 318, R89-R97.
- Zydlewski, J., McCormick, S. D., and Kunkel, J. G. 2003. Late migration and seawater entry is physiologically disadvantageous for American shad juveniles. *J. Fish. Biol.*, 63: 1521-1537.

Appendix 1: Chapter 1 Supplemental Tables

Table S1.1. Treatment conditions and carbon chemistry for CO₂ × temperature experiments shown as mean (±standard deviation) temperature (°C), pH_{NIST}, salinity, P_{O_2} (kPa), P_{CO_2} and f_{CO_2} (μatm), and A_T , DIC, and CO₃²⁻ (μmol kg⁻¹) measured over the course of each experiment for each corresponding target treatment. Measurements are described in the methods section.

Exp.	Target Temp	Measured Temp	Target P_{CO_2}	Measured P_{CO_2}	Measured pH	Sal.	P_{O_2}	A_T	DIC	f_{CO_2}	CO_3^{2-}
1	17	16.9 ± 0.3	400	368 ± 18	8.17 ± 0.12	30	21.2	2038 ± 17	1851 ± 8	367 ± 18	135 ± 6
		16.9 ± 0.3	2200	2037 ± 188	7.49 ± 0.13	30	21.2	2031 ± 12	2058 ± 21	2030 ± 188	32 ± 2
	24	23.5 ± 0.3	400	427 ± 29	8.13 ± 0.09	30	21.2	2042 ± 11	1838 ± 16	426 ± 29	150 ± 7
		23.6 ± 0.3	2200	2190 ± 277	7.49 ± 0.12	30	21.2	2041 ± 11	2048 ± 7	2183 ± 276	5 ± 5
2	17	16.9 ± 0.3	400	341	8.17 ± 0.04	31	22.0 ± 0.4	2059	1852	340.0	147.3
		17.1 ± 0.2	2200	1869	7.47 ± 0.09	31	23.0 ± 0.3	2023	2037	1862.6	35.3
		17.0 ± 0.2	4200	3936	7.22 ± 0.07	31	23.2 ± 0.3	2044	2159	3921.6	17.8
	20	20.2 ± 0.3	400	373	8.13 ± 0.06	31	24.5 ± 0.3	2038	1827	371.5	150.3
		19.9 ± 0.2	2200	2184	7.51 ± 0.05	31	23.8 ± 0.4	2039	2060	2176.7	34.8
		19.9 ± 0.2	4200	3996	7.20 ± 0.09	31	23.4 ± 0.3	2059	2161	3982.0	20.2
	24	24.1 ± 0.2	400	426	8.20 ± 0.05	31	23.6 ± 0.5	2044	1828	424.2	154.9
		23.9 ± 0.2	2200	2043	7.52 ± 0.04	31	23.8 ± 0.5	2022	2020	2036.7	42.6
		24.0 ± 0.3	4200	4310	7.21 ± 0.07	31	24.5 ± 0.4	2031	2127	4295.7	21.6
	3	17	17.4 ± 0.2	400	322 ± 12	8.22 ± 0.01	31	21.5 ± 0.3	2054 ± 8	1838 ± 26	321 ± 12
17.6 ± 0.3			2200	1952 ± 39	7.51 ± 0.01	31	22.4 ± 0.4	2047 ± 20	2066 ± 21	1945 ± 39	35 ± 1
17.4 ± 0.2			4200	4056 ± 204	7.20 ± 0.02	31	22.9 ± 0.4	2053 ± 24	2174 ± 16	4042 ± 203	18 ± 1
20		19.7 ± 0.2	400	345 ± 15	8.20 ± 0.02	31	23.6 ± 0.4	2048 ± 29	1833 ± 3	345 ± 15	160 ± 6
		19.6 ± 0.3	2200	1964 ± 109	7.51 ± 0.03	31	22.6 ± 0.4	2031 ± 14	2039 ± 10	1957 ± 108	38 ± 2
		19.7 ± 0.2	4200	4066 ± 227	7.21 ± 0.02	31	22.1 ± 0.5	2058 ± 6	2153 ± 37	4063 ± 226	20 ± 1
24		23.7 ± 0.2	400	331 ± 14	8.22 ± 0.02	31	22.0 ± 0.5	2044 ± 9	1798 ± 8	330 ± 14	185 ± 5
		23.7 ± 0.3	2200	2157 ± 92	7.49 ± 0.02	31	22.4 ± 0.4	2048 ± 22	2050 ± 25	2151 ± 92	42 ± 1
		23.6 ± 0.2	4200	4339 ± 169	7.20 ± 0.02	31	23.2 ± 0.4	2059 ± 51	2140 ± 8	4325 ± 169	22 ± 1
4	24	24.3 ± 0.4	400	389 ± 23	8.19 ± 0.02	32	21.2	2137 ± 3	1897 ± 13	388 ± 23	175 ± 8
		24.1 ± 0.2	2200	2265 ± 228	7.50 ± 0.04	32	21.2	2151 ± 14	2156 ± 27	2258 ± 227	43 ± 4
		24.2 ± 0.3	4200	4432 ± 180	7.21 ± 0.02	32	21.2	2130 ± 27	2230 ± 25	4418 ± 179	23 ± 1
	28	28.2 ± 0.2	400	350 ± 19	8.23 ± 0.02	32	21.2	2157 ± 24	1857 ± 29	348 ± 19	215 ± 4
		28.1 ± 0.2	2200	2439 ± 84	7.48 ± 0.02	32	21.2	2176 ± 50	2172 ± 48	2431 ± 83	49 ± 2
		28.2 ± 0.3	4200	4720 ± 217	7.20 ± 0.03	32	21.2	2155 ± 20	2244 ± 18	4714 ± 204	26 ± 1

Table S1.2. Treatment conditions and carbon chemistry for CO₂ × oxygen experiments shown as mean (±standard deviation) temperature (°C), pH_{NIST}, *P*_{O₂} (kPa), salinity, *P*_{CO₂} and *f*_{CO₂}, (µatm), and *A*_T, DIC, and CO₃²⁻ (µmol kg⁻¹) measured over the course of each experiment for each corresponding target treatment. Measurements are described in the methods section. The target temperature for all treatments was 24°C.

Exp.	Measured Temp	Target <i>P</i> _{O₂}	Measured <i>P</i> _{O₂}	Target <i>P</i> _{CO₂}	<i>P</i> _{CO₂}	Measured pH	Sal.	<i>A</i> _T	DIC	<i>f</i> _{CO₂}	CO ₃ ²⁻
5	24.7 ± 0.4	24.0	23.7 ± 0.3	400	370 ± 1	8.18 ± 0.02	30	2001 ± 6	1769 ± 5	369 ± 1	165.2 ± 0.5
	24.5 ± 0.3		23.3 ± 0.3	2200	1826 ± 7	7.56 ± 0.1	30	2005 ± 8	1990 ± 8	1821 ± 7	46.7 ± 0.2
	24 ± 0.6		23.1 ± 0.3	4200	4368 ± 19	7.19 ± 0.07	30	1998 ± 9	2099 ± 9	4354 ± 19	20.3 ± 0.1
	24.6 ± 0.4	12.0	12.4 ± 1.2	400	439 ± 2	8.12 ± 0.04	30	1996 ± 7	1793 ± 6	437 ± 2	145.8 ± 0.5
	24.7 ± 0.4		12.1 ± 1.2	2200	2338 ± 5	7.46 ± 0.06	30	2002 ± 4	2015 ± 4	2330 ± 5	37.5 ± 0.1
	24.2 ± 0.5		12.3 ± 0.9	4200	4119 ± 17	7.22 ± 0.03	30	2003 ± 8	2093 ± 8	4105 ± 16	21.7 ± 0.1
	24.9 ± 0.3	7.5	8.2 ± 1.2	400	400 ± 1	8.15 ± 0.05	30	2004 ± 3	1783 ± 3	399 ± 1	157.8 ± 0.2
	24.2 ± 0.5		8.1 ± 1.2	2200	2189 ± 4	7.48 ± 0.08	30	2010 ± 3	2017 ± 3	2182 ± 4	39.3 ± 0.1
	24.3 ± 0.4		7.5 ± 0.9	4200	4337 ± 36	7.2 ± 0.05	30	2014 ± 17	2112 ± 18	4323 ± 36	21 ± 0.2
6	24.4 ± 0.3	24.0	23.5 ± 0.3	400	385 ± 2	8.17 ± 0.08	30	2062 ± 11	1829 ± 10	384 ± 2	167.5 ± 0.9
	24.7 ± 0.3		23.7 ± 0.3	2200	2173 ± 22	7.5 ± 0.07	30	2060 ± 21	2062 ± 21	2166 ± 22	42.2 ± 0.4
	24.4 ± 0.4		23.2 ± 0.3	4200	4539 ± 55	7.19 ± 0.11	30	2064 ± 25	2167 ± 26	4524 ± 55	21.1 ± 0.3
	24.4 ± 0.4	12.0	12.7 ± 0.9	400	505 ± 1	8.07 ± 0.09	30	2046 ± 4	1861 ± 4	503 ± 1	137.2 ± 0.3
	24.4 ± 0.3		12.4 ± 1.2	2200	2157 ± 12	7.5 ± 0.05	30	2055 ± 12	2057 ± 12	2151 ± 12	42 ± 0.2
	24.4 ± 0.4		12.4 ± 1.2	4200	4512 ± 20	7.19 ± 0.07	30	2060 ± 9	2162 ± 10	4498 ± 20	21.2 ± 0.1
	24 ± 0.4	9.0	9.3 ± 1.5	400	520 ± 5	8.06 ± 0.09	30	2050 ± 19	1871 ± 18	518 ± 5	133.3 ± 1.3
	24.1 ± 0.4		9.0 ± 0.9	2200	2151 ± 19	7.5 ± 0.05	30	2039 ± 17	2043 ± 18	2144 ± 18	41 ± 0.4
	24.2 ± 0.5		9.0 ± 0.9	4200	4473 ± 64	7.19 ± 0.06	30	2053 ± 29	2155 ± 31	4459 ± 64	21 ± 0.3

Table S1.3. Coefficient estimates, t-values, and p-values from multiple linear regression models of square-root transformed metabolic rates of *M. menidia* embryos and larvae.

Stage	Predictor Variable	Coefficient Estimate (\pm s.e.m.)	t	p
Embryos	P_{CO_2}	1.5e-5 (\pm 8.2e-6)	1.82	0.070
	Temp	4.5e-3 (\pm 9.0e-4)	4.99	<0.001
	$P_{\text{CO}_2} \times \text{Temp}$	-6.8e-7 (\pm 3.6e-7)	-1.90	0.059
Larvae	P_{CO_2}	-1.8e-5 (\pm 1.4e-5)	-1.32	0.189
	Temp	1.3e-2 (\pm 1.6e-3)	7.86	<0.001
	$P_{\text{CO}_2} \times \text{Temp}$	7.6e-7 (\pm 6.2e-7)	1.22	0.224
Embryos	P_{CO_2}	-7.2e-6 (\pm 4.0e-6)	-1.81	0.071
	P_{O_2}	-2.1e-4 (\pm 6.9e-4)	-0.301	0.763
	$P_{\text{CO}_2} \times P_{\text{O}_2}$	7.1e-7 (\pm 2.4e-7)	2.94	0.004
Larvae	P_{CO_2}	-1.4e-6 (\pm 1.4e-5)	-0.245	0.807
	P_{O_2}	5.9e-4 (\pm 2.3e-3)	0.264	0.792
	$P_{\text{CO}_2} \times P_{\text{O}_2}$	2.7e-7 (\pm 8.1e-7)	0.336	0.737

Table S1.4. Bootstrapped means and 95% confidence interval lower (LL) and upper (UL) limits obtained by sampling from Q_{10} values calculated using metabolic rates for every possible pairing of individuals reared in 17°C and 28°C treatments, for *M. menidia* embryos and larvae across three P_{CO_2} treatments.

Stage	P_{CO_2} (μ atm)	LL	Mean	UL
Embryo	400	2.33	2.47	2.61
	2200	1.83	1.95	2.07
	4200	1.28	1.36	1.44
Larvae	400	2.51	2.65	2.79
	2200	3.00	3.32	3.67
	4200	3.04	3.26	3.49

Appendix 2: Chapter 2 Supplemental Table

Table S2.1. Mean and standard error of measured salinity, temperature (°C), pH, total alkalinity (TA, $\mu\text{mol kg}^{-1}$), and dissolved inorganic carbon (DIC, $\mu\text{mol kg}^{-1}$), and calculated partial pressure of CO_2 ($p\text{CO}_2$, μatm), fugacity of CO_2 ($f\text{CO}_2$, μatm), concentration of bicarbonate ($[\text{HCO}_3^-]$, $\mu\text{mol kg}^{-1}$), concentration of carbonate ($[\text{CO}_3^{2-}]$, $\mu\text{mol kg}^{-1}$), aragonite saturation state (Ω_{arag}), and calcite saturation state (Ω_{calc}) for each treatment within Experiments 1 and 2. Means and standard errors were calculated across repeat samples and replicate tanks. One measured DIC sample within Experiment 2, High CO_2 treatment was sealed improperly and could not be used, so measured pH was instead used to calculate DIC and the other calculated carbonate chemistry parameters.

Exp.	Treatment	Salinity	Temp.	$p\text{CO}_2$	pH	TA	DIC	$f\text{CO}_2$	$[\text{HCO}_3^-]$	$[\text{CO}_3^{2-}]$
1	Ambient	27.6 (± 1.0)	23.8 (± 0.1)	680 (± 62)	7.94 (± 0.07)	2135 (± 59)	1989 (± 37)	678 (± 62)	1851 (± 25)	118 (± 15)
	Moderate	28.0 (± 1.0)	23.8 (± 0.1)	1683 (± 186)	7.41 (± 0.06)	2178 (± 68)	2146 (± 60)	1678 (± 186)	2036 (± 57)	59 (± 8)
	High	27.7 (± 1.0)	23.8 (± 0.1)	3609 (± 219)	7.13 (± 0.08)	2162 (± 64)	2232 (± 59)	3597 (± 219)	2095 (± 58)	28 (± 3)
2	Ambient	27.9 (± 0.8)	22.4 (± 0.1)	442 (± 41)	8.08 (± 0.01)	2097 (± 32)	1921 (± 27)	440 (± 41)	1742 (± 16)	146 (± 14)
	Moderate	28.0 (± 0.8)	22.3 (± 0.1)	2299 (± 243)	7.39 (± 0.06)	2130 (± 40)	2137 (± 43)	2292 (± 242)	2037 (± 45)	38 (± 2)
	High	28.1 (± 0.8)	22.2 (± 0.3)	4531 (± 431)	7.09 (± 0.04)	2140 (± 47)	2217 (± 45)	4516 (± 430)	2091 (± 48)	20 (± 1)

Appendix 3: Chapter 4 Supplemental Figure

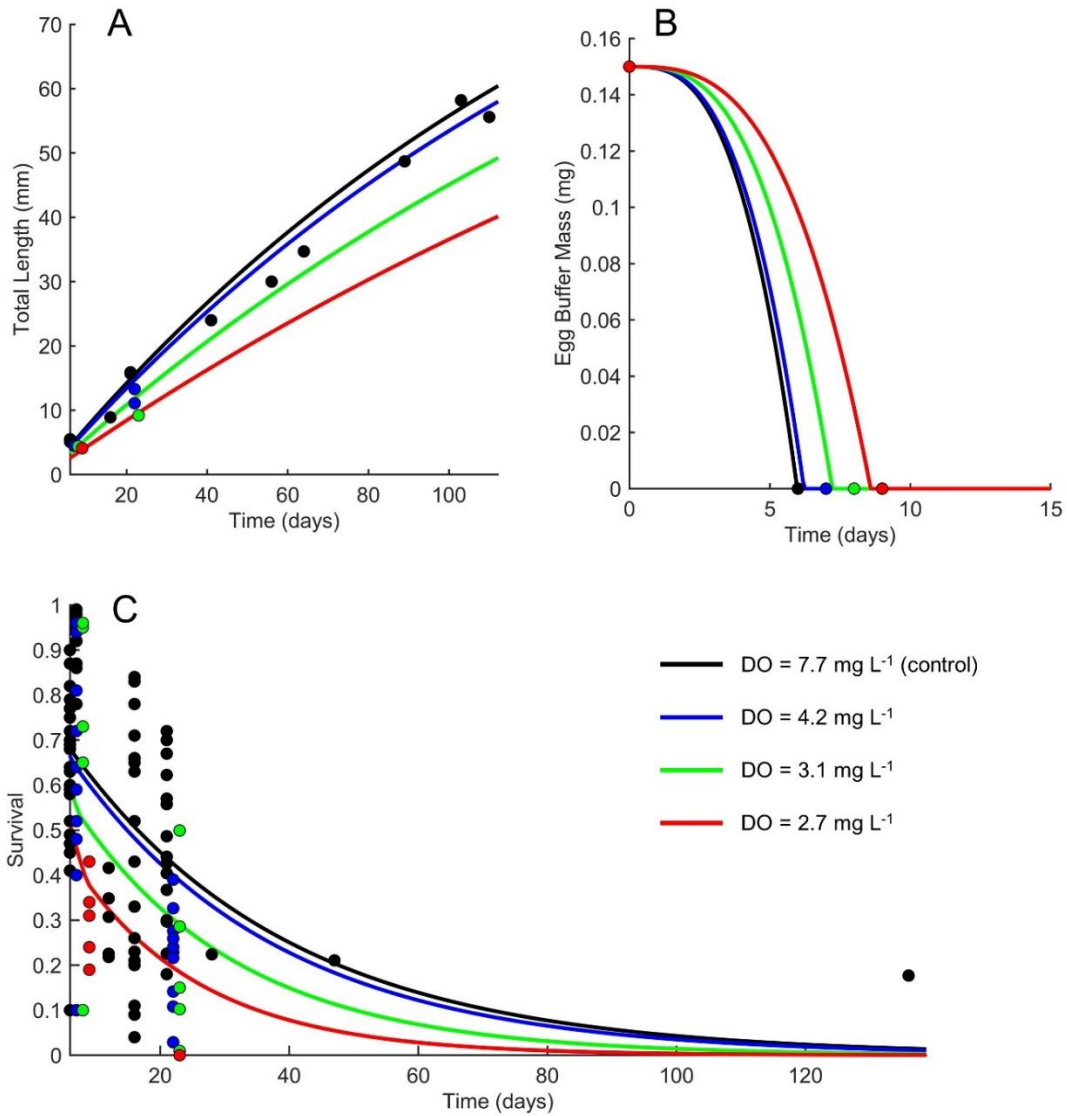


Figure S4.1. Best fit of DEBkiss model to all experimental data from four DO levels. The best fitting model was selected based on a combination of initial criteria that all three response variables' predicted values are affected by the hypoxia correction factor and $\Delta AICc$. (A) is total length (mm) over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time (days).