UNIVERSITY OF CALIFORNIA

Santa Barbara

Feeding for resilience: how nutrition mediates organismal responses to temperature

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology Evolution and Marine Biology

by

Emily Adams Hardison

Committee in charge:

Professor Erika J. Eliason, Chair

Professor Gretchen E. Hofmann

Dr. Christopher L. Jerde, Assistant Researcher

Professor Elizabeth G. Wilbanks

September 2023

The dissertation of Emily Adams Hardison is approved.

_____
Christopher L. Jerde

_____
Gretchen E. Hofmann

_____
Elizabeth G. Wilbanks

_____
Erika J. Eliason, Committee Chair

September 2023

Feeding for resilience: how nutrition mediates organismal responses to temperature

ACKNOWLEDGEMENTS

I am very grateful for the immense amount of support, help, encouragement, and mentorship I have received and that has made this degree possible. To start, I owe a special thank you to my wonderful advisor, Erika Eliason. Erika, you are an incredible mentor, mother, scientist, and advisor. You care so deeply for the people who work in your lab and it really shows – we all feel it. Thank you for everything you have taught me over the last six years and for your endless support, enthusiasm for science, and help in making every aspect of my PhD research possible. It means the world to me to have been able to work in your lab and I am going to miss being in the Eliason lab a lot. To the rest of my wicked smart and inspiring committee, thank you for everything you have taught me over the last six years. Lizzy, I feel like I didn't understand metabolism until I TA'd (while basically also taking) your cell physiology class and microbial physiology courses. Thank you for letting me be the fly on the wall that didn't know anything about microbes. I learned so much. Gretchen, thank you for pushing me to always consider the big picture and insert environmental realism into my experimental designs. I really admire the way you think about and tackle questions in ecological physiology. Chris, thank you for making statistics less intimidating and helping me to feel empowered in the work I am doing. Also, for making time to meet (even when you were super busy) to answer stats questions, talk through experimental designs, and brainstorm ideas. I always left your office feeling like I had a plan and was on the right track. Your support has been invaluable to me.

Thank you to all the undergraduate students that made this research happen. I owe a special thank you to the students who worked tirelessly with me on chapter 2 and 4 (Mason Tittle, Vincent Han Lee, Tina Nyugen, Eileen Schauerman, Madeleine Yung) – not only

would the work not have been possible without all of you, it also would not have been as much fun. Thank you for bringing joy and curiosity to the lab.

I am deeply grateful to the the administrative and facilities staff at UCSB for making sure our lab was operational: Andrea Jorgensen, Cathi Arnold, Azure Stewart, Brittney Dinelli, Nate Angeles-Molina, Mengshu Ye, Haley Martin, Christoph Pierre, Mickey O'connell, Christian Orsini, Davis Davis, Ryan Langlo, Ken Irwin. Being in a new lab came with a whole host of challenges (both administrative and infrastructure related); we had to build new equipment, obtain permits for the first time, and then be the guinea pigs testing everything on critical experiments. I simply could not have successfully completed any of the experiments in this dissertation without you.

Most of the fish studied in this thesis were caught 1 by 1, using hook and line. Thank you to everyone that took time out of their busy schedules to come down to the harbor, brave the hordes of opinionated tourists and loose men, and catch these tiny fish with me: Bartholomew Difiore, Joseph Curtis, Sevan Esaian, Nicholas Lee, Hope Hardison, Osborne Hardison, Will Hardison, Katie Carrillo, Brendan Shanny, Alexander Lill, Terra Dressler, Alexander Little, Anhadh Jassal, Claire Anderson, Jennay Argiris, Garret Parsons, Tyler Parsons, Erika Eliason, Alecia Dezzani, Bashir Ali, Jessica Madden, Madaleine Yung, Madison Heard, Jacey Van Wert, Krista Kraskura, Jasmine Childress, Gail Schwieterman, Kate Becker, Kai Kopecky, Yvette Gaytan, Samantha Csik.

I owe a special thank you to my fellow Eliason Lab friends and colleagues, both past and present (Krista Kraskura, Terra Dressler, Jacey Van Wert, Gail Schwieterman, Jasmine Childress, Kim Birnie-Gauvin, Jess Madden, Matt Drenner, Naomi Pleizer, Alex Little, Bashir Ali, Madison Heard). Thank you for your advice, feedback, brainstorming sessions,

help in the lab and the field. To Krista, Terra, Jacey, Jasmine, Gail, and Kim I am so grateful to have gone through this degree with people that are not just incredible scientists, but also such supportive and caring friends. Working with you all has been a dream. From, Chilis margs of the month, to fishing at the harbor, tidepooling and snorkeling at Mohawk and Coal Oil, all the drag, and the many times we scream sang my favorite songs -- it has been a magical adventure.

To my Santa Barbara family: Sam Csik, Bart Difiore, Joe Curtis, Brendan Shanny, Allison Difiore, Desmond Ho, Michal Ross, Sevan Esaian -- our family dinners, walks on the beach, weekend trips, and game nights have kept me grounded. One of the best things to happen to me in the last 6 years has been meeting friends like you. To my sweet Sam Csik, thank you for being such an incredible and supportive friend. You have been my rock since day 1 of graduate school and I am so lucky to have you in my life. I also owe a special thank you to my oldest friends: Jennah Feeley, Hannah Cundall, Erica Ferrer, Amber Khan. You are all doing incredible things in your careers and your resilience, ambition, grit, and kindness inspire me daily. I love you all dearly and am very thankful to have you in my life.

To my family, thank you for your unconditional love, advice, and support even though I am in not a machine. Thank you to the Lill's: Heike, Thorsten, and Jonas for welcoming me into your family and for supporting my dreams. To my grandparents Bubby and Nana: I love you and would not have been even close to going to graduate school if it weren't for everything you've done for me over my entire life. To my brother Will, you are a renaissance man who never fails to make me laugh. Thank you for all of your support. Mom and dad – I love you SO much. I do not know how to put into words how appreciative I am to have you in my life. Thank you for talking through challenges, for your helpful advice, for visiting, for

all the fun we have had together playing games, adventuring, and being silly. A special shout out to Lords of water deep and the >120 hours we played online together during covid.

Finally, to my Alex and Moggie. I love you both dearly. Alex, you are my sunshine and have kept me grounded, fed, having fun, and healthy throughout this degree. It's been the adventure of a lifetime to build a home in Goleta with you and I can't wait to see what we do next.

VITA OF EMILY ADAMS HARDISON

September 2023

EDUCATION ----------

University of California, Santa Barbara **2017-2023**
PhD Candidate, Department of Ecology Evolution and Marine Biology (EEMB)
Graduate Advisor: Dr. Erika Eliason

University of California, Santa Cruz **2010-2014**
BS in Molecular Cell and Developmental Biology, Highest Honors in Major
BS in Marine Biology, Highest Honors in Major
magna cum laude, Phi Beta Kappa

RESEARCH EXPERIENCE ----------

Graduate Student Researcher, Lab of Dr. Erika Eliason **2017-2023**
Department of Ecology Evolution and Marine Biology, UC Santa Barbara

Junior Specialist, Lab of Dr. Peter Raimondi **2016-2017**
Department of Ecology and Evolutionary Biology, UC Santa Cruz

Junior Specialist, Lab of Dr. Matthew Krummel **2015-2016**
Department of Pathology, UC San Francisco

Undergraduate Researcher, Lab of Dr. Alan Zahler **2013-2014**
Department of Molecular Cell and Developmental Biology, UC Santa Cruz

ACADEMIC FELLOWSHIPS AND AWARDS ----------

Fellowships

National Science Foundation Postdoctoral Research Fellowship in Biology	Beginning 2023
Santa Barbara Coastal LTER Graduate Student Researcher (4 quarters)	2022-2023
Regents Fellow. EEMB University of California Santa Barbara	2020-2021
National Science Foundation Graduate Research Fellow	2017-2022

Awards and Grants

Company of Biologists Travelling Fellowship (£3000)	2023
Society of Experimental Biology Conference Grant (£800)	2023
Journal of Experimental Biology 2021 Outstanding Paper Prize (£1000)	2022
UCSB Doctoral Student Travel Grant ($1500)	2022
UCSB Associated Students Coastal Fund Major Grant ($10100)	2022
Worster Award ($5000). EEMB UC Santa Barbara	2022
Schamberg-Burley Award ($500). EEMB UC Santa Barbara	2022
Company of Biologists Travel Grant (£500)	2022
Storke Award ($2500). EEMB UC Santa Barbara	2021
Block grant in aid of research ($4400). EEMB UC Santa Barbara	2018-2021

Edward C Raney Award ($1000). American Society of Ichthyologists and Herpetologists 2018

PUBLICATIONS ----------

Submitted

13. **Emily A. Hardison**; Erika J. Eliason. Diet effects on ectotherm thermal performance. In review at *Biological Reviews*.

12. Gail D. Schwieterman; **Emily A. Hardison**; Georgina K. Cox; Jacey Van Wert; Kim Birnie-Gauvin; Erika J. Eliason. Mechanisms of cardiac collapse at high temperature in a marine teleost (*Girella nigrians*). In press at Journal of Comparative Biochemistry and Physiology Part A.

11. Krista Kraskura, **Emily A. Hardison**, Erika J. Eliason. Body size and temperature affect metabolic and cardiac thermal tolerance in fish. In revision at Scientific Reports.

Published

10. **Emily A. Hardison**, Gail D. Schwieterman, Erika J. Eliason (2023). Diet changes thermal acclimation capacity, but not acclimation rate in a marine ectotherm (*Girella nigricans*) during warming. Proceedings of the Royal Society B 290: 20222505. https://doi.org/10.1098/rspb.2022.2505

9. Samantha R. Csik, Bartholomew P. DiFiore, Krista Kraskura, **Emily A. Hardison**, Joseph S. Curtis, Erika J. Eliason, and Adrian C. Stier (2023). The metabolic underpinnings of temperature-dependent predation in a key marine predator. Frontiers in Marine Science 10:1072807. doi: 10.3389/fmars.2023.1072807

8. Alexander G. Little, Tanya S. Prystay, **Emily A. Hardison**, Terra Dressler, Krista Kraskura, Steven J. Cooke, David A. Patterson, Scott G. Hinch, Erika J. Eliason. (2022). Evaluating Cardiac Oxygen Limitation as a Mechanism for Female-Biased Mortality in Coho Salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology. https://doi.org/10.1139/cjz-2022-0072.

7. Gail D. Schwieterman, **Emily A. Hardison**, Erika J. Eliason (2022). Effect of thermal variation on the cardiac thermal limits of a eurythermal marine teleost (*Girella nigricans*). Current Research in Physiology (special issue). https://doi.org/10.1016/j.crphys.2022.02.002.

6. **Emily A. Hardison**, Krista Kraskura, Jacey Van Wert, Tina Nguyen†, Erika J. Eliason. (2021). Diet mediates thermal performance traits: implications for marine ectotherms. Journal of Experimental Biology. doi: 10.1242/jeb.242846. https://doi.org/10.1242/jeb.242846. ***Winner of JEB outstanding paper prize and featured in 'Inside JEB' and the UCSB current.***

5. Krista Kraskura*, **Emily A. Hardison***, Alexander G. Little, Terra Dressler, Tanya S. Prystay, Brian Hendriks, Tony P. Farrell, Steve J. Cooke, David A. Patterson, Scott G. Hinch, and Erika J. Eliason. (2021). Sex-specific differences in swimming, aerobic scope, and recovery from exercise in adult coho salmon (*Oncorhynchus kisutch*) across ecologically relevant temperatures. Conservation Physiology, 9(1), 1–22. https://doi.org/10.1093/conphys/coab016. ***Featured in 'Outside JEB'. *co-first author***

4. Alexander G. Little, **Emily Hardison**, Krista Kraskura, Terra Dressler, Tanya Prystay, Brian

Hendriks, Jonathon Pruitt, Tony P. Farrell, Steve J. Cooke, David Patterson, Scott G. Hinch, and Erika Eliason. (2020). Reduced lactate dehydrogenase activity in the heart and suppressed sex hormone levels are associated with female-biased mortality during thermal stress in Pacific salmon. Journal of Experimental Biology. doi: 10.1242/jeb.214841.

3. Alexander G. Little*, Terra Dressler*, Krista Kraskura, **Emily Hardison**, Tony P. Farrell, Scott J. Hinch, David A. Patterson, Erika J. Eliason. (2020). Maxed out: Optimizing accuracy, precision and power for field measures of maximum metabolic rate in fishes. Physiological and Biochemical Zoology. https://doi.org/10.1086/708673.

2. Mikhail Binnewies*, Adriana M. Mujal*, Joshua L. Pollack, Alexis J. Combes, **Emily A. Hardison**, Megan K. Ruhland, Kevin C. Barry, Patrick Ha, Vincent Chan, Edward W. Roberts, Matthew F. Krummel. (2019) Unleashing Type-2 Dendritic Cells to Drive Protective Antitumor CD4+ T Cell Immunity. Cell. https://doi.org/10.1016/J.CELL.2019.02.005.

1. Matthew F. Krummel, Jagdish N. Mahale, Lion F. K. Uhl, **Emily A. Hardison**, Adriana M. Mujal, Julie M. Mazet, Robert J. Weber, Zev J. Gartner, and Audrey Gérard (2018). Paracrine costimulation of IFN-γ signaling by integrins modulates CD8 T cell differentiation. Proceedings of the National Academy of Sciences, 115(45), 11585 LP-11590. https://doi.org/10.1073/pnas.1804556115.

Other Publications

Ocean acidification causes some fishy side effects. (2023) Outside JEB. J. Exp. Biol.
Climate change leaves urchins on a rock in a hard place to live. (2023) Outside JEB. J. Exp. Biol.
Does turning up the heat affect how salmon eat? (2023) Outside JEB. J. Exp. Biol.

PRESENTATIONS AND POSTERS ----------

Talk

11. Effects of food quantity and dietary fat quality on thermal plasticity and tolerance of a marine fish. Society of Experimental Biology. (In Person; 2023).

10. Feeding for resilience: how food quality and quantity affects marine fish in a changing climate. InsideOut gut workshop hosted by the company of Biologists. Ashford, UK. (In Person; 2023).

9. ‘Food for thought: how a fish's diet affects its ability to handle extreme temperatures’. Co-presented with undergraduate mentee (Eileen Schauerman). Worster Award Symposium at UC Santa Barbara. (In person; 2023).

8. ‘The roles of diet in determining thermal acclimation rate and capacity for maximum heart rate in a marine ectotherm (Girella nigricans).’ Society of Experimental Biology. (In person; 2022).

7. ‘The roles of diet and associated fatty acids in determining thermal acclimation rate and capacity in a marine ectotherm (Girella nigricans).’ Canadian Society of Zoology Annual Meeting. (Virtual; 2022).

6. ‘Diet alters thermal performance traits in a marine omnivorous fish (Girella nigricans).’ Society of Experimental Biology Annual Meeting. (Virtual; 2021)

5. ‘Diet alters thermal performance traits in a marine omnivorous fish (Girella nigricans).’ Canadian Society of Zoology Annual Meeting.’ (Virtual; 2021).

4. ‘Analyzing Aquatic Respirometry Data.’ Respirometry Workshop for Government Scientists, Stakeholders, and Academics. UC Santa Barbara. (Virtual; 2020)

Poster

3. ‘You are what you eat! Effects of food quality and quantity on cardiac thermal tolerance in a local marine fish (Girella nigricans).’ Santa Barbara Coastal LTER Annual Meeting. (2022)

2. ‘Effects of food quality and quantity on cardiac thermal tolerance in a temperate marine fish (Girella nigricans).’ American Physiological Society. (2022)

1. ‘The effects of diet on the energetics of opaleye (Girella nigricans).’ Western Society of Naturalist Annual Meeting. (2019)

TEACHING AND MENTORSHIP ----------

Teaching Assistant

Fields Studies in Marine Ecological Physiology Lab (EEMB165). UC Santa Barbara.	Winter 2023
Biology of Fishes Lab (EEMB 106). UC Santa Barbara.	Fall 2021
Cell Physiology (EEMB 157). UC Santa Barbara.	Fall 2018

Guest Lectures

UC Santa Barbara SciTrek. ‘Ecological physiology in action’.	Winter 2021
Animal Physiology (EEMB 157B). Lecture on digestive physiology.	Winter 2020

Research Mentoring

Eileen Schauerman (Worster Award, Dean Bazzi Scholarship, senior honors project)	2022-2023
Madeleine Yung (Undergraduate researcher, summer research technician)	2022-2023
Madison Heard (Masters Student)	2022-2023
Jaden Harding (Undergraduate researcher)	Spring, 2022
Bella Giglio (Undergraduate researcher, senior honors project)	2019-2022
Vincent Han lee (Undergraduate researcher)	2019-2020
Mason Tittle (Summer research technician)	Summer 2019
Tina Nguyen (UC Leads REU)	Summer 2019
Anhadh Jassal (Undergraduate researcher)	2018-2019
Jennay Argiris (Undergraduate researcher)	2018-2019
Elaine Chan (High school volunteer)	Summer 2016

SERVICE AND COMMUNITY CONTRIBUTIONS ----------

University and Departmental Service

Member. Departmental Working group on Recruitment, Retention, and Accountability	2022-2023
Member. EEMB Graduate Student Advisory Council	2021-2022
Student Mentor. 1st-Year Graduate Student EEMB Mentorship Program	2020-2021
Member. EEMB Diversity Equity Inclusion and Wellness Committee	2020-2023

Public Education and Volunteer Work

Science Advisor. Marshmallow Minds.	Fall 2021-Present
Panelist. UC Santa Barbara Methods in Aquatic Community Ecology Lab.	Fall 2022
'Feeding Nemo.' UC Santa Barbara Grad Slam Presentation (Virtual). ***Finalist.***	2021
Lab Volunteer at Undergrad Research Open House. UC Santa Barbara.	Spring 2022
Guest Panelist. UC Santa Barbara Biology of Fishes Class (EEMB 106).	Fall 2019, 2020
World Oceans Day Volunteer. Santa Barbara Sea Center.	June 2018, 2019
CURIE-osity Lab Tour Volunteer. Girls INC.	Spring 2019, 2020
Rainforest Aquarium Volunteer. California Academy of Science.	2015-2016
Exhibit Guide Volunteer. Seymour Marine Discovery Center.	2012-2014

Reviewer for Scientific Publications

Current Research in Physiology, Physiological and Biochemical Zoology, Conservation Physiology

WORKSHOPS ATTENDED ----------

- InsideOut Gut Workshop hosted by the Company of Biologists. Ashford, UK.
- 'Mentoring the Mentors' (5 x 1 h workshops on growth vs fixed mindsets, building soft skills, working with distressed students, intersectionality panel, skills to share with students). UC Santa Barbara. April 3, 2020. May 31st – June 3rd, 2022.
- 'Respirometry Workshop for Government Scientists, Stakeholders, and Academics.' UC Santa Barbara. April 3, 2020.
- 'Respirometry Workshop for Government Scientists, Stakeholders, and Academics.' UC Santa Barbara. October 23-25, 2018.
- 'Scientific Programming in R.' UC Santa Barbara. May 4-5, 2018.

ABSTRACT

Feeding for resilience: how nutrition mediates organismal responses to temperature

by

Emily Adams Hardison

Temperate fishes thrive in dynamic thermal environments, where water temperature can vary dramatically across days, seasons, and years. To survive in these variable environments, they must acquire essential energy and nutrients to support their physiological responses to temperature change. However, climate change is expected to impact the nutritional landscape (prey options, quality, and quantity) for many marine animals, which may limit their ability to respond effectively to co-occurring environmental changes, like temperature rise. In my PhD thesis, I studied how an abundant kelp forest fish, opaleye (*Girella nigricans*), is affected by simultaneous changes in their diet and environmental temperature.

One way opaleye may respond to temperature change is by adjusting their diet selection or ingestion rate to modify their nutritional status. However, my results demonstrate that diet and temperature have inconsistent effects on the thermal sensitivity of ecologically important traits and that opaleye are prone to making diet choices that have negative impacts on their performance in warm water. I also explored the untested hypothesis that nutritional status can affect the rate of thermal plasticity but found that opaleye's rate of plasticity was insensitive to diet. However, their capacity for plasticity was diet-sensitive and could be partially explained by their fatty acid assimilation. Finally, I found that opaleye energetics and thermal

tolerance are impacted by the combined effects of food restriction and dietary fat composition, where the quality of the fish's diet can rescue them from the harmful effects of food restriction. Overall, this dissertation demonstrates that diet mediates thermal plasticity in ectotherms, like opaleye.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Chapters 2 and 3 are from previously published works.

Chapter 2: Emily A. Hardison, Krista Kraskura, Jacey Van Wert, Tina Nguyen†, Erika J. Eliason. (2021). Diet mediates thermal performance traits: implications for marine ectotherms. Journal of Experimental Biology, 224(21), jeb242846. https://doi.org/10.1242/jeb.242846

Chapter 3: Emily A. Hardison, Gail D. Schwieterman, Erika J. Eliason (2023). Diet changes thermal acclimation capacity, but not acclimation rate in a marine ectotherm (*Girella nigricans*) during warming. Proceedings of the Royal Society B 290: 20222505. https://doi.org/10.1098/rspb.2022.2505

Brief introduction to chapters

Temperature profoundly impacts ectotherm physiology, ecology, and biogeography through its thermodynamic effects on biological rates. Despite this, many ectotherms thrive in dynamic thermal environments, like the intertidal and kelp forests of California, where temperature can change >6°C in a day and >12°C across seasons. To survive in these variable environments, the local ectotherms must be adept at thermal plasticity. However, plastic thermal responses are energetically and nutritionally demanding, suggesting that diet may mediate their success. In this thesis, I studied the combined influence of diet and temperature on the thermal plasticity of a temperate omnivorous fish, opaleye (*Girella nigricans*). I conducted three large-scale manipulative laboratory experiments on wild-caught opaleye, where the fish were acclimated to different environmentally relevant diet and temperature treatments in factorial designs, before being tested for ecologically relevant traits. **My overall objective was to evaluate how variation in diet quality, quantity, and preference impact thermal plasticity and thermal tolerance in ectotherms.**

In **Chapter 1**, I conducted a comprehensive review which identified several mechanisms by which diet can influence thermal plasticity and tolerance in ectotherms; each varying in their empirical support. I also found that several species, across taxonomic groups, change their diet preference directly in response to temperature. This suggests that some ectotherms alter their diet to improve their performance across temperatures. I discussed this along with other diet x temperature implications on ectotherm ecology, farming, and biogeography.

In **Chapter 2**, I measured the effect of different diets (designed to mimic natural diet options of wild opaleye) on the thermal sensitivity of biological rates involved in energetics

and thermal tolerance (e.g., whole-animal metabolism, sprint speed, heart rate, enzyme activity, growth). The results contradicted the common assumption that biological rates should scale similarly with temperature across levels of biological organization, making individual measures of performance scalable to ecosystem level effects. Further, this study revealed that diet influences the thermal sensitivity of biological rates in trait-specific ways, suggesting that different diets may produce performance trade-offs. Ectotherms with broad diets may be able to regulate their thermal responses through diet selection.

In **Chapter 3**, I tested the role of diet in determining the rate of cardiac plasticity in opaleye exposed to a simulated warming event. Cardiac function is implicated as the organ system setting thermal limits in fishes because of its critical role in oxygen, metabolite, metabolic waste, and immune factor transport. Here, I found that opaleye acclimated rapidly after warming, but their thermal limits and acclimation rate were not affected by their diet. However, the fish's acclimation capacity for maximum heart rate was sensitive to their diet treatment. To explore the mechanisms driving observed differences in heart rate, I measured the fish's ventricle fatty acid composition, which differed depending on what the fish had eaten and was significantly related to cardiac performance in ways consistent with homeoviscous adaptation. These results demonstrate that diet not only impacts thermal plasticity, but does so on environmentally relevant timescales, and that fatty acid composition is an important mechanism by which diet may mediate ectotherm thermal responses.

In **Chapter 4**, I tested the interacting influence of changes in 1) food quantity and 2) dietary lipid quality on metabolism and thermal tolerance in opaleye. The global availability of essential omega-3 fatty acids, DHA and EPA, is predicted to decrease with climate change, which is a major concern for human health. However, DHA/EPA have low melting

points and may reduce warm tolerance in ectotherms. As fish gain most of their energy from fats, they need to balance assimilating fatty acids against metabolizing them to meet their metabolic demand. The results indicate that dietary lipids are especially important to consider in resource poor environments across temperatures, and that diets lower in DHA/EPA improve opaleye aerobic performance but reduce warm tolerance in these scenarios.

Overall, this dissertation examines when, why, and how diet can influence thermal plasticity in ectotherms. My results demonstrate that we must consider diet when evaluating how ectotherms will respond to climate change, as both diet quality and quantity can interactively affect thermal plasticity. These interactions vary depending on the timescale of temperature exposure and the trait of interest. Given how understudied this topic is and the broad implications of the work, I strongly encourage future work examining diet effects on ectotherm phenotypic plasticity.

I. Chapter 1

1.0 Abstract

The environment is rapidly changing, and considerable research is aimed at understanding the capacity of organisms to respond. Changes in environmental temperature are particularly concerning as most animals are ectothermic, and so temperature is considered a master factor governing their ecology, biogeography, behavior and physiology. The ability of ectotherms to persist in an increasingly warm, variable, and unpredicTable future is supported by their nutritional status. Nutritional resources (e.g., food availability, quality, options) vary across space and time and in response to environmental change, but animals also have the capacity to alter how much they eat and what they eat, which may help them improve their performance under climate change. In this review, we discuss the state of knowledge in the intersection between animal nutrition and temperature. We take an integrative approach to describe nutritional mechanisms by which diet may impact thermal performance and discuss the current knowns/unknowns about them. We performed a comprehensive review of the literature which revealed that diet quality (i.e., macronutrients, lipid composition, and micronutrients) can affect ectotherm thermal plasticity, thermoregulatory behavior, diet preference, and thermal tolerance. We finish by describing how this topic can inform ectotherm biogeography, behavior, and aquaculture research.

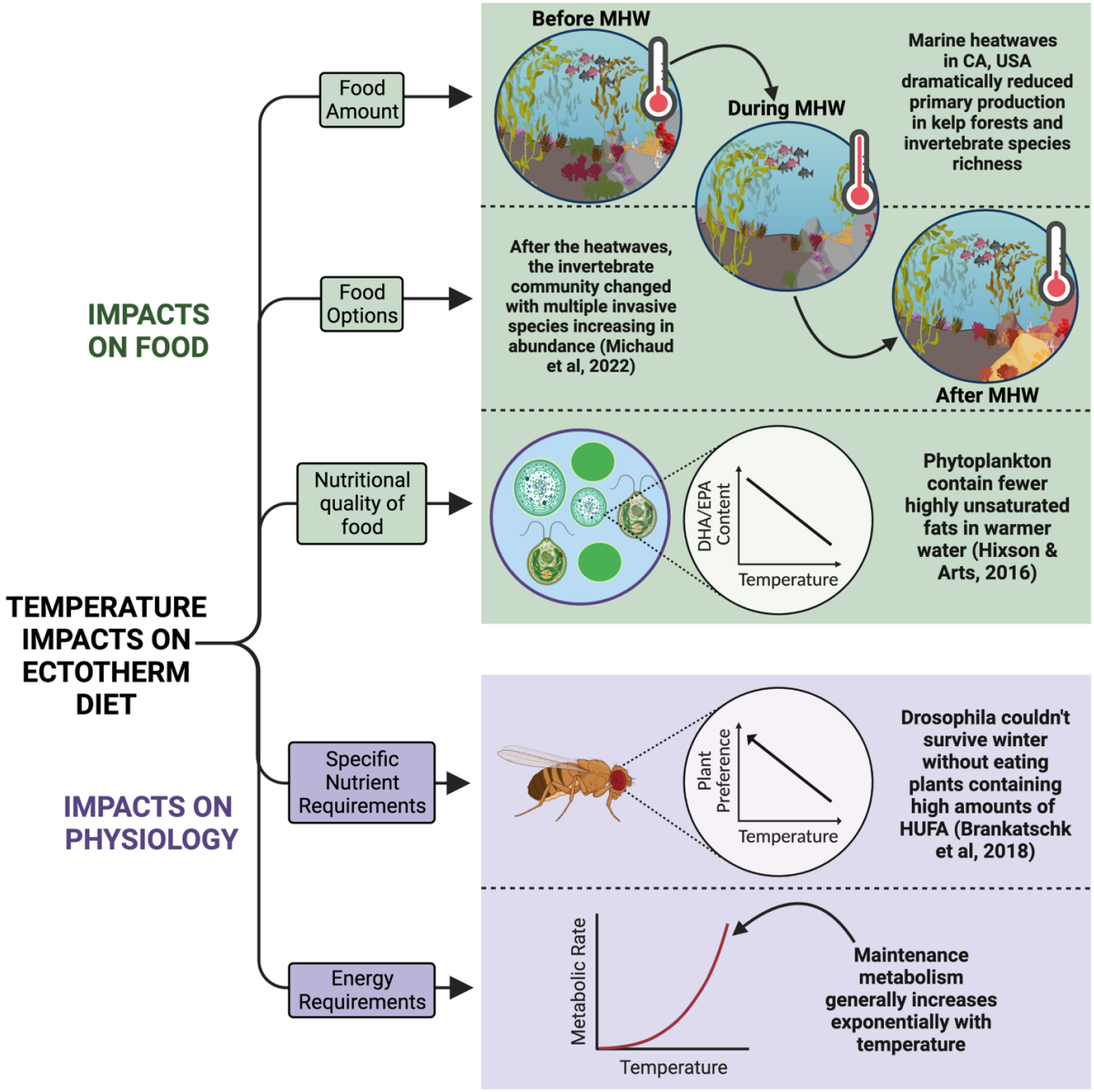

Figure 1.1 Conceptual Figure illustrating ways in which temperature can influence ectothermic animal nutrition and examples of each scenario. Temperature may impact ectotherm nutrition by directly impacting food resources (e.g., food availability, food options, food quality) or by affecting the nutritional requirements of the animal (e.g., specific nutrient requirements, energy requirements). Importantly, in many cases, animals can respond to changes in their food landscape or nutritional needs by adjusting how, when, what they forage for and consume. This can occur through changes in the total amount they consume or their diet selection.

1.1 Introduction

The Anthropocene is dramatically altering the nutritional landscape of many ectothermic animals (Poloczanska *et al.*, 2019). Human-impacts like habitat destruction, overfishing, pollution, drought, and changing temperatures impact what food animals have available to them and the nutritional quality of their prey. Food provides the energy and nutrients that animals need to thrive, grow, reproduce, and respond to environmental stressors. Changes in food availability and nutritional quality could profoundly impact animal performance, particularly in the face of environmental change. However, animals have the capacity to modify their diet to regulate their nutritional intake, and by doing so, they may be able to improve their performance under suboptimal conditions. This review focuses specifically on how diet impacts thermal performance in ectotherms, as temperature is one of the most concerning environmental stressors influencing ectotherm physiology and ecology (Brown *et al.*, 2004; Fry, 1971; Hochachka & Somero, 2002). We address the state of knowledge, critical gaps, principal physiological and biochemical mechanisms underlying diet effects on thermal performance, and the implications of this work on ectotherm biology.

1.2 Ectotherm diets can change with temperature

Global climate change is altering ectotherm nutrition through changes in their food availability, food options, prey quality, inter- and intraspecific interactions, and diet selection (Figure 1; Birnie-Gauvin *et al.*, 2017; Cross *et al.*, 2015; Poloczanska *et al.*, 2019; Rosenblatt & Schmitz, 2016; Zhang *et al*., 2020). *Food availability* varies spatially and temporally (Alton *et al.*, 2020; Arnold *et al.*, 2010; Birnie-Gauvin *et al.*, 2017; Ho, Pennings & Carefoot, 2010). Specific prey items can decline on local or global scales, and

temperature-induced changes in ecosystem structure can alter animal food options. These changes are often environment and location-specific, so we are still learning the many ways in which this occurs in nature. However, there are several reported predictions and examples that have important conservation implications for ectotherms.

For example, marine heatwaves in the North American Pacific between 2014-2016 are implicated as the drivers of a wasting disease outbreak that decimated several sea star species abundance, and reduced canopy forming kelp species in several regions (Beas-Luna *et al.*, 2020; Cavole *et al.*, 2016). Extreme events, like this, can have lasting impacts on community composition and allow for species invasions or range shifts; thus, altering food options (Cavole *et al.*, 2016; Michaud, Reed & Miller, 2022). In tropical systems, multiple anthropogenic stressors, such as overfishing of herbivores and omnivores, invasive species, extreme weather events, and temperature rise, can shift reefs from coral dominated to algae dominated, which impacts resource availability throughout the ecosystem (Burkepile & Hay, 2006). In terrestrial and freshwater systems, multiple stressors (including rising temperatures) are reducing Pacific salmon returns, which is lowering seasonal nutrient influxes along the pacific northwest of North America (Holtgrieve & Schindler, 2011). On land, temperature rise is related to shifts in species abundance across tundra, grassland, and high latitude ecosystems (Grim *et al.*, 2013). Other human impacts may also affect food availability in correlation with changes in temperature. For example, introductions of mosquitofish in California, USA have caused dramatic declines in wetland zooplankton and macroinvertebrates (Preston *et al.*, 2017; Rodgers *et al*., 2019). Anthropogenic change may also alter animal's foraging success and/or opportunities (Huey & Kingsolver, 2019). These

examples represent just a few of the many ways in which food availability and food options are shifting globally, but in context dependent ways.

The *nutritional quality* of the same diet item can also change with temperature (Figure 1). For example, the global availability of the essential omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are expected to decline with increasing temperatures due to climate change (Hixson & Arts, 2016; see section IV: Lipids). Warming may also affect dietary fat content. For example, in Tseng *et al.* (2021), algae raised in warmer water had lower amounts of neutral lipids. In another example, the nutritional quality of giant kelp decreased with rising temperatures in California, USA (Lowman *et al*., 2021), shifting from low carbon:nitrogen to high carbon:nitrogen (Lowman *et al*., 2021). Changes in nutritional quality are not limited to primary producers. For example, warmer temperatures are associated with higher cholesterol content in copepods in Maine, USA (Hassett & Crockett, 2009), and lower carbon: phosphorus in *Daphnia sp.* (Prater, Wagner & Frost, 2018).

While temperature can directly impact the nutritional landscape for animals, it also can affect their nutritional requirements (Figure 1, e.g., energetic needs or specific nutrient requirements). Importantly, some ectotherms change their *diet preference and/or selection* to compensate for changes in their food quantity, food options, food quality, or their nutritional needs (Boersma *et al.,* 2016b; Carreira *et al.*, 2016; Jang *et al.*, 2015; Rho & Lee, 2017; Schmitz & Rosenblatt, 2017; Vejříková *et al*., 2016). Temperature may also impact diet selection by altering the animal's intra/inter-specific interactions, or ability to distinguish prey items (Schmitz & Rosenblatt, 2017). Much of the focus of diet preference by temperature research has explored why specific nutrient requirements in some ectotherms

appear to be temperature dependent. For example, some species prefer more carbohydrates relative to protein in their diet under warmer conditions (Lee *et al*., 2015; Rho & Lee, 2017; see section IV: macronutrient ratios). In some cases, omnivorous aquatic ectotherms also increase the proportion of plant to animal prey in their diet with temperature (Zhang *et al*., 2020). Further, overwintering insects may consume higher amounts of unsaturated fats prior to winter (Brankatschk *et al*., 2018; see section IV: Lipids). Clearly, diet can change in correlation with or directly in response to temperature. These changes may have organismal level consequences that scale to population, species, and ecosystem level effects. In the next section we discuss the evidence for diet effects on ectotherm thermal performance.

1.3 Physiological Consequences of changing diet with temperature

To assess the state of knowledge of the intersection between diet and temperature on ectotherm biology, we performed a comprehensive literature search (i.e., using multiple iterative searches and including papers from reference lists of relevant literature). We looked for experimental studies with >1 diet treatment and >1 temperature treatment in a factorial design. Only diets that varied in nutritional composition (i.e. not only ration size or energetic content) were considered. Studies with >1 diet treatment but only 1 temperature treatment were also included if thermal limits or behavioral thermoregulation were measured. We found 146 studies that fit these criteria. The results of this literature search are summarized in Figure 2, 3.

Most studies were on juvenile fish (74 total fish studies: 50.7%), and assessed the effects of lipid quality (37 studies; 25.3%) or macronutrient ratios (15 studies; 10.3%) on fish thermal responses. Larval insects were the second highest studied age group and taxa (31

total insect studies; 21.2%), with most of the research centered on how macronutrient ratios and temperature impact growth, consumption rates, and development rate. There is little information about diet and temperature effects on other invertebrates, such as mollusks, echinoderms, and crustaceans (Figure 2). While findings varied across studies, our search revealed clear evidence that diet and temperature can interact to influence thermal performance in ectotherms. To evaluate this evidence, we classified it into two categories: thermal limits and sublethal effects (Figure 4 and 5).

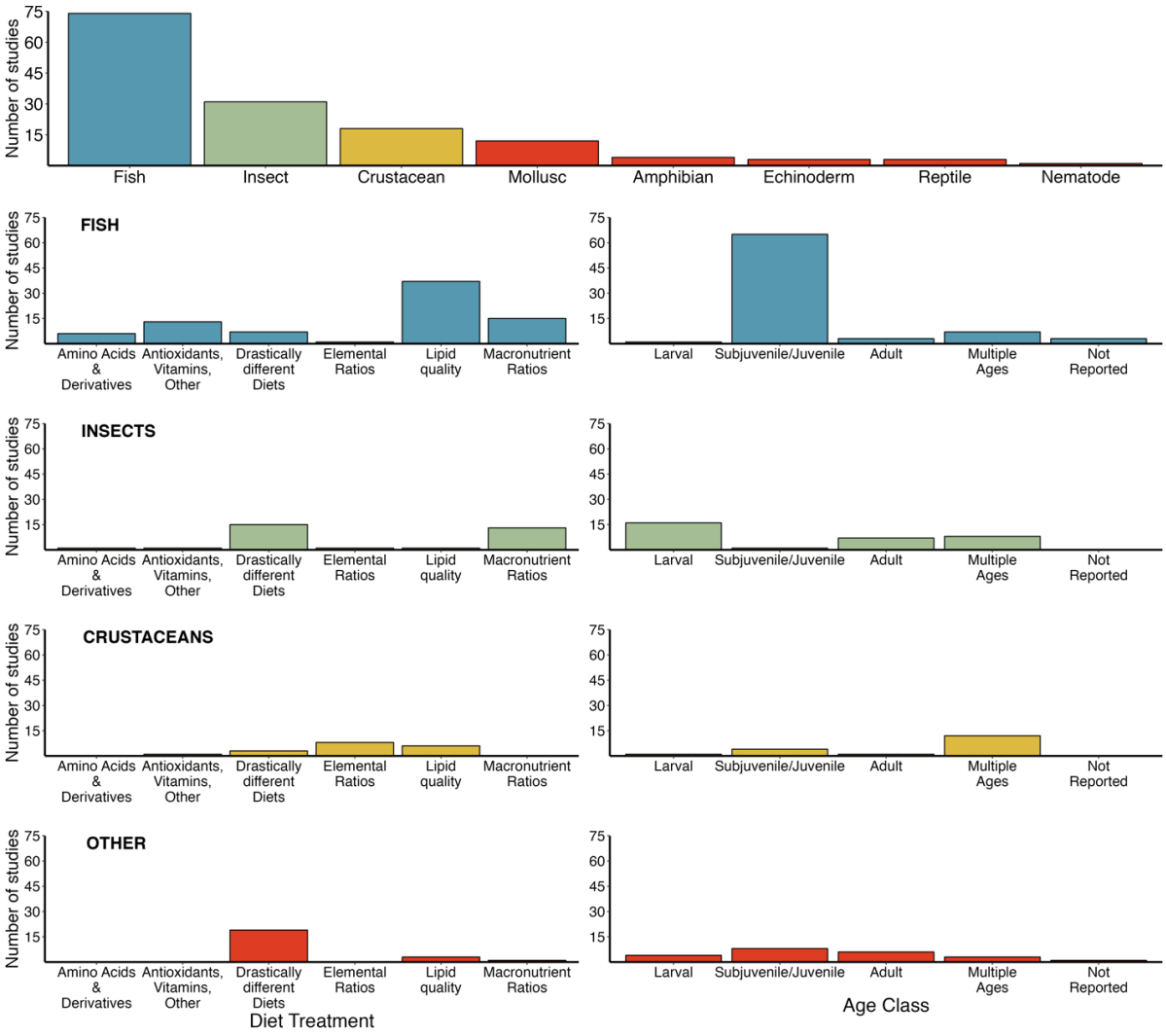

Figure 1.2 Breakdown of the diet by temperature literature. The top graph shows the breakdown of the literature by taxa. The left panel of graphs show the types of diet

manipulations that were made across studies, while the right panel shows the breakdown by age class examined.

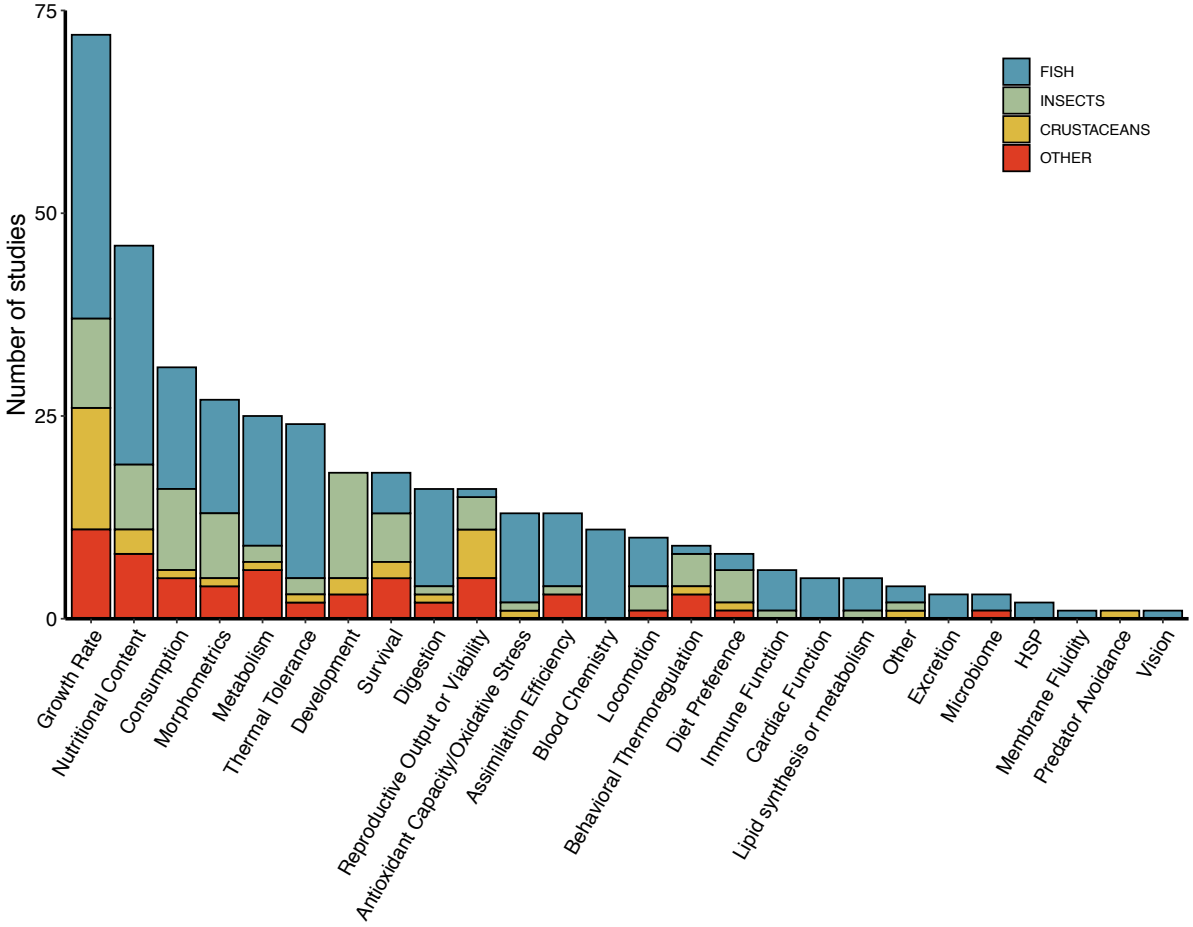

Figure 1.3 Breakdown of the diet x temperature literature based on what trait(s) were measured. Colors indicate specific taxonomic groups.

1.3.1 Thermal limits

Studies of extreme thermal limits date back to Fry and colleague's work in the 1940s on the zone of thermal tolerance, which defines the range of temperatures over which ectotherms can survive for short periods of time (Brett, 1944; Fry, Brett & Clawson, 1942; Fry, 1957). Since its introduction, researchers have conducted thousands of thermal limit studies across diverse taxa. Meta-analyses of these data have revealed global scale patterns that have aided researchers and managers alike in identifying species, environments, and populations most at risk under global climate change (Deutsch *et al.,* 2008, Sunday *et al.*,

2014; Sunday *et al.*, 2019). Through this work, we have learned that resistance to extreme temperatures generally increases with acclimation temperature, but that variation in thermal tolerance and acclimation capacity exists across populations, congeners, and taxa (Stillman, 2003; Somero, 2010). Ectotherms living in more variable environments tend to have a higher thermal breadth, while upper and lower thermal limits tend to be higher in ectotherms living in warmer areas, close to the equator, compared to species living in temperate and polar regions (Sunday, Bates, & Dulvy, 2011). As climate change increases the mean and maximum temperatures that ectotherms experience (Poloczanska *et al.*, 2019), those living in some environments will be at greater risk of exposure to their thermal limits (e.g., intertidal invertebrates, freshwater species, polar species, tropical species; Hofmann & Todgham, 2010). Despite the massive effort to define thermal limits across taxa, life stages, latitudes, and environments, there has been surprisingly little research on the impact of diet on thermal limits (Figure 1.4 and 1.5).

Thermal limits must be interpreted within the context of the exact method used because the thermal history, rate of temperature change, and endpoint definition can alter the results (Lutterschmidt & Hutchison, 1997). Very few studies have measured diet effects on thermal limits with 9 papers using a standard LT or LT50 (endpoint: lethal temperature for 50% of study subjects) and 10 using a critical thermal limit test (endpoint: loss of equilibrium; Figure 1.3-1.5; note that some papers used both LT and CT test). A small number of studies measured static thermal tolerance, where ectotherms were exposed to a heat or cold shock temperature, and their mortality rates (e.g., Harpaz, Becker & Blum, 1999; Hoar & Cottle, 1952) or rates of immobilization (Coggins, Pearson & Yampolsky, 2021)

were measured through time (not included in Figure 1.5). More recent studies have examined cardiac

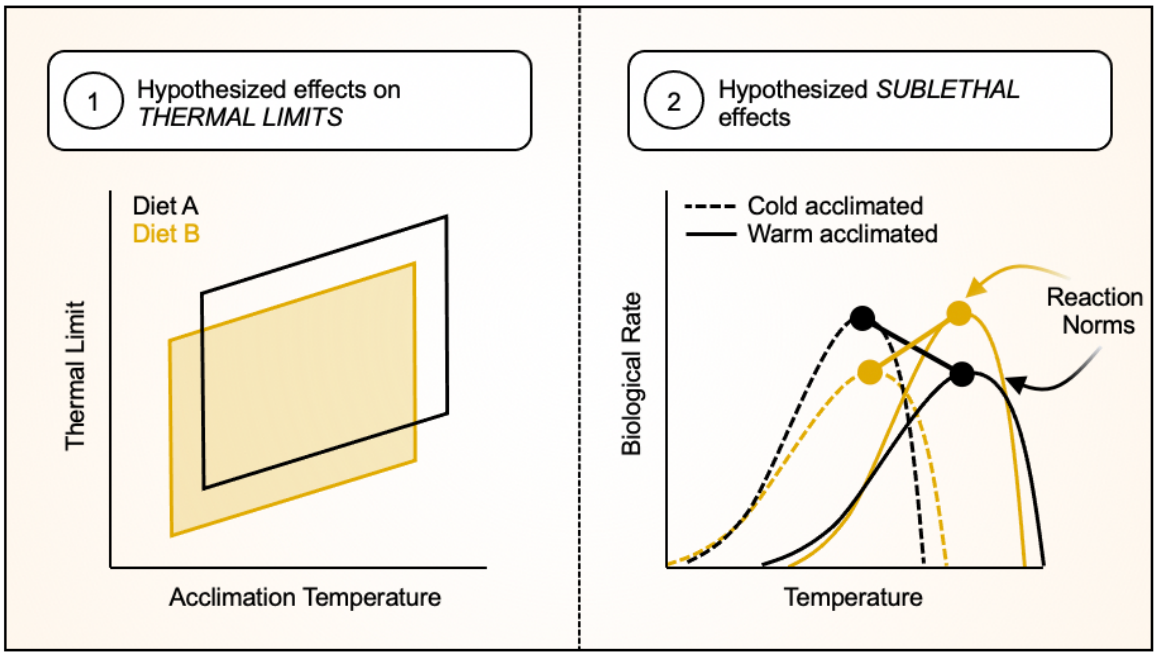

Figure 1.4 Hypothesized ways in which diet may influence thermal limits (left panel) or other traits (right panel) in ectotherms. Left: diet may influence the "zone of thermal tolerance" by increasing or decreasing cold and warm tolerance limits. Right: diet could impact ectotherms sub-lethally by affecting the height, breadth, or placement of thermal performance curves for their biological rate processes (e.g., growth, locomotion, reproduction, heart rate, digestion).

thermal tolerance using an Arrhenius breakpoint test for maximum heart rate (Dixon *et al.*, 2023; Hardison *et al.*, 2021; Hardison, Schwieterman & Eliason, 2023; Papadopoulou *et al.*, 2022; not included in Figure 1.5).

It is difficult to make generalizations about diet effects on thermal limits based on the limited body of literature. Of the studies that have measured thermal limits, only one tested them at more than one acclimation temperature (Hardison *et al.*, 2021). Most of the available data is on aquaculture fish using formulated diets that varied in lipid composition. There is evidence that lipid composition can modify thermal limits in fishes (Abdel-Ghany *et al.*, 2019; Craig, Neil & Gatlin, 1995; Gomez Isaza *et al.*, 2019). For *cold thermal limits*, dietary

oil type (e.g., coconut, menhaden fish oil, saturated fish oil) altered LT50 in juvenile red drum (*Sciaenops ocellatus*) by 5.5°C (from 9.4 to 3.9°C; Craig *et al.*, 1995). Abdel-Ghany et al. (2019) found similar results of oil type (e.g., fish oil, corn oil, coconut oil, oil mix) on cold thermal limits in Nile Tilapia (*Oreochromis niloticus*) fingerlings, where LT50 ranged from 8.5 (corn oil diet) to 15°C (coconut oil diet). Coconut oil is higher in saturated fats than corn and fish oil. Highly unsaturated fats are associated with greater membrane fluidity, while saturated fats are associated with higher membrane stability. This suggests that dietary lipid composition can alter membrane thermal performance; a mechanism discussed in detail in Section IV: Lipids.

Evidence for diet-mediated plasticity in *upper thermal limits* is equivocal, although only a few studies have assessed it. Total lipid composition (10 vs. 20%) had a significant effect on CT_{max} in juvenile barramundi (Gomez Isaza *et al*., 2019). However, the magnitude of that difference was only 0.48°C (Gomez Isaza *et al*., 2019). Similarly, flies (*Pseudosarcophaga afinis*) fed a diet high in saturated fats had a longer time to LT50 when exposed to elevated temperatures compared to those fed a diet high in unsaturated fats (House, Riordan & Barlow, 1958). Dietary supplementation of L-tryptophan and levan increased CT and LT_{max} in *Cirrhinus mrigala* and *Labeo rohita*, respectively (Gupta *et al*., 2010; Tejpal *et al.*, 2014). Dietary *β-alanine* supplementation, which decreased cardiomyocyte taurine uptake, led to an increase in CT_{max} in Brook Char (*Salvelinus fontinalis*; Dixon *et al.*, 2023). In contrast, CT_{max} and cardiac thermal limits were unaffected by a brine shrimp or a brine shrimp with algae diet treatment after acclimation to 12 or 20°C in opaleye (*Girella nigricans*) (Hardison *et al.*, 2021) and oil type did not influence upper or lower thermal limits in juvenile angelfish (Ikeda *et al.*, 2011). Given the value of measuring

thermal limits in ectotherms and the multiple potential mechanisms where diet may alter them (see section IV), it is critical that we address this knowledge gap. We may be overestimating thermal limits in ectotherms with reduced diet options due to anthropogenic impacts. Alternatively, we may be underestimating thermal limits of ectotherms with higher quality diets or more diet flexibility in the wild than what has been measured in the lab (Figure 1.4).

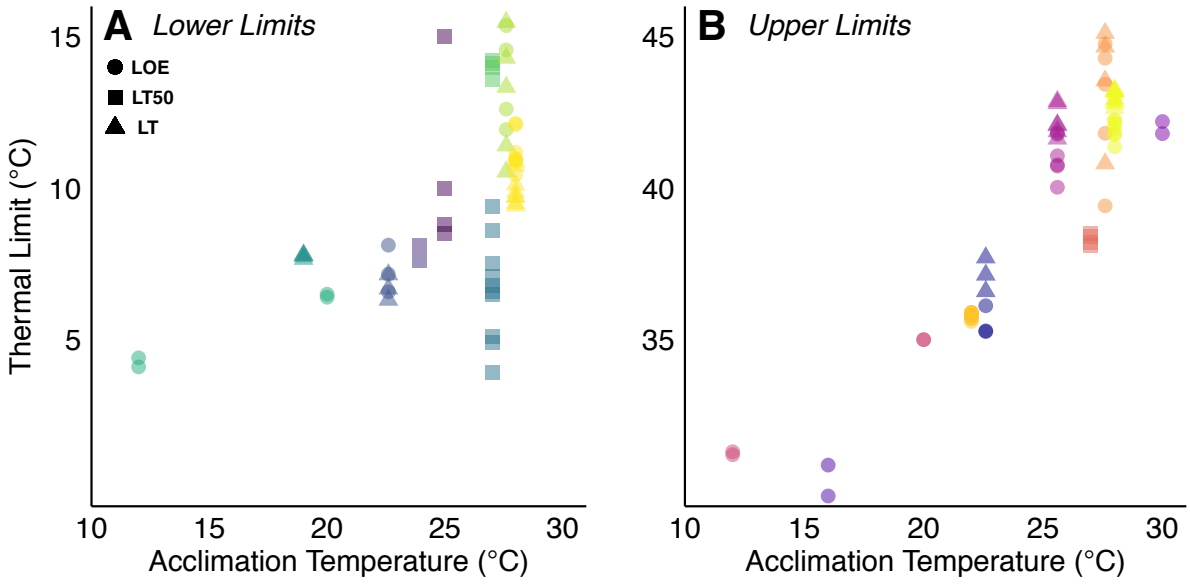

Figure 1.5 Thermal limit data from all fish studies where >1 diet treatment was used and thermal tolerance was assessed using standardized methodology (for upper thermal limits: CTmax, LTmax, LT50; for lower thermal limits: CTmin, LTmin). Values are plotted against their acclimation temperature. Colors indicate a specific study and methodology for the end point is indicated by the shape (either loss of equilibrium (LOE), lethal temperature 50 (LT50) or lethal temperature (LT)).

1.3.2 Sublethal effects of diet and temperature

While thermal limits define the extreme temperatures ectotherms can survive for short periods of time, they do not capture sublethal effects of temperature on ectotherm physiology and ecology. Thermal limits and maximum rate capacities are considered less plastic than performance traits partitioned within those bounds (e.g., resting/standard

metabolic rate, growth, consumption); a hypothesis termed '*plastic floors and concrete ceilings'* (Araujo *et al.,* 2013; Hoffman & Sgrò, 2012; Sandblom *et al.,* 2016). These traits provide insight into how population growth, species interactions, and behavior shift with temperature. The higher plasticity of these performance traits also suggests that diet may have a greater impact on them compared to thermal limits and maximum rate capacities.

Figure 1.3 summarizes the performance traits that have been measured across taxa in diet by temperature studies. Most of what we know about diet by temperature effects is from long term growth studies. The combined effects of diet and temperature on other fitness-enhancing traits, such as metabolism, cardiac function, cognitive function, locomotory performance, and immune function are understudied. Temperature effects on biological rates can be described by acute thermal performance curves (TPC), where the rate typically increases with an acute temperature increase until a peak, after which performance rapidly declines with any further increases in temperature (Figure 1.4). TPCs provide valuable data that can be used to inform models and management efforts for ecologically and environmentally valuable species. However, there are several limitations involved in extrapolating individual performance curves to larger ecological processes, including that factors like diet, which are often not considered, can alter the shape (e.g., width, height) of thermal performance curves and thermal sensitivity of reaction norms (performance at acclimation conditions; Carreira *et al.*, 2020; Hardison *et al.*, 2021; Huey & Buckley, 2022, Sinclair *et al.*, 2016, Figure 1.4). Further, different performance traits can be characterized by unique TPCs (Clark, Sandblom & Jutfelt, 2013), which may create some important performance trade-offs if a diet can maximize the performance of one trait at the cost of maintaining high performance in another (Hardison *et al.*, 2021). Diet may also alter how

quickly ectotherms are able to mount an acclimation response (Hardison *et al.*, 2023) which may be an important, but often overlooked factor in ectotherm thermal biology (Burton, Ratikainen & Einum, 2022).

Physiological responses differ across taxa, age, biome, environment, and population, making it difficult to generalize how diet interacts with temperature to affect ectotherm physiology across traits. Below, we discuss these physiological responses and the potential mechanisms driving diet impacts on thermal performance. By understanding why and how diet affects thermal performance, we can make more accurate predictions about the sublethal consequences of changing diet with temperature on important physiological processes.

1.4 Mechanisms of interaction between diet and temperature on ectotherm physiology

1.4.1 Macronutrient Ratios

As temperature rises, so does baseline metabolism. To meet a rising metabolic demand, ectotherms must take in more fuel. One way to accomplish this is by increasing food intake, which has been demonstrated in many ectotherms across optimal temperatures (Jutfelt *et al.*, 2021). If only food intake changes with temperature, the overall energy intake increases while macronutrient ratios, or the ratio of protein to lipid to carbohydrate in the diet, remain the same. Interestingly, many ectotherms change their preferred macronutrient ratios, in addition to consumption rates, with temperature (Devries & Appel, 2014; Lee *et al*., 2015; Rho & Lee, 2017; Schmitz, Rosenblatt & Smylie, 2016).

The macronutrient ratio hypothesis (MRH) is a leading proposed explanation for why ectotherms alter macronutrient preferences with temperature. MRH suggests that ectotherms

preferred protein:carbohydrate changes in response to temperature (Devries & Appel, 2014; Lee *et al*., 2015; Lemoine & Shantz, 2016; Schmitz *et al*., 2016; Rho & Lee, 2017; Rowe *et al*., 2018); specifically, protein:carbohydrate decreases as temperature increases. Studies on MRH emphasize a trade-off between high protein (thought to maximize growth) versus high carbohydrate diets (thought to maximize energy pay-offs). MRH argues that as metabolic demand increases with temperature, ectotherms require more efficient, easy to digest sources of energy. Proponents of this hypothesis reason that carbohydrates are more efficient to digest in warm conditions relative to protein because protein is not readily stored as fuel in the body (it is more of a "use it or lose it" nutrient) and contains nitrogen (see section IV: *Limitations and future outlooks)*, while carbohydrates can easily be stored as glycogen and do not contain nitrogen. (Lee *et al*., 2015; Rho & Lee, 2017).

Some studies have found support for MRH (Lee *et al*., 2015; Rho & Lee, 2017). For example, Lee *et al*. (2015) tested the effect of variable protein:carbohydrate in caterpillar (*Spodoptera litura*) diets across three temperatures (20, 25, 30°C). As the temperature increased, the caterpillars preferentially ate higher carbohydrate diets. The researchers compared performance under fixed-ratio diets to a choice diet and found that across temperatures the choice diet generally maximized performance (in this case, development time, pupal mass, and survival rate).

An alternative to the MRH is that higher-protein diets will be preferred as temperatures increase, to maintain maximal growth and protein turnover in response to an individual's rising metabolic demands (Devries & Appel, 2014; Lemoine & Shantz, 2016; Schmitz *et al*., 2016; Rowe *et al*., 2018). In support of this hypothesis, Schmitz *et al*. (2016) found that juvenile grasshoppers (*Melanoplus femurrubrum*) preferred higher

protein:carbohydrate at hot temperatures and lower protein:carbohydrate in response to increased predation risk (due to a higher metabolic demand). They hypothesized that juvenile grasshoppers consumed more protein at higher temperatures to maximize growth and thereby, minimize predation risk, since adult grasshopper experience less predation risk compared to juveniles. This was supported by their finding that differences in protein:carbohydrate preference between predator exposed and non-exposed grasshoppers decreased as temperature increased. Similarly, Devries & Appel (2014) found that silverfish insects (*Lepisma saccharina*) preferred proportionally higher amounts of protein and lipid over carbohydrates in their diet as temperature increased.

Recent studies have proposed a derivative of the MRH, called temperature metabolic stoichiometry hypothesis (TMS hypothesis), which argues that ectotherms will preferentially consume higher carbon diets (relative to nitrogen and phosphorus) due to their rising metabolic demands (at the direct cost of growth) as temperatures increase (Boersma *et al*., 2016b; Malzahn*,* Doerfler & Boersma, 2016; Zhang *et al*., 2020). The TMS hypothesis follows similar logic to the MRH; however, empirical evidence for this hypothesis is limited (Boersma *et al*., 2016b; Malzahn *et al*., 2016). Anderson *et al*. (2017) tested the TMS hypothesis using a modeling approach and found no evidence that zooplankton diets shift to a higher carbon:nitrogen ratio with temperature. Anderson *et al.* (2017) discussed how previous stoichiometric models assumed that consumption rates would not change with temperature. When Anderson *et al.* (2017) did not account for changes in consumption with temperature, their model supported the TMS hypothesis, but once they incorporated temperature effects on consumption rates, the models predicted that optimal carbon:nitrogen would not change with temperature. Importantly, the researchers still supported the

hypothesis that ectotherms require more carbon as temperature increases. However, they reasoned that the effect of having higher consumption rates in warmer conditions leads to enough excess carbon in the diet that ectotherms do not have to change their preferred stoichiometric ratio (Anderson *et al.*, 2017). For additional reading, Cross et al. (2015) wrote a review of how stoichiometric ratios and temperature may interact, using ecological stoichiometry and metabolic theory to explain how interactions of stoichiometry and temperature on an individual level can scale to whole ecosystem effects.

Ruiz *et al*. (2020) also expanded on the TMS hypothesis, contending that stoichiometric preference follows a U-shaped response with lower carbon to phosphorus ratios (corresponding with higher growth) being advantageous at optimal temperatures for growth. Outside the optimal thermal range for growth, the need to consume high carbon relative to phosphorus increases. Ruiz *et al*. (2020) tested this hypothesis using a modeling and experimental approach in *Daphnia* sp. and found support for the U-shaped response. Other traits are also affected by macronutrient concentrations and may create important performance trade-offs for varying dietary protein:carbohydrate outside of those predicted by the MRH and TMS hypothesis (Kutz, Sgrò & Mirth, 2019). Using a nutritional geometry framework, Kutz *et al.* (2019) demonstrated that developmental temperature interacted with dietary protein:carbohydrate in trait-specific ways in larval *Drosophila melanogaster*. They generated an impressive 36 diets that varied in protein:carbohydrate and energetic content and measured development time, viability, and adult size and morphology after acclimation to 25 or 28°C. At the current summer temperature (25°C), fly performance was optimal across a wide nutritional space. However, when flies were reared under a climate-change scenario temperature (28°C), there were fewer dietary protein:carbohydrate ratios that

maximized fly performance. This suggests that suboptimal temperatures may limit the diet options capable of maximizing an animal's performance. Similarly, cold acclimated *Daphnia sp*. had a wide and flattened growth curve in response to being fed a range of carbon:phosphorus in the diet (Laspoumaderes *et al.*, 2022). However, in warm, the water fleas' growth was maximal across a smaller number of diets (Laspoumaderes *et al.*, 2022). The author's contend that the optimal ratio of carbon:phosphorus depends on the thermal sensitivity of metabolic rate, growth, and ingestion, where a higher carbon:phosphorus is required when an animal's thermal sensitivity for metabolism exceeds that of ingestion or growth (Laspoumaderes *et al.*, 2022). A similar nutritional framework was used in Rowe *et al.* (2018) to determine how dietary protein:carbohydrate impacted resting and active metabolic rate in Damselfish (*Abudefduf vaigiensis*). While the nutritional geometry framework is experimentally challenging due to the large number of treatments, it provides a more complete picture of how nutrition and temperature may interact and allows for determination of complex and multi-optima nutritional effects.

1.4.2 Limitations and future outlooks:

In addition to mixed support, there are several noteworthy limitations to the MRH and TMS hypothesis. For example, most studies have been conducted on ectotherms in the larval or juvenile stage. Growth rates change throughout life history, so the cost and benefit of higher growth compared to higher energy yielding diets may differ with age. Second, MRH focuses on dietary protein:carbohydrate but does not discuss the effect of lipid composition, which is also undoubtably important, especially in marine environments, where lipids are a more bioavailable energy source than carbohydrates and some aquatic animals have lower

glucose tolerance (Steinberg, 2022; Turchini *et al.,* 2022). Overall, there is a clear need for more macronutrient by temperature research across environments, taxa, and age classes.

A third limitation is that these hypotheses do not account for variation in nitrogen excretion across taxa. Protein is the only metabolizable macronutrient class that contains nitrogen. This nitrogen is released as ammonia during protein catabolism which becomes toxic if left to accumulate and thus must be excreted or converted to less toxic compounds (e.g. uric acid, urea). Ammonia production is directly related to the amount of protein catabolized (Randall & Wright, 1987). Consequently, ectotherms consuming high protein diets excrete more nitrogen than those consuming low protein diets. Most terrestrial animals are either ureotelic (excrete urea) or uricotelic (excrete uric acid), while many aquatic organisms are ammoniotelic (excrete ammonia). The production and excretion of urea and uric acid are much more energy expensive than the excretion of ammonia (Randall & Wright, 1987). This difference in nitrogen waste removal may reduce the cost of consuming high protein diets at high temperatures in ammoniotelic animals and could explain some of the observed differences in macronutrient selection across taxa.

1.4.3 Lipids

Lipid quality can impact thermal limits and other sublethal thermal performance traits in ectotherms (Abdel-Ghany *et al.*, 2019; Craig *et al.,* 1995; Geiser, Firth & Seymour, 1992; Hassett & Crockett, 2009; Hoar & Cottle, 1952). Lipids play a key role in cell membranes, cell signaling, gene regulation, energetics, and more. As membrane fluidity rises with temperature, ectotherms undergo a process called homeoviscous adaptation to optimize their membrane performance. This can occur through multiple mechanisms, including remodeling the components of their cell membranes by exchanging phospholipids, inserting cholesterol

into membranes, modifying lipids through processes like acetylation or altering membrane-bound protein concentrations (Hochachka & Somero, 2002). A common mechanism of homeoviscous adaptation is to adjust the composition of fatty acids in the membrane. As fatty acid tail length and degree of saturation increases, so does the interaction strength (due to van der waals interactions) between acyl chains in membranes (Hochachka & Somero, 2002). This means that longer and more saturated fatty acids in the diet decrease membrane fluidity, while unsaturated fatty acids and fatty acids with short acyl chains in the diet increase membrane fluidity (Hochachka & Somero, 2002).

There is evidence to support the hypothesis that dietary fatty acid composition can influence thermal performance in ways predicted by homeoviscous adaptation. For example, Hardison *et al*. (2023) found that opaleye fish (*Girella nigricans*) acclimated to 12 or 20°C and fed brine shrimp, algae or both, assimilated different fatty acids into their hearts. Fish with more ventricular unsaturated fats had greater maximum heart rates (i.e. improved performance) under cold acclimation, but lower maximum heart rates under warm acclimation (Hardison *et al.,* 2023). Brankatschk et al. (2018) also found evidence of homeoviscous adaptation affecting diet preference in *Drosophila* sp. Interestingly, the flies preferred plants, which were high in unsaturated fats, compared to yeast at cold "winter" temperatures (12°C). Plant consumption was associated with higher cold tolerance (e.g., greater mobility, higher overwinter survival) and greater tissue PUFA content. Here, the flies preferentially chose a diet based on fatty acid composition and did so in ways that maximized survival overwinter. These results are consistent with those from other overwintering insects, where they increase unsaturated fatty acid composition in winter to ensure sufficient fluidity for lipid metabolism to occur and membrane composition to stay functional (Sinclair &

Marshall, 2018). These findings beg the untested question of whether dietary generalist species have a wider thermal breadth than specialists with constrained diet options. Further, the incentive to adjust diet preference to assimilate different lipids with temperature may be stronger for ectotherms living in environments that experience extreme daily or seasonal temperatures.

It can be energetically expensive to alter the length and degree of saturation for fatty acids or synthesize lipids de novo (Secor, 2009), and many animals cannot synthesize certain lipids and must assimilate them from their diet (for relevant review in fish: Turchini *et al.,* 2022; in insects: Sinclair & Marshall, 2018). As such, ectotherm tissue lipid composition often differs depending on its dietary lipid composition (examples: Alhazzaa *et al.,* 2013; Atwood *et al.,* 2003; Craig *et al.,* 1995; Farkas *et al.,* 1980; House *et al.,* 1958; Vagner *et al.,* 2019). However, many ectotherms are capable of mounting a high degree of lipid regulation and tissues can be differentially affected by dietary lipid deficiencies (Skalli *et al*., 2006). For instance, Skalli *et al*. (2006) found that dietary deficiency of certain essential fatty acids had a larger impact on the fatty acid content of muscle, liver, and gills than that of the eye and brain tissue in juvenile European sea bass (*Dicentrarchus labrax*). While many ectotherms have some capacity to alter acyl chains through desaturase or elongation activity or prioritize lipid deposition to specific tissues, there is still a strong energetic incentive for ectotherms to alter their diet strategy if they are placed in temperatures where their current diet has a sub-optimal lipid profile, as seen in Brankatschk *et al*. (2018). This energetic trade-off of synthesizing versus assimilating specific lipids can have consequences on growth. For example, dietary deficiency of omega -3 fatty acids in Atlantic salmon (*Salmo salmar*) resulted in higher bioaccumulation of DHA (i.e., the fish had to spend energy to convert

ALA to DHA) and consequently lower growth (Závorka *et al*., 2021). These types of energetic trade-offs may be exacerbated in extreme conditions, such as overwinter, where survival is contingent on successful accumulation and regulation of energy stores.

Other lipids, aside from fatty acids, can provide important performance enhancements at suboptimal temperatures. Hassett & Crockett (2009) found that copepod growth was limited by dietary cholesterol content in both their cold (6°C) and warm (25°C) treatment temperatures. Hassett & Crockett (2009) also sampled the cholesterol content of 9 species of copepods across a range of habitat temperatures and found a positive correlation between the amount of cholesterol in the copepod and its habitat temperature as well as the species upper thermal tolerance.

While we have discussed the effects of dietary lipid ***quality*** on ectotherm thermal performance, there is no consensus on how optimal dietary lipid ***quantity*** changes with temperature. In Jonsson *et al*. (2013), Atlantic salmon consuming diets with higher a proportion of lipids and a lower proportion of indigestible materials (e.g., cellulose, rutin) exhibited higher growth rates, faster development times, and quicker maturation times regardless of the treatment temperature. However, in a similar study on Rohu, the optimal lipid concentration changed with temperature (Mishra & Samantaray, 2004). In yet another study by Sealey *et al*. (2012), juvenile suckerfish fed high lipid diets had higher growth rates than those fed low lipid diets across temperatures and dietary protein content. However, the fish fed the highest protein and lipid diet had reduced thermal tolerance at the warmest treatment temperature. Other studies have found similar trends, where high lipid content does not necessarily confer high performance (Guerreiro *et al*., 2012).

1.4.4 Limitations and future outlooks

Previous studies have revealed that at least some ectotherms are capable of preferentially selecting diets based on their lipid composition at different temperatures and that lipid composition can (but does not always) affect performance in ways predicted by homeoviscous adaptation. However, there are several gaps in our understanding of dietary lipid effects on ectotherm thermal biology. Most lipid research has focused on the effects of fatty acids on growth and thermal limits. These studies were most often performed on juvenile aquaculture fish using formulated diets that varied in dietary oil type (see section V: Aquaculture). However, it is important to consider the diverse roles lipids play in cell biology. Fatty acids are the backbone of many different lipid classes that perform a host diverse of cellular and organ level functions. For example, phospholipids and sterols are key components of biological membranes, triacylgycerides are commonly used in energy storage, certain LC-PUFA can regulate metabolic gene expression by binding to various transcription factors (Hochachka & Somero, 2002; Turchini *et al*., 2022). Lipids are also a primary energy source for most fishes, but digestive energetics differs across lipid classes (Turchini *et al*., 2022). The more hydrophobic a lipid is, the more difficult it is to digest and assimilate, meaning saturated fatty acids, longer chain fatty acids, and wax esters, are less digestible than polyunsaturated fatty acids, short-chain fatty acids and triacylglycerides (especially at lower temperatures) (Bogevik *et al*., 2010). Lipids also vary in their energy density, with longer chain saturated fats providing greater energy per unit molecule than short chain or unsaturated fats. From what we know about homeoviscous adaptation, and the energetics of lipid biosynthesis, and assimilation, it follows that ectotherms limited to diets containing sub-optimal lipid quality and quantity at a given temperature may have a reduced capacity for

membrane plasticity and impaired metabolism, which could scale to whole animal performance.

It is also important to consider the timescale with which dietary lipids affect performance, especially for ectotherms living in variable environments. Homeoviscous adaptation occurs rapidly in some ectotherms (Williams & Hazel, 1994; Wodtke & Cossins, 1991). For example, Williams & Somero (1996) found that *Mytilus californianus* (an intertidal mussel native to the West Coast of the United States) remodeled its phospholipid membrane during tidal cycles in a matter of hours and did so repeatedly. This acute response suggests that lipid remodeling may be an everyday coping mechanism used by ectotherms living in highly variable environments. If ectotherms do not have access to the pool of lipids needed to elicit these responses, then rapid membrane plasticity may either not be possible, or require de novo synthesis using desaturases or elongases. Ectotherms in variable environments may prefer diets with high lipid content and diversity to facilitate rapid membrane plasticity, although this remains to be tested.

Finally, lipid composition may affect metabolic signaling and stress at suboptimal temperatures. Reactive oxygen species (ROS) can cause damage to cell membranes through a process called lipid peroxidation (LPO; oxidative degradation of lipids). Fatty acids vary in their vulnerability to LPO, where polyunsaturated fatty acids are especially vulnerable and are associated with shortened lifespans in some species (Hubert, 2008; Munro & Blier, 2012), and increased activity of antioxidant enzymes (Gourtay *et al.*, 2020). Higher concentrations of protein and lipid in the diet are associated with higher ROS production, and ROS production often increases with temperature (Coggins *et al*., 2017; Hwang & Lin, 2002). Importantly, antioxidants, like vitamin C and vitamin E, can counteract ROS and

reduce LPO (see Section IV: Other dietary nutrients, supplements and signaling molecules). The known effects of temperature and dietary antioxidants are discussed in the next section. However, the interactions between temperature, dietary lipid quality, antioxidant concentrations and capacity in the diet are unknown and require further investigation.

1.4.5 Other dietary nutrients, supplements and signaling molecules

Beyond broad macronutrients, numerous other dietary components (e.g. vitamins, minerals, specific nutrients, signaling molecules) could potentially alter thermal performance in ectotherms. There are a handful of studies that have explored the interactive effects of temperature and one specific nutrient or signaling molecule on performance, with mixed results. The primary motivation for this work has been to inform aquaculture feeding regimes for economically valuable species. Much of this research has explored antioxidant content and amino acids or their derivatives on growth and tissue quality (for flavor) across temperatures.

Antioxidants *(i.e., Glutathione, Vitamin C, Vitamin E, Spermine, etc.)* scavenge reactive oxygen species (ROS). As temperatures rise, ROS production generally increases (Coggins *et al*., 2017; Hwang & Lin, 2002). Extreme acute and prolonged cold exposure can also induce oxidative stress (Lu *et al.*, 2019). Therefore, diets with higher antioxidant concentrations may benefit organisms that are under thermal stress by increasing their antioxidant capacity. This has been tested in a small number of studies. Coggins *et al*. (2017) examined the effect of the antioxidant, glutathione, supplementation on thermal tolerance, antioxidant capacity, and lipid peroxidation (LPO) in *Daphnia* sp. and found that as glutathione concentration increased, so did *Daphnia* sp. antioxidant capacity. However, glutathione supplementation did not alter the flies LPO or thermal tolerance. Similarly, Ilham

& Fotedar (2016) found that selenium supplementation (Se is an element in glutathione peroxidase, which is a vital antioxidant enzyme in red blood cells) increased growth at lower temperatures and red blood cell count and glutathione peroxidase activity across temperatures in yellowtail kingfish (*Seriola lalandi*). Further, Hwang & Lin (2002) tested the effects of vitamin C supplementation on TBARS (a by-product of LPO) at 25 and 35°C. The 35°C treatment caused higher LPO, which was reduced by dietary addition of vitamin C. Finally, Lu *et al.* (2019) found that antioxidant supplementation in zebrafish (*Danio rerio*) diets increased the fish's survival and liver catalase activity during a cold shock experiment relative to control diets. In contrast to these studies, Olsen, Løvaas & Lie (1999) tested the interactive effects of the antioxidant spermine, dietary PUFA content, Vitamin E concentration, and acclimation temperature (0.6 and 12°C) on Arctic Char (*Salvelinus alpinus*) and found no evidence that increasing vitamin E above baseline levels or supplementing the diet with spermine altered oxidative stress (measured using TBARS assay) across temperatures. While there is evidence that antioxidant supplementation can increase antioxidant capacity, the impacts of this on performance, oxidative stress and thermal tolerance were less consistent across studies and may depend on whether the acclimation temperature fell within the optimal range for the species.

Other studies have investigated how dietary concentrations of essential and non-essential amino acids *(e.g., L-Carnitine, L-Tryptophan, β-alanine)* alter performance in ectotherms. However, there are only a few studies that have assessed whether these dietary requirements change with temperature. For example, Akhtar *et al*. (2013) found that dietary supplementation with L-tryptophan mitigated the effects of thermal and salinity stress in the fish, Rohu (*Labeo rohita*). In another example, Lamb & Loschiavo (1981) observed an

interactive effect of dietary lysine concentration and temperature on development rates in larval beetles (*Tribolium confusum*), with high lysine plus high temperature treatments conferring the fastest development times. Finally, in Dixon *et al.* (2023), brook char (*Salvelinus fontinalis*) fed a *β-alanine* supplementation to reduce cardiomyocyte taurine uptake, experienced a reduction in maximum heart rate across a thermal gradient, and improved upper thermal limits. There are many amino acid derivatives which could influence thermal acclimation responses in ectotherms. For example, L-carnitine (important in lipid metabolism) supplementation in cichlid fish (*Pelvicachromis pulcher*) diets increased survival rates during a cold shock experiment (Harpaz *et al.,* 1999). In contrast, Dzikowski *et al*. (2001) tested the effect of L-carnitine supplementation on guppy reproductive success across temperatures and found no effect of the diet on reproduction.

1.4.6 Limitations and future outlooks

It is resource and time intensive to study how the dietary concentration of one nutrient at a time impacts thermal performance. The best model species for this type of work are ones where large numbers of replicates and treatments can easily be maintained (e.g., insects and small crustaceans). However, it is also essential that similar studies are conducted on vertebrate and larger invertebrate taxa to account for inter-taxon variation in nutritional and thermal biology. Importantly, different performance traits can be characterized by unique thermal performance curves (Clark *et al.*, 2013; Hardison *et al.*, 2021). Therefore, the optimal concentration of certain amino acids (or their derivatives) for growth may not be the optimal concentration for thermal tolerance or fecundity. One sector that may have the resources and incentive for this work is aquaculture (see section V: Aquaculture). Clearly,

more research is needed regarding the combined effects of temperature and vitamins, minerals, and other specific nutrients.

Another unexplored area is the relationship between dietary hormones and temperature. In ectotherms, endogenous hormone production and activity are temperature sensitive (Farrell, 2011), and exogenous hormones from food can be assimilated and may have interactive effects with environmental conditions. To illustrate this, consider thyroid hormone, which regulates thermal acclimation responses in zebrafish (*Danio rerio)* and cannot be synthesized de novo by some invertebrates, such as some larval echinoderms (Little *et al*., 2013; Little *&* Seebacher*,* 2014). Dietary thyroid hormone alters growth and proximate body composition (i.e., lipid, water, protein, ash content) in red drum (*Sciaenops ocellatus*) (Moon, MacKenzie & Gatlin, 1994) as well as the development rate and metabolism in many marine invertebrates (Little *&* Seebacher, 2014). Therefore, thermal acclimation responses may be affected by dietary thyroid hormone concentration, especially in ectotherms that do not synthesize thyroid hormone de novo. Thyroid hormone is just one of many hormones that could be assimilated from the diet and interact with temperature to alter performance in ectotherms.

Finally, dietary microRNAs (miRNAs) could potentially impact thermal performance in ectotherms, though there is minimal research exploring this possibility to date. MiRNAs are small, non-coding RNAs that act by silencing genes and thus play a key role in many biological processes such as cell differentiation, apoptosis, immune function, reproduction, and the stress response (Raza *et al.*, 2022). In fish, miRNAs have been discovered to be differentially expressed in response to cold and warm temperatures (Raza *et al.*, 2022) and have been suggested to potentially target regulatory enzymes for glucose and lipid

metabolism (Blödorn *et al.*, 2021). Traditionally, miRNAs were considered to be synthesized and utilized endogenously; however, there is increasing evidence that miRNAs can be incorporated from exogenous sources to act as cross-kingdom gene regulation (Zempleni *et al.,* 2015; Zhang *et al.,* 2019). For example, the parasitic plant *Cuscuta campestris* can transport miRNAs to their host plants to silence host genes and enhance *C. campestris* growth (Shahid *et al.*, 2018). MiRNAs from food can also be absorbed through the digestive system, into the circulatory system and delivered to tissues (Zhang *et al.,* 2019). However, evidence of dietary miRNAs playing a functional role is equivocal in humans and animals (Zhang *et al.,* 2019), and thus, dietary miRNAs remain an exciting avenue for future investigation.

1.4.7 Digestive Physiology

Ectotherm digestive systems are diverse and vary as a function of age, sex, diet strategy, species, taxa, environmental conditions, body size, and more (Andrade *et al.*, 2005; Starck, 2003). However, the multifunctional digestive system is ubiquitously important for nutrient uptake, osmoregulation, immune function and represents a substantial proportion of ectothermic energy budgets (Andrade *et al.*, 2005; Grosell, Farrell & Brauner, 2010). For example, the gut in some fish is estimated to receive 25% of cardiac output in non-digesting individuals (Farrell, 2016). While the separate effects of diet and temperature on digestive physiology have been extensively studied in ectotherms, their combined effects remain poorly understood.

The metabolic cost of digestion, or specific dynamic action (SDA), is the increase in metabolism that occurs during the consumption, digestion, absorption, and assimilation of a meal (Secor, 2009). It is well documented that SDA is positively correlated with the

energetic content of a meal and meal size (Secor, 2009). However, other diet-specific impacts on SDA are uncertain due to discrepancies in findings across studies. For example, protein digestion, assimilation, and synthesis are considered to make up a large portion of the SDA response, with several papers supporting this hypothesis (for an extensive review see Secor, 2009; Andrade *et al.*, 2005); however, there are also studies that report no differences in SDA in response to changes in dietary protein concentration (using isocaloric diets with varying protein to lipid ratios in rainbow trout; Eliason, Higgs & Farrell, 2007). This makes determining the ultimate cause for differences in SDA even more challenging for diet and temperature effects. To date, one study measured SDA for different meal types across temperatures (Pérez-Casanova, Lall & Gamperl, 2010). Pérez-Casanova *et al*. (2010) measured SDA in Atlantic cod and haddock (*Gadus morhua L. & Melannogramus*) in response to different dietary protein to lipid ratio at 2, 6, and 11°C. While diet did not affect the SDA response, temperature increased resting metabolism and total SDA. Notably, the researchers used isoenergetic diets with relatively small differences in macronutrient content (11 versus 16% lipid), and measured SDA on fish fed to satiation (i.e., meal size was not standardized to body mass; Pérez-Casanova *et al*., 2010).

While diet and temperature can separately alter the overall SDA response, it is difficult to resolve the mechanism underlying those differences or predict their interactive effects. The digestion and assimilation efficiency of specific nutrients/macronutrients can change with temperature in complex ways. For instance, Plasman *et al*. (2019) measured the overall SDA response in lizards (*Agama atra*) held at 20, 25, or 32°C. Consistent with known temperature effects, peak SDA increased and gut passage rates decreased at higher temperatures (although total SDA did not change). While total SDA was the same, protein

assimilation increased, and both lipid and protein were oxidized on different timescales, with proteins being metabolized first across temperatures (Plasman *et al*., 2019). In Clissold, Coggan & Simpson (2013), locusts (*Locusta migratoria*) had completely different patterns of macronutrient digestibility when fed kangaroo grass (*Themeda triandra*) or wheat seedlings (*Triticum aestivum*) after acclimation to 32 or 38°C. Carbohydrate absorption was higher at 32°C, while protein absorption was higher at 38°C, but only in locusts fed kangaroo grass. In wheat seed fed locusts, the ratio of carbohydrate to protein absorbed did not change with temperature.

We identified a small number of studies that measured diet by temperature effects on other measures of digestive physiology, with mixed results. For example, Castañeda *et al.* (2006) did not find a difference in intestinal morphology or multiple digestive enzymes (i.e., aminopeptidase-N and maltase) between Chilean Giant Frog tadpoles (*Caudiverbera caudiverbera*) fed rat chow or spinach. However, temperature altered digestive enzyme activity and higher temperatures were associated with shorter gut lengths compared to cold treated tadpoles (Castañeda *et al*., 2006). Similarly, inclusion of prebiotic fructooligosaccharides did not affect most digestive enzymes (e.g., lipase, a-Amylase) or gut morphology in Gilhead sea bream (*Sparus aurata*) held at 18 or 25°C, with an exception of an interactive effect of diet and temperature on total alkaline protease activity in the posterior gut (Guerreiro *et al.*, 2016). In contrast, Mazumder *et al.* (2018) found that diet (pellet vs. shrimp) and temperature had separate (but not interactive) effects on relative gut length and pepsin activity in malabar blood snapper (*Lutjanus malabaricus*), where pepsin activity was higher in the shrimp diet and relative gut length was lower. These results demonstrate how

complex the combined effects of diet and temperature are on digestive physiology, and how little we currently know about when and how interactions between the two manifest.

1.4.8 Limitations and future outlooks

Our search revealed that there is an urgent need for more research on diet and temperature effects on digestive physiology in ectotherms. A few considerations are listed below. First, some animals can supplement consumption with nutrient absorption directly from the environment (Blewett & Goss, 2017; Glover, Blewett & Wood, 2016). For example, some animals can absorb amino acids through their skin (Glover *et al*., 2016), or gills (Blewett & Goss, 2017), which should be considered within their energy budget across temperatures. Second, energetic investments in digestive tissue maintenance and function vary with diet strategy. For example, herbivores have longer guts than closely related omnivores, and carnivorous (Caruso & Sheridan, 2011; Horn, 1989). Gut morphology and surface area are also plastic traits that can change in response to both diet and temperature (Behrens & Lafferty, 2012; Mazumder *et al*., 2018). In herbivores, the cost of digestive tissue maintenance may be higher than that of carnivores. These hypotheses have yet to be tested but highlight avenues to explore. We should also pursue integrative studies that assess several measures of digestive physiology across levels of biological organization (from cellular to metabolism to growth). This will help to clarify diet and temperature effects on digestion, assimilation, and SDA relative to an individual's overall energy budget.

Ectotherms may also modify their diet selection or ingestion rate to ensure that their overall energy budget is not constrained. As temperatures warm, standard metabolic rate (i.e., the energy required for basic maintenance functions such as protein turn-over, respiration) typically increases exponentially while maximum metabolic rate (i.e., the peak metabolism

possible) increases to a point and then usually plateaus or declines. The difference between maximum and standard metabolic rate is termed the aerobic scope, which represents the aerobic capacity of an animal to perform fitness-enhancing tasks (e.g., locomotion, digestion, reproduction). During post-prandial metabolism, oxygen uptake increases until peak SDA (which scales with meal size). Any remaining aerobic scope during digestion is termed the post-prandial aerobic scope, and represents the remaining capacity of animals to allocate oxygen to other activities (e.g. exercise, vigilance against predators, foraging, etc). At suboptimal temperatures, an animal's aerobic scope can decrease, while simultaneously, it's energetic capacity to effectively digest/assimilate a meal and perform other fitness-enhancing activities becomes constrained. In this case, animals may consume smaller meals to conserve their energetic capacity for other fitness enhancing functions (Eliason, Van Wert & Schwieterman, 2022; Jutfelt *et al.*, 2021, Norin & Clark, 2017). Alternatively, a novel idea that remains to be explored is that under thermal stress, animals may alter their diet choices to less energetically expensive options (i.e., to reduce peak SDA) and conserve their post-prandial aerobic capacity. It should be noted, though, that 1) at extreme temperatures, animals often cease feeding entirely and 2) there are noTable exceptions to these ideas, like lionfish (*Pterois* sp.), which appear to be released from predation pressure and eat maximally even under thermal stress (e.g., Steell *et al.*, 2019).

1.4.9 Microbiome

The microbiome has wide-ranging effects on host physiology, with studies demonstrating a role for microbiota in host digestive performance, health, locomotory performance, cardiovascular function, growth, development, behavior, and even thermal tolerance (Fontaine, Mineo & Kohl, 2022; Henry & Colinet, 2018; Kohl & Carey, 2016;

Moeller *et al.,* 2020; Semova *et al*., 2012; Schretter *et al*., 2018). Ectotherm intestinal microbiomes are fascinating communities because they must tolerate the same changes in environmental conditions as their host (e.g., temperature, salinity), while simultaneously coping with variation in nutrient availability (i.e., host diet), and habitat structure (i.e., intestinal morphology). There is ample evidence that both temperature and diet can separately affect gut microbiome composition, but their combined effects remain poorly understood (Ayayee *et al*., 2020; Bestion *et al*., 2017; Fontaine, Novarro & Kohl, 2018; Piazzon *et al*., 2017; Ramsby *et al*., 2018).

Diet accounts for a significant proportion of observed variability in microbiome composition in wild animals (Clements *et al*., 2014; Kohl & Carey, 2016; Piazzon *et al*., 2017). Microbes can alter host physiology through metabolite signaling (i.e., SCFA's, amino acid derivatives, vitamins, etc.), and the activity of the microbiome and production of these molecules depends on host nutrition (Kohl & Carey, 2016). For temperature, ectotherm microbiomes are subject to the same temperature changes as their hosts, which can have profound impacts on microbial physiology and microbiome community composition and thus host physiology (Trevelline *et al*., 2019; Amphibian: Fontaine *et al*., 2018; reptile: Bestion *et al*., 2017; Moeller *et al.*, 2020; Sponge: Ramsby *et al*., 2018).

There is evidence that diet and temperature mediated changes in gut microbiome composition can have functional consequences on animal performance, although much of this evidence is correlative. For example, Ayayee *et al*. (2020) found that diet altered both standard metabolic rate and microbiome composition in cockroaches, with evidence that the microbiome's function also changed in response to diet. Aquaculture studies have demonstrated a more direct role of diet in modifying microbiome function (Piazzon *et al*.,

2017). For temperature, lizards (*Zootoca vivipara*) held under a natural or climate change scenario temperature treatment and found that increased temperature caused a 34% reduction in the gut microbiome's species diversity (Bestion *et al*., 2017). Notably, survival rates among the lizards over a year later were positively correlated with microbiome species diversity. Similarly, wild-caught western fence lizard (*Sceloporus occidentalis*) microbiome composition changed with experimental warming and microbiome composition was associated with heat tolerance in the lizards (Moeller *et al.,* 2020). Fontaine *et al*., (2018) found similar effects of increasing temperature on the gut microbiome of salamanders (*Plethodon cinereus*), where lower microbial diversity was observed at sub-optimal temperature treatments. For organisms like corals, whose health is reliant on microbial symbionts, temperature alters coral health directly through coral bleaching, where the coral rejects its symbiotic partners in response to elevated temperatures. Interestingly, Rosado *et al*. (2019) found that corals (*Pocillopora damicornis*) inoculated with beneficial microbes had lower levels of bleaching at high temperatures and reduced bleaching in response to pathogen challenge. Similarly, the aphid, Acyrthosiphon pisum, had thermal limits that were determined by the lethal temperature of their symbiotic *Buchnera aphidicola* (Dunbar *et al*., 2007).

Our search identified only three studies that explored the simultaneous effects of diet and temperature on microbiome composition. One study independently assessed diet and temperature effects on abalone (*Haliotis discus hannai and H. discus hannai × H. fulgens hybrids*) microbiomes (i.e., non-factorial design; Wang *et al*., 2020). The core microbiome in their study remained sTable, but there were some differences in microbiome composition in response to diet and temperature. In Guerreiro *et al.* (2016), dietary inclusion of

prebiotic fructo-oligosaccharides did not influence microbiome composition in Gilhead sea bream (*Sparus aurata*) held at 18 or 25°C. A third study assessed how dietary lipid composition and environmental temperature (20 and 26°C) impacted microbiome composition in yellowtail kingfish *(Seriola lalandi*) (Soriano *et al*., 2018). Diet and temperature interactively influenced microbiome composition, where the diet with the highest lipid content rescued the harmful effects that the lower (20°C) temperature treatment had on microbial diversity. It should be noted that the results of this study demonstrated a stronger effect of temperature than diet, but the authors argue that this was because their diet treatments were quite similar.

1.4.10 Limitations and future outlooks

If sub-optimal temperatures negatively affect microbiome composition and function and anthropogenic stress causes changes in food availability and quality, then the interaction of these two environmental stressors could be detrimental to animal health. It is essential that researchers continue to investigate how microbiomes are affected by a multi-stress environment, how these changes alter host physiology, and what we can do to mitigate these effects. Understanding the functional consequences of these community changes is technically challenging, especially in wild animals. However, researchers have been able to explore how changes in the microbiome impact wild animal physiology using some combination of metabolomics, microbiome manipulations, gnotobiotic animals, RNAseq, proteomics, metagenomics, imaging, and more. For example, recent studies by Kohl and coauthors using microbiome manipulations offer further evidence of the importance of the gut microbiome in ectotherm plasticity and diet and temperature mediating these changes (Fontaine *et al.*, 2022; Trevelline & Kohl, 2022). In one study, microbiome disruption

lowered critical thermal maxima and raised thermal minima in wild green frog tadpoles (*Lithobates clamitans*) (Fontaine *et al.*, 2022). In Trevelline & Kohl (2022) gut microbiome composition altered diet selection in mice (*Mus musculus*). These results highlight how the microbiome is a central player in ectotherm ecological physiology.

1.4.11 Quantity vs. Quality

Food restriction can change an animal's energy balance and nutrient assimilation, which may have downstream impacts on its environmental tolerance and plasticity. Increases in temperature may force ectotherms to use their diminishing nutrient supply on fueling their rising metabolic demand at the cost of maximizing other physiological functions (see section 1V: Digestive Physiology for more on this). Some animals may also behaviorally thermoregulate to compensate for food restriction by moving into cooler areas that reduce their metabolic demand; a response termed "behavioral hypothermia" (Rodgers *et al.*, 2019). These interactions may be further complicated by simultaneous changes in prey nutritional quality or an animal's diet selection. To limit the scope of this review, we did not include food ration as a factor in our literature search, however, it is essential to consider within the context of diet by temperature effects and thus, we highlight some noTable discussion points and examples below.

Food ration size can influence thermal tolerance (e.g., CT_{max}, cardiac breakpoint temperature), behavior (e.g., foraging time, behavioral thermoregulation), and the thermal sensitivity of several performance traits (e.g., growth, metabolism, cardiac performance, etc.). Some studies have shown reductions in thermal tolerance after food restriction (e.g., Lee *et al.*, 2016; Nyamukondiwa & Terblanche, 2009; Woiwode & Aidelman, 1992), and others have not found any influence (Lee *et al.*, 2016; Mclean & Todgham, 2015; Rodgers *et*

al., 2019). For sublethal traits, growth typically decreases with food restriction, and the optimal temperature for growth and thermal breadth is also expected to decrease when animals eat less (Brett, Shelbourn & Shoop, 1969; Brett, 1971; Huey & Kingsolver, 2019). Traits can be differentially influenced by food ration and temperature. For example, in larval white sturgeon (*Acipenser transmontanus*), growth was antagonistically influenced by cold water exposure and food restriction, while CT_{max} increased with acclimation temperature, but was unaffected by food ration (Rodgers *et al.*, 2019). Similarly, dungeness crabs (*Metacarcinus magister*) had lower growth but maintained cardiac thermal tolerance under food restriction across summer and winter water conditions (Mclean & Todgham, 2015). The extent to which food restriction impacts performance is likely influenced by some combination of the animal's energy stores, metabolic rate, ontogeny (actively growing or investing in reproduction), and the extent to which the trait matters for the animal's fitness.

Several behavioral responses are impacted by food restriction and temperature. For example, food restricted animals often decrease their preferred temperature, presumably to reduce their maintenance costs (e.g., Gilbert & Miles, 2016; relevant review: McCue, 2010). Additionally, in Lienart *et al.* (2014), juvenile damselfish (*Pomacentrus chrysurus*) did not respond to predator cues when exposed to simultaneous heat and food stress but showed risk-averse responses when food was abundant or in cooler water. This indicated that when conditions were stressful, the fish were more likely to put themselves at risk to meet their energy needs. In these cases, higher quality foods may rescue some of the negative impacts of food restriction on behavior.

1.5 Ecological and environmental consequences of changing diet with temperature

The interactions between the amount of food animals consume, the quality of that food, and environmental temperature can have significant impacts on ectotherm physiology, behavior, environmental tolerance, and ultimately, ecology. The extent to which these factors influence performance depends on how much ingestion is reduced, the exact nutritional differences in the diet(s), the animal's prior nutritional state, and the surrounding environmental conditions. In this section, we discuss some of the broader ecological impacts of these factors.

1.5.1 Species distributions and range shifts

As temperature profiles and environmental landscapes shift, previously occupied habitats may become unsuiTable, while others may become more favorable (Deutsch *et al.*, 2008; Pinsky, Comte & Sax, 2022). Species distributions have been evaluated through ecological and thermal biology lenses, which has offered insight into ectotherm biogeography (Deutsch *et al.*, 2008; 2015; Fredston *et al.*, 2021; Pinsky *et al.,* 2019; 2022; Sunday *et al.,* 2011; 2014; 2019). Physiological thermal tolerance and species interactions (e.g., competition, predator-prey) are both important for determining species range limits. Diet interacts with both axes – it can alter thermal performance and is dependent upon the species that can co-exist under those conditions. Thus, diet strategy and nutrition may also be critical factors determining a species success and range movements.

For example, consider the distribution of herbivorous and omnivorous fishes in the Northern hemisphere, which is characterized by high biodiversity (as measured by species richness) at the equator, but diminishes as latitude increases and water temperature decreases (González-bergonzoni *et al.*, 2012; Floeter *et al.,* 2005; Vejříková *et al*., 2016; for critique: Clements *et al*., 2009). One of leading explanations of this phenomenon is that cold water

limits herbivorous fish digestion efficiency (through limitations on their microbiome function) to the point where they are unable to meet their metabolic demand on a plant-based diet (temperature physiological constraint hypothesis or TPCH). TPCH suggests that as water warms, herbivorous fishes ranges may shift towards higher latitudes (i.e., following warmer water) and omnivorous fish may consume greater proportions of algae in their diets, which could dramatically alter subtropical and temperate ecosystems (Behrens *et al.*, 2007; Behrens & Lafferty, 2012; Floeter *et al.,* 2005; González-bergonzoni *et al.*, 2012; Vejříková *et al*., 2016).

This hypothesis has been debated, with critics arguing that fish in the southern hemisphere do not follow the same distribution pattern as those in the Northern hemisphere, (Clements *et al*., 2009). For example, the common herbivorous fish, *Odax pullus*, did not have diet choice or species distribution trends consistent with this hypothesis in the wild (Johnson *et al*., 2020; Trip *et al*., 2014). Additionally, a recent meta-analysis did not find support for TPCH in governing herbivorous fish digestion across temperatures (Knight *et al.,* 2021). These studies discuss alternative hypotheses, such as evolutionary constraints, that could also explain observed distribution patterns. Clearly, examining these mechanistic links is challenging and requires input from nutritional ecologists, evolutionary biologists, and comparative physiologists. While an active area of research, this example highlights how species distributions may be limited in some ways by their nutritional resources, digestive physiology, or environmental conditions.

1.5.2 Behavioral thermoregulation

When faced with unfavorable temperatures, ectotherms can acclimate (within-generation), use transgenerational plasticity (between generations), adapt (takes many

generations), or move to a more suiTable habitat (Hofmann & Togdham, 2010). Moving requires a heterogenous environment where preferred temperature(s) are accessible to the animal. There is evidence that some ectotherm's temperature preference changes when fed different diet treatments in the lab (Brzeziński & Von Elert, 2015; Clissold *et al*., 2013; Coggan, Clissold & Simpson, 2011). For example, Brzeziński & Von Elert (2015) demonstrated that certain *Daphnia* sp. only performed diel migrations from warm water (associated with higher predators) to cold water (associated with lower predators) when fed a diet supplemented with EPA, but not in response to cholesterol supplementation. Similarly, the lizard, *Tiliqua rugosa*, lowered its selected body temperature by 3-5°C after being fed a diet high in unsaturated fats (compared to lizards fed a diet high in saturated fats; Geiser *et al.,* 1992). Differences in lipid quality seem to affect behavioral thermoregulation in ways predicted by homeoviscous adaptation.

Other animals change their selected body temperature when fed diets that differ in macronutrient composition; presumably, to optimize their energetics. For example, Coggan *et al.* (2011) held locusts (*Locusta migratoria*) at 32°C and fed them diets consisting of equal protein:carbohydrate that differed in the total amount of protein and carbohydrate in the diet (from 15-42%) to vary the diet's energy density. As nutritional quality (i.e., higher gross amount of protein and carbohydrates, more energy available) increased, locusts preferred warmer temperatures that facilitated higher growth rates. In contrast, under nutrient limited conditions, the locusts chose cooler temperatures that conferred high assimilation efficiency but lower growth. In another study, locusts fed kangaroo grass had higher protein absorption in warm conditions but higher carbohydrate absorption at cold (Clissold *et al*., 2013). However, when the locusts were fed a different diet of wheat grass, their protein and

carbohydrate absorption did not change with temperature. When subsequently deprived of carbohydrates for a few days, locusts preferred colder temperatures (maximized carbohydrate absorption), but only after being fed kangaroo grass (Clissold *et al*., 2013). Locusts fed wheat seedling preferentially chose warmer temperatures, irrespective of prior nutritional state, which maximized their growth. These results indicate that locusts were able to alter temperature preference based on prior nutritional history to maximize performance on diets that varied in their digestibility (Clissold *et al*., 2013).

Most of the diet studies that measured behavioral thermoregulation are on terrestrial species. In heterogenous environments where the capacity for thermoregulation can be higher, selecting for the best temperature to maximize digestive energetics and growth may be more important than finding a diet that maximizes performance at a given temperature. In other words, there may be a greater incentive for generalist diet strategies in heterogenous thermal environments, although this idea has yet to be tested. Lipid quality, in particular, seems to mediate several ecologically important behaviors and species interactions, such as predator avoidance (Brzeziński & Von Elert, 2015) and behavioral thermoregulation (Geiser *et al.,* 1992) in ways that are consistent with homeoviscous adaptation. In contrast, ectotherms fed different macronutrient ratios appear to select body temperatures that optimize their energetics, with cooler preferred temperatures reducing growth but slowing metabolism, digestion and increasing or not changing assimilation efficiency (Clissold *et al*., 2013; Coggan *et al.*, 2011).

1.5.3 Aquaculture

A substantial portion of the diet by temperature literature is focused on aquaculture, as there is a vested interest in determining the best aquafeed ingredients to maintain optimal

feed conversion ratios, health, and growth under a changing climate. Aquaculture is an important and ever-growing food sector, but one that must deal with a variety of sustainability and logistical challenges. These challenges include, but are not limited to, disease, parasites, interbreeding with native fish, introductions as invasive species, depletion of forage fish for fishmeal and fish oil, and pollution (Froehlich *et al*., 2022; Jobling, 2016; Lafferty *et al*., 2015; Stentiford *et al.,* 2021). Each of these issues may be exacerbated by suboptimal nutrition and temperature.

One of the challenges to aquaculture of fed fish (i.e., animals requiring aquafeeds as opposed to filter feeding animals) is creating sustainable aquafeeds (Bowyer, Qin & Stone, 2013). Forage fish have been commonly harvested for fish meal and oil production, which is unsustainable as a primary protein source given the rising demand for farmed fish (Oyinlola *et al.*, 2021). Farmers are interested in finding alternative protein and fat sources to supplement forage fish. Over time, aquaculture feeds have shifted to include proportionally more terrestrial ingredients (e.g., soybeen, wheat, nuts, etc) which has effectively lowered the trophic level of farmed seafood relative to their wild counterparts over the last 20 years (Cottrell *et al.*, 2021). Plant-meal is generally lower in protein and lacking in the complete amino acid profile required for carnivorous fish; thus, these feeds require additional nutritional supplementation (Bowyer *et al*., 2013; Cottrell *et al.*, 2021). Any differences in nutrition of farmed vs wild fish may influence their plasticity and environmental tolerance. As discussed above, an ectotherm's optimal dietary macronutrient ratios may change with temperature, their dietary lipid composition can influence their thermal limits, and specific dietary amino acids and micronutrients may impact their thermal performance (see Section IV).

When considering the costs and benefits of alternative protein and fat sources in aquaculture, these interactions require close examination. For example, lipid composition generally differs between terrestrial plant and fish oil, with fish oil being higher in certain Omega-3 fatty acids (e.g., EPA and DHA). Further, some plants contain anti-nutrient compounds, some of which can be inactivated upon heating and will not be discussed in detail here (For comprehensive discussion of nutrition in aquaculture feeds, we recommend "Fish Nutrition" textbook; Jobling, 2016). Microbiome "engineering" and prebiotic feed additives may be able to enhance fish digestion efficiency of plant protein and lipid sources or improve their immune response, antioxidant capacity, and thermal tolerance at suboptimal temperatures (for review: Naiel *et al*., 2022). Farmers may also be able to adjust fish feed with the seasons to maximize thermal performance in farmed fish. Exploring these interactions is no small feat given the time and resources needed to undergo feeding and growth studies on farmed animals. Additionally, reliance on terrestrial crops has its own environmental costs that should be weighed against alternative ingredients (Cottrell *et al.*, 2021); although, the environmental impacts of feeding farmed seafood is estimated to be lower than their terrestrial counterparts (Froehlich *et al*., 2018). All of this requires further investigation, but ultimately could help advance sustainable aquaculture practices in a changing climate.

1.5 Concluding Thoughts

Ectotherm diets are changing due to anthropogenic stress, which profoundly impacts their thermal performance. When an ectotherm's food availability, food options or dietary nutritional quality change, the animal may respond by adjusting their diet or temperature

selection. Temperature may also directly impact an animal's nutritional needs, which could influence its consumption, foraging behavior, performance, and ultimately, it's ecological role. Our literature search revealed that diet quality (i.e., macronutrients, lipid composition, and micronutrients) can affect ectotherm thermal plasticity, thermoregulatory behavior, and thermal tolerance. Additionally, the microbiome is emerging as a critical player in thermal biology, digestive physiology, and diet selection. These findings underscore the importance of considering diet when evaluating how ectotherms will respond to temperature change. Given the broad implications of this topic and the limited amount of work that has been done, we strongly encourage future work examining diet effects on ectotherm thermal performance. By understanding when and why diet and temperature interact, we can make more accurate and far-reaching predictions across taxa. More broadly, diet may help or hinder animals' ability to tolerate several other anthropogenic stressors (e.g., salinity, desiccation, pH, hypoxia, pollution, etc.), and these interactions require further examination. This type of research requires extensive diet and nutritional information for wild animals, which is notoriously difficult to obtain. Thus, this effort will undoubtably involve close collaboration among physiologists and ecologists to identify species and environments of interest and to uncover the mechanisms driving observed ecological trends.

II. . Chapter 2;

2.0 Abstract

Thermal acclimation is a key process enabling ectotherms to cope with temperature change. To undergo a successful acclimation response, ectotherms require energy and nutritional building blocks obtained from their diet. However, diet is often overlooked as a factor that can alter acclimation responses. Using a temperate omnivorous fish, opaleye (*Girella nigricans*), as a model system, we tested the hypotheses that (1) diet can impact the magnitude of thermal acclimation responses and (2) traits vary in their sensitivity to both temperature acclimation and diet. We fed opaleye a simple omnivorous diet (*ad libitum Artemia* sp. and *Ulva* sp.) or a carnivorous diet (*ad libitum Artemia* sp.) at two ecologically relevant temperatures (12 and 20°C) and measured a suite of whole-animal (growth, sprint speed, metabolism), organ (cardiac thermal tolerance) and cellular-level traits (oxidative stress, glycolytic capacity). When opaleye were offered two diet options compared with one, they had reduced cardiovascular thermal performance and higher standard metabolic rate under conditions representative of the maximal seasonal temperature the population experiences (20°C). Further, sprint speed and absolute aerobic scope were insensitive to diet and temperature, while growth was highly sensitive to temperature but not diet, and standard metabolic rate and maximum heart rate were sensitive to both diet and temperature. Our results reveal that diet influences thermal performance in trait-specific ways, which could create diet trade-offs for generalist ectotherms living in thermally variable environments. Ectotherms that alter their diet may be able to regulate their performance at different environmental temperatures.

2.1 Introduction

Understanding the full range and maximum capacity of ectotherm physiological responses to environmental change is essential to predict species' vulnerability to global climate change (Huey *et al.*, 2012; Somero, 2011; Stillman, 2003). Temperature is a critical environmental factor governing ectotherm physiology, behavior and ecology (Brett, 1971). The current paradigm suggests that ectotherms have three options when faced with unfavorable temperatures: they can move to a more suiTable habitat, adapt over multiple generations or acclimate to the new conditions (Daufresne *et al.*, 2009; Glanville & Seebacher, 2006; Hofmann & Todgham, 2010; Somero, 2011). Thermal acclimation is an essential survival mechanism for ectotherms living in variable environments and a critical coping mechanism against global climate change (Bernhardt and Leslie, 2013; Jackson *et al.*, 2021; Seebacher *et al.*, 2015). During thermal acclimation, ectotherms undergo reversible phenotypic changes that improve their performance at a given temperature (Figure 2.1; e.g. enzyme activity, membrane composition, mitochondrial density, oxygen transport, organ morphology and function; Anttila *et al.*, 2014; Chung & Schulte, 2020; Ekström *et al.*, 2016; Little *et al.*, 2020a; Seebacher *et al.*, 2015). It is often assumed that ectotherms will achieve the same level of performance after repeated exposures to a temperature, as long as all other environmental conditions (i.e. salinity, pH, dissolved oxygen) are held the same (Sinclair *et al.*, 2016). To undergo a successful acclimation response, ectotherms require energy and nutritional building blocks obtained from their diet. Diets vary considerably in nutritional and energetic content, which suggests that different diets may mediate distinct thermal acclimation responses (Figure 2.1).

Food quality and availability can change seasonally, with global climate change and across habitats (Alton *et al.*, 2020; Arnold *et al.*, 2010; Birnie-Gauvin *et al.*, 2017; Ho *et al.*, 2010). Many ectotherms are also generalists and vary their diet to meet their nutritional requirements or maximize energy-use efficiency (Jobling, 2016; Johnson *et al.*, 2017; Kaiser & Hughes, 1993; Raubenheimer *et al.*, 2005; Rubio *et al.*, 2003, 2009; Sánchez-Vázquez *et al.*, 1998). Some ectotherms also change their diet with temperature (Boersma *et al.*, 2016a; Carreira *et al.*, 2016; Jang *et al.*, 2015; Rho and Lee, 2017; Schmitz and Rosenblatt, 2017; Vejříková *et al.*, 2016). For example, multiple omnivorous fishes consume higher proportions of algae as water temperatures increase (e.g. Behrens & Lafferty, 2012; Emde *et al.*, 2016; González-Bergonzoni *et al.*, 2016; Guinan *et al.*, 2015; Prejs, 1984). The exact reasons for these diet shifts are unknown, with some suggesting that the optimal dietary protein to carbohydrate ratio for ectotherms differs across temperatures (Lee *et al.*, 2015; Rho & Lee, 2017; Zhang *et al.*, 2020), or that cold temperature constrains the digestive physiology of herbivores and omnivores (Floeter *et al.*, 2005; González-Bergonzoni *et al.*, 2012). These proposed explanations hint at a broader hypothesis: that omnivores consume different proportions of plants and animals to regulate their physiological responses to changing temperatures. More broadly, any changes in an ectotherm's diet that coincide with a change in environmental temperature (through differences in dietary preference, availability or nutrient composition) might alter its thermal performance.

To understand the interaction between diet and temperature, we must consider how traits critical to survival may be differentially affected (Figure 2.1). Measuring thermal limits in conjunction with vital biological rates provides comprehensive insight into ectotherm thermal biology in variable and changing environments (Magozzi & Calosi, 2015). A

common assumption in thermal biology is that biological rates have the same thermal sensitivity and that aerobic capacity and baseline metabolism can be used as proxies for many performance traits (Fry, 1947; Brett, 1971; Claireaux and Lefrançois, 2007; Pörtner, 2001, 2010; for a critique, see Clark *et al.*, 2013; Schulte, 2015). However, there is growing support for a multiple-performance, multiple-optima model, which states that thermal sensitivity differs across biological rates (i.e. absolute aerobic scope, standard metabolic rate, growth rate, sprint speed) and is not always predicTable based on aerobic capacity or baseline metabolism (Clark *et al.*, 2013; Dell *et al.*, 2011; Kellermann *et al.*, 2019; Seebacher *et al.*, 2015). For example, Healy and Schulte (2012) demonstrated that specific growth rate was negative at temperatures where absolute aerobic scope (i.e. maximum−standard metabolic rate) was maximal in killifish. This model has been challenging to test empirically as few performance traits are usually measured per study and these traits are often considered separately from upper and lower thermal limits (Magozzi & Calosi, 2015). If diet and temperature together influence biological rates and thermal limits, ectotherms may be incentivized to make diet choices that improve their thermal responses. However, if fitness-enhancing traits are differentially affected by diet and temperature, there could be important performance consequences associated with an ectotherm's ultimate diet choices.

Opaleye (*Girella nigricans*) are temperate omnivorous fish that consume a greater proportion of algae in warmer water across their geographic range (Behrens and Lafferty, 2012), which makes them an ideal model for exploring diet effects on thermal acclimation responses. Here, we tested the hypothesis that when offered a simple choice omnivorous diet (*ad libitum Artemia* sp. and *Ulva* sp.) versus a carnivorous diet (*ad libitum Artemia* sp. only) at two ecologically relevant temperatures (12 and 20°C), opaleye would make diet choices

that altered their thermal acclimation responses in trait-specific ways. As juveniles, opaleye live in the intertidal zone where they face many challenges, including escaping predators, maintaining growth rates and dealing with high daily thermal variation (Somero, 2010). Therefore, we adopted an integrative approach and assessed the opaleye's thermal acclimation responses at the whole-animal (growth, sprint speed, metabolism, critical thermal limits), organ (cardiac thermal limits) and cellular (glycolytic capacity, oxidative stress) levels to compare ecologically and physiologically relevant performance traits and thermal limits for the fish in their juvenile life stage and identify any trade-offs associated with the treatment diets. We hypothesized that biological rates would increase with temperature but have different thermal sensitivities depending on the diet treatment. Specifically, we predicted that there would be costs to consuming the omnivorous diet (e.g. higher digestive infrastructure costs resulting in higher maintenance metabolism or reduced growth from lower protein diet) that would be offset by increases in the performance of other traits (e.g. thermal limits, sprint performance, glycolytic capacity, oxidative stress).

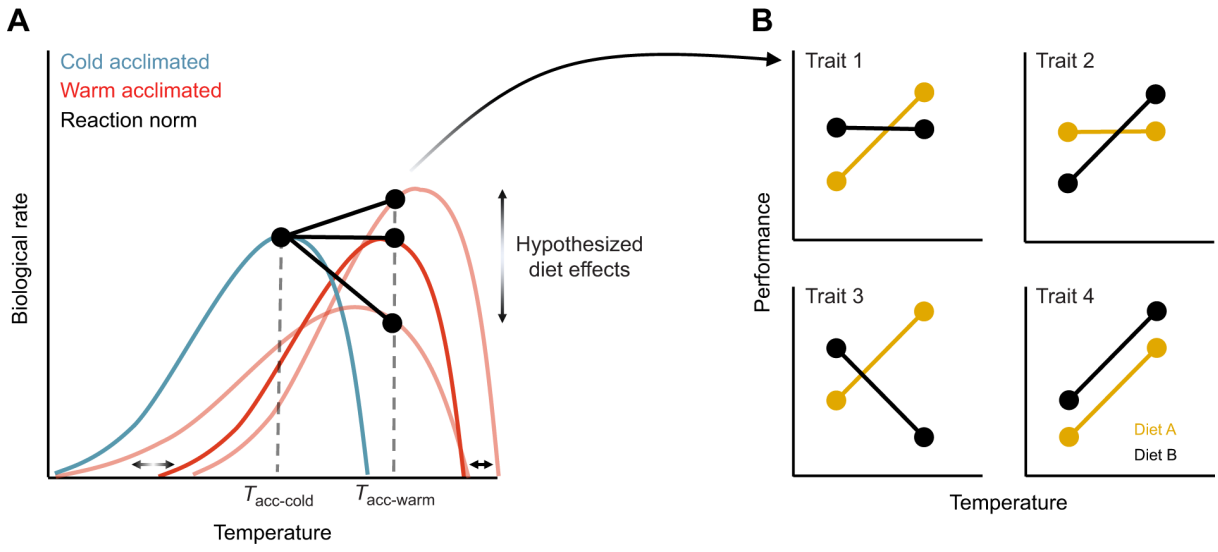

Figure 2.1 Conceptual graphs illustrating how diet may affect thermal acclimation responses and how those effects could be trait specific. (A) Diet can affect thermal performance. The graph shows how acute thermal performance curves (TPC) shift towards the acclimation

temperature (T_{acc}), where blue is the acute TPC after acclimation to cold and red is the acute TPC after acclimation to warm conditions. Note that both cold and warm acclimation may be affected by diet, but only potential effects of warm acclimation are displayed for simplicity. Diet may influence the shape (height and breadth) of those acute TPC (indicated by pink curves) or the location of the curve along the x-axis (i.e. temperature of peak performance). These effects could influence the slope of the line between reaction norms (performance at acclimation conditions, indicated by black lines). (B) Diet and temperature may interact and have trait-specific effects. The graphs are a series of hypothetical reaction norm plots for various traits. These traits can have different diet and temperature sensitivities, which could create performance trade-offs for ectotherms consuming different diets.

2.2 Methods

2.2.1 Fish collection

Juvenile opaleye, *Girella nigricans* (Ayres 1860), were collected in spring 2019 [experiment 1: respirometry, sprint, growth, critical thermal maxima (CT_{max}/CT_{min}); *N*=144, mean±s.d. body mass (BM) 14.75±3.53 g and total length (TL) 9.42±0.76 cm] and in winter 2020 (experiment 2: Arrhenius breakpoint test; *N*=126, BM 19.5±6.1 g and TL 10.5±1.1 cm) by seine or hook and line from Santa Barbara Harbor, CA, USA (34.40829, −119.691389). Fish were transported in coolers (>70% air saturation) to the University of California, Santa Barbara and held in 95 l fiberglass flow-through seawater tanks (9–12 fish per tank). Prior to the start of acclimation, fish were held at ambient conditions (mean±s.d. experiment 1: 13.9±1.1°C and experiment 2: 16.2±0.6°C) and fed *ad libitum* omnivorous diets (*Ulva* sp. and *Artemia* sp.). All protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara.

2.2.2 Acclimation and diet treatments

Fish were randomly assigned to one of two ecologically relevant temperatures (12 and 20°C, representative of the low and high seasonal temperatures experienced in Santa Barbara, CA, USA; Figure 2.2) and fed one of two *ad libitum* diets (omnivorous: *Artemia* sp.

and *Ulva* sp.; and carnivorous: *Artemia* sp.) in a factorial design with 3–4 replicate tanks per treatment. *Ulva* sp. (collected by hand from Goleta Beach, Goleta, CA, USA) and *Artemia* sp. (brineshrimpdirect.com) were replaced every morning. Diets were selected based on stomach content information from Barry and Ehret (1993), which demonstrated that both *Ulva* sp. and small crustaceans constitute a significant portion of wild opaleye diets, and Behrens and Lafferty (2007), which used *Ulva* sp. as a representative herbivorous diet for a lab study on opaleye. Preliminary data demonstrated that *Ulva* sp.-only diets were not nutritionally sufficient. Dietary proximate analysis is provided in Table S2.1.

Food consumption rates were assessed during preliminary trials. All tanks were fasted for 24 h, and then the fish were offered their pre-weighed diet treatment for 1 h. The remaining *Artemia* sp. and *Ulva* sp. were removed and weighed. However, we were unable to obtain any measurable estimates of *Ulva* sp. consumption during these brief 1 h trials. At 20°C, *Artemia* sp. consumption was ~23% lower in the omnivorous treatment (7.39% body mass) than in the carnivorous treatment (9.58% body mass), suggesting that the fish in the omnivorous treatment were supplementing their diet with *Ulva* sp. This is consistent with our visual observations that the fish readily consumed *Ulva* sp. in the warm treatment (though they consistently ate more *Artemia* sp. compared with *Ulva* sp.). In contrast, at 12°C, *Artemia* sp. consumption was only ~10% lower in the omnivorous treatment (3.65% body mass) compared with the carnivorous (4.07% body mass) and we did not observe the fish consuming *Ulva* sp. at this temperature. This suggests that the fish either were not consuming *Ulva* sp. or were doing so in small amounts at the cold acclimation treatment. Further, opaleye ate less at 12°C compared to 20°C. Overall, *Artemia* sp. consumption was ~55% lower in the cold treatment compared with the warm.

Temperature and dissolved oxygen content were monitored 1–2 times daily by hand using a Digi-sense Traceable Singe RTD thermometer (Cole Palmer, IL, USA) and an OxyGuard handy Polaris 2 dissolved oxygen meter (OxyGuard International A/S, Farum, Denmark). Oxygen was maintained at >80% air saturation throughout the study. The average temperature per treatment was 20.0±0.4 and 12.2±0.4°C in the two experiments (mean±s.d.; determined from in-tank Thermochron 4 K iButtons programmed to record every 20 min). Fish were acclimated to treatment conditions for 3 weeks prior to experimentation (14 h:10 h light:dark cycle). All individuals were fasted for 36–40 h prior to respirometry, thermal limit and sprint testing. All tests were performed at acclimation temperatures unless otherwise noted.

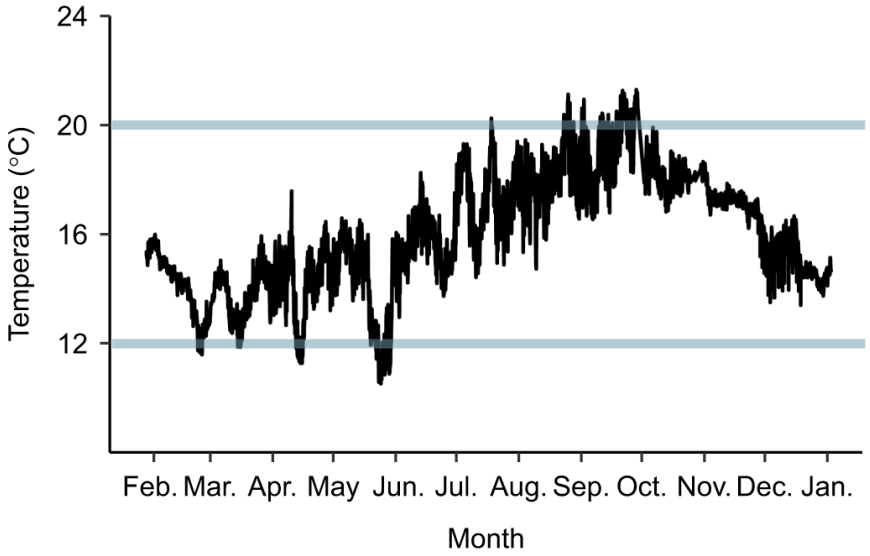

Figure 2.2 Temperature data collected in 2019 at Stearns Wharf in Santa Barbara, CA, USA (located next to Santa Barbara Harbor, where fish were collected). The temperature sensor was mounted at 2 m depth and set to record every 4 min. Blue lines indicate treatment temperatures (12 and 20°C). Data source: Washburn, 2021 and Southern California Coastal Ocean Observing System (SCCOOS; https://www.sccoos.org/data/autoss/timeline/?main=single&station=stearns_wharf).

2.2.3 Intermittent flow respirometry

Respirometry was conducted using 12 respirometers in one of three sizes: 349, 579 and 711 ml. Water was flushed and recirculated through the chamber at a rate of 2.5 l min^{-1} (Eheim Universal 300 pumps, Eheim, Germany). Dissolved oxygen was measured continuously in each respirometer using a robust oxygen probe and Firesting optical oxygen meter (Pyroscience, Germany).

Fish were transferred with minimal air exposure (<10 s) to a cylindrical chase tank (20 l), where they were chased by hand for 5 min and then immediately placed in the respirometers to obtain an estimate of their maximum metabolic rate (MMR) (this chase protocol was the most effective at eliciting MMR in opaleye; data not shown). Chases occurred between 10:30 h and 14:30 h and were followed by ~20 h of automated measurement cycles (15 min total flush/recirculation cycle). Tanks were covered in shade cloth to minimize potential disturbance. Fish were held at either 20.1±0.6 or 11.8±0.4°C (mean±s.d.) across all tests. Background respiration was measured before and after each test for ≥3 full measurement cycles.

After 20 h in the respirometers, fish were removed and anesthetized in 80 mg l^{-1} MS-222 buffered with 80 mg l^{-1} $NaHCO_3^-$ (Sigma Aldrich Co., St Louis, MO, USA). Each fish was weighed (mass in g), measured for TL, and tagged with a unique color code using Visible Implant Elastomer Tags (Northwest Marine Fisheries Inc., Seattle, WA, USA).

2.2.4 Data analysis for respirometry data

All oxygen consumption data were analyzed in R (version 3.5.1) using best practices as outlined in Rosewarne *et al.* (2016) and Chabot *et al.* (2016b) (http://www.R-project.org/). The data were used to calculate four metabolic rate metrics which define an individual's aerobic energy budget. Standard metabolic rate (SMR) is the baseline metabolic rate needed

to survive, and MMR is the maximum rate of energy expenditure. The difference between these two metabolic rates is the absolute aerobic scope (AAS=MMR−SMR), which is representative of the aerobic energy budget a fish has to perform all critical biological processes (Clark *et al.*, 2013). Factorial aerobic scope (FAS=MMR/SMR) is another estimate of the aerobic energy budget and represents the scope for increasing metabolic rate proportional to SMR (Clark *et al.*, 2013). Only fish for which >75% of measurements followed a linear decrease with R^2>0.9 were included in SMR analysis (i.e. >60 $\dot{M}_{O2}$ measurements total per fish; N=6–10 per treatment). SMR was calculated as the lowest 15% quantile of all recorded measurement cycles (Chabot *et al.*, 2016b). MMR was calculated as the steepest 120 s slope during the first measurement period (Little *et al.*, 2020b; N=6–11 per treatment). All presented metabolic rate measurements are body mass specific (i.e. presented as mg O_2 kg^{-1} min^{-1}). We tested for the need to account for body size scaling but given the small size range in the study (8–39 g), we did not find evidence of a scaling relationship when the data were plotted on log–log plots (data not shown). Given the lack of knowledge on a species-specific scaling exponent, we did not scale the metabolism to a common body size using an allometric scaling slope. Background respiration was assessed and, when applicable, a linear regression was fitted between the pre- and post-background measurements and subtracted from the corresponding $\dot{M}_{O2}$ values (Rodgers *et al.*, 2016; Rosewarne *et al.*, 2016).

2.2.5 Sprint speed

We used a modified protocol based on Kraskura & Nelson (2018). The setup included a custom-built acrylic sprint chamber (128 cm×30 cm×30 cm, L×W×H; water height 10 cm; water volume 41.6 l) with a camera (Canon EOS Rebel T4i) positioned above the sprint

chamber to ensure full view of the work area. Each sprint trial was recorded at 60 frames s^{-1} and saved for digital analysis. Fish were placed behind a gated and shaded area in a sprint chamber and habituated to the chamber for 10 min. The gate was removed and the fish were motivated to sprint by manual chasing. Each fish performed ≤7 trials with 5 min of recovery between trials. Only trial videos where the fish had undergone a sprint with (1) a straight path of >20 cm, (2) an unobstructed view of 50 cm of the chamber, (3) active and continued bursts, and (4) no back and forth swimming around the chamber were used in subsequent analysis. Temperature was monitored throughout all trials and remained within ±1°C of the test temperature. All videos used in analysis were visualized and validated by ≥2 researchers. Videos were tracked in ImageJ/MtrackJ and subsequently analyzed in R to determine the fastest 5 frames of sprinting (~10 cm) per trial; 2–3 trials were analyzed per fish and averaged to determine each fish's sprint performance (cm s^{-1}) (Figs S1 and S2; *N*=8–10 per treatment). All fish were given at least 3 weeks of recovery between respirometry or thermal limit experiments and the sprint test.

2.2.6 Thermal limits: CT_{max} and CT_{min} testing

We used a standardized critical thermal maxima and minima tests (CT_{max} and CT_{min}, respectively) as described in Beitinger & Lutterschmidt (2011). CT_{max} and CT_{min} represent the most extreme temperatures a fish can survive at for a short period of time. Briefly, 3–4 fish per tank were transferred to the testing tank (78 cm×61 cm×18 cm, L×W×H; water height 12 cm, water volume 57 l) at their treatment temperature with minimal air exposure (<10 s; total *N*=8–12 per treatment). After a brief habituation period (~5 min), the test tank was heated (CT_{max}; 1500 W immersion heater, Process Technology, Willoughby, OH, USA) or cooled (CT_{min}; 1 HP AquaEuro chiller, AquaEuroUSA, Los Angeles, CA, USA) at a rate

of ~0.3°C min^{-1} until fish lost the ability to maintain their righting response. Temperature was recorded at the loss of equilibrium and the fish were immediately placed in a recovery tank at an intermediate temperature before being returned to their treatment tanks. All fish fully recovered following the critical thermal tests.

2.2.7 Growth

All fish were weighed and measured for length prior to acclimation and again after 8 weeks under treatment conditions (note that some had been randomly removed for tissue sample collection, see below) to estimate growth across tanks (i.e. average weight gain per week).

2.2.8 Dissections and frozen tissue assays

Fish from each treatment (*N*=6 per treatment) were euthanized by cerebral concussion followed by severing of the spinal cord on day 27–34 of acclimation after the aerobic scope trials were complete. Morphometrics (body mass and TL) were measured, and a white muscle and liver sample were flash frozen in liquid nitrogen and stored at −80°C for future analysis. Stomach and remaining gut contents were emptied and leftover fish remains were stored at −20°C for proximate analysis (Table S2.1).

For proximate analysis, frozen fish remains were homogenized using a Fisher Brand Bead Mill 24 and subsamples of the homogenate were weighed and freeze dried (Labconco Lyophilizer). *Protein:* Protein content was estimated in triplicate (intra-assay CV% <10%) using a BCA assay with a 72% TCA precipitation (Pierce BCA kit, ThermoFisher Scientific, MA, USA), where absorbance was measured at 562 nm. *Lipids:* Lipid content was estimated using a chloroform:methanol extraction as described in Mann & Gallager (1985) and Johnson

et al. (2017). Lipids from 50 mg of freeze-dried homogenized sample were extracted using 100 ul milliQ water and 1.5 ml chloroform:methanol (1:2) (vortexed, incubated at 4°C, centrifuged at 4000 rpm for 5 min). The supernatant was removed and remaining sample was re-extracted in 1.5 ml chloroform:methanol (2:1). The supernatants were pooled, mixed with 950 ul NaCl (0.7%), incubated at 4°C for 30 min, then centrifuged (4000 rpm, 5 min), and the volume of the bottom layer was measured. Dried subsamples of the bottom layer were used to extrapolate lipid content to the entire sample. *Ash Content:* Ash content was determined by drying freeze-dried samples overnight at 100°C to account for any moisture that returned during sample storage. Samples were then weighed (~30 mg) before being combusted in a muffle furnace at 450°C for 12 h and then re-weighed.

2.2.9 Lactate dehydrogenase activity

Lactate dehydrogenase activity assays were performed as outlined in Little *et al.* (2020a) as a proxy for glycolytic capacity in white muscle, where high levels of lactate dehydrogenase activity indicate that the animal has a high capacity to support anaerobic ATP production to fuel burst swimming or hypoxia tolerance (Little *et al.*, 2020a). Briefly, white muscle samples were homogenized in homogenization buffer (0.1% Triton, 50 mmol l^{-1} Hepes, 1 mmol l^{-1} EDTA, pH 7.4 buffer) before being run in triplicate (intra-assay coefficient of variation, CV <15%) on a SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices) using a wavelength of 340 nm to measure the disappearance of NADH. The assay was repeated at 8, 12, 20, 26 and 32°C.

2.2.10 lipid peroxidation assay

Thiobarbituric acid reactive substances were quantified in the liver to estimate lipid peroxidation as a proxy for oxidative stress using a commercially available fluorometric assay kit (Cayman Chemical). Here, higher levels of lipid peroxidation are indicative of greater oxidative damage to cellular components (Castro *et al.*, 2012). Samples were homogenized in RIPA buffer and treated according to the manufacturer's instructions before being run in duplicate at an excitation wavelength of 544 nm and emission wavelength of 590 nm.

2.2.11 Thermal limits: Arrhenius breakpoint temperature test

Arrhenius breakpoint temperature (ABT) tests on the heart were conducted (N=6–14 per test and per treatment) as outlined in Casselman *et al.* (2012) and Gilbert *et al.* (2020). Briefly, fish were anesthetized in seawater containing 80 mg l^{-1} MS-222 buffered with 1 g l^{-1} $NaHCO_3^-$ before being placed ventral side up in an experimental sling in the test tank (10 l seawater containing buffered 65 mg l^{-1} MS-222). Water was circulated past the gills to irrigate them. Stainless Steel Needle Tip Electrodes (ADInstruments Inc., Colorado Springs, CO, USA) were inserted just under the skin to detect an ECG signal, which was amplified using a Dual Bio Amp amplifier (ADInstruments Inc.) and filtered (filters: 60 Hz Notch filter; mains filter; low pass: 2 kHz; high pass: 10 Hz; range: 2 mV).

After a 30 min equilibration period at the acclimation temperature (Ferreira *et al.*, 2014; Hansen *et al.*, 2017), atropine sulfate was injected intraperitoneally (1.2 mg kg^{-1} in 0.9% NaCl) to block vagal tone followed by isoproterenol (4 μg kg^{-1} in 0.9% NaCl) to maximally stimulate β-adrenoreceptors. Any fish that did not respond to the drug injections or for which experimental error occurred (e.g. water pump failure) were removed from the analysis and not considered further. These drug concentrations were tested prior to

experimentation to ensure doubling the concentration did not further increase heart rate (f_H; beats min^{-1}). Fifteen minutes after isoproterenol injection, water temperature was heated (warm ABT test) or cooled (cold ABT test) at 1°C every 6 min (Polystat recirculating heater/chiller; Cole-Palmer, Vernon Hills, IL, USA). At each 1°C interval, f_H and temperature were stabilized to record a value for f_H. This procedure was repeated until the onset of cardiac arrythmia (T_{arr}), as indicated by a transition from rhythmic to arrhythmic beating or a missed QRS peak resulting in a precipitous drop in f_H (Casselman *et al.*, 2012) or until the known average CT_{max} for the species (generally 0–5°C higher than T_{arr}; Chen *et al.*, 2015; Muñoz *et al.*, 2014; Safi *et al.*, 2019), to ensure that curves could later be fitted to the data for comparisons of acute thermal performance curves across treatments. All fish were immediately euthanized at the end of the test.

2.2.12 Data analysis for ABT tests

All ECG analyses were performed in LabChart software (www.adinstruments.com). f_H was calculated for each temperature increment from 15 continuous seconds of measurements using automated ECG analysis software in LabChart (Gradil *et al.*, 2016). The heart may set upper thermal tolerance temperatures in fish (Anttila *et al.*, 2014; Muñoz *et al.*, 2014). The warm ABT test measures three sublethal thermal limits on cardiac function (T_{AB}, T_{peak}, T_{arr}). Each of these limits indicates transition temperatures where the heart's capacity to transport oxygen, nutrients and immune cells becomes compromised (Anttila *et al.*, 2014; Muñoz *et al.*, 2014). The first thermal limit (T_{AB}) is highly correlated with the thermal optimum for aerobic scope in other teleosts (Anttila *et al.*, 2013; Ferreira *et al.*, 2014). T_{AB} was calculated by performing ABT tests on the rising phase of the thermal performance curve for f_H using *segmented* (v1.1-0; Muggeo, 2008) in R. The

temperature corresponding to the breakpoint in f_H was defined as T_{AB} (warm ABT test) or $T_{AB\text{-}cold}$ (cold ABT test). Overall maximum heart rate ($f_{H,max}$) was defined as the highest f_H recorded during the 15 s measurement phases in the warm ABT test and minimum heart rate ($f_{H,min}$) was defined as the lowest f_H recorded during the 15 s measurement phases during the cold ABT test. Peak temperature (T_{peak}) was the temperature corresponding to $f_{H,max}$.

2.2.13 Statistical analysis

All data were statistically analyzed using R (version 3.5.1). All metrics were investigated for normality using Shapiro–Wilk tests and quantile–quantile plots, and for heteroscedasticity using Levene's test. Data that were not normally distributed were log-transformed before statistical analysis (only FAS). Data are displayed with untransformed values. All data were statistically analyzed (significance level α=0.05) using a 2-way ANOVA (*Car* v3.0-2; Fox & Weisberg, 2011) with *post hoc* Tukey HSD, except for the thermal limits from the cold ABT, which were analyzed using a *t*-test. Differences between treatments were also assessed using Cohen's *D*-tests (*effsize*; https://CRAN.R-project.org/package=effsize). Note that 12°C fish were not tested for the cold ABT because of complications surrounding COVID-19 forcing the early shutdown of the experiment. In all 2-way ANOVA tests, significance of interaction between diet and temperature was tested for and excluded when non-significant. Polynomial curves were fitted to f_H data and compared using Akaike information criterion (AIC), where the fit with the lowest AIC score was assigned the best fit model, but all models with ΔAIC<2 were considered (Burnham and Anderson, 2002). Measures of thermal sensitivity for all biological rates were calculated for each diet treatment using Q_{10} values, where:

$$Q_{10} = \frac{R_2}{R_1}^{(10/(T_2-T_1))},$$

R_1 is the treatment mean at 12°C, R_2 is the treatment mean at 20°C, T_1 is 12°C and T_2 is 20°C.

2.3 Results

2.3.1 Metabolic rates

Metabolism was influenced by diet and temperature, but each metabolic rate responded differently (Figs 3 and 4). SMR was 28% higher in the 20°C omnivorous diet treatment compared with the carnivorous diet treatment, resulting in a significant interaction between diet and temperature (Figure 2.3A, Table 2.1). This was further supported by a large effect size between the 20°C treatments (Cohen's *D*-test). In contrast, MMR significantly increased with acclimation temperature, but did not differ across diet treatments (Figure 2.3B,C, Table 2.1). There was a marginal, but not significant diet and temperature effect on AAS (Figure 2.3C, Table 2.1). This was likely driven by the high individual variability in MMR, as the effect of diet on AAS had a *P*-value of 0.087 and medium effect sizes between diet treatments at 20°C. In contrast, FAS in the 20°C omnivorous diet treatment was significantly lower than that in the carnivorous diet treatment (diet: d.f.=1, F=5.796, P=0.023), which was largely driven by a 44% higher FAS (with a large effect size) in the carnivorous versus omnivorous diet (Figure 2.3D, Table 2.1).

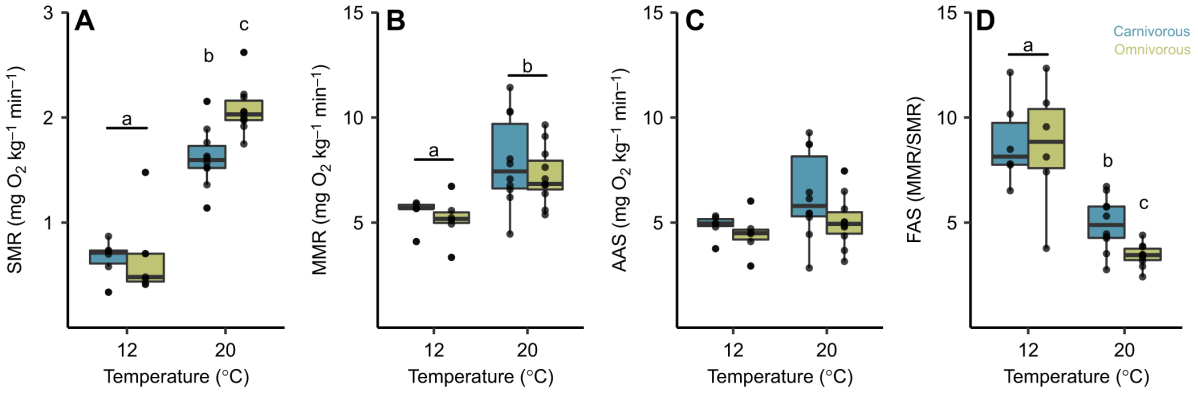

Figure 2.3 Oxygen uptake rate (mg O_2 kg^{-1} min^{-1}) after an exhaustive chase protocol in opaleye acclimated to 12 or 20°C and fed either a carnivorous or omnivorous diet. (A) Standard metabolic rate (SMR), (B) maximum metabolic rate (MMR), (C) absolute aerobic scope (AAS; MMR−RMR) and (D) factorial aerobic scope (FAS; MMR/SMR). Blue, carnivorous diet (Artemia sp.); green, omnivorous diet (Artemia sp. and Ulva sp.). Lowercase letters indicate significant differences ($P<0.05$) between treatment groups where applicable (see Table S2.1 for 2-way ANOVA outputs). Boxplots represent interquartile ranges (boxes and whiskers), median values (solid lines) and outliers (>1.5 beyond interquartile range) plotted as data points outside the whiskers.

Table 2.1 Summary statistics for biological rates

		Results				Statistical parameters								
		Carnivorous		Omnivorous		Diet			Temperature			Diet×Temperature		
Biological rate	Temp. (°C)	*n*	Mean±s.e.m.	*n*	Mean±s.e.m.	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
SMR (mg O_2 kg^{-1} min^{-1})	12	6	0.66±0.07	7	0.67±0.14	1	8.577	0.007	1	147.093	<0.001	1	5.282	0.029
	20	10	1.62±0.09	10	2.08±0.07									
MMR (mg O_2 kg^{-1} min^{-1})	12	6	5.49±0.28	6	5.15±0.45	1	1.061	0.311	1	16.542	<0.001			
	20	10	7.89±0.69	11	7.23±0.40									
AAS (mg O_2 kg^{-1} min^{-1})	12	6	4.84±0.23	6	4.46±0.41	1	3.131	0.087	1	3.656	0.066			
	20	10	6.27±0.66	10	5.05±0.40									
FAS (MMR/SMR)	12	6	8.81±0.83	6	8.65±1.21	1	5.796	0.023	1	52.242	<0.001			
	20	10	4.94±0.41	10	3.43±0.17									
Growth (g $week^{-1}$)	12	3	0.03±0.19	3	0.08±0.01	1	0.003	0.958	1	23.498	<0.001			
	20	3	0.75±0.15	3	0.69±0.16									
Sprint speed (cm s^{-1})	12	10	112.36±4.88	9	110.85±5.26	1	0.099	0.756	1	0.456	0.504			
	20	8	116.10±5.46	8	114.26±6.01									

Means±s.e.m. for each test group and ANOVA results are presented. SMR, standard metabolic rate; MMR, maximum metabolic rate; AAS, absolute aerobic scope; FAS, factorial aerobic scope; Temp., temperature, d.f., degrees of freedom.

2.3.2 Other biological rates and traits

Growth rate, sprint speed, lipid peroxidation and lactate dehydrogenase activity were inconsistently affected by diet and temperature (Figure 2.4). Growth was significantly higher at 20°C but did not differ across diets (Figure 2.4, Table 2.1; Figure S2.2). Unexpectedly,

the Q_{10} value for growth was higher than that for all other rates (28.05) and not close to any of the Q_{10} values for metabolic rate (range 1.28–4.12; Figure 2.4, Table 2.1; Figure S2.2). Proximate analyses for whole tissue did not differ between treatment groups (Table S2.1).

In contrast with growth, maximum sprint speed did not differ in response to thermal acclimation (Q_{10}=1.04) or diet treatment (Figure 2.4, Table 2.1; Figs S2.1 and S2.2). Similarly, lipid peroxidation in liver tissue did not differ in response to temperature and showed a marginal but insignificant effect of diet (Figure 2.4; Figure S2.2; temperature: d.f.=1, F=0.318, P=0.579; diet: d.f.=1, F=3.260, P=0.085). Lactate dehydrogenase activity in white muscle was moderately affected by temperature acclimation (Q_{10}=2.10), being higher at 20°C compared with 12°C, but did not differ across diets (Figure 2.4; Figure S2.3). Lactate dehydrogenase activity also increased with acute temperature exposure (Figure S2.3). Overall, Q_{10} values for the reaction norms differed dramatically across biological rates, with sprint speed having a Q_{10} of 1.04 (insensitive to temperature), while growth rate had a Q_{10} of 28.05 (highly sensitive to temperature) (Figure 2.4).

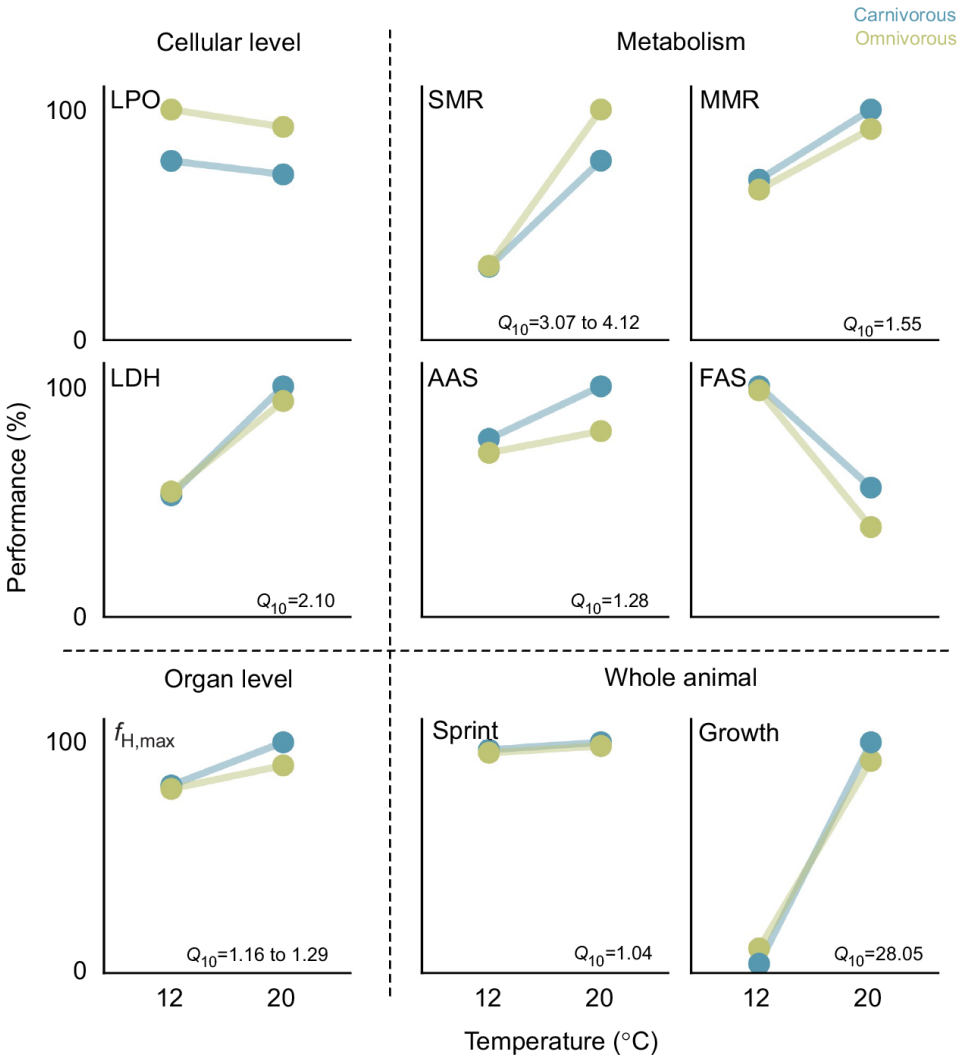

Figure 2.4 Reaction norms plotted across all measured biological rates and traits. Average data from each treatment, scaled to the maximum average treatment value (i.e. the maximum treatment value is equal to 100%). All values are from fish tested at their acclimation temperature. Graphs are arranged by level of biological organization (cellular, organ, whole animal) and labelled by trait: SMR, MMR, AAS (MMR–SMR), FAS (MMR/SMR), maximum overall heart rate ($f_{H,max}$), maximum sprint speed, growth rate, lipid peroxidation (LPO) and lactate dehydrogenase activity (LDH). Q_{10} values are listed for all biological rate measurements (i.e. everything except LPO and FAS), as a range when the diet treatments were statistically different and as individual values when the diet treatments were not statistically different. Lines and circles indicate reaction norms and colors indicate diet treatment (blue, carnivorous diet; green, omnivorous diet).

Table 2.2 Summary statistics for all thermal limits

		Results				Statistical parameters					
		Carnivorous		Omnivorous		Diet			Temperature		
Thermal limit	Temp. (°C)	*n*	Mean±s.e.m.	*n*	Mean±s.e.m.	d.f.	*F* (or *t**)	*P*	d.f.	*F*	*P*
CT_{min}	12	9	4.4±0.2	10	4.1±0.2	1	1.984	0.168	1	226.206	<0.001
	20	12	6.5±0.1	8	6.4±0.1						
T_{AB}	12	6	20.1±0.7	7	19.0±0.9	1	2.127	0.153	1	110.743	<0.001
	20	13	25.4±0.3	14	24.9±0.4						
T_{peak}	12	6	25.7±1.2	7	26.4±0.5	1	0.612	0.439	1	46.990	<0.001
	20	13	30.4±0.3	14	29.5±0.4						
T_{arr}	12	6	27.5±1.1	7	28.6±0.5	1	0.021	0.886	1	33.766	<0.001
	20	13	31.8±0.4	14	31.2±0.5						
CT_{max}	12	10	31.2±0.2	10	31.3±0.2	1	0.547	0.465	1	500.078	<0.001
	20	8	35.0±0.2	10	35.0±0.1						
$f_{H,max}$	12	6	166.6±12.5	7	163.5±6.2	1	5.243	0.028	1	17.669	<0.001
	20	13	204.8±4.4	14	184.4±5.4						
$T_{arr\text{-}cold}$	12	NA	NA	NA	NA	18	1.790*	0.090			
	20	9	5.8±0.3	11	4.7±0.5						
$T_{AB\text{-}cold}$	12	NA	NA	NA	NA	14	–0.493*	0.630			
	20	8	11.0±1.0	8	11.7±0.8						
$f_{H,min}$	12	NA	NA	NA	NA	18	2.407*	0.027			
	20	9	44.5±1.9	11	37.4±2.2						

Means±s.e.m. for each test group and ANOVA results are presented. *Note that *t*-test results are reported on the results of the cold ABT test, as this test was only run on 20°C acclimation fish because of complications surrounding COVID-19. CT_{min}, critical thermal minimum; T_{AB}, breakpoint temperature of the heart; T_{peak}, temperature corresponding to maximum heart rate; T_{arr}, temperature at the onset of cardiac arrhythmia; CT_{max}, critical thermal maximum; $f_{H,max}$, maximum heart rate across entire warm ABT test; $f_{H,min}$, minimum heart rate during cold ABT test.

2.3.3 Thermal tolerance

All thermal limits increased with acclimation to 20°C (Figure 2.5, Table 2.2). Upper thermal limits (CT_{max}, T_{AB}, T_{peak}, T_{arr}) increased by 2.6–5.3°C and CT_{min} increased by 2.1–2.3°C with warm acclimation (Figure 2.5, Table 2.2). Surprisingly, $f_{H,max}$ was significantly lower in the omnivorous treatments, which was driven by a 10% lower $f_{H,max}$ in the 20°C omnivorous treatment relative to the carnivorous treatment (Figure 2.5, Table 2.2). As expected, f_H followed the shape of an acute thermal performance curve (TPC), where it increased with an acute temperature increase until T_{peak}, at which point f_H began declining with temperature until the onset of cardiac arrhythmia (T_{arr}; Figure 2.5A). Thermal acclimation to 20°C shifted the acute TPC for f_H to the right of the TPC at 12°C (Figure 2.5A). There was evidence to support an effect of diet on model selection for f_H in the warm ABT test, where the best fit model by AIC was a third-order polynomial curve that incorporated an interaction of acclimation temperature,

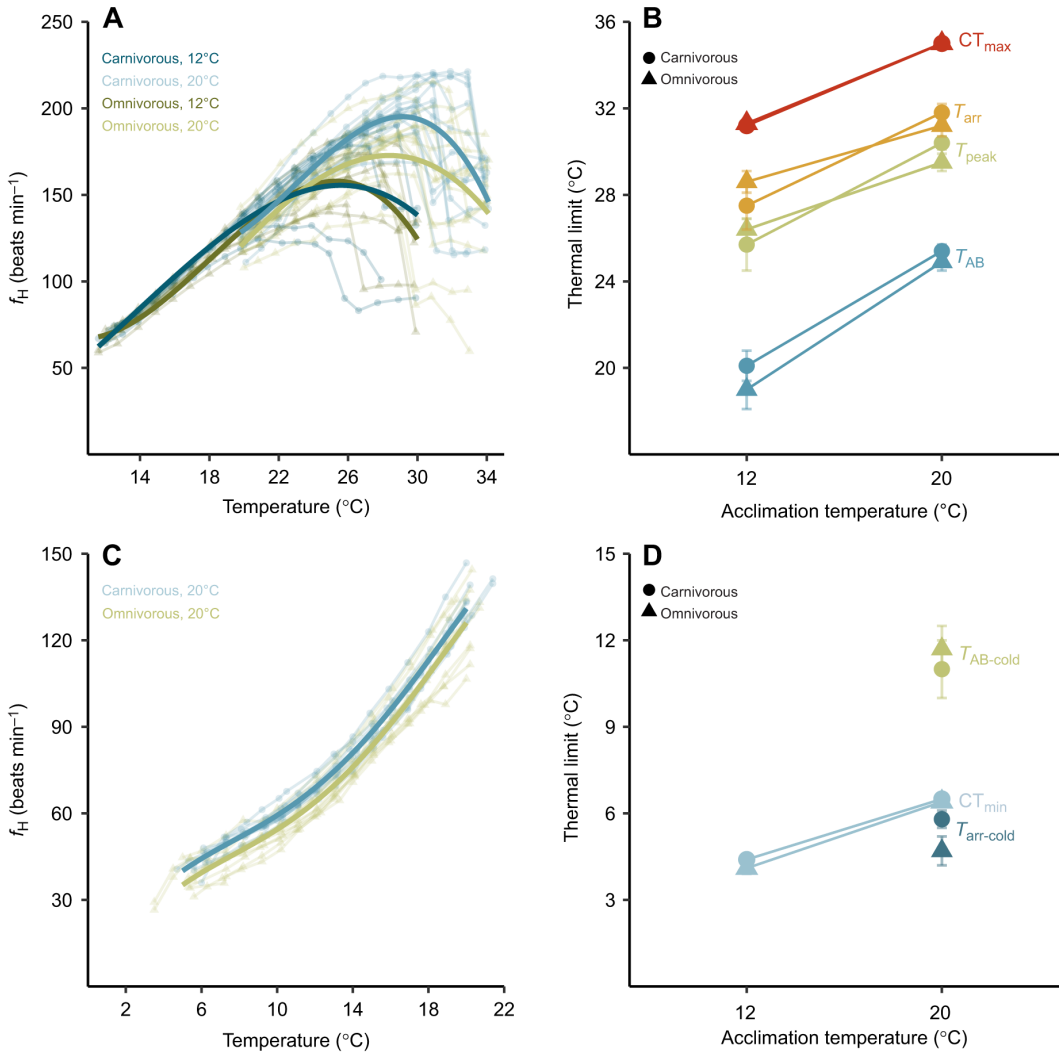

Figure 2.5 Cardiac thermal performance. (A) Individual- and treatment-level responses of heart rate (f_H) of opaleye during acute warming from 12°C (dark blue, carnivorous diet, N=6; and dark green, omnivorous diet, N=7) or 20°C (light blue, carnivorous diet, N=13; and light green, omnivorous diet, N=14). Curves are third-order polynomials that account for the interaction diet×acclimation temperature×acute temperature. This was determined to be the best fit model by AIC (Table S2.2). (B) Upper thermal limits across diet and temperature treatments in opaleye. Circles and triangles indicate mean (±s.e.m.) values for the carnivorous and omnivorous diet treatments, respectively. (C) Individual- and treatment-level responses in f_H from opaleye during acute cooling from 20°C (light blue, carnivorous diet, N=9; and light green, omnivorous diet, N=11). Curves are fourth-order polynomials that account for a fixed effect of diet and acute temperature. This was determined to be the best fit model by AIC (Table S2.2). (D) Lower thermal limits across diet and temperature treatments in opaleye. Circles indicate mean (±s.e.m.) values for the carnivorous diet treatment and triangles indicate mean (±s.e.m.) values for the omnivorous diet treatments. Note that f_H limits are missing from the 12°C treatments because of issues surrounding COVID-19. T_{arr}, temperature at the onset of cardiac arrhythmia; T_{AB}, breakpoint temperature of the heart;

T_{peak}, temperature corresponding to maximum heart rate; CT_{max}, critical thermal maximum; CT_{min}, critical thermal minimum.

acute test temperature and diet (Figure 2.5A; Table S2.2). These models demonstrated that the acute TPC for f_H was lower across temperatures in the 20°C choice treatment compared with 20°C carnivorous treatment, which was consistent with the observed differences in $f_{H,max}$ (Figure 2.5A). Altogether, these results demonstrate that for the 20°C acclimated fish, the omnivorous diet reduced overall cardiac function across temperatures but did not affect the upper thermal tolerance limits on the heart.

The results of the cold ABT test showed similar evidence of an effect of diet on f_H and $f_{H,min}$. Here, diet did not have a significant effect on any cold thermal limits (CT_{min}, $T_{AB\text{-}cold}$, $T_{arr\text{-}cold}$), but $f_{H,min}$ was lower (16%) in the omnivorous treatment compared with the carnivorous treatment at 20°C. There was also evidence to support an effect of diet on model selection, where the best fit model by AIC was a fourth order polynomial curve that incorporated an effect of acute test temperature and diet (Figure 2.5C; Table S2.2).

2.4 Discussion

Using an omnivorous fish, opaleye, as a model species, we found evidence that diet can influence thermal performance in ectotherms and does so in trait-specific ways. This has critical implications for our understanding of species responses to temperature change. Specifically, we examined three concepts outlined in Figure 2.1: (1) whether diet and temperature acclimation can affect thermal performance and limits, (2) whether ecologically important traits vary in their diet and temperature sensitivity (integrating multiple levels of biological organization) and (3) whether trait-specific variation in diet and temperature responses creates performance trade-offs for opaleye fed an omnivorous diet compared with a carnivorous diet.

2.4.1 Thermal limits increased with temperature but did not differ across diet treatments

We assessed thermal limits using a standard and commonly used critical thermal (CT) test, as well as an ABT test, which measures thermal limits of the heart (Casselman *et al.*, 2012). The heart may be a primary regulator of functional thermal tolerance in fishes. It is responsible for oxygen, immune cell, metabolite and waste transport around the body and is thought to be the first organ system to fail at extreme temperatures (Christen *et al.*, 2018; Eliason & Anttila, 2017). The heart starts showing declines in performance (at T_{AB}) ~10–20°C lower than CT_{max} and fails (T_{arr}) at temperatures ~0–5°C lower than CT_{max} in fishes (Chen *et al.*, 2015; Muñoz *et al.*, 2014; Safi *et al.*, 2019). As expected, all thermal limits increased with temperature acclimation and CT_{max} was 2.7–3.8°C higher than T_{arr}. Opaleye showed a highly plastic acclimation response across all thermal limits, consistent with other temperate marine ectotherms (Vinagre *et al.*, 2016). However, their thermal limits did not differ between diets.

While we did not observe a diet difference here, diet quality and quantity can alter thermal limits in ectotherms (fishes: Hoar & Cottle, 1952; Craig *et al.*, 1995; Abdel-Ghany *et al.*, 2019; Gomez Isaza *et al.*, 2019; Lee *et al.*, 2016; Turko *et al.*, 2020; Woiwode & Adelman, 1992). Most previous studies used formulated diets varying in lipid composition; thus, dietary lipid composition may be a primary factor affecting thermal limits. This is critical to consider in the context of aquaculture, where animal feeds should be designed to ensure farmed animals have adequate thermal performance and resistance to suboptimal temperatures. To our knowledge, this is the first study to test the effect of quasi-natural diets on the thermal acclimation of critical and cardiac thermal limits in ectotherms. Research with natural diets is ecologically relevant for ectotherms that consume broad diets and especially

for fish such as opaleye that seem to change their diet in response to temperature (Behrens & Lafferty, 2007, 2012; Emde *et al.*, 2016; González-Bergonzoni *et al.*, 2016; Guinan *et al.*, 2015; Vejříková *et al.*, 2016). Climate change is altering the nutritional landscape for many aquatic ectotherms (Birnie-Gauvin *et al.*, 2017; Huey and Kingsolver, 2019). Managers and biologists should consider the effects of this on ectotherm thermal limits. Thus, future research should explore whether other natural diets can influence thermal limits in ectotherms, as this has critical implications for how they may respond to global climate change.

2.4.2 Thermal acclimation responses did not scale with temperature and diet equally across biological rates

The biological rates assessed here did not scale with diet and temperature equally. Thermal sensitivity differed dramatically across biological rates, where sprint speed did not differ with diet and temperature acclimation (Q_{10}=1.04), lactate dehydrogenase activity was moderately affected by temperature (Q_{10}=2.10), but insensitive to diet, and growth was highly temperature sensitive (Q_{10}=28.05), but also not sensitive to the diet treatments used in this study. These results are consistent with large scale meta-analyses, which have revealed that Q_{10} values differ across biological rates (Dell *et al.*, 2011; Seebacher *et al.*, 2015). Diet sensitivity was also inconsistent across rates, where SMR and $f_{H,max}$ were sensitive to diet, but all other rates were not. This was surprising for rates that are known to be impacted by diet, such as growth rate. Diet has been shown to have interactive effects with temperature on growth in other ectotherms (Sengalese sole: Guerreiro *et al.*, 2012; rohu: Mishra and Samantaray, 2004; yellowtail kingfish: Ilham & Fotedar, 2016; crustaceans: Malzahn *et al.*, 2016; Persson *et al.*, 2011; Ruiz *et al.*, 2020; Starke *et al.*, 2020; insects: Kingsolver *et al.*,

2006; Lee & Roh, 2010). However, *Ulva* sp. supplementation has had mixed effects on growth in aquaculture fish, with some studies showing modest amounts of *Ulva* sp. reducing growth, while others finding positive or no effect of *Ulva* sp. supplementation on growth (Wan *et al.*, 2019). Future work should explore how broader diet differences affect growth in relation to other important biological rates.

The effects of temperature on sprint speed in comparison to lactate dehydrogenase activity were similarly unexpected. Sprinting in fish is primarily driven by glycolytic fast twitch white muscle (McDonald *et al.*, 1998; Kraskura & Nelson, 2018). Lactate dehydrogenase is a critical enzyme in lactic acid fermentation during glycolysis. Thus, we expected that sprint performance would have a comparable thermal sensitivity to lactate dehydrogenase activity. Lactate dehydrogenase activity increased with temperature acclimation, suggesting the opaleye had a greater anaerobic capacity at 20°C (McDonald *et al.*, 1998). However, this did not translate to increases in sprint performance. Other enzymes in glycolysis could be rate-limiting steps (e.g. phosphofructokinase; McDonald *et al.*, 1998) and more highly correlated with sprint speed. Sprint speed has been examined in one other diet×temperature study in fishes, which measured macronutrient selection and temperature effects on damselfish (Rowe *et al.*, 2018). Macronutrient selection did not change with temperature, but sprint speed was highest in the colder temperature treatment (Rowe *et al.*, 2018). Fish use two different swimming modes: anaerobically powered burst swimming and sustained aerobically powered swimming. These swimming modes may be differentially affected by diet and temperature. The effect of diet and temperature on aerobically powered swimming and maximum swim speeds (i.e. maximum swimming speed, U_{max}, and critical swimming speed, U_{crit}) have been assessed in grey mullet (Vagner *et al.*, 2015, 2019), with

no observed effect of diet, but an effect of temperature on U_{max} and no effect of diet or temperature on U_{crit}. Given that two metabolic traits, SMR and FAS, were affected by diet and temperature in this study, future work should explore how aerobically powered swimming is impacted by different levels of omnivory.

The diverse diet and temperature responses that we observed across biological rates resulted in no consistent pattern in the thermal sensitivity of any metabolic rates or levels of biological organization. The small number of papers that have measured the interactive effects of diet and temperature on metabolic rates in other ectotherms have found mixed results (i.e. insects: Alton *et al.*, 2020; Schmitz & Rosenblatt, 2017; fish: Pérez-Casanova *et al.*, 2010; Vagner *et al.*, 2015). For example, Vagner *et al.* (2015) found an interactive effect of dietary fatty acid composition and temperature on the AAS of juvenile grey mullet. In contrast, Pérez-Casanova *et al.* (2010) did not find any effects of macronutrient ratios on metabolism in juvenile Atlantic cod and haddock. Our results were especially surprising for SMR, as it is often assumed that maintenance metabolism is coupled to all other biological rates and should have the same thermal sensitivity (Gillooly *et al.*, 2001; Brown *et al.*, 2004). Aerobic scope is also considered a master physiological factor that determines the capacity for all other aerobically powered functions (Fry, 1947; Brett, 1971; Claireaux & Lefrançois, 2007; Pörtner, 2010), although this idea has been heavily debated (Clark *et al.*, 2013; Schulte, 2015). Here, AAS did not differ in response to diet or temperature (although FAS did) and there was also no observed pattern between AAS, SMR and other biological rates. These results indicate that caution should be taken when using AAS and SMR as indicators of overall performance or proxies for other biological rates, especially when predicting the effects of multiple factors, such as diet and temperature.

2.4.3 Performance costs and benefits for diet choice at different acclimation temperatures

Contrary to our hypothesis, we did not find evidence of a performance trade-off to the omnivorous diet used in this study. Instead, opaleye that consumed the omnivorous diet displayed several higher costs: lower f_H across a thermal gradient, higher SMR and reduced FAS when acclimated to 20°C. Given that the thermal limits of the heart did not change with diet, it was remarkable that diet downshifted the thermal performance curve for $f_{H,max}$. A reduction in $f_{H,max}$ indicates a reduced capacity to transport oxygen, metabolites, immune cells and waste around the body. There are several potential mechanisms that could have driven this reduction in $f_{H,max}$ across acute temperatures. For example, differences in the lipid composition of the diet can impact membrane remodeling, which can affect cardiac function in fish (Chatelier *et al.*, 2006; McKenzie, 2001). Further, although *Ulva* sp. contains no known herbivore deterrents, our results suggest some sort of anti-nutrient effect of *Ulva* sp. supplementation, which could have caused the observed reductions in cardiac thermal performance.

The higher SMR observed in the 20°C omnivorous treatment was expected as omnivorous and herbivorous animals generally have higher digestive infrastructure costs than carnivores (e.g. broader digestive enzymes, higher gut surface area; Caruso and Sheridan, 2011; Clements *et al.*, 2009; Horn, 1989). Consistent with the higher SMR at 20°C, the opaleye in the omnivorous treatment had a reduced FAS (3.43) that was less than half that at 12°C (8.65). In contrast, the carnivorous diet maintained a 44% higher FAS at 20°C (4.94) compared with the omnivorous diet. FAS is indicative of the amount of scope available to perform critical biological functions that scale proportional to SMR (Farrell, 2016).

Digestion generally requires at least a doubling of SMR in many fishes (i.e. FAS>2; Chabot *et al.*, 2016a; Farrell, 2016; McCue, 2006). This suggests that the opaleye in the omnivorous treatment at 20°C may have been on the threshold of having limited scope for activities beyond digestion. As climate change increases the seasonal extreme temperatures and the frequency of marine heatwaves in the rocky intertidal zone (IPCC, 2019), the opaleye's FAS will decrease further, which could limit other important biological functions, such as digestion, and exacerbate energetic tradeoffs associated with different diets. Thus, another avenue for future research will be to untangle diet×temperature effects on digestive costs (i.e. specific dynamic action) in relation to AAS and digestion efficiency (Jutfelt *et al.*, 2021).

Given that opaleye and other omnivorous fishes eat more plants in warmer water in the wild, we expected there to be a performance advantage to the omnivorous diet. We measured a suite of traits to test for any performance benefits that may offset the costs of the omnivorous treatment. Specifically, we explored how diet and temperature affected (1) thermal tolerance, (2) maximum sprint speed, (3) glycolytic capacity and (4) oxidative stress. We predicted that because opaleye eat more plants in warmer water (Behrens and Lafferty, 2012), the omnivorous diet would result in higher thermal limits than the carnivorous diet at 20°C. However, we did not find evidence of any benefits to the omnivorous diet. We also expected that the higher lipid and protein content of the carnivorous treatment would raise oxidative stress relative to the omnivorous diet. Analysis of lipid peroxidation in liver tissue revealed a marginal trend of higher oxidative stress in the omnivorous diet treatment. However, antioxidant capacity could still have been higher in the omnivorous treatment. For example, Coggins *et al.* (2017) examined the effect of dietary glutathione supplementation on thermal limits, antioxidant capacity and lipid peroxidation in *Daphnia* sp. As the glutathione

concentration increased, so did total antioxidant capacity, but glutathione supplementation did not alter lipid peroxidation or thermal limits (Coggins *et al.*, 2017). In contrast, Castro *et al.* (2012) examined the effect of macronutrient ratios (45% and 55% protein) and temperature acclimation (12 and 18°C) on multiple antioxidant enzymes and lipid peroxidation in juvenile Senegalese sole. Lipid peroxidation differed across temperature treatments and was highest in the 55% protein diet (Castro *et al.*, 2012). The limited amount of research on these interactions and inconclusiveness across studies indicate that more research is needed to elucidate the role of dietary antioxidants and macronutrient ratios in regulating oxidative stress across temperatures and taxa.

While it was not within the scope of this study, other important performance traits may have differed between the diet treatments. These include, but are not limited to, differences in microbiome diversity and function, visual acuity, cognitive ability, digestion efficiency and digestive costs relative to aerobic scope, immune function, aerobic swimming performance and cardiac stroke volume (Glencross & Rutherford, 2010; Koven *et al.*, 2018; Vagner *et al.*, 2014, 2019). It remains unclear whether opaleye and other omnivorous ectotherms consume different proportions of plant to animal to regulate their thermal responses. Here, the opaleye's omnivorous diet did not maximize their performance compared with a more specialized carnivorous diet. However, many other factors govern diet choice in the wild, including life history (Zhang *et al.*, 2020), competition (Pfenning, 1990), predation (Schmitz *et al.*, 2016), food availability (MacArthur & Pianka, 1966) and habitat structure (Behrens & Lafferty, 2007). Therefore, the ecological benefits of consuming an omnivorous diet may outweigh the physiological costs.

Many ectotherms change their diets with temperature; either directly because their diet preference changes in response to temperature (Boersma *et al.*, 2016b; Carreira *et al.*, 2016; Devries & Appel, 2014; Lee *et al.*, 2015; Lemoine *et al.*, 2014; Rho and Lee, 2017; Schmitz *et al.*, 2016; Vejříková *et al.*, 2016) or indirectly because food availability or the nutritional content of a diet item changes with temperature (Alton *et al.*, 2020; Boersma *et al.*, 2016b; Cross *et al.*, 2015; Ho *et al.*, 2010). In either scenario, generalist ectotherms that have the capacity to adjust their diet may be at an advantage compared with those with more specialized inflexible diets. However, the ultimate diet choices that ectotherms make may not always be 'better' or 'worse' because diet and temperature can interact and have trait-specific effects. Not all diet choices are necessarily adaptive. Irrespective of the reasons why an ectotherm eats what it eats, our work here demonstrates that diet choices have consequences. These consequences have far-reaching implications, including whether diet choice can facilitate geographic range expansion, or colonization of warmer or cooler habitats; and further, whether specialist diets constrain thermal niches or whether diet can facilitate differences in acclimation rates or performance under fluctuating temperatures. Overall, diet should be treated as an interacting factor that has the capacity to modify the thermal responses of ectotherms.

2.5 Concluding remarks

Thermal acclimation is a key mechanism that ectotherms employ to maintain performance across a range of temperatures. Acclimation requires energy and nutritional building blocks that ectotherms obtain from their diet. Here, we explored whether different diets mediated distinct thermal acclimation responses in an omnivorous fish, opaleye. We found clear evidence that diet influences thermal acclimation responses. However, there was

no consistent pattern in how different biological rates responded to the temperature and diet treatments, with Q_{10} values ranging from 1 to 28. When confronted with a seasonal warm temperature (20°C), the opaleye in the omnivorous diet treatment had inferior performance (higher SMR, lower FAS and lower cardiac performance) relative to the opaleye fed a carnivorous diet treatment. Global climate change is already changing the average and extreme temperatures that marine ectotherms experience as well as their nutritional landscape (IPCC, 2019; Birnie-Gauvin *et al.*, 2017). These environmental changes are likely to interact and alter many ectotherms' thermal performance in the wild. Incorporating multiple interacting factors into our understanding of species' responses to global climate change is the next step in ensuring that researchers capture the resilience of different species and populations (Jackson *et al.*, 2021). Accordingly, diet is essential to consider when predicting ectotherm performance in variable environments and in response to global climate change.

III. . Chapter 3

3.0 Abstract

Global climate change is increasing thermal variability in coastal marine environments and the frequency, intensity and duration of marine heatwaves. At the same time, food availability and quality are being altered by anthropogenic environmental changes. Marine ectotherms often cope with changes in temperature through physiological acclimation, which can take several weeks and is a nutritionally demanding process. Here, we tested the hypothesis that different ecologically relevant diets (omnivorous, herbivorous, carnivorous) impact thermal acclimation rate and capacity, using a temperate omnivorous fish as a model (opaleye, *Girella nigricans*). We measured acute thermal performance curves for maximum heart rate because cardiac function has been observed to set upper thermal limits in ectotherms. Opaleye acclimated rapidly after raising water temperatures, but their thermal limits and acclimation rate were not affected by their diet. However, the fish's acclimation capacity for maximum heart rate was sensitive to diet, with fish in the herbivorous treatment displaying the smallest change in heart rate throughout acclimation. Mechanistically, ventricle fatty acid composition differed with diet treatment and was related to cardiac performance in ways consistent with homeoviscous adaptation. Our results suggest that diet is an important, but often overlooked, determinant of thermal performance in ectotherms on environmentally relevant time scales.

3.1 Introduction

Understanding the mechanisms driving thermal tolerance in ectotherms is a primary focus in ecological physiology. Temperature is one of the most important environmental

factors governing the physiology, behaviour and ecology of ectotherms (Hochachka & Somero, 2002; Schulte, 2015). Temperature profiles vary substantially across environments, and thus endemic ectotherms are adapted to tolerate their local thermal regimes (Somero, 2010). Temperate marine environments are especially thermally dynamic, with changes in temperature occurring on acute (diurnal), intermediate (seasonal upwelling and marine heatwaves) and long-term (annual–decadal) time scales (Hobday *et al.*, 2016; Hoshijima & Hofmann, 2019; Kroeker *et al.*, 2019). Importantly, climate change is expected to increase the severity and duration of natural (i.e. upwelling, diurnal cycles) and extreme (i.e. heat waves) sources of temperature variation (Kroeker *et al.*, 2019; Oliver *et al.*, 2018). To survive in these dynamic thermal environments, ectotherms must be able to cope with changes in temperature across all relevant time scales (Kroeker *et al.*, 2019; Sandblom *et al.*, 2014; Schulte *et al.*, 2011). As such, these ectotherms generally possess the capacity for high levels of thermal plasticity, otherwise known as thermal acclimation capacity.

Thermal acclimation occurs through a series of phenotypic changes across levels of biological organization that optimize biological rates, minimize waste production or cellular damage or conserve energy (Schulte *et al.*, 2011; Dalhoff & Somero, 1993; Anttila *et al.*, 2014; Ekström *et al.*, 2016). While these changes are occurring, the animal's performance changes as a function of how long they have been exposed to the new conditions. Thus, acclimating quickly may be just as, if not more important than full acclimation capacity (Sandblom *et al.*, 2014; Sidell *et al.*, 1973; Hazel & Prosser, 1974; Johansen *et al.*, 2021), especially in the context of an increasingly variable environment. As the heart is essential for ensuring blood circulation, which is critical for transporting nutrients, waste, immune cells and oxygen, around the body, it is thought to govern thermal tolerance in several ectotherms

(Eliason & Anttila, 2017) and has been shown to rapidly acclimate to high temperatures in some temperate fish (Ekström *et al.*, 2016; Gilbert *et al.*, 2022). Given that acclimation is a remodelling process, successful and efficient acclimation requires energy and nutrients. Thus, the rate of acclimation and overall acclimation capacity for cardiac thermal tolerance may be dependent on the quality and quantity of what an ectotherm has eaten.

Climate change is predicted to alter food availability, prey nutritional quality, and diet preference for several ectotherms (Birnie-Gauvin *et al.*, 2017; Cross *et al.*, 2015; Rosenblatt & Schmitz, 2016; Zhang *et al.*, 2020), yet the interaction of diet and temperature on thermal performance remains largely unknown. Animals have a remarkable capacity to vary their diet to meet their nutritional requirements through changes in their consumption rate or diet selection (Jobling, 2016; Johnson *et al.*, 2017; Raubenheimer *et al.*, 2017). Generalist ectotherms, like omnivores, can choose between foraging for plants or animals to meet their nutritional needs (Zhang *et al.*, 2020). Interestingly, some omnivorous aquatic ectotherms increase the ratio of plant to animal in their diet as temperature increases, suggesting that the nutritional needs of these ectotherms are temperature dependent (Zhang *et al.*, 2020). The physiological consequences of these diet shifts are not well understood but may be associated with altered performance and thermal tolerance (Carreira *et al*., 2020; Chapter 2; Brankatschk *et al.*, 2018). An out- standing question is if the ratio of plant to animal in an omnivore's diet affects their thermal plasticity. More specifically, can diet affect thermal acclimation rates and capacity, and if so, what mechanisms underly these differences.

Diet quality varies dramatically across prey types. For omnivores, plants tend to be higher in mineral content, antioxidants and complex carbohydrates, while animal diets tend

to have higher protein and lipid content. In general, fatty acid (FA) composition also differs between plants, algae and animals, although the exact composition is also dependent on the environment (i.e. terrestrial, marine, freshwater) and conditions (e.g. environmental oxygen, temperature; Turchini *et al.*, 2022). For example, the global availability of essential omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are expected to decline due to increasing temperatures associated with climate change (Hixson & Arts, 2016). FA composition can influence membrane fluidity, thermal limits (Abdel-Ghany *et al.*, 2019; Gomz Isaza *et al.*, 2019), cardiac performance (Vagner *et al.*, 2019), swimming performance (McKenzie *et al.*, 1998), growth (Gourtay *et al.*, 2018), cognitive function (Závorka *et al.*, 2021) and diet preference (Brankatschk *et al.*, 2018), suggesting that variation in FA composition may be an important mechanism underlying thermal performance differences in ectotherms whose diet changes with temperature.

The objective of our study was to determine whether diet impacts the (1) rate and (2) capacity of cardiac thermal acclimation in a temperate omnivorous fish, opaleye (*Girella nigricans*), during a simulated warming event. We measured thermal performance of maximum heart rate (f_{Hmax}) as it is thought to limit thermal tolerance in fishes, and heart rate is governed by processes occurring across biological lipid membranes (Eliason & Anttila, 2017). Opaleye were fed either a fully herbivorous (ad lib red algae, *Gracilaria pacifica*), carnivorous (ad lib brine shrimp, *Artemia* sp.), or omnivorous (free choice between algae and *Artemia* sp.) diet for two weeks at 12°C. We then increased temperature to 20°C, simulating an acute marine warming event and periodically measured cardiac thermal performance over the next two weeks while the fish were acclimating to 20°C. We predicted that the lower energy, lipid and protein content in the algae compared to *Artemia* sp. would result in lower

acclimation capacity, slower acclimation rates, and reduced upper thermal limits compared to the carnivorous treatment. Further, that fish in the omnivorous treatment would have the highest performance due to the greater diversity of nutrients available, high energetic content of the diet, and flexibility to choose between the algae and *Artemia* sp. To examine the mechanisms underlying observed differences in cardiac performance, we measured the FA composition of the fish's ventricles and related FA composition to heart rate and thermal tolerance.

3.2 Methods

3.2.1 Fish Collection

Juvenile opaleye (estimated to be <1 year old; Bredvik *et al.*, 2011) were collected in May-August, 2021 by hook and line from Santa Barbara Harbor, California, USA (34.40829, -119.691389). Fish were transported in coolers (>70% air saturation) to the University of California, Santa Barbara and held in 95 L fiberglass flow-through seawater tanks (N = 224 fish total, 14 fish per tank, 4x turnover h^{-1}). Prior to the start of acclimation, fish were held at ambient conditions (ranged ~12.5-17.5°C throughout the study) for <3 weeks and fed *ad libitum* omnivorous diets (*Gracilaria pacifica* from the cultured abalone farm in Goleta, CA and *Artemia* sp. from brineshrijmpdirect.com). All protocols were approved by the Institutional Animal Care and Use Committee at the University of California, Santa Barbara (permit No. 935).

3.2.2 Acclimation and Diet Treatments

Opaleye were held at 12ºC and fed *ad libitum* one of the three diet treatments for two weeks. The diet treatments were 1) carnivorous (*Artemia* sp.), 2) herbivorous (red macro

algae native to California called *Gracilaria pacifica*), and 3) choice omnivorous (*Artemia* sp. and algae). Consistent with other aquatic omnivorous ectotherms, opaleye increase algae relative to prey consumption in the warmer part of their geographic range (Behrens & Lafferty, 2012). After two weeks, fish were subjected to a cardiac thermal tolerance test called an Arrhenius breakpoint temperature test (ABT test; outlined below). One to two days later, temperature in the holding tanks was raised over ~10-12 h from 12 to 20ºC (representative of the low and high seasonal temperatures experienced in Santa Barbara, CA). As temperate intertidal fish, this rate of temperature change is high for Santa Barbara, however not unheard of given the ecology of the species and a common rate of change in more Southern parts of their range. Following the temperature change, individuals from each treatment were tested using an independent sampling design on day 1 (post-change), 3, 7, and 14-15 for their upper cardiac thermal limits using the same ABT test. The experiment was replicated 5 times to ensure adequate sample sizes for each timepoint (final N=7-15 fish per diet treatment per time point). The 5th replicate was only run until day 3 post temperature change to increase the sample sizes at earlier timepoints. We also included one tank of fish that were acclimated to 20°C for 4 weeks and tested on week 2 and 4 of acclimation (Figure S1).

At the time that ABT tests were conducted, body mass and total length were 36.42 ± 16.12 g. and 12.18 ± 1.87 cm. (mean ± SD), respectively and did not differ with diet treatment. However, conditions factor varied slightly across diet treatments (herbivorous = 1.79 ± 0.13, carnivorous = 1.87 ± 0.18, and omnivorous = 1.97 ± 0.26 g/cm^3; $p < 0.001$; $\chi 2 = 25.015$). Temperature and dissolved oxygen content were monitored daily by hand using an Omega Thermocouple (Omega Engineering INC, Norwalk, CT, USA) and an Oxyguard

handy Polaris 2 (OxyGuard International A/S, Farum, Denmark). Oxygen was maintained at >80% air saturation throughout the study. Average temperature per treatment was 12.2 ± 0.5°C and 20.0 ± 0.6°C across replicates (mean ± SD; determined using Thermochron 4K iButtons programmed to record every 20 minutes; accuracy ± 1°C and resolution to 0.5°C). Replicate level temperature data is provided in Table S3.1. Fish were held under a 14:10 h light: dark cycle.

3.2.3 Direct Field Sampling

We examined how our results compared to seasonally warm-acclimatized opaleye in the wild. Juvenile opaleye were collected in the August of 2021 (avg. temp for 2-weeks prior was 18.4 ± 1.0°C; mean ± SD; N = 10) from Santa Barbara Harbor using hook and line. ≤ 4 fish were caught per day for 3 days. Fish were immediately transported back to the University of California, Santa Barbara and an Arrhenius breakpoint temperature test followed by dissection (described below) was conducted within 3 h of being caught using water from the harbor.

3.2.4 Dissections and Stomach Contents

After each ABT test, fish were euthanized and dissected. Liver and ventricle were weighed for hepatosomatic index (HSI) and relative ventricular mass (RVM). The ventricle was flash frozen for later analysis. Fish were not fasted before testing so that we could evaluate their stomach contents. Fish in the carnivorous treatment had similar results to fasted opaleye fed brine shrimp and acclimated to 12 and 20°C in Chapter 2. Thus, we do not anticipate that post-prandial increases in the oxygen uptake rate had a major impact on our

findings. Stomach contents were weighed and sorted in fish from the omnivorous treatment to estimate the proportion of algae to brine shrimp (see Table S3.2).

3.2.5 Proximate Analysis

Frozen fish remains on day 14 (after 2 weeks at 20°C and 4 weeks on the treatment diet; stored at -20°C) were homogenized using a Fisher Brand Bead Mill 24 and subsamples of the homogenate were weighed and freeze dried (Labconco Lyophilizer). Subsamples of algae and *Artemia* sp. were also freeze dried for proximate analysis (see Table S3.3). Protein, lipid and ash content were estimated as described in Chapter 2. Total fatty acid composition was determined on ventricles (N = 9 per treatment and timepoint; stored at -80°C) from fish acclimated 2 weeks at 12°C and 2 weeks at 20°C and diet samples (N = 6 per diet) by gas chromatography/mass spectroscopy (GC-MS) at the University of California, San Diego Lipidomics Core using methods outlined in Quehenberger *et al.* (2010) (see Table S3.4, Table S3.5). Fatty acids that were "not detected" in the sample were assumed to be 0.

3.2.6 Thermal Limits: Arrhenius Breakpoint Test

ABT tests were conducted as outlined Chapter 2 and Schwieterman *et al.* (2022) (see Table S3.6 for specific values). Briefly, fish were anesthetized in seawater containing 80 mg L^{-1} buffered MS-222 and then placed in an experimental sling in a 10 L test tank which contained a maintenance dose of buffered 65mg L^{-1} MS-222. Water was circulated continuously past the gills throughout the test and stainless-steel needle tip electrodes (ADInstruments INC, Colorado Springs, CO, USA) were shallowly inserted under the skin above the heart to detect an ECG signal. The signal was amplified using a Dual Bio Amp

(ADInstruments INC, Colorado Springs, CO, USA) and filtered (Filters: 60 hz Notch filter; Mains filter; Low-Pass: 1 Kz; High Pass: 10 hz; Range: 1-2 mV).

After a 15 min equilibration period at the acclimation temperature, atropine sulfate was injected intraperitonially (1.2 mg kg^{-1} in 0.9% NaCl) to block vagal tone. 15 minutes later, isoproterenol was injected intraperitonially (4 ug kg^{-1} in 0.9% NaCl) to maximally stimulate ß-adrenoreceptors. In 20°C acclimated fish, water temperature was cooled from 20 to 18°C before the start of the test to provide additional f_{Hmax} data for breakpoint temperature calculations. After isoproterenol injection, fish were given a 30-minute equilibration period. Then water temperature was heated (warm ABT test) at 10°C h^{-1} (Polystat recirculating heater/chiller; Cole-Palmer, Vernon Hills, IL, USA) while continuously recording an ECG trace. At each 1°C interval, f_{Hmax} and temperature were stabilized for 30 seconds to record an average value for heart rate at the temperature. This procedure was repeated until the onset of cardiac arrythmia (T_{ARR}). The fish were kept in the test until the next 1°C interval after T_{ARR} (<6 min) to ensure that the decrease in heart rate following cardiac arrythmia was captured for fitting thermal performance curves (TPC). Fish were omitted from the analysis when experimental error occurred (e.g., water pump failure, drug injection complications, or ECG signal was too noisy for software to interpret; 11% of individuals tested). Cold ABT tests were only performed on the last day of testing (i.e., day 14/15 post temperature switch; N = 5-7 per diet). The same rate of temperature change (decrease by 10°C h^{-1}) was targeted; however, below 11°C, the rate of decrease in temperature was harder to maintain and deviated occasionally from the desired rate (~0.3-1 °C every 6 min). We do not anticipate that this impacted the results, as Casselman *et al.* (2012) found that slower rates of temperature change did not impact ABT test results.

3.2.7 Data Analysis for Arrhenius Breakpoint Test

At each 1°C temperature increment, f_{Hmax} was calculated from 15 continuous seconds of ECG recordings using automated ECG analysis software in LabChart (AD Instruments; www.adinstruments.com) (Chapter 2). Three thermal limits were calculated from the warm ABT test (T_{AB}, T_{PEAK}, T_{ARR}). The lowest thermal limit (T_{AB}) represents when the heart first starts showing signs of impairment due to temperature. For example, T_{AB} was comparable to the optimal temperature window for aerobic scope in Pacific salmon (Cassselman et al., 2012), rainbow trout (Anttila et al., 2013), and goldfish (Ferreira et al., 2014). T_{AB} was defined as the temperature corresponding to a breakpoint in a plot of $\log(f_{Hmax})$ against the Arrhenius temperature. This breakpoint analysis was conducted using *segmented* package (v1.1-0; Muggeo, 2008) in R. Note that T_{AB} may be artificially high in the early time points because the test started at a higher temperature (18 instead of 12°C). For the warm ABT test, the highest f_{Hmax} recorded during any 15 s measurement period was designated as the overall peak heart rate (Peak f_{Hmax}). The temperature in which peak f_{Hmax} occurred was defined as the peak temperature (T_{PEAK}). This indicates an important transition temperature where cardiac arrythmia usually follows shortly thereafter. Finally, T_{ARR} indicates the temperature where the heart is no longer able to maintain rhythmic beating. At 12 and 20°C, T_{ARR} occurs at temperatures ~2.7-3.8°C lower than critical thermal maxima (CT_{max}) in opaleye and thus, represents a more functional upper thermal limit for these fish Chapter 2.

3.2.8 Statistical analysis

All data were statistically analyzed using R (version 3.5.1). All metrics were investigated for normality using Shapiro–Wilk tests and quantile–quantile plots, and for heteroscedasticity using Levene's test. All data were sufficiently normal with equal variance.

Acclimation rate data were statistically analyzed (significance level α=0.05) by fitting a linear mixed effect model where timepoint and diet were included as fixed effects and replicate number was included as a random effect (lme4 v1.1-19; Fox, 2011). We ran a 2-way ANOVA on each model followed by a post hoc Tukey test when significant main effects were detected. In all 2-way ANOVA tests, the interaction between diet and timepoint was tested for and excluded when non-significant. Polynomial curves were fitted to acclimation capacity f_{Hmax} data (i.e., data from fish held for 2 weeks at 12°C and 2 weeks at 20°C) and compared using Schwarz Information Criterion (SIC), where the fit with the lowest SIC score was assigned the best fit model, but all models with ΔSIC<7 were considered (Table S3.7; Jerde *et al.*, 2019). Replicate number and individual were included as random effects.

To simplify fatty acid analysis, the DBI and average chain length were calculated for each sample. DBI = $\sum_1^n n \times (proportion\ of\ FA\ with\ n\ double\ bonds)$, where N = the number of double bonds in a type of fatty acids. Average chain length = $\sum_1^L L \times (proportion\ of\ FA\ with\ n\ double\ bonds)$, where L = the number of carbons in the fatty acid chain. Linear regressions between DBI or average chain length and all cardiac parameters (f_{Hmax}, peak f_{Hmax}, T_{ARR}, T_{PEAK}, T_{AB}) were assessed in fish acclimated for 2 weeks at 12°C and 2 weeks at 20°C. Residual plots of each linear regression model were visually assessed in R to investigate normality and heteroscedasticity. Complete dietary and tissue sample FA analysis (i.e., mean ± SEM for all FA's measured) is provided in the supplement (Table S3.4, Table S3.5).

3.3 Results

3.3.1 Acclimation Capacity

The thermal acclimation capacity for f_{Hmax} was influenced by diet in opaleye (Figure 3.1, Table S3.8). Model selection revealed strong evidence of an interactive effect of diet and temperature on the acute TPC for f_{Hmax} in the warm ABT test (Table S3.7). The best fit model by SIC was a third-order polynomial curve that incorporated an interaction of acclimation temperature (12 or 20°C), diet (herbivorous, omnivorous, carnivorous), and acute test temperature (Table S3.7). The acute TPC for f_{Hmax} followed a traditional shape with a negative skew, where f_{Hmax} increased with temperature until T_{AB}, at which point f_{Hmax} began leveling off until T_{PEAK}. T_{ARR} generally occurred shortly after T_{PEAK}.

Upper thermal limits (T_{AB}, T_{PEAK}, T_{ARR}) increased by an average of 3.3-3.9°C (irrespective of diet) when switched from 12 to 20°C (Figure 3.2, Table S3.6). While diet did not affect any thermal limits (diet effect for T_{AB} $p = 0.1647$, T_{PEAK} $p = 0.2269$, T_{ARR} $p = 0.1998$, Table S3.6, S3.7), it did impact the height of the acute TPC (diet effect $p < 0.001$). For the omnivorous and carnivorous treatments, peak f_{Hmax} increased during acclimation from 12 to 20°C (Figure 3.3). In contrast, the herbivorous treatment did not alter peak f_{Hmax} in response to warm acclimation ($p = 0.9856$ from Tukey HSD comparing herbivore treatment at 12°C to the herbivore treatment after 2-weeks at 20°C). Accordingly, the herbivorous treatment had a lower Δ peak f_{Hmax} (peak $f_{Hmax\ at\ 20°C}$ - peak $f_{Hmax\ at\ 12°C}$) of 12.62 bpm compared to 55.10 and 43.96 bpm for the omnivorous and carnivorous treatments, respectively.

3.3.2 Acclimation Rate

The majority of the acclimation response for thermal limits occurred in the first 3 days post temperature change, with an average of 69.2% of the response for T_{AB}, 60.6% for T_{PEAK}, and 60% for T_{ARR} occurring in the first 3 days at 20°C, irrespective of diet (Table S3.9). Diet

did not impact any thermal limit at any timepoint during acclimation from 12 to 20°C (Figure 3.2, Table S3.8). However, there was an interactive effect of diet and timepoint on peak f_{Hmax} (diet×timepoint p = 0.0128), where it was lower in the herbivorous treatment compared to the omnivorous (23.3% higher) and carnivorous (16.5% higher) treatments after the 2-week acclimation to 20°C (Figure 3.3, Table S8).

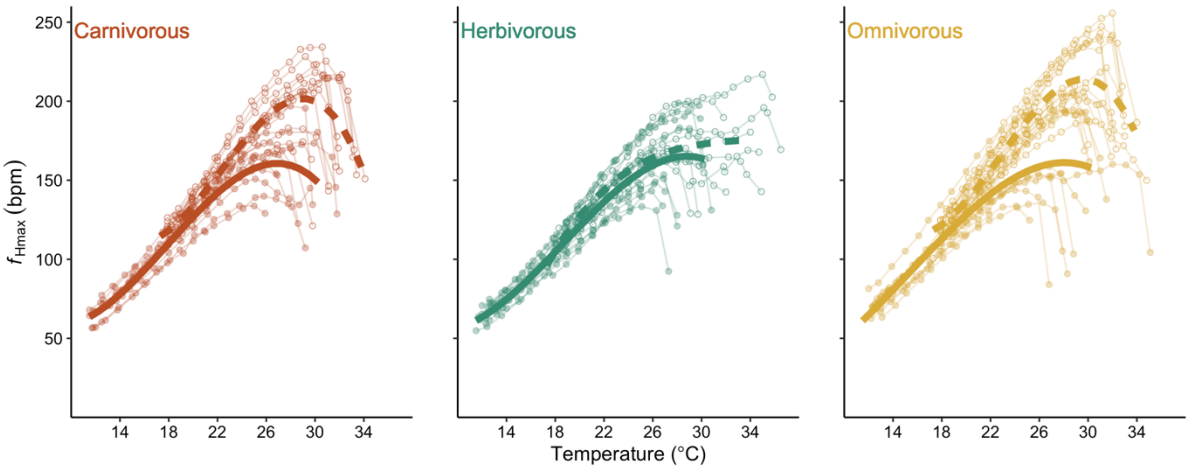

Figure 3.1 Dietary effects on cardiac thermal acclimation capacity. Individual- and treatment-level responses of max heart rate (f_{Hmax}) of opaleye during acute warming. Panels show fish from different diet treatments (from left to right: carnivorous, herbivorous, omnivorous) that were tested after a 2-week acclimation to 12°C (closed circles) and 20°C (open circles). Curves indicate treatment level effects after 12°C (solid line) and 20°C (dashed line) acclimation and are third-order polynomials that account for the interaction diet×timepoint×acute temperature with random effects of individual fish id and replicate. This was determined to be the best fit model by SIC (Table S7).

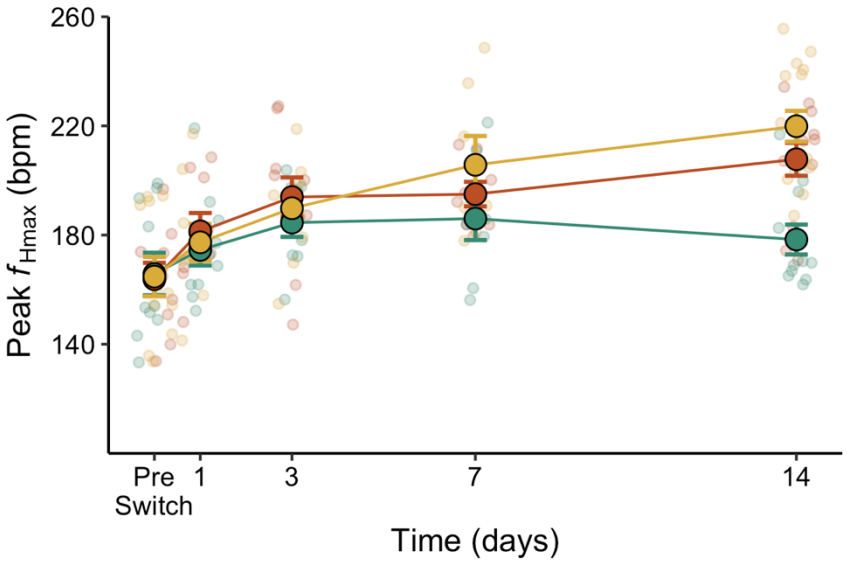

Figure 3.2 Dietary effects on thermal acclimation rate for cardiac thermal limits. Individual- and treatment-level acclimation rate responses for upper thermal limits (T_{AB}, T_{PEAK}, T_{ARR}) across diet treatments and timepoints in opaleye. Colors distinguish diet treatments (red: carnivorous, green: herbivorous, yellow: omnivorous). Individual responses are indicated by the translucent points, and treatment level mean (± SEM) are overlayed with connecting lines. Different letters indicate significant differences across time points ($p < 0.05$) by Tukey HSD. Each thermal limit was analyzed independently. There were no significant effects of diet on any thermal limit.

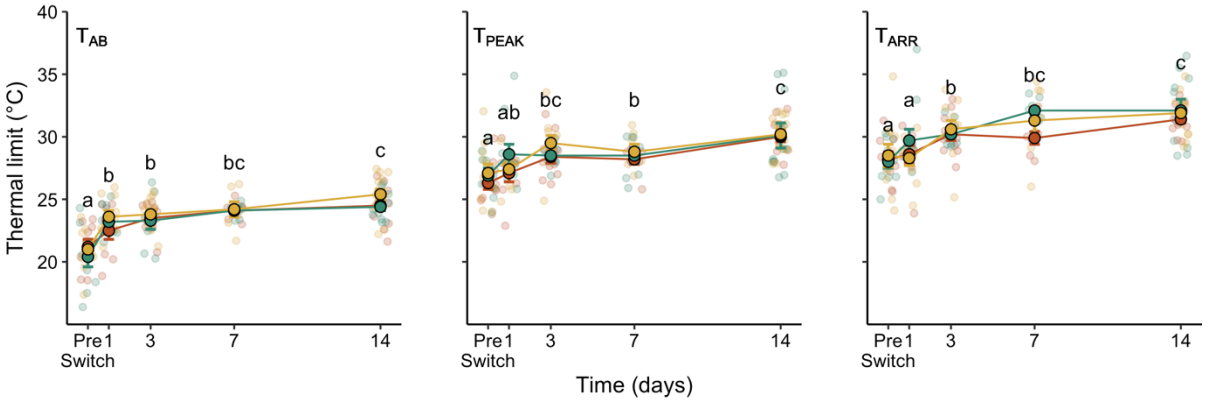

Figure 3.3 Dietary effects on thermal acclimation rate for peak f_{Hmax}. Individual- and treatment-level acclimation rate responses in peak f_{Hmax} across diet treatments and timepoints in opaleye. Colors distinguish diet treatments (red: carnivorous, green: herbivorous, yellow: omnivorous). Individual responses are indicated by the translucent points. Treatment level mean (± SEM) are overlayed with connecting lines. There was a significant interactive effect of diet and timepoint ($P = 0.0128$; Table S3.8) on peak f_{Hmax}.

3.3.3 Warm versus cold thermal limits and seasonally acclimatized fish

A group of carnivorous fish were tested after an extended acclimation period to 20°C (2 and 4 weeks) (Figure S3.1). Peak f_{Hmax} increased from 2 to 4 weeks while T_{PEAK} and T_{ARR} did not change, indicating the opaleye could have continued acclimating a bit more if the acclimation time had been extended to 4-weeks across treatments (Figure S3.1). The fish tested in the acclimation rate experiment had an average peak f_{Hmax} of 207.78 ± 6.04 bpm and 31.4 ± 0.3°C T_{ARR} after 14 days at 20°C compared to 213.20 ± 5.94 bpm and 32.7 ± 0.4°C from opaleye held 4 weeks at 20°C. Thus, the majority of the opaleye's acclimation response was complete by week 2 post-temperature change, which is a more representative timescale for what they experience in the wild.

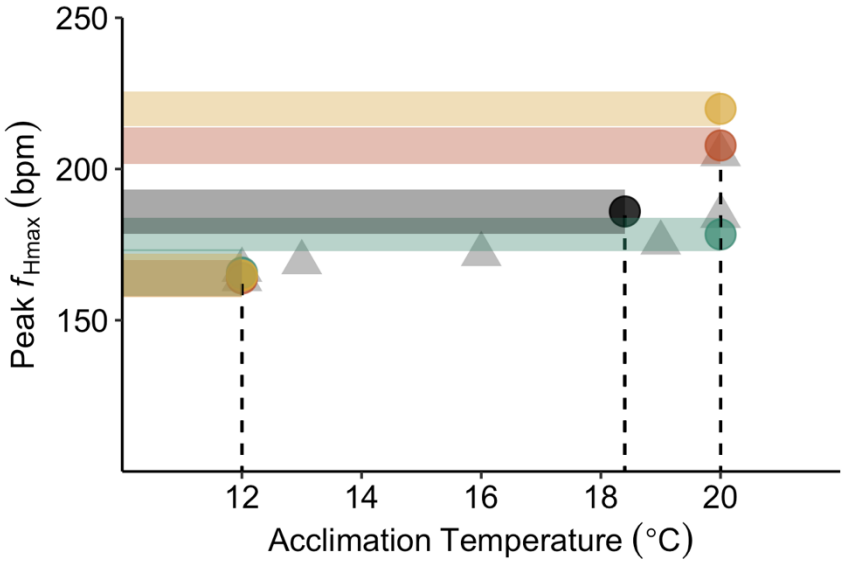

Figure 3.4 Comparison of treatment to opaleye literature values in peak f_{Hmax}. Comparing mean peak f_{Hmax} values from current treatments to seasonally acclimatized opaleye as well as all other available literature values on opaleye (Chapter 2; Schwieterman *et al.*, 2022). Light Gray filled triangles indicate mean values from other studies on opaleye. Colors distinguish diet treatments used in this study (red: carnivorous, green: herbivorous, yellow: omnivorous). Dark gray circle indicates the mean for seasonally acclimatized opaleye. Shaded rectangles indicate ± SEM for each treatment in this study. Dashed lines indicate acclimation or acclimatization temperature (from prior 2 weeks) in this study.

On the last timepoint (day 14 at 20°C), both upper and lower thermal limits for f_{Hmax} were assessed. Diet did not affect either upper (see Table S8) or lower thermal limits (Diet effect on $T_{ARR\text{-}cold}$ $\chi^2 = 0.660$, df = 2, pr>$\chi^2 = 0.719$). For the cold ABT test, $T_{ARR\ -\ COLD}$ was 5.3 ± 0.2°C for the herbivorous, 5.5 ± 0.5°C for the omnivorous, and 5.8 ± 0.6°C for the carnivorous.

Seasonally acclimatized opaleye (i.e., tested immediately following wild capture) experienced an average intertidal temperature of 18.4 ± 1.0°C (mean ± SD) in the 2 weeks prior to field sampling. Fish sampled in the late summer (August) had cardiac and morphometric performance that was closest to the herbivorous treatment at 20°C (Figure 3.4), with an average peak f_{Hmax} of 185.96 ± 7.29 bpm and T_{AB} of 23.4 ±0.3°C, T_{PEAK} of 28.9 ± 0.6°C, and T_{ARR} of 30.6 ± 0.7°C. Further, HSI was an average of 1.1% and RVM was 0.059%, where HSI was most similar to the herbivorous treatment at 12 and 20°C (1.1 and 0.8%, respectively).

3.3.4 Cardiac Fatty Acid Analysis

Diets and ventricles differed in their fatty acid composition (Table S4, Table S5, Figure S2). The algae had lower fatty acids overall and displayed higher proportions of saturated fats compared to brine shrimp (Table S3.4). In the ventricle, however, the DBI was highest in the 20°C herbivorous treatment, which was largely driven by differences in DHA content at that time (Table S3.5, Figure S3.2). Fish in the herbivorous treatment also had lower amounts of fatty acids with 18 carbon chains (e.g., oleic, linoleic, γ-linolenic) compared to the carnivorous and omnivorous treatments (Table S3.5, Figure S3.2). Variation in f_{Hmax} could be partially explained by differences in ventricle DBI. For example, 12°C acclimated fish showed a significant positive relationship between DBI vs. f_{Hmax} at 12°C (p =

0.018; $R^2 = 0.25$; Figure 3.5a), while 20°C acclimated fish displayed a significant negative relationship between ventricle DBI vs. f_{Hmax} at 20°C ($p = 0.009$; $R^2 = 0.24$; Figure 3.5) and ventricle DBI vs. peak f_{Hmax} ($p = 0.002$; $R^2 = 0.32$; Figure 3.5c). There was no relationship between DBI vs. peak f_{Hmax} in 12°C acclimated fish ($p = 0.182$; $R^2 = 0.07$; Figure 3.5c). DBI and average chain length were highly positively correlated, irrespective of diet ($p < 0.001$; $R^2 = 0.98$). Thus, similar trends in average chain length vs cardiac parameters were observed. Several other significant correlations existed between cardiac parameters and specific fatty acid content (Figure 3.5d; Figure S3.3).

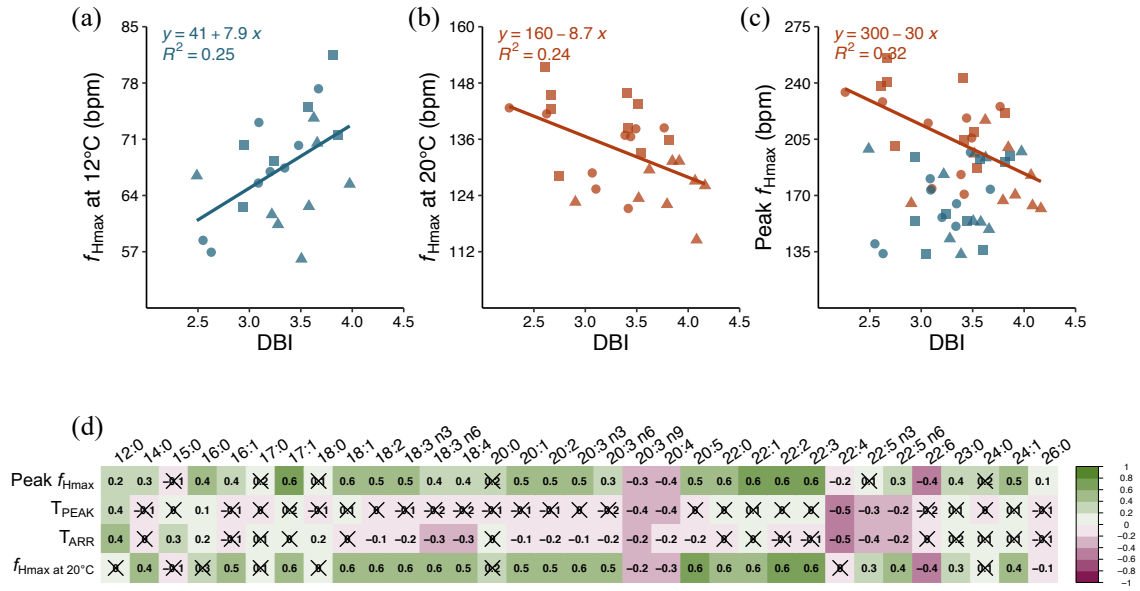

Figure 3.5 Fatty acid analysis from opaleye ventricles**.** Plots a-c show linear regressions between DBI and f_{Hmax} from opaleye across all diet treatments, where shapes indicate different diet treatments (carnivorous: circle, herbivorous: triangle, omnivorous: square). a) f_{Hmax} at 12°C from 12°C acclimated opaleye b) f_{Hmax} at 20°C from 20°C acclimated opaleye c) Absolute peak f_{Hmax} from 12°C (blue) and 20°C (red) acclimated opaleye. Equations are presented for significant linear regressions between parameters ($p<0.05$). There was no significant relationship between peak f_{Hmax} and DBI for 12°C acclimated fish. d) Correlations between cardiac parameters and individual fatty acids or fatty acid summary metrics from 20°C acclimated fish ventricles (made using 'Corrplot' verson 0.84 in R). Numbers and colors indicate the correlation coefficient. Non-significant correlations are crossed out (alpha < 0.05).

3.4 Discussion

Diet is an understudied factor that could impact the rate and capacity for thermal acclimation in ectotherms. Here, we measured how diet impacted the time course of cardiac thermal plasticity in a temperate omnivorous fish, opaleye, during a simulated warming event. We found that diet did influence the acclimation capacity for f_{Hmax} but not the rate of acclimation. We also examined how cardiac thermal performance related to ventricle fatty acid composition and found significant relationships between fatty acid composition and f_{Hmax}. This has important implications for our understanding of species vulnerability to rising temperatures.

3.4.1 Thermal acclimation capacity was diet dependent

In the face of ongoing anthropogenic changes to temperature and food resources (Oliver *et al.*, 2018), it is critical to consider if nutrition can mediate ectotherm resilience. Here, diet clearly impacted cardiac remodeling for f_{Hmax}, but it did not alter the acclimation capacity of thermal limits (T_{AB}, T_{PEAK}, or T_{ARR}). In all diet treatments, thermal limits increased with warm temperature acclimation, consistent with cardiac responses observed in opaleye and many other fishes (Anttila *et al.*, 2014; Chapter 2). For this generalist omnivorous species, a short-term shift to a lower energy, lipid, and protein diet did not constrain the fish's thermal limits or their ability to acclimate them to a higher temperature. Further, the more nutritionally diverse (omnivorous) diet treatment here did not result in higher thermal limits than the other diet treatments.

Instead, the height of the acute TPC for f_{Hmax}, but not the breadth of the TPC, was sensitive to diet. Consequently, diet determined thermal plasticity for f_{Hmax}, with fish in the herbivorous treatment undergoing the lowest change in peak f_{Hmax} between 12 and 20°C.

Similar diet effects on TPCs for f_{Hmax} have been found in two other studies (Chapter 2; Papadopoulou *et al.*, 2022). In Chapter 2, *Ulva* sp. consumption lowered peak f_{Hmax}, but not T_{AB}, T_{PEAK}, or T_{ARR} in opaleye at 20°C (Figure 4). In Papadopoulou *et al.* (2022), allicin supplementation reduced f_{Hmax} across temperatures in exercise trained trout. Our data add to growing evidence that the amplitude of the acute TPC is more plastic than the breadth of the curve. While our results are from measures of cardiac performance, other performance metrics may follow similar patterns. For example, in some cases aerobic capacity may be more sensitive in amplitude, rather than breadth (Norin *et al*., 2014; Scheuffele *et al*., 2022). Differences in the amplitude of acute TPCs can alter functional thermal tolerance. Even though thermal limits were not impaired by diet here, the reduced f_{Hmax} in the herbivorous treatment indicates that f_{Hscope} (max – resting heart rate) could have a narrower thermal breadth if there were not corresponding decreases in resting heart rate (Eliason *et al.*, 2013). In this case, performance under realistic temperature scenarios, or functional thermal tolerance, may be impaired, while more commonly measured extreme thermal limits (e.g., CT_{max}, LT_{50}, T_{ARR}) remain the same. Notably, our findings on fed opaleye here were similar to results on opaleye that were fasted before testing, indicating that being fed did not have a major impact on f_{Hmax} (Chapter 2). However, metabolism (and thus heart rate) increase during digestion and the metabolic cost of digestion can differ depending on an animal's diet (Eliason *et al.*, 2008). Thus, the scope for heart rate during digestion may also vary with diet and could limit functional thermal tolerance in opaleye.

Opaleye are generalists that feed on a mixture of macro-algae, and small invertebrates (e.g., small crustacea, hydroids), but are known to eat proportionally more prey in the colder part of their geographic range (Behrens & Lafferty, 2012). At least in the short term, algae

consumption in this study was not associated with any decrease in cardiac thermal performance at cold temperatures (12°C). Notably, opaleye in the herbivorous treatment had lower HSI (1.1%) than the carnivorous (3.1%) and omnivorous (3.0%) treatments at 12°C, indicative of an energetic disadvantage. However, more research is needed to determine the energetic and performance costs associated with herbivory in active fishes at cold temperatures and how the ecological role of herbivorous and omnivorous fish will change with an increasingly warm and more variable thermal environment.

3.4.2 Thermal acclimation rate did not change with diet

In temperate environments, temperature often changes on shorter timescales than it takes for full thermal acclimation to occur (Hoshijima & Hofmann, 2019). For the endemic ectotherms, plasticity within the first few days after a temperature shift may be more important than overall acclimation capacity. However, most thermal performance data is collected on ectotherms that are fully acclimated to treatment conditions (generally ≥ 2 weeks), leading to overestimations of real-world thermal limits and performance. If diet can alter the rate of acclimation, this could incentivize temperate fish to modify their diet with temperature.

As expected, thermal limits increased throughout the 2-week acclimation to 20°C, but surprisingly, there were no differences between diets. We did not observe any differences in acclimation rate despite HSI being higher in the carnivorous and omnivorous treatments (indicative of greater energy stores) after the initial 2-week acclimation to 12°C. All thermal limits showed a similar logarithmic relationship with time, consistent with acclimation rate studies using a critical thermal maxima test to estimate thermal limits (Fangue *et al*., 2014; Healy & Schulte, 2012). These results add further evidence that consuming a lower quality

diet (i.e., algae) did not impair thermal limits in opaleye, at least on timescales that are environmentally relevant.

Thermal limits were not affected by diet, but peak f_{Hmax} was, where fish from early timepoints had similar cardiac performance irrespective of diet, but performance diverged between the herbivorous and carnivorous/omnivorous treatments during the last week of acclimation to 20°C. Given the greater nutritional diversity (e.g., availability of micro and macronutrients), and flexibility in the omnivorous treatment and that opaleye, and many other aquatic omnivores (Zhang *et al*., 2020), appear to consume greater proportions of algae in their diet in warmer water, we suspected the fish in the omnivorous treatment would have faster acclimation rates relative to those in the carnivorous and herbivorous treatments. However, we did not observe this. While fish in the omnivorous treatment had higher f_{Hmax} across temperatures than those in the herbivorous treatment after 2 weeks at 20°C, they had similar performance to the carnivorous treatment across all metrics and timepoints.

Notably, the fish at 12°C were much less likely to have food in their stomachs at the time of sampling, so it was not possible for us to estimate the exact ratio of algae to brine consumption at that time. However, we only started detecting small amounts of algae consumption on day 3 after raising the tank temperature to 20°C. The lack of differences between the carnivorous and omnivorous treatments may have resulted from fish not eating algae long enough for it to impact their heart rate. While not within the scope of this study, it would be interesting to extend out to longer timepoints and see if prolonged acclimation to the omnivorous treatment at 20°C results in even greater algal consumption and higher cardiac performance. In Chapter 2, opaleye acclimated to three weeks on an omnivorous diet (consisting of *Ulva* sp and brine shrimp) had slightly reduced cardiac thermal performance

relative to a carnivorous treatment, indicating that 3-4 weeks at a given temperature may be necessary to observe subtle diet effects. Consistent with this, Sandblom *et al.* (2014) demonstrated that the metabolism during digestion (or specific dynamic action) and standard metabolic rate took between 4 to 8 weeks to fully acclimate after switching from 10 to 16°C in shorthorn sculpin (*Myoxocephalus scorpius*).

There are a small number of studies that have measured how quickly cardiac performance acclimates in fish, without a diet manipulation (Ekström *et al.*, 2016; Gilbert *et al.*, 2022; Sutcliffe *et al.*, 2020). Interestingly, all studies found that temperate fish rapidly acclimate, with most of their response occurring within 3 days. Ekström *et al.* (2016) measured f_{Hscope} in rainbow trout (*Oncorhynchus mykiss*) acclimated from 9 to 16°C and tested at 16°C. f_{Hmax} increased after 1 day at 16°C, and then began decreasing as acclimation occurred. These results were consistent with data from the herbivorous treatment, where f_{Hmax} at 20°C increased within 3 days of acclimation, but went back down by 2-weeks at 20°C. In contrast, the f_{Hmax} for the carnivorous and omnivorous treatments remained high throughout acclimation to 20°C. Gilbert *et al.* (2022) used the ABT test to determine how f_{Hmax} changed during acclimation from 10 to 18°C in rainbow trout. In line with our results here, trout also underwent >50% of their acclimation response for f_{Hmax} in the first 72 hours post temperature change.

The speed with which temperate fishes, like trout and opaleye, mount an acclimation response indicates that acclimation rate may be a conserved and selected upon factor, although this has yet to be tested. This work demonstrates how essential it is that we integrate time of exposure into our modelling of thermal tolerance and predictions of species responses to climate change. While these fish rapidly acclimate, they are still most vulnerable in the

first few days after a temperature change, as their thermal tolerance has not fully compensated, and they are actively remodeling. It is logistically challenging to gather these data, but once in hand, they can be used to predict performance in complex environments, under variable, more realistic conditions.

3.4.3 Fatty acid composition

Fatty acids serve many important functions, including as the phospholipid tails in biological membranes. Changes in fatty acids and membrane performance can impact cellular processes occurring across them, such as membrane bound enzyme activities, membrane potential, and ion movement (Hochachka & Somero, 2002; Turchini *et al*., 2022), which can have consequences for organ and whole-animal performance. Although membrane fatty acid composition is tightly regulated, composition and content differ greatly across diets and animal fatty acid composition can be related to fatty acid composition of the diet (Farkas *et al.*, 1980; Chatelier *et al.*, 2006). Consequently, dietary fatty acid composition can influence cardiac function, whole-animal metabolism, and swimming performance in fish (Vagner et a;., 2019; McKenzie et al., 1998; Chatelier *et al.*, 2006). For example, in Chatelier *et al.* (2006), seabass (*Dicentrarchus labrax*) had lower cardiac output and aerobic scope when fed a diet containing fish oil as opposed to canola oil or palm oil. The effect of dietary lipids on CT_{max} and lethal thermal limits has also been assessed in a handful of studies, with mixed results. Oil type did not influence upper thermal limits in juvenile angelfish (Ikeda *et al.,* 2011), but total lipid composition (10 vs. 20%) had a significant effect on CT_{max} in juvenile barramundi (Gomez Isaza *et al.*, 2019). The effect of dietary fats on cardiac thermal plasticity in fish is relatively unknown. In our study, ventricle fatty acid composition was related to f_{Hmax} and acclimation temperature.

DBI is a measure of how "unsaturated" a tissue sample is on average, with higher DBI indicating a greater presence of double bonds in the fatty acid tails (Winnikoff *et al.*, 2011). Here, cold-acclimated fish had higher heart rates (improved cardiac performance) when their ventricle fatty acids had more double bonds (DBI), or more fluid membranes (thus counteracting the cold). The opposite trend was observed for warm-acclimated fish: they had improved cardiac performance when their ventricles had fewer double bonds, or reduced membrane fluidity. In particular, saturated fatty acid (SFA) content appeared to drive the relationship between f_{Hmax} and DBI in 12°C acclimated fish, while highly unsaturated fatty acids (HUFA) appeared to be the major driver of the negative relationship between DBI and f_{Hmax} /peak f_{Hmax} in 20°C acclimated fish. DBI and average chain length were also positively correlated, meaning that fish with more unsaturated fats in their ventricle also had longer FA chains. While adding double bonds lowers the melting point and increases membrane fluidity, lengthening the carbon chain raises the melting point and increases membrane stability (Hochachka & Somero, 2002). Thus, the fish may have increased the carbon chain length to partially compensate for their higher DBI.

Even though the algal dietary fatty acid composition had a higher proportion of saturated fats, the herbivorous treatment at 20°C had the highest ventricle DBI, which was largely driven by differences in the HUFA, DHA. DHA is an essential fatty acid critical to cognitive function and development (Pilecky *et al*., 2021). However, it has six double bonds, resulting in a melting point of -44°C. It is unclear why the herbivorous fish ventricles contained more HUFAs, like DHA, relative to SFAs at 20°C. One potential explanation is that the herbivorous fish were metabolizing the SFAs or prioritizing their deposition in other tissues, as SFA have higher energy density compared to HUFAs of the same length (although

digestibility is lower). Given the lower lipid content of the herbivorous diet, the fish could have prioritized meeting their metabolic demand over optimizing their membrane fluidity.

Given the high degree to which cells can regulate membrane performance and the many other functions for lipids in the body, it is not surprising that only ~20-30% of the variation in f_{Hmax} could be explained by fatty acid composition. Christen *et al.* (2020) found significant negative linear relationships between thermal limits (time to CT_{max}) and omega-3 fatty acid composition in *Salvelinus alpinus, Salvelinus fontinalis,* and their hybrids. Here, there were some significant correlations between specific fatty acids and cardiac thermal limits, although there was no significant linear regression found between overall DBI and cardiac thermal limits. While not evaluated here, other factors, such as sterol composition (Hassett & Crockett, 2009), phospholipid class (Guderley, 2004) and phospholipid fatty acid composition (Guderley et al., 2008), are known influencers of membrane performance that may have differed between the fish ventricles at the time of sampling and could explain more of the variation in heart rate across treatments.

The global availability of the essential omega-3 fatty acids, DHA and EPA, are expected to decrease with increasing temperatures associated with climate change (Hixson & Arts, 2016; Pilecky *et al.*, 2021). While there is concern over the predicted reduction in the global availability of omega-3 FAs, the results here indicate that higher tissue SFA is associated with improved cardiac performance in warm for opaleye. In other words, shifts in global FA composition may be of concern for human nutrition and other measures of fish performance (e.g., fish cognitive health), but could also mediate cardiac upper thermal tolerance in fishes.

3.5 Conclusion

Our results demonstrate that for generalist species, like opaleye, variation in diet is an important modulator of thermal plasticity on environmentally relevant timescales. While diet did not affect the fish's thermal limits for f_{Hmax} or acclimation rate, it did impact the height of their acute TPC. Thus, ectotherm thermal performance changes as a function of exposure time, and the amplitude of acute TPCs can be influenced by the animal's nutrition. Mechanistically, fatty acids were related to heart performance, indicating that they may be a primary regulator of cardiac thermal performance in ectotherms. While temperate fish, like opaleye, have a remarkable capacity for rapid thermal plasticity, they are still most vulnerable in the first few days after a warming event, as they have yet to fully compensate for changes in temperature. These results highlight the importance of accounting for changes in nutrition, and thermal exposure time when assessing ectotherm thermal tolerance.

IV Chapter 4

4.1 Introduction

Climate change is altering food availability and the nutritional quality of plants and animals in marine food webs, which may exacerbate the harmful effects of other environmental stressors on marine life (Chapter 1). For example, in 2014-2016, a series of marine heatwaves known as "the blob" reduced primary production in several of the Pacific Northwest's iconic giant kelp forests (Michaud *et al.*, 2022). Reductions in food, along with the unprecedented heat, are implicated as the cause behind rapid declines in many sessile invertebrate species, with several forests suffering lasting changes in community composition and animals range shifts (Michaud *et al.*, 2022; Sanford *et al.*, 2019; Beas-Luna *et al.*, 2020). In other systems, like coral reefs, variation in nutrient availability, due to thermal stratification in the water column and nutrient pollution from terrestrial run-off, exacerbate the harmful effects of extreme temperature events on coral health, causing devastating impacts to the entire reef community (Ezzat et al., 2019; Zaneveld *et al.*, 2016). In order to predict how ecosystem-level changes will manifest in future extreme temperature and food-stress events, it is critical that we better understand the organismal consequences of changing ocean temperature and animal nutrition.

In addition to changes in food availability, ocean warming is expected to reduce the abundance of omega-3 poly unsaturated fats (n-3 PUFA), EPA and DHA, in aquatic food webs (IPCC, Hixson & Arts, 2016; Tan *et al.*, 2022). DHA and EPA can be synthesized to some degree in vertebrates from the essential fatty acid ALA (18:3 n3), but the process is energetically expensive and so dietary sources of these nutrients are critical for marine fish to maintain high levels of tissue n-3 PUFA (Pilecky *et al*., 2021). Given that DHA and EPA are

made almost exclusively in aquatic environments, humans rely heavily on seafood for these nutrients. While these predictions are alarming from a human health and nutrition standpoint, they are also concerning from an animal physiology perspective, as EPA and DHA are critical for animal growth, cognition, vision, membrane bound protein function, metabolism, and more (Pilecky *et al.*, 2021; Tan *et al*., 2022). However, there is an important trade-off here that must also be considered. Many n-3 PUFA have low melting points and raise membrane fluidity when assimilated into cell membranes due to lower bonding strength to neighboring fatty acids (Hochachka & Somero, 2002). Cells must maintain a precise balance between membrane fluidity and stability to ensure optimal cross-cell communication, signaling, metabolism, action potentials, and performance of other cellular functions (Hochachka & Somero, 2002). Thus, high dietary n-3 PUFA content may improve membrane performance in cold, while reducing performance in warm conditions.

In line with this prediction, under cold conditions, high PUFA diets are often beneficial. For example, drosophila could not survive overwinter without eating a high PUFA diet (Brankatscht *et al.*, 2018) and several species have improved cold tolerance after being fed diets with more n-3 PUFA, compared to those with higher saturated fatty acids (SFA) (Abdel-Ghany *et al.*, 2019; Craig *et al.,* 1995). In contrast, juvenile Atlantic salmon (*Salmo salar*) fed diets deficient in n-3 PUFA had lower mitochondrial efficiency and growth in cold and warm water (13 and 18°C; Závorka *et al.*, 2021). Therefore, while abundance of dietary n-3 PUFA has been shown to improve cold performance in animals, reduction in dietary n-3 PUFA does not necessarily confer them with greater warm performance. These trade-offs may be exacerbated by concurrent changes in food availability and environmental temperature.

As temperature rises in ectothermic animals, like fish, so does their metabolic demand (Schulte *et al.*, 2015). While many animals compensate for their rising metabolism by simply eating more, this may not always be possible if food availability is simultaneously changing or temperatures are too warm for the animal to efficiently forage, ingest, or digest a meal (Jutfelt *et al.*, 2021). Many fish use fats as a primary fuel source (Turchin *et al.*, 2022), and so increases in temperature or reductions in food availability can affect how the fish uses its dietary fats; whether it be as fuel to meet their metabolic demand or in assimilating the fats into tissues for growth, reproduction, or maximizing thermal tolerance and performance. This suggests that when food is abundant, fat quality might have a lesser impact on performance and animals may easily meet their metabolic demand while simultaneously maximizing their performance of other traits.

Ultimately, when an animal's nutritional status changes it may impair their energy balance and ability to tolerate co-occurring stressors and environmental extremes. Here, we examined the combined effects food restriction, dietary n-3 PUFA composition, and temperature on the energetics, thermal tolerance, and cardiac thermal performance of the temperate omnivorous fish, opaleye (*Girella nigricans*). We collected wild-caught juvenile opaleye and acclimated them in a factorial design to two environmentally relevant temperatures (presumed optimal: 15°C and climate-change scenario: 22°C), while simultaneously manipulating the amount of food they were offered (either a high or low ration), and the amount of n-3 PUFA in their diet (n-3 PUFA enriched high fat diet or n-3 PUFA poor lower fat diet). Then, we measured their metabolism using aquatic respirometry, warm and cold tolerance using an Arrhenius Breakpoint Temperature (ABT) test for cardiac performance and critical thermal minima (CT_{MIN}) test, and nutritional status (from organ

morphometrics and whole fish fat and protein composition). We hypothesized that (1) opaleye would be able to eat more to improve their thermal tolerance and aerobic performance, especially in the climate-change scenario temperature, (2) dietary fat quality would have a greater impact on opaleye performance when their food was restricted, and (3) under food restriction, the n-3 PUFA enriched diet would improve the fish's cold tolerance and give them an energetic advantage (due to the high fat composition of the diet), possibly at the cost to maintaining a high warm tolerance in the fish. By understanding how these various co-occurring changes interact, we can better predict the impacts of environmental change on marine life and food web dynamics.

4.2 Methods

4.2.1 Collections

Juvenile opaleye were collected using hook and line in the summer of 2022 from Santa Barbara Harbor, Santa Barbara, CA, USA and brought back to the Eliason seawater lab at the University of California, Santa Barbara in well oxygenated coolers (>70% air saturation). Once in the lab, they were held in a flow-through system using 95 l fiberglass tanks. The fish were grouped by size class to avoid bullying and distributed equally across treatments (7-9 fish per tank; 3 replicate tanks per treatment; total N = 200 fish). Before acclimations began, fish were held in ambient ocean temperature conditions (14.8 ± 0.5°C; mean ± SD) and fed *Artemia* sp. (brine shrimp).

4.2.2 Treatments

Opaleye were held at two environmentally relevant temperatures (presumed optimal: 15°C and climate change-scenario: 22°C) and fed on one of two ration levels (high ration: 20% bm day^{-1} in wet weight or low ration: 8% bm day^{-1} in wet weight) and two brine shrimp types (n-3 PUFA enriched brine shrimp or normal brine shrimp; Hakari brand). Standard brine shrimp is generally low in n-3 PUFA, thus, we classified these diets as 1) High ration n-3 PUFA enriched, 2) Low ration n-3 PUFA enriched, 3) High Ration n-3 PUFA-poor, 4) Low ration n-3 PUFA-poor. The two brine shrimps also differed in their overall lipid composition, with the n-3 PUFA enriched diet having greater lipid composition overall (lipid = 11 vs. 17 % of dry weight, protein = 38% of dry weight in both diets). Most of the differences in fat composition were driven by differences in n-3 PUFA, however, a few other fatty acids that made up a smaller proportion of dietary fats (e.g., 16:0, 16:1) also differed between the two diets. The exact differences in fatty acid composition between the diets are presented in Table 4.1. The ration sizes were set to be a high growth ration under the current summer extreme temperature of 22°C vs a maintenance ration at 22°C. We chose to standardize the amount of food offered across acclimation temperatures to maximize the ecological relevance of the diets. If we had reduced the amount of food in the colder treatment, this would suggest that food availability decreases at moderate temperatures (15°C), which is not the case in this system.

Fish were fed across two daily feedings: morning (~10-11:00 am) and afternoon (~3:00-4:00 pm). During feeding, the standpipe was covered in a fine mesh filter that ensured no brine shrimp was lost down the drain. The fish were allowed to feed freely for 30 minutes with the filter on before it was removed and replaced with the usual standpipe cover (which had larger holes to avoid clogging and subsequent tank overflow from fecal matter and

leftover food). Across most treatments, fish generally ate all of the brine shrimp in the first 10-15 minutes of the feeding, with exception of the 15°C-acclimated high ration treatments, where the fish always had leftover food in the tank.

Temperature was heated and cooled at the building level. The 22°C treatment also had one 800-Watt immersion heater (Finnex, McCook, IL, USA) placed inside the tank to ensure it was maintained at 22°C. Temperature and oxygen were monitored daily with an Omega Thermocouple (Omega Engineering, Norwalk, CT, USA) and Oxygaurd Handy Polaris (OxyGuard International A/S, Farum, Denmark). Temperature was also continuously monitored using ibuttons (iButtonLink LLC, Whitewater, WI, USA) programmed to record every 15-20 minutes. Average temperatures for each tank are reported in Supplemental Table 4.1, but the overall average across all tanks throughout the study was 15.2 ± 0.6 and 21.9 ± 0.4 (mean ± SD).

4.2.3 Timeline of testing

Following >2 weeks of acclimation, we tested the fish's metabolism using intermittent flow aquatic respirometry (testing occurred during week 2-3.5 of acclimation), warm tolerance using an Arrhenius breakpoint test (week 3.5-5 of acclimation), and cold tolerance using a CT_{MIN} test (week 5-5.5 of acclimation). Fish were weighed every ~1.5-2 weeks to update ration sizes if they grew. After 3-5 weeks of acclimation some fish in the high omega-22°C treatments began developing signs of disease. Thus, the remaining fish in this treatment were humanely euthanized and CT_{MIN} tests were not performed on this treatment. Only visibly healthy fish from all treatments were included in the dataset.

Table 4.1. Dietary fatty acid composition

Fatty Acid (carbon chain length: # of double bonds)	Concentration of fatty acid in Artemia sp. diets (pmol/mg wet tissue)	
	n-3 PUFA deplete	n-3 PUFA enriched
12:0	1.733 ± 1.002	3.5 ± 0.2
14:0	34.167 ± 20.611	91.7 ± 13.061
15:0	23.233 ± 11.441	26.233 ± 10.058
16:0	590.133 ± 302.617	846.3 ± 48.257
16:1	134.167 ± 64.443	426.3 ± 75.771
17:0	29.333 ± 6.301	37.333 ± 9.356
17:1	0.500 ± 0.100	2.7 ± 1.819
18:0	245.400 ± 101.001	528.6 ± 111.221
18:1	1467.033 ± 783.804	1320.067 ± 79.79
18:2	240.067 ± 145.201	451.133 ± 29.272
18:3 n3	881.567 ± 395.995	2271.567 ± 414.865
18:3 n6	69.967 ± 48.700	88.5 ± 7.318
18:4	517.667 ± 308.732	963.933 ± 483.335
20:0	3.133 ± 1.501	12.967 ± 3.683
20:1	16.500 ± 7.275	53.367 ± 10.536
20:2	5.433 ± 2.589	17.267 ± 1.29
20:3 n3	13.733 ± 3.787	74.6 ± 40.428
20:3 n6	6.467 ± 3.950	15.7 ± 1.808
20:3 n9	0.167 ± 0.058	2.333 ± 1.097
20:4	70.567 ± 37.838	111.633 ± 42.394
20:5	276.933 ± 31.089	1541 ± 680.309
22:0	1.133 ± 0.603	2.333 ± 0.551
22:1	0.667 ± 0.153	5 ± 1.249
22:2	0 ± 0	0.667 ± 0.153
22:3	0.133 ± 0.058	1.267 ± 0.153
22:4	0.767 ± 0.586	4.967 ± 1.801
22:5 n3	2.767 ± 1.234	172 ± 73.904
22:5 n6	6.467 ± 5.348	24.933 ± 9.465
22:6	36.000 ± 15.997	1405.9 ± 673.577
23:0	0.167 ± 0.058	0.367 ± 0.058
24:0	0.333 ± 0.058	0.9 ± 0.346
24:1	0.167 ± 0.058	2.867 ± 0.321
26:0	0.267 ± 0.058	0.567 ± 0.379

Represented are means ± SD of fatty acids in the two different *Artemia* sp. diets. Fatty acids are listed as the carbon chain length: number of double bonds and n followed by a number indicates the placement of a double bond when multiple options for the placement are possible. Shaded rows denote omega-3 fatty acids, with 18:3 n3 = alpha-linolenic acid; 18:4 = stearidonic acid; 20:3 n3 = dihomo-alpha-linolenic acid; 20:5 = eicosapentaenoic acid; 22:3 DTrE; 22:5 n3 = docosapentaenoic acid; 22:6 = docosahexaenoic acid.

4.2.4 Metabolism: Set up and trials

Metabolism was estimated using aquatic intermittent flow respirometry and best practices as described in Chapter 2. Methods are described using guidelines outlined in Killen *et al.*, 2021. Fish were placed in plastic respirometers (0.57-1.90 L based of fish size; calculation includes recirculation loop tubing volume; fish size to chamber volume ratios ranged = 16-46; mean ± SD = 29 ± 7). Each respirometer was connected to a recirculating pump (Eheim Universal 300 pumps, Eheim, Germany) via clear PVC tubing and calibrated to a flow 150 L/hour using flow valves set in the recirculation line. All respirometers were also attached to a flush pump (Eheim Universal 300) set to flush at a rate of 150 L/hour, which was connected to a timer which turned it on and off. The combined flush and recirculation cycle lasted 15 minutes, but the exact timing of each measurement phase varied depending on temperature and animal size to ensure adequate O_2 levels were maintained throughout the trials, while simultaneously capturing a linear decrease in O_2 with a high R^2 value. Oxygen was recorded continuously throughout the trials using Pyroscience Firesting fiberoptic oxygen probes and meter (Pyroscience, Germany), which recorded every ~1 second. The probe was set in the recirculation loop. Oxygen probes were calibrated using a 2-point calibration following manufacturer's instructions (0% made by mixing sodium sulfite in freshwater and 100% using aerated seawater) before the trials. The calibration was checked periodically throughout the trials to ensure recalibration was not necessary over the short duration that trials occurred (~1.7 weeks).

During the trials, the fish were placed in the respirometers overnight for ~20 hours to determine standard metabolic rate (SMR). The tanks were covered in shade-cloth lids and all trials occurred in isolated environmental chambers to ensure minimal disturbance to the animals. Animals were fasted 24 hours prior to the start of the trial. The trial tanks were plumbed to the UC Santa Barbara flow-through seawater system, with seawater that was gravity sand filtered to 20 microns. Heating was maintained using in bath submersible heaters (Finnex, McCook, IL, USA) and was maintained between 14.25-16.27 or 21.04-22.86 °C (max and min temperature recorded across trials in each acclimation treatment). The following morning, the fish were removed from the chambers and chased for five minutes before being immediately returned to the respirometer to capture MMR (Chapter 2).

4.2.5 Metabolism: Data analysis

SMR was calculated as the low 15% quantile of all measurement cycles. MMR was calculated as the steepest slope from the first measurement cycle lasting 120 s. However, during spontaneous activity overnight a few fish reached higher MO2 values. In this case, that highest value recorded was used. Absolute aerobic scope (AAS) was MMR – SMR, and Factorial Aerobic scope was MMR/SMR. To calculate these metrics, the following pipeline was conducted: The first and last 30 seconds of each measurement phase were removed from analysis to avoid any mixing or pump delays. Background respiration was estimated before and after each trial in at least 3 measurement cycles. In instances where background respiration was present, we assumed a linear relationship between the pre- and post-trial background measurements and subtracted the subsequent estimates of background from the according MO_2 values (ranged from 0-48% of signal in a single measurement, but the average background across any overnight trial was always <32%). We statistically

confirmed that there was no relationship between the amount of background in a trial and the estimate of SMR for that trial. While oxygen was well maintained during trials (at well over 80% air saturation for >95% of the time in the chamber), during bouts of overnight spontaneous activity, oxygen occasionally and briefly dipped below 80% air saturation; generally, when the lights turned on or off in the environmental chamber where the fish were tested. Individuals were excluded from the dataset if oxygen fell below 65% air saturation during any bouts of activity (n = 3 fish). We tested for evidence of body size scaling by comparing mixed effect models (using Schwarz information criterion; SIC) that varied in their scaling coefficient (e.g., global fish coefficient 0.89, isometric scaling, or slope of the log-log plot) and did not find sufficient evidence of an allometric scaling relationship to transform the data (Jerde *et al.*, 2019). All respirometry data and relevant meta data (e.g., fish body mass, chamber volume, etc.) will be provided in an online dryad repository upon publication. Sample sizes for each treatment ranged 9-13 and are reported in Table 4.2.

4.2.6 Warm Tolerance (Arrhenius breakpoint temperature - ABT – test)

Warm thermal tolerance was measured as outlined in Chapter 2 and 3. Briefly, the fish were anesthetized in seawater containing buffered MS-222 at their acclimation temperature and placed ventral side up in an experimental sling with water continuously circulating over their gills. To measure heart rate, a stainless-steel needle-tip electrode was inserted just under the skin on the ventral side of the fish above the heart. The ECG signal was amplified using a dual bioamp and filtered as described in Chapter 3.

The fish were given a 30 minute habituation period in the 10L test tank before being injected intraperitonially with atropine (1.2 mg kg-1 in 0.9% NaCl) followed by isoproterenol (4 ug kg-1 in 0.9% NaCl) to induce maximum intrinsic heart rate. After a 15 min

equilibration period, temperature was increased at a rate of 1°C every 6 minutes while continuously recording heart rate. At each 1°C interval, the temperature was stabilized to allow for a 15 second period of ECG at that temperature. The fish were kept in the test until the degree just following the onset of cardiac arrhythmia (T_{ARR}), at which point the fish was removed from the test tank, immediately euthanized and subsequently dissected for organ and body morphometrics.

The data were analyzed in LabChart (www.adinstruments.com). Estimates of maximum heart rate (f_{Hmax}) were taken from 15 s of continuous ECG recordings at each 1°C temperature interval. For each individual, two upper thermal limits were determined, (1) T_{PEAK} was the temperature corresponding to the highest maximum heart rate and (2) T_{ARR} was the temperature at which arrhythmia occurred. Both represent important transition temperatures where further increases in temperature are met with rapid declines in heart performance. Sample sizes for each treatment ranged 7-12 and are reported in Table 4.3.

4.2.7 Cold Tolerance (Critical thermal minimum – CT_{MIN} test)

Cold tolerance was assessed as outlined in Chapter 2 using a standardized critical thermal minimum test (CT_{MIN}). The opposite of CT_{MIN} is CT_{MAX}, which is a measure of warm tolerance (see Chapter 2). Here we opted to use the ABT test to measure warm tolerance instead of CT_{MAX} because it provides information about a fish's heart performance and its warm tolerance limits. Also, T_{PEAK} and T_{ARR} are generally much lower than CT_{MAX}, and are considered more physiologically relevant estimates of the warmest temperatures fish can survive at. However, running the ABT test in reverse (i.e., cooling the water instead of heating it) is a much longer test than CT_{MIN}, but provides more conservative estimates of cold tolerance in opaleye. For example, in Chapter 2, a breakpoint temperature at cold was

not able to be estimated in several fish, and T_{ARR} at cold was lower than CT_{MIN} by 0.7-1.7°C (see Chapter 2). Thus, we opted to use CT_{MIN} for cold tolerance and the ABT test for warm tolerance. To run the CT_{MIN} test, 7-15 fish (larger fish were tested at lower tank density) at a time were placed into the ~57 L experimental tank and given 10 minutes to adjust to the new environment. The water was cooled using a 1 HP AquaEuro chiller (AquaEuroUSA, Los Angeles, CA, USA) and sea ice at a rate of decline of ~0.3°C per minute. CT_{MIN} temperature for each fish was recorded at the exact time when they lost equilibrium for a period of 5 s. Immediately following loss of equilibrium, each fish was removed from the experimental tank and placed into a recovery tank at an intermediate temperature before being returned back to their original tanks. Sample sizes for each treatment ranged 10-14.

4.2.8 Dissections and proximate analysis

Fish that were tested in the ABT test were weighed, measured for total length and dissected for ventricle and liver mass. A subset of the fish were stored at -20°C for whole-fish proximate analysis. Proximate composition (i.e., moisture, lipids, protein, ash) for the diets and whole fish was determined using methods outlined in Chapter 2. Fatty acid composition was determined for the diets at UCSD lipidomics core using methods outlined in Quehenberger *et al.* (2010).

4.2.10 Statistical Analysis

All data were statistically analyzed in R studio (version 4.2.3) For all metrics, we tested for heterogeneity of variance using Levene's test and normality using a shapiro wilke's and visual assessment of quantile-quantile plots. Traits were log transformed before statistical analysis when they did not meet the assumptions (i.e., HSI). A 3-way anova was

run to test for the impacts of acclimation temperature, diet type, and ration size (car package version 3.1-2) followed by a post-hoc Tukey HSD for significant main effects (emmeans package version 1.8.5). We tested for a three-way interaction but dropped the interaction when found to be non-significant. Because we did not obtain CT_{MIN} data on fish fed the n-3 PUFA enriched diet at 22°C, we analyzed the CT_{MIN} data in two parts: first comparing data from 15°C acclimated fish and then comparing high and low ration and acclimation temperature effects, but only in low n-3 PUFA fed fish. Thermal performance curve data was analyzed by comparing various polynomial mixed effects models using SIC, where the best fit model was the one with the lowest SIC value, but all models within 7 of the best fit model were considered. The models we compared varied in their main effects, but all accounted for individual as a random effect.

4.3 Results

4.3.1 Metabolism

Standard metabolic rate increased with acclimation temperature but was not affected by ration size or diet type (Table 4.4, Figure 4.1). Maximum metabolic rate and absolute aerobic scope both increased with acclimation temperature and were impacted by an interactive effect of ration size and diet type (Table 4.4, Figure 4.1). Within a diet type, ration size did not impact maximum metabolic rate or absolute aerobic scope. However, when the fish's food was restricted, the opaleye fed the n-3 PUFA deplete diet had 11-19% higher maximum metabolic rate and 15-23% higher absolute aerobic scope across both acclimation temperatures compared to the fish fed an n-3 enriched diet (Table 4.4, Figure 4.1).

Table 4.2: Summary statistics for respirometry metrics

Temp (°C)	Ration Size	Diet Type	n	SMR (mgO_2 kg^{-1} min^{-1})	MMR (mgO_2 kg^{-1} min^{-1})	AAS (mgO_2 kg^{-1} min^{-1})	FAS (MMR/SMR)
15	low	omega-poor	13	1.71 ± 0.04^{a}	6.68 ± 0.32^{a}	5.30 ± 0.26^{af}	4.12 ± 0.18^{a}
		omega-enriched	11	1.74 ± 0.06^{a}	5.60 ± 0.28^{bc}	4.32 ± 0.19^{bc}	3.50 ± 0.09^{c}
	high	omega-poor	12	1.81 ± 0.07^{a}	6.67 ± 0.17^{ac}	4.86 ± 0.16^{ac}	3.74 ± 0.15^{c}
		omega-enriched	11	1.85 ± 0.08^{a}	6.43 ± 0.32^{ac}	4.69 ± 0.25^{ac}	3.56 ± 0.13^{c}
22	low	omega-poor	11	2.46 ± 0.12^{b}	9.38 ± 0.33^{d}	6.92 ± 0.36^{d}	3.91 ± 0.26^{a}
		omega-enriched	11	2.58 ± 0.12^{b}	8.46 ± 0.36^{e}	6.01 ± 0.29^{ef}	3.38 ± 0.15^{c}
	high	omega-poor	9	2.67 ± 0.11^{b}	8.57 ± 0.34^{de}	5.91 ± 0.29^{def}	3.24 ± 0.11^{c}
		omega-enriched	11	2.60 ± 0.13^{b}	8.77 ± 0.41^{de}	6.16 ± 0.41^{def}	3.44 ± 0.20^{c}

Means ± SEM for each test are presented. MMR, maximum metabolic rate; SMR, standard metabolic rate; AAS, absolute aerobic scope; FAS, factorial aerobic scope. Letters indicate post-hoc results for comparisons made on significant main effects. Note that only significant main effects were run through post hoc analysis.

Factorial aerobic scope was influenced by a similar interactive effect of ration size and diet type, where low ration n-3 PUFA poor diets conferred ~17% higher factorial aerobic scope compared to low ration n-3 PUFA enriched diets (post hoc $p = 0.0038$) and 14% higher than the high ration n-3 deplete and 15% higher than the high ration n-3 PUFA enriched diet (Table 4.4, Figure 4.1). However, acclimation temperature did not impact factorial aerobic scope ($p = 0.052$).

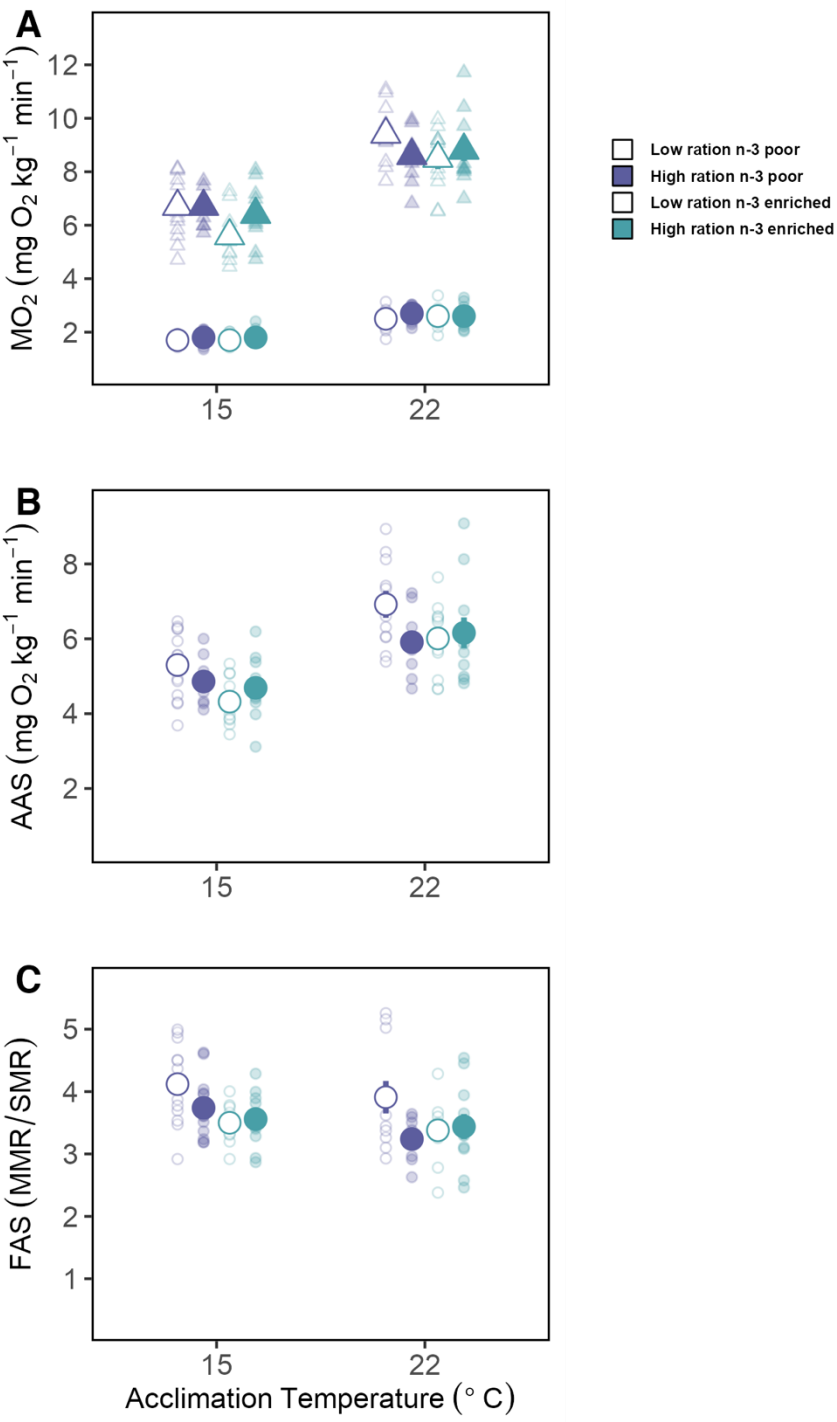

Figure 4.1. Ration, diet type, and temperature effects on whole-animal metabolism. Oxygen uptake rate (mg O_2 kg^{-1} min^{-1}) after an exhaustive chase protocol in opaleye acclimated to 15 or 22°C and fed either a high or low ration diet and n-3 enriched or deplete food source. (A) Standard metabolic rate (SMR) is indicated by circles and maximum metabolic rate (MMR) is indicated by triangles, (B) absolute aerobic scope (AAS; MMR−RMR) and (C) factorial aerobic scope (FAS; MMR/SMR). Open shapes indicate low ration treatments and filled in shapes indicate high ration treatments. Colors indicate diet type, with purple illustrating

responses from fish fed a n-3 PUFA deplete diet and green showing fish fed a n-3 PUFA enriched diet. Individual responses are indicated by the smaller translucent points, and treatment level mean (± SEM) is overlayed in the larger shape.

4.3.2 Cardiac thermal tolerance

Maximum heart rate followed a classic thermal performance curve shape, where it increased with temperature until T_{PEAK}, and then rapidly declined before T_{ARR}. The curves also shifted right on the x-axis with acclimation to the heatwave treatment of 22°C (Figure 4.2). When comparing these thermal performance curves across treatments, the best fit model was a third order polynomial that accounted for an interactive effect of ration size and acclimation temperature with a random effect of fish ID – formula: lmer(bpm~poly(temp,3) * ration size * acclimation temperature (°C) + (1|fish id)), with the next best model having a delta BIC of 28.461 (Figure 4.2). Diet type did not emerge as a factor influencing the overall thermal performance curves; however, it did have an interactive effect on trait values of interest that were calculated from the curves (T_{PEAK}, T_{ARR}, Peak $f_{Hmax;}$ Table 4.5, Figure 4.3). Here, ration size and diet type had significant interactive effects on all traits, where higher ration diet led to higher T_{PEAK}, T_{ARR}, Peak $f_{Hmax,}$ but only in fish fed the diet containing minimal n-3 PUFA. Peak f_{Hmax} did not change with acclimation temperature, but T_{ARR} and T_{PEAK} increased slightly with acclimation to 22°C.

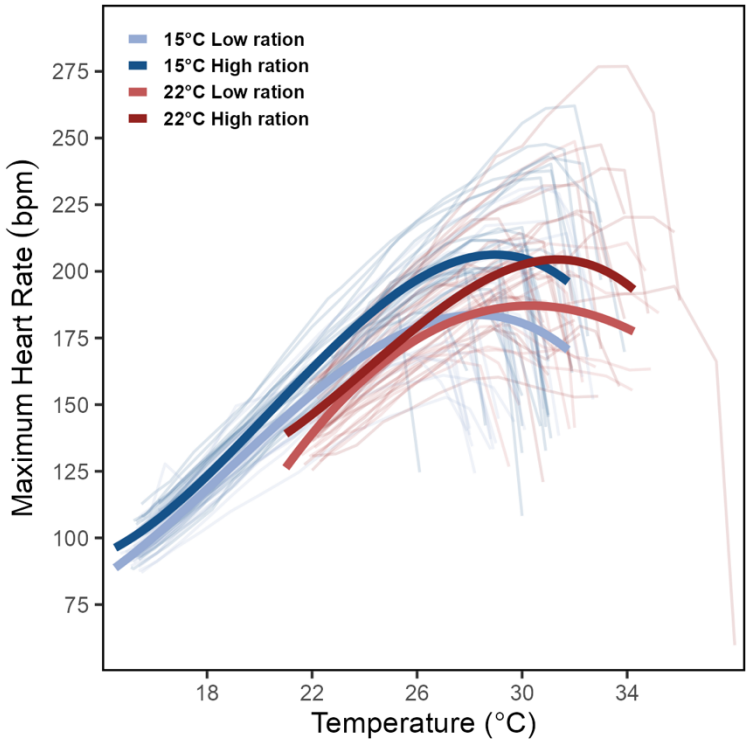

Figure 4.2. Dietary effects on acute TPC for maximum heart rate. Individual- and treatment-level responses of maximum heart rate (f_{Hmax}) of opaleye during acute warming. Colors distinguish fish from different diet treatments (blue: 15°C acclimated; red: 22°C acclimated) and shades indicate ration size (darker colors indicate high ration). The thin lines are data from individual fish and the thicker curves are the treatment level effects determined from model comparison. The best fit model was a third-order polynomial that accounted for an interaction acclimation temperature × ration size with random effects of individual fish id. This was determined to be the best fit model by SIC (Table S4.1).

Table 4.3: Summary statistics for thermal tolerance metrics and morphometrics

Temp (°C)	Ration Size	Diet Type	n	Peak f_{HMAX} (bpm)	T_{PEAK} (°C)	T_{ARR} (°C)	HSI	RVM
15	low	omega-poor	11	184.58 ± 6.18^{a}	27.9 ± 0.5^{a}	29.1 ± 0.4^{a}	1.743 ± 0.112^{ae}	0.056 ± 0.002^{a}
		omega-enriched	12	194.14 ± 6.63^{ab}	28.5 ± 0.5^{abc}	29.9 ± 0.5^{abc}	2.427 ± 0.140^{bg}	0.055 ± 0.002^{a}
	high	omega-poor	12	222.01 ± 5.50$_{b}$	30.1 ± 0.3^{bcd}	31.5 ± 0.3^{bcd}	1.798 ± 0.111^{af}	0.055 ± 0.003^{a}
		omega-enriched	11	208.65 ± 6.83^{b}	28.8 ± 0.5^{acf}	29.6 ± 0.6^{ac}	3.410 ± 0.499^{b}	0.057 ± 0.001^{a}
22	low	omega-poor	9	185.57 ± 4.40^{a}	29.2 ± 0.4^{bcd}	31.6 ± 0.7^{bcd}	0.807 ± 0.05^{c}	0.048 ± 0.001^{b}
		omega-enriched	7	205.83 ± 8.81^{ab}	31.2 ± 0.6^{def}	32.2 ± 0.6^{de}	1.419 ± 0.074^{e}	0.055 ± 0.002^{b}

	high	omega-poor	10	209.55 ± 9.45^{b}	31.4 ± 0.9^{e}	32.8 ± 1.0d^{e}	1.423 ± 0.136^{de}	0.053 ± 0.002^{b}
		omega-enriched	11	205.74 ± 8.38^{b}	30.1 ± 0.6^{bde}	31.9 ± 0.4^{bde}	2.191 ± 0.172^{fg}	0.054 ± 0.002^{b}

Means ± SEM for each test are presented. T_{PEAK}, temperature corresponding to maximum heart rate; T_{ARR}, temperature at the onset of cardiac arrhythmia; Peak f_{Hmax}, maximum heart rate achieved during the warm ABT test; HSI = hepatosomatic index; RVM = relative ventricular mass. Letters indicate post-hoc results. Note that only significant main effects were run through post hoc analysis.

Table 4.4. Statistics outputs from ANOVA for all metrics from the respirometry trials.

Parameter	Factor	Sum Sq	df	F value	P value
SMR	Ration	0.241	1	2.599	0.111
	Diet Type	0.021	1	0.230	0.633
	Temperature	14.109	1	152.464	<0.001
MMR	Ration	0.231	1	0.203	0.653
	Diet Type	6.516	1	5.723	0.019
	Temperature	134.019	1	117.708	<0.001
	Ration x Diet Type	4.758	1	4.179	0.044
FAS	Ration	1.114	1	3.461	0.066
	Diet Type	2.247	1	6.979	0.010
	Ration x Diet Type	1.753	1	5.445	0.022
AAS	Ration	1.065	1	1.204	0.276
	Diet Type	5.068	1	5.728	0.019
	Temperature	47.534	1	53.722	<0.001
	Ration x Diet Type	4.958	1	5.603	0.020

Table 4.5. Statistics outputs from ANOVA for metrics from the ABT test.

Parameter	Factor	Sum sq	df	F value	P value
T_{PEAK}	Ration	19.052	1	5.782	0.019
	Diet Type	0.390	1	0.118	0.732
	Temperature	54.708	1	16.601	<0.001
	Ration x Diet Type	31.450	1	9.544	0.003
T_{ARR}	Ration	13.641	1	3.998	0.049
	Diet Type	3.809	1	1.116	0.294
	Temperature	88.114	1	25.823	<0.001
	Ration x Diet Type	23.770	1	6.966	0.010
Peak f_{Hmax}	Ration	8427.167	1	16.212	<0.001
	Diet Type	43.649	1	0.084	0.773
	Ration x Diet Type	2633.309	1	5.066	0.027

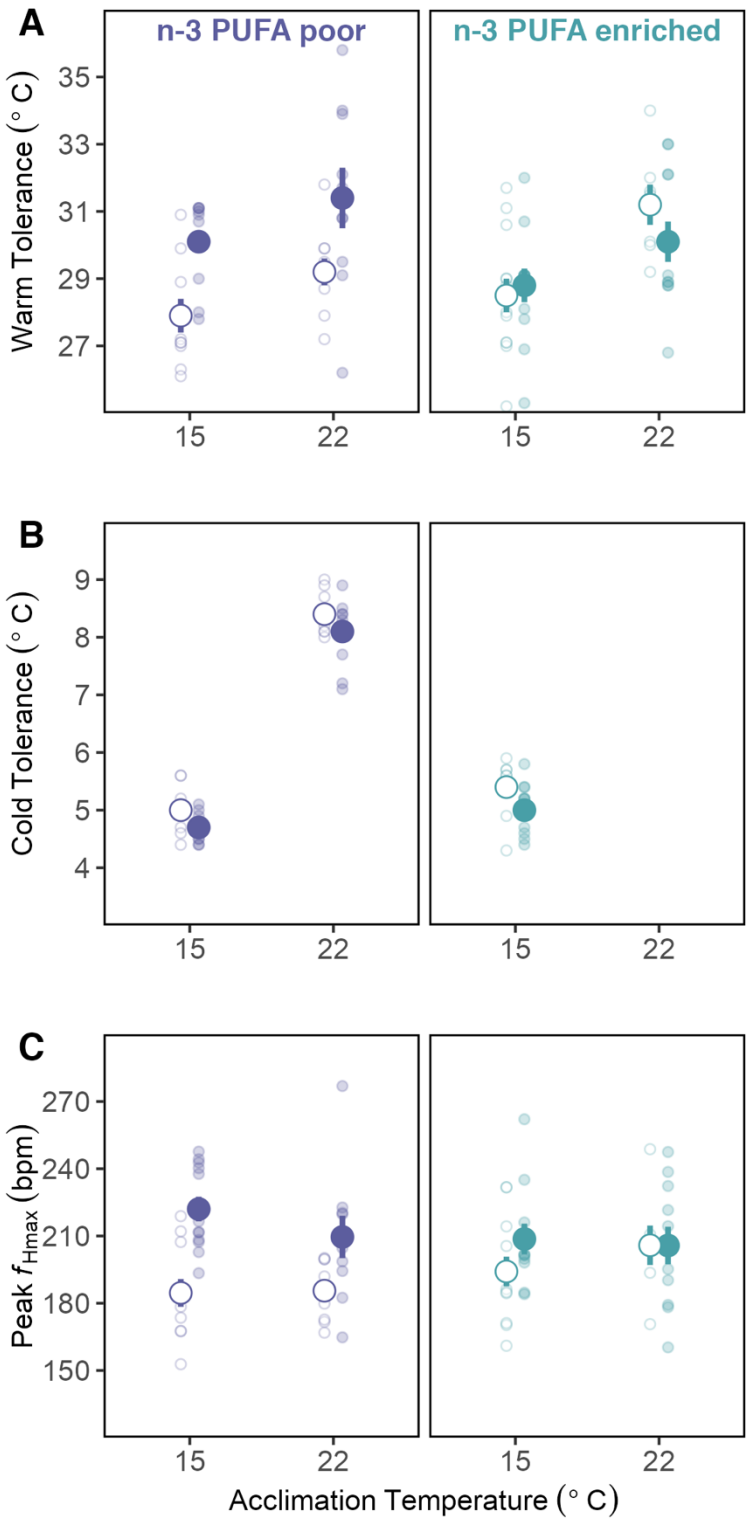

Figure 4.3. Ration, diet type, and temperature effects on cardiac thermal limits and peak f_{Hmax}. For all plots, colors distinguish diet types (purple: n-3 PUFA poor, green: n-3 PUFA enriched), with high ration treatments indicated by the filled in circles and low ration indicated by the open circles. Individual responses are indicated by the smaller translucent

points, and treatment level mean (± SEM) is overlayed. **(A)** Individual- and treatment-level responses for warm tolerance (T_{PEAK}) across diet treatments and acclimation temperatures in opaleye. **(B)** Individual- and treatment-level responses for cold tolerance (CT_{MIN}) across diet treatments and acclimation temperatures in opaleye. **(a)** Individual- and treatment-level responses for peak f_{Hmax} across diet treatments and acclimation temperatures in opaleye.

4.3.3 CT_{MIN}

CT_{MIN} decreased with acclimation to 15°C, indicating an improved cold tolerance at the colder acclimation temperature (ration: df = 1, f-stat = 8.046, p = 0.007; temperature: df = 1, f-stat = 821.811, p < 0.001; Figure 4.3). CT_{MIN} was also impacted by ration size, where high ration diets slightly improved the fish's cold tolerance by ~0.3°C. Finally, fish fed n-3 PUFA enriched diets had surprisingly higher CT_{MIN} (worse cold tolerance) across both ration sizes in the 15°C-acclimation treatment by ~0.3°C (ration: df = 1, f-stat = 11.628, p = 0.0013; diet type: df = 1, f-stat = 11.261, p = 0.0016; Figure 4.3).

4.3.4 Morphometrics

Opaleye had slightly higher RVM after acclimation to 15°C (~6% higher than at 22°C; p = 0.036), but their RVM was not influenced by diet (p = 0.165). However, HSI was affected by all three treatment variables (diet type: df = 1, f-stat = 68.657, p < 0.001; ration size: df = 1, f-stat = 27.309, p < 0.001; acclimation temperature: df = 1, f-stat = 67.341, p < 0.001; note that HSI data were log transformed for analysis) and an interactive effect of ration size and acclimation temperature (df = 1, f-stat = 8.318, p = 0.005). The heatwave temperature led to lower HSI across diet treatments and the enriched n-3 PUFA diet resulted in higher HSI (Table 4.5). Additionally, the higher ration diet resulted in higher HSI, but only at 22°C.

4.4 Discussion

4.4.1 Opaleye can eat more to improve their thermal performance across temperatures

We found strong evidence that higher food availability improves performance across a wide range of temperatures in opaleye, regardless of their dietary fat composition. Fish that were fed more had higher maximum heart rates across a thermal gradient, improved cold tolerance (CT_{MIN}), and higher HSI at 22°C. Food restriction can alter ectotherm behavioural thermoregulation, risk-taking behavior, growth, metabolism, and environmental tolerance (Rodgers *et al.*, 2019; Huey & Kingsolver, 2019; Gilbert & Miles, 2016; McCue, 2010; Lienart *et al.*, 2014). For example, food restriction can reduce thermal tolerance in some species (Lee *et al.*, 2016; Nyamukondiwa & Terblanche, 2009; Woiwode & Aidelman, 1980), although not all species react to food restriction in the same way across traits (Rodgers *et al.*, 2018; Mclean & Todgham, 2015; Lee *et al.*, 2016). For our results, food restriction universally impaired cold tolerance, but only impacted warm tolerance in fish fed a n-3 PUFA deplete diet. This demonstrates the importance of considering both quality and quality in concert.

Interestingly, we did not observe any benefits to aerobic performance resulting from the higher ration treatments. Food restriction often leads to metabolic depression in animals, which causes reductions in their SMR and can lead to artificially high aerobic scope (see Burton *et al.*, 2011 for relevant review). However, we did not observe this here, potentially because our most restricted treatment was still a maintenance ration for these fish. Further food restriction would likely result in metabolic depression in this species, however more research is necessary to determine the threshold ingestion rate that would elicit this response in these fish, and how that threshold varies depending on prey quality.

We originally predicted that ration size would have a greater impact on performance under the marine heatwave treatment of 22°C. The rations were standardized to be a high growth and maintenance ration for the 22°C acclimated fish. At 15°C, the fish in the low ration treatments were being fed more than a maintenance ration and the fish in the high ration treatments never finished all the food that they were offered. Thus, we expected the fish's performance between high and low ration treatments to converge at 15°C. Instead, we found the exact opposite. The opaleye displayed dramatic differences in maximum heart rate at both acclimation temperatures, indicating that even small differences in their food intake can have large thermal performance consequences at both optimal and suboptimal temperatures.

4.4.2 Dietary fat quality had a greater impact on opaleye thermal performance when their food was restricted

Outside of HSI, diet type had no impact on any thermal tolerance or metabolic traits in high ration fed fish. However, in support of our hypothesis, diet quality had several impacts on the fish's performance when they were food limited. Fish in the n-3 PUFA poor treatments had higher maximum metabolic rates, absolute and factorial aerobic scope at both acclimation temperatures when compared to their n-3 PUFA enriched counterparts, indicating that this diet led to greater aerobic performance in food restricted opaleye. In contrast, T_{PEAK}, peak f_{HMAX} and T_{ARR} were impacted by an interaction between ration size and diet type, where n-3 PUFA enriched diets rescued the impacts of eating less on the fish's heart thermal performance. In other words, high and low ration diets conferred the same warm tolerance in fish fed the n-3 PUFA enriched diet, but not in the fish fed the n-3 PUFA poor diet. Fish fed the low ration n-3 PUFA deplete diet had impaired warm tolerance.

It is not clear why the lower fat/n-3 PUFA deplete diet caused higher aerobic performance in the fish compared to the higher fat/higher n-3 PUFA diet (in food restricted individuals). Interestingly, these results were driven by differences in maximum, not standard metabolic rate and were observed across both acclimation temperatures. To better understand the mechanism underpinning these trends, our future work will investigate phospholipid profiles in ventricles from these fish. These data will uncover how the pressures placed on these fish in their respective treatments impacted their nutrient assimilation, and whether variation in nutrient assimilation to metabolically critical organs like the heart, can explain variation observed in cardiac performance and whole animal metabolism. Overall, these results highlight an idea described in detail in Chapter 2 of this thesis - that different diets may produce thermal performance trade-offs, where they may maximize the performance of one trait while minimizing performance of another (See Chapter 2 Figure 1.1).

4.4.3 Under food restriction, the n-3 PUFA enriched diet did not confer greater cold tolerance

In contrast to our hypothesis, n-3 PUFA enriched diets impaired cold tolerance compared to the n-3 PUFA poor diets. This was a highly unexpected finding that we have yet to fully understand. Given that 15°C is not a "cold" temperature for this species, it may be that the opaleye did not assimilate n-3 PUFA in large amounts regardless of diet type. Running comprehensive lipidomic analyses on tissues of interest (i.e., heart, brain, etc.) would help resolve some of these outstanding mechanistic questions. Although, fat composition and assimilation can differ depending on tissue type (Skalli *et al*., 2006), so any future work would need to consider how nutrient assimilation may vary across tissues and impact thermal tolerance of different organ systems independently. Especially, given that

CT_{MIN} is caused by neural disfunction, while T_{PEAK} and T_{ARR} are measures of heart thermal tolerance.

We also anticipated that warm tolerance may be higher in the n-3 PUFA poor diet during acclimation to 22°C, especially in the high ration treatment. However, we did not observe this. Given that the diets differed in fat amount and composition, it may be that the n-3 PUFA enriched diets had enough saturated fats to maintain high warm thermal tolerance and metabolized or used the n-3 PUFA in other organ systems besides the heart. A next step will be to conduct follow up studies using custom research diets which vary in (1) their fat amount, but have the same ratios of fatty acids, and (2) the fatty acid composition, but have the same total amount of fat in the diet. Work has been done in other species, where oil type or oil inclusion was manipulated in aquaculture fish pelleted diets and subsequently, warm or cold thermal tolerance was measured (Abdel-Ghany *et al.* 2019; Craig *et al.*, 1995; Gomez *et al.*, 2019). However, these studies were almost exclusively conducted at one acclimation temperature and used high ration feeding regimes. To better understand how diet impacts thermal performance in wild animals, it is essential to consider quantity and quality effects simultaneously. This means we need to continue diverting resources to food web research as well as studies on the ingestion rates of wild animals to ground our experiments in realistic diet scenarios.

4.4.4 Dietary effects on warm vs cold tolerance

Diet had a much greater impact on warm tolerance than it did on cold tolerance in opaleye. Temperature acclimation only increased the fish's warm tolerance by 1.3-2.7°C (T_{PEAK} change across diets) while ration size caused a ~2.2°C increase in warm tolerance in the n-3 PUFA poor fed fish. In contrast, acclimation temperature caused a ~3.4°C change in

the fish's cold tolerance, while diet altered their cold tolerance by only 0.3-0.4°C. These differences could be because we used two different assessments of thermal tolerance (heart thermal tolerance vs CT test), or it may indicate the fish's cold tolerance is more sensitive to temperature acclimation, while their warm tolerance is more sensitive to their diet. 22°C is especially warm for this population (see Chapter 2), and the fish may have reduced capacity to increase their upper thermal limits and may be nearing a thermal ceiling at that temperature (Sandblom *et al.*, 2016).

4.5 Conclusion

As marine fish gain most of their energy from fats, they must balance assimilating them into tissues against metabolizing them to meet their metabolic demand. Their capacity to maintain an optimal balance between the two may be impaired by reductions in food, declines in valuable nutrients, and co-occurring environmental stressors, such as rising temperature. Here, we assessed the impacts of changes in the amount of food, along with the amount of omega-3 fatty acids in the diet, on opaleye metabolism and thermal tolerance after acclimation to two environmentally relevant temperatures and found that dietary fat quality had a greater impact on opaleye performance when the fish were fed *less* across both temperatures. Specifically, diets low in n-3 PUFA conferred higher aerobic scope but led to lower upper thermal limits. Ration size was also an important determinant of thermal performance, where fish fed high ration diets had higher maximum heart rates across temperatures and slightly better cold tolerance. These results demonstrate how important it is to consider diet when evaluating ectotherm responses to climate change, as diet quality and quantity can interactively affect thermal plasticity and tolerance.

References

ABDEL-GHANY, H.M., EL-SAYED, A.F.M., EZZAT, A.A., ESSA, M.A. & HELAL, A.M. (2019) Dietary lipid sources affect cold tolerance of Nile tilapia (Oreochromis niloticus). *Journal of Thermal Biology* **79**, 50–55.

AKHTAR, M.S., PAL, A.K., SAHU, N.P., CIJI, A., MEENA, D.K. & DAS, P. (2013) Physiological responses of dietary tryptophan fed Labeo rohita to temperature and salinity stress. *Journal of Animal Physiology and Animal Nutrition* **97**, 1075–1083.

ALTON, L.A., KUTZ, T.C., BYWATER, C.L., BEAMAN, J.E., ARNOLD, P.A., MIRTH, C.K., SGRÒ, C.M. & WHITE, C.R. (2020) Developmental nutrition modulates metabolic responses to projected climate change. *Functional Ecology* **34**, 2488–2502.

ALHAZZAA, R., BRIDLE, A.R., NICHOLS, P.D. & CARTER, C.G. (2013) Coping with sub-optimal water temperature: Modifications in fatty acid profile of barramundi as influenced by dietary lipid. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **165**, 243–253.

ANDERSON, T.R., HESSEN, D.O., BOERSMA, M., URABE, J. & MAYOR, D.J. (2017) Will invertebrates require increasingly carbon-rich food in a warming world? *American Naturalist* **190**, 725–742.

ANDRADE, D. V, CRUZ-NETO, A.P., ABE, A.S. & WANG, T. (2005) Specific dynamic action in ectothermic vertebrates: a review of the determinants of postprandial metabolic response in fishes, amphibians, and reptiles. *Physiological and ecological adaptations to feeding in vertebrates*. Science Publishers Enfield, NH.

ANTTILA, K., CASSELMAN, M.T., SCHULTE, P.M. & FARRELL, A.P. (2013) Optimum temperature in juvenile salmonids: connecting subcellular indicators to tissue function and whole-organism thermal optimum. *Physiological and biochemical zoology* **86**, 245–256.

ANTTILA, K., COUTURIER, C.S., ØVERLI, Ø., JOHNSEN, A., MARTHINSEN, G., NILSSON, G.E. & FARRELL, A.P. (2014) Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nature Communications* **5**, 6–11.

ARAÚJO, M.B., FERRI-YÁÑEZ, F., BOZINOVIC, F., MARQUET, P.A., VALLADARES, F. & CHOWN, S.L. (2013) Heat freezes niche evolution. *Ecology letters* **16**, 1206–1219.

ARNOLD, K.E., RAMSAY, S.L., HENDERSON, L. & LARCOMBE, S.D. (2010) Seasonal variation in diet quality: Antioxidants, invertebrates and blue tits Cyanistes caeruleus. *Biological Journal of the Linnean Society* **99**, 708–717.

ATWOOD, H.L., TOMASSO, J.R., WEBB, K. & GATLIN, D.M. (2003) Low-temperature tolerance of Nile tilapia, Oreochromis niloticus: effects of environmental and dietary factors. *Aquaculture Research* **34**, 241–252.

AYAYEE, P.A., KINNEY, G., YARNES, C., LARSEN, T., CUSTER, G.F., VAN DIEPEN, L.T.A. & MUÑOZ-GARCIA, A. (2020) The role of the gut microbiome in mediating standard metabolic rate after dietary shifts in the viviparous cockroach, Diploptera punctata. *The Journal of Experimental Biology* **223**, jeb.218271.

BARRY, J.P. & EHRET, M.J. (1993) Diet , food preference , and algal availability for fishes and crabs on intertidal reef communities in southern California. *Environmental Biology of Fishes* **37**, 75–95.

BEAS-LUNA, R., MICHELI, F., WOODSON, C.B., CARR, M., MALONE, D., TORRE, J., BOCH, C., CASELLE, J.E., EDWARDS, M., FREIWALD, J., HAMILTON, S.L., HERNANDEZ, A., KONAR,

B., KROEKER, K.J., LORDA, J., ET AL. (2020) Geographic variation in responses of kelp forest communities of the California Current to recent climatic changes. *Global Change Biology* **26**, 6457–6473.

BEHRENS, M.D. & LAFFERTY, K.D. (2007) Temperature and diet effects on omnivorous fish performance: implications for the latitudinal diversity gradient in herbivorous fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 867–873.

BEHRENS, M.D. & LAFFERTY, K.D. (2012) Geographic Variation in the Diet of Opaleye (Girella nigricans) with Respect to Temperature and Habitat. *PLOS One* **7,** e45901.

BEITINGER, T. & LUTTERSCHMIDT, W. (2011). Temperature Measures of thermal tolerance. In *Encyclopedia of Fish Physiology: From Genome to Environment* (ed. A. P. Farrell), pp. 1695-1702. Elsevier Ltd.

BERNHARDT, J.R. & LESLIE, H.M. (2013) Resilience to climate chnge in coastal marine ecosystems. *Annual Review of Marine Science* **5**, 371–392.

BESTION, E., JACOB, S., ZINGER, L., DI GESU, L., RICHARD, M., WHITE, J. & COTE, J. (2017) Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. *Nature Ecology and Evolution* **1**, 1–3.

BIRNIE-GAUVIN, K., PEIMAN, K.S., RAUBENHEIMER, D. & COOKE, S.J. (2017) Nutritional physiology and ecology of wildlife in a changing world. *Conservation Physiology* **5**, cox030.

BLEWETT, T.A. & GOSS, G.G. (2017) A novel pathway of nutrient absorption in crustaceans: Branchial amino acid uptake in the green shore crab (Carcinus maenas). *Proceedings of the Royal Society B: Biological Sciences* **284**, 1–6.

BLÖDORN, E.B., DOMINGUES, W.B., NUNES, L.S., KOMNINOU, E.R., PINHAL, D. & CAMPOS, V.F. (2021) MicroRNA roles and their potential use as selection tool to cold tolerance of domesticated teleostean species: A systematic review. *Aquaculture* **540**, 736747.

BOERSMA, M., MATHEW, K.A., NIEHOFF, B., SCHOO, K.L., FRANCO-SANTOS, R.M. & MEUNIER, C.L. (2016a) Temperature-driven changes in the diet preference of omnivorous copepods: no more meat when it's hot? A response to Winder et al. *Ecology Letters* **19**, 1386–1388.

BOERSMA, M., MATHEW, K.A., NIEHOFF, B., SCHOO, K.L., FRANCO-SANTOS, R.M. & MEUNIER, C.L. (2016b) Temperature driven changes in the diet preference of omnivorous copepods: no more meat when it's hot? *Ecology Letters* **19**, 45–53.

BOGEVIK, A.S., HENDERSON, R.J., MUNDHEIM, H., WAAGBØ, R., TOCHER, D.R. & OLSEN, R.E. (2010) The influence of temperature on the apparent lipid digestibility in Atlantic salmon (Salmo salar) fed Calanus finmarchicus oil at two dietary levels. *Aquaculture* **309**, 143–151.

BOWYER, J.N., QIN, J.G. & STONE, D.A.J. (2013) Protein, lipid and energy requirements of cultured marine fish in cold, temperate and warm water. *Reviews in Aquaculture* **5**, 10–32.

BRANKATSCHK, M., GUTMANN, T., KNITTELFELDER, O., PALLADINI, A., PRINCE, E., GRZYBEK, M., BRANKATSCHK, B., SHEVCHENKO, A., COSKUN, Ü. & EATON, S. (2018) A Temperature-Dependent Switch in Feeding Preference Improves Drosophila Development and Survival in the Cold. *Developmental Cell* **46**, 781-793.

BREDVIK, J.J., BOERGER, C. & ALLEN, L.G. (undated) Age and Growth of Two Herbivorous, Kelp Forest Fishes, the Opaleye (Girella nigricans) and Halfmoon (Medialuna californiensis). *Bulletin, Southern California Academy of Sciences* **110**, 25–34.

BRETT, J.R. (1944). Some lethal temperature relations of AIgonquin Park fishes. *Publication of the Ontario Fisheries Research Laboratory* **63**, 1-49.
BRETT, J.R. (1971) Energetic Responses of Salmon to Temperature. A Study of Some Thermal Relations in the Physiology and Freshwater Ecology of Sockeye Salmon (Oncorhynchus nerka). *American Zoologist* **11**, 99–113.
BRETT, J.R., SHELBOURN, J.E. & SHOOP, C.T. (1969) Growth Rate and Body Composition of Fingerling Sockeye Salmon, Oncorhynchus nerka, in relation to Temperature and Ration Size. *Journal of the Fisheries Research Board of Canada* **26**, 2363–2394.
BRZEZIŃSKI, T. & VON ELERT, E. (2015) Predator evasion in zooplankton is suppressed by polyunsaturated fatty acid limitation. *Oecologia* **179**, 687–697.
BROWN, J.H., GILLOOLY, J.F., ALLEN, A.P., SAVAGE, V.M. & WEST, G.B. (2004) Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.
BURKEPILE, D.E. & HAY, M.E. (2008) Herbivore species richness and feeding complementarity affect community structure and function on a coral reef **105**, 16201–16206.
BURTON, T., KILLEN, S.S., ARMSTRONG, J.D. & METCALFE, N.B. (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences* **278**, 3465–3473.
BURTON, T., RATIKAINEN, I.I. & EINUM, S. (2022) Environmental change and the rate of phenotypic plasticity. *Global Change Biology* **28**, 5337–5345.
CARREIRA, B.M., SEGURADO, P., LAURILA, A. & REBELO, R. (2020) Heat waves trigger swift changes in the diet and life-history of a freshwater snail. *Hydrobiologia* **847**, 999–1011.
CARREIRA, B.M., SEGURADO, P., ORIZAOLA, G., GONÇALVES, N., PINTO, V., LAURILA, A. & REBELO, R. (2016) Warm vegetarians? Heat waves and diet shifts in tadpoles. *Ecology* **97**, 2964–2974.
CARUSO, M.A. & SHERIDAN, M.A. (2011) Gut anatomy and morphology | Pancreas. *Encyclopedia of Fish Physiology* **2**, 1276–1283.
CASSELMAN, M.T., ANTTILA, K. & FARRELL, A.P. (2012) Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon Oncorhynchus spp. *Journal of Fish Biology* **80**, 358–377.
CASTAÑEDA, L.E., SABAT, P., GONZALEZ, S.P. & NESPOLO, R.F. (2006) Digestive plasticity in tadpoles of the Chilean giant frog (Caudiverbera caudiverbera): Factorial effects of diet and temperature. *Physiological and Biochemical Zoology* **79**, 919–926.
CASTRO, C., PÉREZ-JIMÉNEZ, A., GUERREIRO, I., PERES, H., CASTRO-CUNHA, M. & OLIVA-TELES, A. (2012) Effects of temperature and dietary protein level on hepatic oxidative status of Senegalese sole juveniles (Solea senegalensis). *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **163**, 372–378.
CAVOLE, L.M., DEMKO, A.M., DINER, R.E., GIDDINGS, A., KOESTER, I., PAGNIELLO, C.M.L.S., PAULSEN, M.L., RAMIREZ-VALDEZ, A., SCHWENCK, S.M., YEN, N.K., ZILL, M.E. & FRANKS, P.J.S. (2016) Biological impacts of the 2013–2015 warm-water anomaly in the northeast Pacific: Winners, Losers, and the Future. *Oceanography* **29**, 273–285.
CHABOT, D., KOENKER, R. & FARRELL, A.P. (2016a) The measurement of specific dynamic action in fishes. *Journal of Fish Biology* **88**, 152–172.

CHABOT, D., STEFFENSEN, J.F. & FARRELL, A.P. (2016b) The determination of standard metabolic rate in fishes. *Journal of Fish Biology* **88**, 81–121.
CHATELIER, A., MCKENZIE, D.J., PRINET, A., GALOIS, R., ROBIN, J., ZAMBONINO, J. & CLAIREAUX, G. (2006) Associations between tissue fatty acid composition and physiological traits of performance and metabolism in the seabass (Dicentrarchus labrax). *Journal of Experimental Biology* **209**, 3429–3439.
CHEN, Z., DEVLIN, R.H. & FARRELL, A.P. (2015) Upper thermal tolerance of wild-type, domesticated and growth hormone-transgenic coho salmon Oncorhynchus kisutch. *Journal of Fish Biology* **87**, 763–773.
CHRISTEN, F., DUFRESNE, F., LEDUC, G., DUPONT-CYR, B.A., VANDENBERG, G., FRANÇOIS, N.R.L., TARDIF, J.C., LAMARRE, S. & BLIER, P.U. (2020) Thermal tolerance and fish heart integrity: Fatty acids profiles as predictors of species resilience. *Conservation Physiology* **8**, coaa108.
CHRISTEN, F., DESROSIERS, V., DUPONT-CYR, B.A., VANDENBERG, G.W., LE FRANÇOIS, N.R., TARDIF, J.C., DUFRESNE, F., LAMARRE, S.G. & BLIER, P.U. (2018) Thermal tolerance and thermal sensitivity of heart mitochondria: Mitochondrial integrity and ROS production. *Free Radical Biology and Medicine* **116**, 11–18.
CHUNG, D.J. & SCHULTE, P.M. (2020) Mitochondria and the thermal limits of ectotherms. *Journal of Experimental Biology* **223**, jeb227801.
CLAIREAUX, G. & LEFRANÇOIS, C. (2007) Linking environmental variability and fish performance: integration through the concept of scope for activity. *Philosophical Transactions of the Royal Society B: Biological Sciences* **362**, 2031–2041.
COGGINS, B.L., COLLINS, J.W., HOLBROOK, K.J. & YAMPOLSKY, L.Y. (2017) Antioxidant capacity, lipid peroxidation, and lipid composition changes during long-term and short-term thermal acclimation in Daphnia. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* **187**, 1091–1106.
COGGINS, B.L., PEARSON, A.C. & YAMPOLSKY, L.Y. (2021) Does geographic variation in thermal tolerance in Daphnia represent trade-offs or conditional neutrality? *Journal of Thermal Biology* **98**, 102934.
CLARK, T.D., SANDBLOM, E. & JUTFELT, F. (2013) Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology* **216**, 2771–2782.
CLEMENTS, K.D., ANGERT, E.R., MONTGOMERY, W.L. & CHOAT, J.H. (2014) Intestinal microbiota in fishes: What's known and what's not. *Molecular Ecology* **23**, 1891–1898.
CLEMENTS, K.D., RAUBENHEIMER, D. & CHOAT, J.H. (2009) Nutritional ecology of marine herbivorous fishes: ten years on. *Functional Ecology* **23**, 79–92.
CLISSOLD, F.J., COGGAN, N. & SIMPSON, S.J. (2013) Insect herbivores can choose microclimates to achieve nutritional homeostasis. *Journal of Experimental Biology* **216**, 2089–2096.
COGGAN, N., CLISSOLD, F.J. & SIMPSON, S.J. (2011) Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. P*roceedings of the Royal Society, B* **278**, 2745–2752.
COTTRELL, R.S., METIAN, M., FROEHLICH, H.E., BLANCHARD, J.L., SAND JACOBSEN, N., MCINTYRE, P.B., NASH, K.L., WILLIAMS, D.R., BOUWMAN, L., GEPHART, J.A., KUEMPEL, C.D., MORAN, D.D., TROELL, M. & HALPERN, B.S. (2021) Time to rethink trophic levels in aquaculture policy. *Reviews in Aquaculture* **13**, 1583–1593.

CRAIG, S.R., NEILL, W.H. & GATLIN, D.M. (1995) Effects of dietary lipid and environmental salinity on growth, body composition, and cold tolerance of juvenile red drum (Sciaenops ocellatus). *Fish Physiology and Biochemistry* **14**, 49–61.

CROSS, W.F., HOOD, J.M., BENSTEAD, J.P., HURYN, A.D. & NELSON, D. (2015) Interactions between temperature and nutrients across levels of ecological organization. *Global Change Biology* **21**, 1025–1040.

DAHLHOFF, E.A. & SOMERO, G.N. (1993) Effects of temperature on mitochondria from abalone (genus Haliotis): adaptive plasticity and its limits. *Journal of Experimental Biology* **185**, 151–168.

DAUFRESNE, M., LENGFELLNER, K. & SOMMER, U. (2009) Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 12788–12793.

DELL, A.I., PAWAR, S. & SAVAGE, V.M. (2011) Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 10591–10596.

DEUTSCH, C., FERREL, A., SEIBEL, B., PÖRTNER, H.-O. & HUEY, R.B. (2015) Climate change tightens a metabolic constraint on marine habitats. *Science* **348**, 1132–1135.

DEUTSCH, C.A., TEWKSBURY, J.J., HUEY, R.B., SHELDON, K.S., GHALAMBOR, C.K., HAAK, D.C. & MARTIN, P.R. (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 6668–6672.

DEVRIES, Z.C. & APPEL, A.G. (2014) Effects of temperature on nutrient self-selection in the silverfish lepisma saccharina. *Physiological Entomology* **39**, 217–221.

DIXON, T.M., RHYNO, E.M., EL, N., MCGAW, S.P., OTLEY, N.A., PARKER, K.S., BULDO, E.C., PABODY, C.M., SAVOIE, M., COCKSHUTT, A., MORASH, A.J., LAMARRE, S.G. & MACCORMACK, T.J. (2023) Taurine depletion impairs cardiac function and affects tolerance to hypoxia and high temperatures in brook char (Salvelinus fontinalis) **226**, jeb245092.

DUNBAR, H.E., WILSON, A.C.C., FERGUSON, N.R. & MORAN, N.A. (2007) Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biology* **5**, 1006–1015.

DZIKOWSKI, R., HULATA, G., KARPLUS, I. & HARPAZ, S. (2001) Effect of temperature and dietary L-carnitine supplementation on reproductive performance of female guppy (Poecilia reticulata). *Aquaculture* **199**, 323–332.

EKSTRÖM, A., HELLGREN, K., GRÄNS, A., PICHAUD, N. & SANDBLOM, E. (2016) Dynamic changes in scope for heart rate and cardiac autonomic control during warm acclimation in rainbow trout. *Journal of Experimental Biology* **219**, 1106–1109.

ELIASON, E.J. & ANTTILA, K. (2017) 4 - Temperature and the Cardiovascular System. In *Fish Physiology* (eds A.K. GAMPERL, T.E. GILLIS, A.P. FARRELL & C.J. BRAUNER), pp. 235–297. Academic Press.

ELIASON, E.J., CLARK, T.D., HINCH, S.G. & FARRELL, A.P. (2013) Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. *Conservation Physiology* **1**, cot008.

ELIASON, E.J., HIGGS, D.A. & FARRELL, A.P. (2007) Effect of isoenergetic diets with different protein and lipid content on the growth performance and heat increment of rainbow trout **272**, 723–736.

ELIASON, E.J., HIGGS, D.A. & FARRELL, A.P. (2008) Postprandial gastrointestinal blood flow, oxygen consumption and heart rate in rainbow trout (Oncorhynchus mykiss). *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **149**, 380–388.

ELIASON, E.J., VAN WERT, J.C. & SCHWIETERMAN, G.D. (2022) *Applied aspects of the cardiorespiratory system*. In *Conservation Physiology for the Anthropocene – A Systems Approach Part A* p., 1st edition. Elsevier Inc.

EMDE, S., KOCHMANN, J., KUHN, T., DÖRGE, D.D., PLATH, M., MIESEN, F.W. & KLIMPEL, S. (2016) Cooling water of power plant creates "hot spots" for tropical fishes and parasites. *Parasitology Research* **115**, 85–98.

EZZAT, L., MAGUER, J.F., GROVER, R., ROTTIER, C., TREMBLAY, P. & FERRIER-PAGÈS, C. (2019) Nutrient starvation impairs the trophic plasticity of reef-building corals under ocean warming. *Functional Ecology* **33**, 643–653.

FANGUE, N.A., WUNDERLY, M.A., DABRUZZI, T.F. & BENNETT, W.A. (2014) Asymmetric thermal acclimation responses allow sheepshead minnow cyprinodon variegatus to cope with rapidly changing temperatures. *Physiological and Biochemical Zoology* **87**, 805–816.

FARKAS, T., CSENGERI, I., MAJOROS, F. & OLÁH, J. (1980) Metabolism of fatty acids in fish. III. Combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, Cyprinus carpio Linnaeus 1758. *Aquaculture* **20**, 29–40.

FARRELL, A.P. (2011) *Encyclopedia of fish physiology: from genome to environment*. Academic press.

FARRELL, A.P. (2016) Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *Journal of Fish Biology* **88**, 322–343.

FERREIRA, E.O., ANTTILA, K. & FARRELL, A.P. (2014) Thermal optima and tolerance in the eurythermic goldfish (Carassius auratus): Relationships between whole-animal aerobic capacity and maximum heart rate. *Physiological and Biochemical Zoology* **87**, 599–611.

FLOETER, S.R., BEHRENS, M.D., FERREIRA, C.E.L., PADDACK, M.J. & HORN, M.H. (2005) Geographical gradients of marine herbivorous fishes: patterns and processes. *Marine Biology* **147**, 1435–1447.

FONTAINE, S.S., MINEO, P.M. & KOHL, K.D. (2022) Experimental manipulation of microbiota reduces host thermal tolerance and fitness under heat stress in a vertebrate ectotherm. *Nature Ecology and Evolution* **6**, 405–417.

FONTAINE, S.S., NOVARRO, A.J. & KOHL, K.D. (2018) Environmental temperature alters the digestive performance and gut microbiota of a terrestrial amphibian. *The Journal of Experimental Biology* **221**, jeb187559.

FOX, J., WEISBERG, S. (2019). *An R Companion to Applied Regression*, Third edition.

FREDSTON, A., PINSKY, M., SELDEN, R.L., SZUWALSKI, C., THORSON, J.T., GAINES, S.D. & HALPERN, B.S. (2021) Range edges of North American marine species are tracking temperature over decades. *Global Change Biology* **27**, 3145–3156.

FROEHLICH, H.E., RUNGE, C.A., GENTRY, R.R., GAINES, S.D. & HALPERN, B.S. (2018) Comparative terrestrial feed and land use of an aquaculture-dominant world. *Proceedings of the National Academy of Sciences of the United States of America* **115**, 5295–5300.

FROEHLICH, H.E., KOEHN, J.Z., HOLSMAN, K.K. & HALPERN, B.S. (2022) Emerging trends in science and news of climate change threats to and adaptation of aquaculture. *Aquaculture* **549**, 737812.
FRY, F.E.J. (1947). *Effects of the Environment on Animal Activity*, Vol. 68, pp. 1-52. Toronto: University of Toronto Press.
FRY, F.E.J. (1957) The lethal temperature as a tool in taxonomy *Année Biologique* **33**, 205-219.
FRY, F.E.J. (1971) 1 - The Effect of Environmental Factors on the Physiology of Fish. In *Environmental Relations and Behavior* (eds W.S. HOAR & D.J.B.T.-F.P. RANDALL), pp. 1–98. Academic Press.
FRY, F.E.J., BRETT, J.R. & CLAWSON, G.H. (1942) Lethal limits of temperature for young goldfish. *Review of Canadian Biology* **1**, 50–56.
GEISER, F., FIRTH, B.T. & SEYMOUR, R.S. (1992) Polyunsaturated dietary lipids lower the selected body temperature of a lizard. *Journal of Comparative Physiology B* **162**, 1–4.
GILBERT, M.J.H., ADAMS, O.A. & FARRELL, A.P. (2022) A sudden change of heart: Warm acclimation can produce a rapid adjustment of maximum heart rate and cardiac thermal sensitivity in rainbow trout. *Current Research in Physiology* **5**, 179-183.
GILBERT, A.L. & MILES, D.B. (2016) Food, temperature and endurance: effects of food deprivation on the thermal sensitivity of physiological performance. *Functional Ecology* **30**, 1790–1799.
GILBERT, M.J.H., HARRIS, L.N., MALLEY, B.K., SCHIMNOWSKI, A., MOORE, J.-S. & FARRELL, A.P. (2020) The thermal limits of cardiorespiratory performance in anadromous Arctic char (Salvelinus alpinus): a field-based investigation using a remote mobile laboratory. *Conservation Physiology* **8**, coaa036.
GILLOOLY, J.F., BROWN, J.H., WEST, G.B., SAVAGE, V.M. & CHARNOV, E.L. (2001) Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251.
GLANVILLE, E.J. & SEEBACHER, F. (2006) Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. *Journal of Experimental Biology* **209**, 4869–4877.
GLENCROSS, B. & RUTHERFORD, N. (2010) Dietary strategies to improve the growth and feed utilization of barramundi, Lates calcarifer under high water temperature conditions. *Aquaculture Nutrition* **16**, 343–350.
GLOVER, C.N., BLEWETT, T.A. & WOOD, C.M. (2016) Determining the functional role of waterborne amino acid uptake in hagfish nutrition: a constitutive pathway when fasting or a supplementary pathway when feeding? *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* **186**, 843–853.
GOMEZ ISAZA, D.F., CRAMP, R.L., SMULLEN, R., GLENCROSS, B.D. & FRANKLIN, C.E. (2019) Coping with climatic extremes: Dietary fat content decreased the thermal resilience of barramundi (Lates calcarifer). *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology* **230**, 64–70.
GONZÁLEZ-BERGONZONI, I., JEPPESEN, E., VIDAL, N., TEIXEIRA-DE MELLO, F., GOYENOLA, G., LÓPEZ-RODRÍGUEZ, A. & MEERHOFF, M. (2016) Potential drivers of seasonal shifts in fish omnivory in a subtropical stream. *Hydrobiologia* **768**, 183–196.
GONZÁLEZ-BERGONZONI, I., MEERHOFF, M., DAVIDSON, T.A., TEIXEIRA-DE MELLO, F., BAATTRUP-PEDERSEN, A. & JEPPESEN, E. (2012) Meta-analysis Shows a Consistent and

Strong Latitudinal Pattern in Fish Omnivory Across Ecosystems. *Ecosystems* **15**, 492–503.

GOURTAY, C., CHABOT, D., AUDET, C., LE DELLIOU, H., QUAZUGUEL, P., CLAIREAUX, G. & ZAMBONINO-INFANTE, J.L. (2018) Will global warming affect the functional need for essential fatty acids in juvenile sea bass (Dicentrarchus labrax)? A first overview of the consequences of lower availability of nutritional fatty acids on growth performance. *Marine Biology* **165**, 143.

GOURTAY, C., CHABOT, D., AUDET, C., MADEC, L., HUELVAN, C., DUCROS, L., CLAIREAUX, G., MAZURAIS, D. & ZAMBONINO-INFANTE, J.L. (2020) Effect of thermal and nutritional conditions on fatty acid metabolism and oxidative stress response in juvenile European sea bass (Dicentrarchus labrax). *Marine Biology* **167**, 144.

GRADIL, K.J., GARNER, S.R., WILSON, C.C., FARRELL, A.P. & NEFF, B.D. (2016) Relationship between cardiac performance and environment across populations of Atlantic salmon (Salmo salar): a common garden experiment implicates local adaptation. *Evolutionary Ecology* **30**, 877–886.

GRIMM, N.B., CHAPIN III, F.S., BIERWAGEN, B., GONZALEZ, P., GROFFMAN, P.M., LUO, Y., MELTON, F., NADELHOFFER, K., PAIRIS, A., RAYMOND, P.A., SCHIMEL, J. & WILLIAMSON, C.E. (2013) The impacts of climate change on ecosystem structure and function. *Frontiers in Ecology and the Environment* **11**, 474–482.

GROSELL, M., FARRELL, A.P. & BRAUNER, C.J. (2010) *Fish physiology: The multifunctional gut of fish*. Academic Press.

GUDERLEY, H. (2004) Metabolic responses to low temperature in fish muscle. *Biological Reviews of the Cambridge Philosophical Society* **79**, 409–427.

GUDERLEY, H., KRAFFE, E., BUREAU, W. & BUREAU, D.P. (2008) Dietary fatty acid composition changes mitochondrial phospholipids and oxidative capacities in rainbow trout red muscle. *Journal of Comparative Physiology B* **178**, 385–399.

GUERREIRO, I., PERES, H., CASTRO-CUNHA, M. & OLIVA-TELES, A. (2012) Effect of temperature and dietary protein/lipid ratio on growth performance and nutrient utilization of juvenile Senegalese sole (Solea senegalensis). *Aquaculture Nutrition* **18**, 98–106.

GUERREIRO, I., SERRA, C.R., ENES, P., COUTO, A., SALVADOR, A., COSTAS, B. & OLIVA-TELES, A. (2016) Effect of short chain fructooligosaccharides (scFOS) on immunological status and gut microbiota of gilthead sea bream (Sparus aurata) reared at two temperatures. *Fish and Shellfish Immunology* **49**, 122–131.

GUINAN, M.E., KAPUSCINSKI, K.L. & TEECE, M.A. (2015) Seasonal diet shifts and trophic position of an invasive cyprinid, the rudd Scardinius erythrophthalmus (Linnaeus, 1758), in the upper Niagara river. *Aquatic Invasions* **10**, 217–225.

GUPTA, S.K., PAL, A.K., SAHU, N.P., DALVI, R.S., AKHTAR, M.S., JHA, A.K. & BARUAH, K. (2010) Dietary microbial levan enhances tolerance of Labeo rohita (Hamilton) juveniles to thermal stress. *Aquaculture* **306**, 398–402.

HANSEN, A.K., BYRIEL, D.B., JENSEN, M.R., STEFFENSEN, J.F. & SVENDSEN, M.B.S. (2017) Optimum temperature of a northern population of Arctic charr (Salvelinus alpinus) using heart rate Arrhenius breakpoint analysis. *Polar Biology* **40**, 1063–1070.

HARDISON, E.A., KRASKURA, K., VAN WERT, J., NGUYEN, T. & ELIASON, E.J. (2021) Diet mediates thermal performance traits: implications for marine ectotherms. *Journal of Experimental Biology* **224**, jeb242846.

HARDISON, E.A., KRASKURA, K., VAN WERT, J., NGUYEN, T. & ELIASON, E.J. (2021) Data from: Diet mediates thermal performance traits: implications for marine ectotherms. *Dryad digital repositor,* doi:10.25349/D98P66

HARDISON, E.A., SCHWIETERMAN, G.D. & ELIASON, E.J. (2023) Diet changes thermal acclimation capacity, but not acclimation rate, in a marine ectotherm (Girella nigricans) during warming. *Proceedings of the Royal Society B* **290**, 20222505.

HARDISON, E.A., SCHWIETERMAN, G.D. & ELIASON, E.J. (2023) Data from: Diet changes thermal acclimation capacity, but not acclimation rate, in a marine ectotherm (Girella nigricans) during warming. *Dryad digital repository*, doi:10.25349/D9Q905.

HARPAZ, S., BECKER, K. & BLUM, R. (1999) The effect of dietary L-carnitine supplementation on cold tolerance and growth of the ornamental cichlid fish Pelvicachromis pulcher - Preliminary results. *Journal of Thermal Biology* **24**, 57–62.

HASSETT, R.P. & CROCKETT, E.L. (2009) Habitat temperature is an important determinant of cholesterol contents in copepods. *Journal of Experimental Biology* **212**, 71–77.

HAZEL, J.R. & PROSSER, C.L. (1974) Molecular mechanisms of temperature compensation in poikilotherms. *Physiological Reviews* **54**, 620–677.

HEALY, T.M. & SCHULTE, P.M. (2012) Factors affecting plasticity in whole-organism thermal tolerance in common killifish (Fundulus heteroclitus). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* **182**, 49–62.

HENRY, Y. & COLINET, H. (2018) Microbiota disruption leads to reduced cold tolerance in Drosophila flies. *Science of Nature* **105**, 59.

HIXSON, S.M. & ARTS, M.T. (2016) Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. *Global change biology* **22**, 2744–2755.

HO, C.K., PENNINGS, S.C. & CAREFOOT, T.H. (2010) Is diet quality an overlooked mechanism for Bergmann's rule? *American Naturalist* **175**, 269–276.

HOAR, W.S. & COTTLE, M.K. (1952) DIETARY FAT AND TEMPERATURE TOLERANCE OF GOLDFISH. *Canadian Journal of Zoology* **30**, 41–48.

HOBDAY, A.J., ALEXANDER, L. V., PERKINS, S.E., SMALE, D.A., STRAUB, S.C., OLIVER, E.C.J., BENTHUYSEN, J.A., BURROWS, M.T., DONAT, M.G., FENG, M., HOLBROOK, N.J., MOORE, P.J., SCANNELL, H.A., SEN GUPTA, A. & WERNBERG, T. (2016) A hierarchical approach to defining marine heatwaves. *Progress in Oceanography* **141**, 227–238.

HOCHACHKA, P.W. & SOMERO, G.N. (2002) Biochemical adaptation: Mechanism and process in physiological evolution. Oxford University Press, New York.

HOFFMANN, A.A. & SGRÒ, C.M. (2011) Climate change and evolutionary adaptation. *Nature* **470**, 479–485.

HOFMANN, G.E. & TODGHAM, A.E. (2010) Living in the Now: Physiological Mechanisms to Tolerate a Rapidly Changing Environment **72**, 127–145.

HOLTGRIEVE, G.W. & SCHINDLER, D.E. (2011) Marine-derived nutrients, bioturbation, and ecosystem metabolism: reconsidering the role of salmon in streams. *Ecology* **92**, 373–385.

HORN, M.H. (1989) Biology of marine herbivorous fishes. *Oceanography and Marine Biology Annual Reviews* **27**, 167–272.

HOSHIJIMA, U. & HOFMANN, G.E. (2019) Variability of seawater chemistry in a kelp forest environment is linked to in situ transgenerational effects in the purple sea urchin, Strongylocentrotus purpuratus. *Frontiers in Marine Science* **6**, 62.

HOUSE, H.L., RIORDAN, D.F. & BARLOW, J.S. (1958) EFFECTS OF THERMAL CONDITIONING AND OF DEGREE OF SATURATION OF DIETARY LIPIDS ON RESISTANCE OF AN INSECT TO A HIGH TEMPERATURE. *Canadian Journal of Zoology* **36**, 629–632.

HUEY, R.B. & BUCKLEY, L.B. (2022) Designing a seasonal acclimation study presents challenges and opportunities. *Integrative Organismal Biology* **4**, obac016.

HUEY, R.B., KEARNEY, M.R., KROCKENBERGER, A., HOLTUM, J.A.M., JESS, M. & WILLIAMS, S.E. (2012) Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367**, 1665–1679.

HUEY, R.B. & KINGSOLVER, J.G. (2019) Climate warming, resource availability, and the metabolic meltdown of ectotherms. *American Naturalist* **194**, E140–E150.

HULBERT, A.J. (2008) The links between membrane composition, metabolic rate and lifespan. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **150**, 196–203.

HWANG, D.F. & LIN, T.K. (2002) Effect of temperature on dietary vitamin C requirement and lipid in common carp. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology* **131**, 1–7.

IKEDA, A.K., ZUANON, J.A.S., SALARO, A.L., FREITAS, M.B.D., PONTES, M.D., SOUZA, L.S. & SANTOS, M. V. (2011) VegeTable oil sources in diets for freshwater angelfish (Pterophyllum scalare, Cichlidae): Growth and thermal tolerance. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* **63**, 670–677.

ILHAM, I. & FOTEDAR, R. (2016) Growth, antioxidant capacity and muscle histochemistry of yellowtail kingfish (Seriola lalandi Valenciennes 1883): Selenium and temperature interaction. *Animal Feed Science and Technology* **217**, 76–86.

IPCC (2021) Climate Change 2021 - The Physical Science Basis - Summary for Policymakers. *Climate Change 2021: The Physical Science Basis.*, 31.

JACKSON, M.C., PAWAR, S. & WOODWARD, G. (2021) The Temporal Dynamics of Multiple Stressor Effects: From Individuals to Ecosystems. *Trends in Ecology & Evolution* **36**, 402–410.

JANG, T., RHO, M.S., KOH, S.H. & LEE, K.P. (2015) Host-plant quality alters herbivore responses to temperature: A case study using the generalist Hyphantria cunea. *Entomologia Experimentalis et Applicata* **154**, 120–130.

JERDE, C.L., KRASKURA, K., ELIASON, E.J., CSIK, S.R., STIER, A.C. & TAPER, M.L. (2019) Strong Evidence for an Intraspecific Metabolic Scaling Coefficient Near 0.89 in Fish. *Frontiers in Physiology* **10**, 1166.

JOBLING, M. (2016) Fish nutrition research: past, present and future. *Aquaculture International* **24**, 767–786.

JOHANSEN, J.L., NADLER, L.E., HABARY, A., BOWDEN, A.J. & RUMMER, J. (2021) Thermal acclimation of tropical coral reef fishes to global heat waves. *eLife* **10**, e59162.

JOHNSON, J.S., CLEMENTS, K.D. & RAUBENHEIMER, D. (2017) The nutritional basis of seasonal selective feeding by a marine herbivorous fish. *Marine Biology* **164**, 201.

JOHNSON, J.S., RAUBENHEIMER, D., BURY, S.J. & CLEMENTS, K.D. (2020) Does temperature constrain diet choice in a marine herbivorous fish? *Marine Biology* **167**, 1–12.

JONSSON, B., JONSSON, N. & FINSTAD, A.G. (2013) Effects of temperature and food quality on age and size at maturity in ectotherms: an experimental test with Atlantic salmon. *Journal of Animal Ecology* **82**, 201–210.
JUTFELT, F., NORIN, T., ÅSHEIM, E.R., ROWSEY, L.E., ANDREASSEN, A.H., MORGAN, R., CLARK, T.D. & SPEERS-ROESCH, B. (2021) 'Aerobic scope protection' reduces ectotherm growth under warming. *Functional Ecology* **35**, 1397–1407.
KAISER, M.J. & HUGHES, R.N. (1993) Factors affecting the behavioural mechanisms of diet selection in fishes. *Marine Behaviour and Physiology* **23**, 105–118.
KELLERMANN, V., CHOWN, S.L., SCHOU, M.F., AITKENHEAD, I., JANION-SCHEEPERS, C., CLEMSON, A., SCOTT, M.T. & SGRÒ, C.M. (2019) Comparing thermal performance curves across traits: How consistent are they? *Journal of Experimental Biology* **222**, jeb193433.
KILLEN, S.S., CHRISTENSEN, E.A.F., CORTESE, D., ZÁVORKA, L., NORIN, T., COTGROVE, L., CRESPEL, A., MUNSON, A., NATI, J.J.H., PAPATHEODOULOU, M. & MCKENZIE, D.J. (2021) Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. *Journal of Experimental Biology* **224**, jeb242522.
KINGSOLVER, J.G., SHLICHTA, J.G., RAGLAND, G.J. & MASSIE, K.R. (2006) Thermal reaction norms for caterpillar growth depend on diet. *Evolutionary Ecology Research* **8**, 703–715.
KNIGHT, N.S., GUICHARD, F. & ALTIERI, A.H. (2021) A global meta-analysis of temperature effects on marine fishes' digestion across trophic groups. *Global Ecology and Biogeography* **30**, 795–810.
KOHL, K.D. & CAREY, H. V. (2016) A place for host-microbe symbiosis in the comparative physiologist's toolbox. *Journal of Experimental Biology* **219**, 3496–3504.
KOVEN, W., NIXON, O., ALLON, G., GAON, A., EL SADIN, S., FALCON, J., BESSEAU, L., ESCANDE, M., VASSALLO AGIUS, R., GORDIN, H. & TANDLER, A. (2018) The effect of dietary DHA and taurine on rotifer capture success, growth, survival and vision in the larvae of Atlantic bluefin tuna (Thunnus thynnus). *Aquaculture* **482**, 137–145.
KRASKURA, K. & NELSON, J.A. (2018) Hypoxia and Sprint Swimming Performance of Juvenile Striped Bass, Morone saxatilis. *Physiological and biochemical zoology* **91**, 682–690.
KROEKER, K.J., BELL, L.E., DONHAM, E.M., HOSHIJIMA, U., LUMMIS, S., TOY, J.A. & WILLIS-NORTON, E. (2020) Ecological change in dynamic environments: Accounting for temporal environmental variability in studies of ocean change biology. *Global Change Biology* **26**, 54–67.
KUTZ, T.C., SGRÒ, C.M. & MIRTH, C.K. (2019) Interacting with change: Diet mediates how larvae respond to their thermal environment. *Functional Ecology* **33**, 1940–1951.
LAFFERTY, K.D., HARVELL, C.D., CONRAD, J.M., FRIEDMAN, C.S., KENT, M.L., KURIS, A.M., POWELL, E.N., RONDEAU, D. & SAKSIDA, S.M. (2015) Infectious diseases affect marine fisheries and aquaculture economics. *Annual Review of Marine Science* **7**, 471–496.
LAMB, R.J. & LOSCHIAVO, S.R. (1981) DIET, TEMPERATURE, AND THE LOGISTIC MODEL OF DEVELOPMENTAL RATE FOR TRIBOLIUM CONFUSUM (COLEOPTERA: TENEBRIONIDAE). *The Canadian Entomologist* **113**, 813–818.
LASPOUMADERES, C., MEUNIER, C.L., MAGNIN, A., BERLINGHOF, J., ELSER, J.J., BALSEIRO, E., TORRES, G., MODENUTTI, B., TREMBLAY, N. & BOERSMA, M. (2022) A common

temperature dependence of nutritional demands in ectotherms. *Ecology Letters* **25**, 2189–2202.
LEE, K.P., JANG, T., RAVZANAADII, N. & RHO, M.S. (2015) Macronutrient Balance Modulates the Temperature-Size Rule in an Ectotherm. *The American Naturalist* **186**, 212–222.
LEE, S., HUNG, S.S.O., FANGUE, N.A., HALLER, L., VERHILLE, C.E., ZHAO, J. & TODGHAM, A.E. (2016) Effects of feed restriction on the upper temperature tolerance and heat shock response in juvenile green and white sturgeon. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology* **198**, 87–95.
LEE, K.P. & ROH, C. (2010) Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. *Entomologia Experimentalis et Applicata* **136**, 151–163.
LEMOINE, N.P., GIERY, S.T. & BURKEPILE, D.E. (2014) Differing nutritional constraints of consumers across ecosystems. *Oecologia* **174**, 1367–1376.
LEMOINE, N.P. & SHANTZ, A.A. (2016) Increased temperature causes protein limitation by reducing the efficiency of nitrogen digestion in the ectothermic herbivore Spodoptera exigua. *Physiological Entomology* **41**, 143–151.
LENTH, R. (2019) Emmeans: EstimatedMarginal Means, Aka Least-Squares Means. CRAN R package: https://cran.r-project.org/web/packages/emmeans/index.html.
LIENART, G.D.H., MITCHELL, M.D., FERRARI, M.C.O. & MCCORMICK, M.I. (2014) Temperature and food availability affect risk assessment in an ectotherm. *Animal Behaviour* **89**, 199–204.
LITTLE, A., DRESSLER, T., KRASKURA, K., HARDISON, E., HENDRIKS, B., PRYSTAY, T., FARRELL, A.P., COOKE, S.J., PATTERSON, D.A., HINCH, S.G. & ELIASON, E.J. (2020) Maxed out: Optimizing accuracy, precision and power for field measures of maximum metabolic rate in fishes. *Physiological and Biochemical Zoology* **93**, 243–254.
LITTLE, A.G., HARDISON, E., KRASKURA, K., DRESSLER, T., PRYSTAY, T.S., HENDRIKS, B., PRUITT, J.N., FARRELL, A.P., COOKE, S.J., PATTERSON, D.A., HINCH, S.G. & ELIASON, E.J. (2020) Reduced lactate dehydrogenase activity in the heart and suppressed sex hormone levels are associated with female-biased mortality during thermal stress in Pacific salmon. *The Journal of experimental biology* **223**, jeb214841.
LITTLE, A.G., KUNISUE, T., KANNAN, K. & SEEBACHER, F. (2013) Thyroid hormone actions are temperature-specific and regulate thermal acclimation in zebrafish (Danio rerio). *BMC Biology* **11**, 26.
LITTLE, A.G. & SEEBACHER, F. (2014) The evolution of endothermy is explained by thyroid hormone-mediated responses to cold in early vertebrates. *The Journal of experimental biology* **217**, 1642–1648.
LOWMAN, H.E., EMERY, K.A., DUGAN, J.E. & MILLER, R.J. (2021) Nutritional quality of giant kelp declines due to warming ocean temperatures. *Oikos*, 1–14.
LU, D.L., MA, Q., SUN, S.X., ZHANG, H., CHEN, L.Q., ZHANG, M.L. & DU, Z.Y. (2019) Reduced oxidative stress increases acute cold stress tolerance in zebrafish. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology* **235**, 166–173.
LUTTERSCHMIDT, W.I. & HUTCHISON, V.H. (1997) The critical thermal maximum: History and critique. *Canadian Journal of Zoology* **75**, 1561–1574.
MACARTHUR, R.H. & PIANKA, E.R. (1966) On Optimal Use of a Patchy Environment. *The American Naturalist* **100**, 603–609.

MAGOZZI, S. & CALOSI, P. (2015) Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Global Change Biology* **21**, 181–194.
MALZAHN, A.M., DOERFLER, D. & BOERSMA, M. (2016) Junk food gets healthier when it's warm. *Limnology and Oceanography* **61**, 1677–1685.
MANN, R. & GALLAGER, S.M. (1985). Physiological and biochemical energetics of larvae of Teredo navalis L. and Bankia gouldi (Bartsch) (Bivalvia: Teredinidae). *Journal of Experimental Marine Biology and Ecology* **85**, 211-228.
MAZUMDER, S.K., DAS, S.K., RAHIM, S.M. & GHAFFAR, M.A. (2018) Temperature and diet effect on the pepsin enzyme activities, digestive somatic index and relative gut length of Malabar blood snapper (Lutjanus malabaricus Bloch & Schneider, 1801). *Aquaculture Reports* **9**, 1–9.
MCCUE, M.D. (2006) Specific dynamic action: A century of investigation. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **144**, 381–394.
MCCUE, M.D. (2010) Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **156**, 1–18.
MCDONALD, D.G., MCFARLANE, W.J. & MILLIGAN, C.L. (1998) Anaerobic capacity and swim performance of juvenile salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1198–1207.
MCKENZIE, D.J. (2001) Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* **128**, 605–619.
MCKENZIE, D.J., HIGGS, D.A., DOSANJH, B.S., DEACON, G. & RANDALL, D.J. (1998) Dietary fatty acid composition influences swimming performance in Atlantic salmon (Salmo salar) in seawater. *Fish Physiology and Biochemistry* **19**, 111–122.
MCLEAN, K.M. & TODGHAM, A.E. (2015) Effect of food availability on the growth and thermal physiology of juvenile Dungeness crabs (Metacarcinus magister). *Conservation Physiology* **3**, cov013.
MICHAUD, K.M., REED, D.C. & MILLER, R.J. (2022) The Blob marine heatwave transforms California kelp forest ecosystems. *Communications Biology* **5**, 1143.
MISHRA, K. & SAMANTARAY, K. (2004) Interacting effects of dietary lipid level and temperature on growth, body composition and fatty acid profile of rohu, Labeo rohita (Hamilton). *Aquaculture Nutrition* **10**, 359–369.
MOELLER, A.H., IVEY, K., CORNWALL, M.B., HERR, K., REDE, J., TAYLOR, E.N. & GUNDERSON, A.R. (2020) The lizard gut microbiome changes with temperature and is associated with heat tolerance. *Applied and Environmental Microbiology* **86**, e01181-20.
MOON, H.Y.L., MACKENZIE, D.S. & GATLIN, D.M. (1994) Effects of dietary thyroid hormones on the red drum (Sciaenops ocellatus). *Fish Physiology and Biochemistry* **12**, 369–380.
MUGGEO, V. M. R. (2008). Segmented: an R package to fit regression models with broken- line relationships. RNews **8**, 20-25.
MUÑOZ, N.J., ANTTILA, K., CHEN, Z., HEATH, J.W., FARRELL, A.P. & NEFF, B.D. (2014) Indirect genetic effects underlie oxygenlimited thermal tolerance within a coastal

population of chinook salmon. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20141082.

MUNRO, D. & BLIER, P. U. (2012). The extreme longevity of Arctica islandica is associated with increased peroxidation resistance in mitochondrial membranes. *Aging cell*, **11**, 845-855.

NAIEL, M.E., SHEHATA, A.M., EL-KHOLY, A.I., EL-NAGGAR, K., FARAG, M.R. & ALAGAWANY, M. (2022) The mitigating role of probiotics against the adverse effects of suboptimal temperature in farmed fish: A review. *Aquaculture* **550**, 737877.

NORIN, T. & CLARK, T.D. (2017) Fish face a trade-off between 'eating big' for growth efficiency and 'eating small' to retain aerobic capacity. *Biology Letters* **13**, 20170298.

NORIN, T., MALTE, H. & CLARK, T.D. (2014) Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *Journal of Experimental Biology* **217**, 244–251.

NYAMUKONDIWA, C. & TERBLANCHE, J.S. (2009) Thermal tolerance in adult Mediterranean and Natal fruit flies (Ceratitis capitata and Ceratitis rosa): Effects of age, gender and feeding status. *Journal of Thermal Biology* **34**, 406–414.

OLIVER, E.C.J., DONAT, M.G., BURROWS, M.T., MOORE, P.J., SMALE, D.A., ALEXANDER, L. V., BENTHUYSEN, J.A., FENG, M., SEN GUPTA, A., HOBDAY, A.J., HOLBROOK, N.J., PERKINS-KIRKPATRICK, S.E., SCANNELL, H.A., STRAUB, S.C. & WERNBERG, T. (2018) Longer and more frequent marine heatwaves over the past century. *Nature Communications* **9**, 1324.

OLSEN, R.E., LØVAAS, E. & LIE (1999) The influence of temperature, dietary polyunsaturated fatty acids, α-tocopherol and spermine on fatty acid composition and indices of oxidative stress in juvenile Arctic char, Salvelinus alpinus (L.). *Fish Physiology and Biochemistry* **20**, 13–29.

OYINLOLA, M.A., REYGONDEAU, G., WABNITZ, C.C.C., FRÖLICHER, T.L., LAM, V.W.Y. & CHEUNG, W.W.L. (2021) Projecting global mariculture production and adaptation pathways under climate change. *Global Change Biology* **28**, 1315–1331.

PAPADOPOULOU, A., PETTINAU, L., SEPPÄNEN, E., SIKANEN, A. & ANTTILA, K. (2022) The interactive effects of exercise training and functional feeds on the cardiovascular performance of rainbow trout (Oncorhynchus mykiss) at high temperatures. *Current Research in Physiology* **5**, 142–150.

PÉREZ-CASANOVA, J.C., LALL, S.P. & GAMPERL, A.K. (2010) Effects of dietary protein and lipid level, and water temperature, on the post-feeding oxygen consumption of Atlantic cod and haddock. *Aquaculture Research* **41**, 198–209.

PERSSON, J., WOJEWODZIC, M.W., HESSEN, D.O. & ANDERSEN, T. (2011) Increased risk of phosphorus limitation at higher temperatures for Daphnia magna. *Oecologia* **165**, 123–129.

PFENNIG, D. (1990) The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85**, 101–107.

PIAZZON, M.C., CALDUCH-GINER, J.A., FOUZ, B., ESTENSORO, I., SIMÓ-MIRABET, P., PUYALTO, M., KARALAZOS, V., PALENZUELA, O., SITJÀ-BOBADILLA, A. & PÉREZ-SÁNCHEZ, J. (2017) Under control: how a dietary additive can restore the gut microbiome and proteomic profile, and improve disease resilience in a marine teleostean fish fed vegeTable diets. *Microbiome* **5**, 164.

PILECKY, M., ZÁVORKA, L., ARTS, M.T. & KAINZ, M.J. (2021) Omega-3 PUFA profoundly affect neural, physiological, and behavioural competences – implications for systemic changes in trophic interactions. *Biological Reviews* **96**, 2127–2145.
PINSKY, M.L., COMTE, L. & SAX, D.F. (2022) Unifying climate change biology across realms and taxa. *Trends in Ecology and Evolution* **37**, 672–682.
PINSKY, M.L., EIKESET, A.M., MCCAULEY, D.J., PAYNE, J.L. & SUNDAY, J.M. (2019) Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* **569**, 108–111.
PLASMAN, M., MCCUE, M.D., REYNOSO, V.H., TERBLANCHE, J.S. & CLUSELLA-TRULLAS, S. (2019) Environmental temperature alters the overall digestive energetics and differentially affects dietary protein and lipid use in a lizard. *The Journal of Experimental Biology* **222**, jeb194480.
POLOCZANSKA, E., MINTENBECK, K., PORTNER, H.O., ROBERTS, D. & LEVIN, L.A. (2019) The Ocean and Cryosphere in a Changing Climate. A Special Report of the Intergovernmental Panel on Climate Change. *Intergovernmental Panel on Climate Change*, 1–765.
PÖRTNER, H. (2001) Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137–146.
PÖRTNER, H.O. (2010) Oxygen- And capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology* **213**, 881–893.
PRATER, C., WAGNER, N.D. & FROST, P.C. (2018) Seasonal effects of food quality and temperature on body stoichiometry, biochemistry, and biomass production in Daphnia populations. *Limnology and Oceanography* **63**, 1727–1740.
PREJS, A. (1984) Herbivory by temperate freshwater fishes and its consequences. *Environmental Biology of Fishes* **10**, 281–296.
PRESTON, D.L., HEDMAN, H.D., ESFAHANI, E.R., PENA, E.M., BOLAND, C.E., LUNDE, K.B. & JOHNSON, P.T.J. (2017) Responses of a wetland ecosystem to the controlled introduction of invasive fish. *Freshwater Biology* **62**, 767–778.
QUEHENBERGER, O., ARMANDO, A.M., BROWN, A.H., MILNE, S.B., MYERS, D.S., MERRILL, A.H., BANDYOPADHYAY, S., JONES, K.N., KELLY, S., SHANER, R.L., SULLARDS, C.M., WANG, E., MURPHY, R.C., BARKLEY, R.M., LEIKER, T.J., ET AL. (2010) Lipidomics reveals a remarkable diversity of lipids in human plasma1. *Journal of Lipid Research* **51**, 3299–3305.
RAMSBY, B.D., HOOGENBOOM, M.O., WHALAN, S. & WEBSTER, N.S. (2018) Elevated seawater temperature disrupts the microbiome of an ecologically important bioeroding sponge. *Molecular Ecology* **27**, 2124–2137.
RANDALL, D.J. & WRIGHT, P.A. (1987) Ammonia distribution and excretion in fish. *Fish physiology and biochemistry* **3**, 107–120.
RAUBENHEIMER, D., ZEMKE-WHITE, W.L., PHILLIPS, R.J. & CLEMENTS, K.D. (2005) Algal macronutrients and food selection by the omnivorous marine fish Girella tricuspidata. *Ecology* **86**, 2601–2610.
RAZA, S.H.A., ABDELNOUR, S.A., ALOTAIBI, M.A., ALGABBANI, Q., NAIEL, M.A.E., SHOKROLLAHI, B., NORELDIN, A.E., JAHEJO, A.R., ZHANG, H., ALAGAWANY, M. & ZAN, L. (2022) MicroRNAs mediated environmental stress responses and toxicity signs in teleost fish species. *Aquaculture* **546**, 737310.

RHO, M.S. & LEE, K.P. (2017) Temperature-driven plasticity in nutrient use and preference in an ectotherm. *Oecologia* **185**, 401–413.
RODGERS, G.G., TENZING, P. & CLARK, T.D. (2016) Experimental methods in aquatic respirometry: the importance of mixing devices and accounting. *Journal of Fish Biology* **88**, 65–80.
RODGERS, E.M., TODGHAM, A.E., CONNON, R.E. & FANGUE, N.A. (2019) Stressor interactions in freshwater habitats: Effects of cold water exposure and food limitation on early-life growth and upper thermal tolerance in white sturgeon, Acipenser transmontanus. *Freshwater Biology* **64**, 348–358.
ROSADO, P.M., LEITE, D.C.A., DUARTE, G.A.S., CHALOUB, R.M., JOSPIN, G., NUNES DA ROCHA, U., P. SARAIVA, J., DINI-ANDREOTE, F., EISEN, J.A., BOURNE, D.G. & PEIXOTO, R.S. (2018) Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. *The ISME Journal* **13**, 921–936.
ROSENBLATT, A.E. & SCHMITZ, O.J. (2016) Climate Change, Nutrition, and Bottom-Up and Top-Down Food Web Processes. *Trends in Ecology & Evolution* pp. 965–975.
ROSEWARNE, P.J., WILSON, J.M. & SVENDSEN, J.C. (2016) Measuring maximum and standard metabolic rates using intermittent-flow respirometry : a student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. *Journal of Fish Biology* **88**, 265–283.
ROWE, C.E., FIGUEIRA, W., RAUBENHEIMER, D., BIET, S.M.S. & CAPUSKA, G.E.M. (2018) Effects of temperature on macronutrient selection, metabolic and swimming performance of the Indo - Pacific Damselfish (Abudefduf vaigiensis). *Marine Biology* **165**, 178.
RUBIO, V.C., SÁNCHEZ-VÁZQUEZ, F.J. & MADRID, J.A. (2003) Macronutrient selection through postingestive signals in sea bass fed on gelatine capsules. *Physiology and Behavior* **78**, 795–803.
RUBIO, V.C., BOLUDA NAVARRO, D., MADRID, J.A. & SÁNCHEZ-VÁZQUEZ, F.J. (2009) Macronutrient self-selection in Solea senegalensis fed macronutrient diets and challenged with dietary protein dilutions. *Aquaculture* **291**, 95–100.
RUIZ, T., KOUSSOROPLIS, A.-M., DANGER, M., AGUER, J.-P., MOREL-DESROSIERS, N. & BEC, A.
(2020) U-shaped response Unifies views on temperature dependency of stoichiometric requirements. *Ecology Letters* **23**, 860-869.
SÁNCHEZ-VÁZQUEZ, F.J., YAMAMOTO, T., AKIYAMA, T., MADRID, J.A. & TABATA, M. (1998) Selection of macronutrients by goldfish operating self-feeders. *Physiology and Behavior* **65**, 211–218.
SAFI, H., ZHANG, Y., SCHULTE, P.M. & FARRELL, A.P. (2019) The effect of acute warming and thermal acclimation on maximum heart rate of the common killifish Fundulus heteroclitus. *Journal of Fish Biology* **95**, 1441–1446.
SANDBLOM, E., CLARK, T.D., GRÄNS, A., EKSTRÖM, A., BRIJS, J., SUNDSTRÖM, L.F., ODELSTRÖM, A., ADILL, A., AHO, T. & JUTFELT, F. (2016) Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nature Communications* **7**, 11447.
SANDBLOM, E., GRÄNS, A., AXELSSON, M. & SETH, H. (2014) Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: Implications in a thermally extreme future. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20141490.

SANFORD, E., SONES, J.L., GARCÍA-REYES, M., GODDARD, J.H.R. & LARGIER, J.L. (2019) Widespread shifts in the coastal biota of northern California during the 2014–2016 marine heatwaves. *Scientific Reports* **9**, 4216.
SCHEUFFELE, H., RUBIO-GRACIA, F. & CLARK, T.D. (2021) Thermal performance curves for aerobic scope in a tropical fish (Lates calcarifer): flexible in amplitude but not breadth. *Journal of Experimental Biology* **224**, jeb243504.
SCHMITZ, O.J. & ROSENBLATT, A.E. (2017) The temperature dependence of predation stress and prey nutritional stoichiometry. *Frontiers in Ecology and Evolution* **5**, 1–8.
SCHMITZ, O.J., ROSENBLATT, A.E. & SMYLIE, M. (2016) Temperature dependence of predation stress and the nutritional ecology of a generalist herbivore. *Ecology* **97**, 3119–3130.
SCHRETTER, C.E., VIELMETTER, J., BARTOS, I., MARKA, Z., MARKA, S., ARGADE, S. & MAZMANIAN, S.K. (2018) A gut microbial factor modulates locomotor behaviour in Drosophila. *Nature* **563**, 402–406.
SCHULTE, P.M. (2015) The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology* **218**, 1856–1866.
SCHULTE, P.M., HEALY, T.M. & FANGUE, N.A. (2011) Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology* **51**, 691–702.
SCHWIETERMAN, G.D., HARDISON, E.A. & ELIASON, E.J. (2022) Effect of thermal variation on the cardiac thermal limits of a eurythermal marine teleost (Girella nigricans). *Current Research in Physiology* **5**, 109–117.
SEALEY, W.M., GIBSON GAYLORD, T., TONER, M., ILGEN, J., FRASER, W.C., HOOLEY, C.G. & BARROWS, F.T. (2012) Growth and acute temperature tolerance of june sucker juveniles fed varying dietary protein and lipid levels with and without supplemental dicalcium phosphate. *North American Journal of Aquaculture* **75**, 124–132.
SECOR, S.M. (2009) Specific dynamic action: A review of the postprandial metabolic response. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* **179**, 1–56.
SEEBACHER, F., WHITE, C.R. & FRANKLIN, C.E. (2015) Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change* **5**, 61–66.
SEMOVA, I., CARTEN, J.D., STOMBAUGH, J., MACKEY, L.C., KNIGHT, R., FARBER, S.A. & RAWLS, J.F. (2012) Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell Host and Microbe* **12**, 277–288.
SHAHID, S., KIM, G., JOHNSON, N.R., WAFULA, E., WANG, F., CORUH, C., BERNAL-GALEANO, V., PHIFER, T., DEPAMPHILIS, C.W., WESTWOOD, J.H. & AXTELL, M.J. (2018) MicroRNAs from the parasitic plant Cuscuta campestris target host messenger RNAs. *Nature* **553**, 82–85.
SIDELL, B.D., WILSON, F.R., HAZEL, J. & PROSSER, C.L. (1973) Time course of thermal acclimation in goldfish. *Journal of Comparative Physiology* **84**, 119–127.
SINCLAIR, B.J. & MARSHALL, K.E. (2018) The many roles of fats in overwintering insects. *Journal of Experimental Biology* **121,** jeb161836.
SINCLAIR, B.J., MARSHALL, K.E., SEWELL, M.A., LEVESQUE, D.L., WILLETT, C.S., SLOTSBO, S., DONG, Y., HARLEY, C.D.G., MARSHALL, D.J., HELMUTH, B.S. & HUEY, R.B. (2016)

Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters* **19**, 1372–1385.
SKALLI, A., ROBIN, J.H., LE BAYON, N., LE DELLIOU, H. & PERSON-LE RUYET, J. (2006) Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (Dicentrarchus labrax). *Aquaculture* **255**, 223–232.
STARCK, J.M. (2003) Shaping up: how vertebrates adjust their digestive system to changing environmental conditions. *Animal Biology* **53**, 245–257.
STEELL, S.C., VAN LEEUWEN, T.E., BROWNSCOMBE, J.W., COOKE, S.J. & ELIASON, E.J. (2019) An appetite for invasion: digestive physiology, thermal performance, and food intake in lionfish (Pterois spp.). *The Journal of Experimental Biology* **222**, jeb.209437.
STEINBERG, C.E.W. (2022) Glucose Intolerance—'Life's Real Luxury?' *Aquatic Animal Nutrition: Organic Macro-and Micro-Nutrients*, 329–348.
STENTIFORD, G.D., SRITUNYALUCKSANA, K., FLEGEL, T.W., WILLIAMS, B.A.P., WITHYACHUMNARNKUL, B., ITSATHITPHAISARN, O. & BASS, D. (2017) New Paradigms to Help Solve the Global Aquaculture Disease Crisis. *PLoS Pathogens* **13**, 1–6.
STILLMAN, J.H. (2003) Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65.
SOMERO, G.N. (2010) The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology* **213**, 912–920.
SOMERO, G.N. (2011) Comparative physiology: A 'crystal ball' for predicting consequences of global change. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* **301**, 1-14.
SORIANO, E.L., RAMÍREZ, D.T., ARAUJO, D.R., GÓMEZ-GIL, B., CASTRO, L.I. & SÁNCHEZ, C.G. (2018) Effect of temperature and dietary lipid proportion on gut microbiota in yellowtail kingfish Seriola lalandi juveniles. *Aquaculture* **497**, 269–277.
STARKE, C.W.E., JONES, C.L.C., BURR, W.S. & FROST, P.C. (2020) Interactive effects of water temperature and stoichiometric food quality on Daphnia pulicaria. *Freshwater Biology* **00**, 1–10.
SUNDAY, J.M., BATES, A.E. & DULVY, N.K. (2011) Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences* **278**, 1823–1830.
SUNDAY, J.M., BATES, A.E., KEARNEY, M.R., COLWELL, R.K., DULVY, N.K., LONGINO, J.T. & HUEY, R.B. (2014) Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 5610–5615.
SUNDAY, J., BENNETT, J.M., CALOSI, P., CLUSELLA-TRULLAS, S., GRAVEL, S., HARGREAVES, A.L., LEIVA, F.P., VERBERK, W.C.E.P., OLALLA-TÁRRAGA, M.Á. & MORALES-CASTILLA, I. (2019) Thermal tolerance patterns across latitude and elevation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374,** 20190036.
SUTCLIFFE, R.L., LI, S., GILBERT, M.J.H., SCHULTE, P.M., MILLER, K.M. & FARRELL, A.P. (2020) A rapid intrinsic heart rate resetting response with thermal acclimation in rainbow trout, Oncorhynchus mykiss. *Journal of Experimental Biology* **223**, jeb215210.
TAN, K., ZHANG, H. & ZHENG, H. (2022) Climate change and n-3 LC-PUFA availability. *Progress in Lipid Research* **86**, 101161.

TEJPAL, C.S., SUMITHA, E.B., PAL, A.K., SHIVANANDA MURTHY, H., SAHU, N.P. & SIDDAIAH, G.M. (2014) Effect of dietary supplementation of l-tryptophan on thermal tolerance and oxygen consumption rate in Cirrhinus mrigala fingerlings under varied stocking density. *Journal of Thermal Biology* **41**, 59–64.

TREVELLINE, B.K., FONTAINE, S.S., HARTUP, B.K. & KOHL, K.D. (2019) Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences* **286**, 20182448.

TREVELLINE, B.K. & KOHL, K.D. (2022) The gut microbiome influences host diet selection behavior. *Proceedings of the National Academy of Sciences of the United States of America* **119**, 1–8.

TRIP, E.D.L., CLEMENTS, K.D., RAUBENHEIMER, D. & HOWARD CHOAT, J. (2016) Temperate marine herbivorous fishes will likely do worse, not better, as waters warm up. *Marine Biology* **163**, 24.

TSENG, M., DI FILIPPO, C.M., FUNG, M., KIM, J.O., FORSTER, I.P. & ZHOU, Y. (2021) Cascading effects of algal warming in a freshwater community. *Functional Ecology* **35**, 920–929.

TURCHINI, G.M., FRANCIS, D.S., DU, Z.-Y., OLSEN, R.E., RINGØ, E. & TOCHER, D.R. (2022) Chapter 5 - The lipids. In *Fish Nutrition (Fourth Edition)* (eds R.W. HARDY & S.J. KAUSHIK), pp. 303–467 Fourth Edi. Academic Press.

TURKO, A.J., NOLAN, C.B., BALSHINE, S., SCOTT, G.R. & PITCHER, T.E. (2020) Thermal tolerance depends on season , age and body condition in imperilled redside dace Clinostomus elongatus. *Conservation Physiology* **8**, 1–15.

VAGNER, M., PANTE, E., VIRICEL, A., LACOUE-LABARTHE, T., ZAMBONINO-INFANTE, J.-L., QUAZUGUEL, P., DUBILLOT, E., HUET, V., LE DELLIOU, H., LEFRANÇOIS, C. & IMBERT-AUVRAY, N. (2019) Ocean warming combined with lower omega-3 nutritional availability impairs the cardio-respiratory function of a marine fish. *The Journal of Experimental Biology* **222**, jeb187179.

VAGNER, M., ZAMBONINO-INFANTE, J.-L., MAZURAIS, D., IMBERT-AUVRAY, N., OUILLON, N., DUBILLOT, E., LE DELLIOU, H., AKBAR, D. & LEFRANÇOIS, C. (2014) Reduced n-3 highly unsaturated fatty acids dietary content expected with global change reduces the metabolic capacity of the golden grey mullet. *Marine Biology* **161**, 2547–2562.

VAGNER, M., LACOUE-LABARTHE, T., INFANTE, J.L.Z., MAZURAIS, D., DUBILLOT, E., LE DELLIOU, H., QUAZUGUEL, P. & LEFRANÇOIS, C. (2015) Depletion of essential fatty acids in the food source affects aerobic capacities of the golden grey mullet Liza aurata in a warming seawater context. *PLoS ONE* **10**, e0126489.

VEJŘÍKOVÁ, I., VEJŘÍK, L., SYVÄRANTA, J., KILJUNEN, M., ČECH, M., BLABOLIL, P., VAŠEK, M., SAJDLOVÁ, Z., CHUNG, S.H.T., ŠMEJKAL, M., FROUZOVÁ, J. & PETERKA, J. (2016) Distribution of Herbivorous Fish Is Frozen by Low Temperature. *Scientific Reports* **6**, 39600.

VINAGRE, C., LEAL, I., MENDONÇA, V., MADEIRA, D., NARCISO, L., DINIZ, M.S. & FLORES, A.A.V. (2016) Vulnerability to climate warming and acclimation capacity of tropical and temperate coastal organisms. *Ecological Indicators* **62**, 317–327.

WAN, A.H.L., DAVIES, S.J., SOLER-VILA, A., FITZGERALD, R. & JOHNSON, M.P. (2019) Macroalgae as a sustainable aquafeed ingredient. *Reviews in Aquaculture* **11**, 458–492.

WANG, X., TANG, B., LUO, X., KE, C., HUANG, M., YOU, W. & WANG, Y. (2020) Effects of temperature, diet and genotype-induced variations on the gut microbiota of abalone. *Aquaculture* **524**, 735269.

WILLIAMS, E.E. & HAZEL, J. R. (1994). Membrane fluidity and hemilayer temperature sensitivity in trout hepatocytes during brief in vitro cold exposure. *American Journal of Physiology* **266**, R773–R780.

WILLIAMS, E.E. & SOMERO, G.N. (1996) Seasonal-, tidal-cycle- and microhabitat-related variation in membrane order of phospholipid vesicles from gills of the intertidal mussel Mytilus californianus. *Journal of Experimental Biology* **199**, 1587–1596.

WINNIKOFF, J.R., HADDOCK, S.H.D. & BUDIN, I. (2021) Depth- and temperature-specific fatty acid adaptations in ctenophores from extreme habitats. *Journal of Experimental Biology* **224**, jeb242800.

WODTKE, E. & COSSINS, A. R. (1991). Rapid cold-induced changes of membrane order and delta-9 desaturase activity in endoplasmic reticulum of carp liver: a time course study of thermal acclimation. *Biochimica et Biophysica Acta* **1064**, 343–350.

WOIWODE, J.G. & ADELMAN, I.R. (1992) Effects of starvation, oscillating temperatures, and photoperiod on the critical thermal maximum of hybrid striped × white bass. *Journal of Thermal Biology* **17**, 271–275.

ZANEVELD, J.R., BURKEPILE, D.E., SHANTZ, A.A., PRITCHARD, C.E., MCMINDS, R., PAYET, J.P., WELSH, R., CORREA, A.M.S., LEMOINE, N.P., ROSALES, S., FUCHS, C., MAYNARD, J.A. & THURBER, R.V. (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nature Communications* **7**, 11833.

ZÁVORKA, L., CRESPEL, A., DAWSON, N.J., PAPATHEODOULOU, M., KILLEN, S.S. & KAINZ, M.J. (2021) Climate change-induced deprivation of dietary essential fatty acids can reduce growth and mitochondrial efficiency of wild juvenile salmon. *Functional Ecology* **35**, 1960–1971.

ZEMPLENI, J., BAIER, S.R., HOWARD, K.M. & CUI, J. (2015) Gene regulation by dietary microRNAs. *Canadian Journal of Physiology and Pharmacology* **93**, 1097–1102.

ZHANG, L., CHEN, T., YIN, Y., ZHANG, C. & ZHANG, Y. (2019) Dietary microRNA — A Novel Functional Component of Food. *American Society for Nutrition* **1**, 711–721.

ZHANG, P., LEEUWEN, C.H.A. VAN, BOGERS, D., POELMA, M., XU, J. & BAKKER, E.S. (2020) Ectothermic omnivores increase herbivory in response to rising temperature. *Oikos* **129**, 1028–1039.

Appendix

Table S2.1 Dietary and whole-body Proximate composition (% wet weight)

Dietary Proximate composition (% wet weight)				
	Experiment 1		Experiment 2	
	Ulva	*Artemia*	*Ulva*	*Artemia*
% Moisture	82.04 ± 1.63	87.48 ± 0.91	75.33 ± 3.81	86.83 ± 0.38
% Protein	1.47 ± 0.27	4.75 ± 0.51	1.95 ± 0.88	5.59 ± 0.62
% Lipid	0.42 ± 0.05	1.23 ± 0.14	0.55 ± 0.10	1.84 ± 0.08
% Ash	10.71 ± 1.87	1.44 ± NA	9.93 ± NA	1.78 ± 0.04
Whole body Proximate composition (% wet weight)				
	12°C		20°C	
	Carnivorous	Omnivorous	Carnivorous	Omnivorous
% Moisture	70.15 ± 1.15	72.25 ± 1.40	72.98 ± 0.74	71.79 ± 0.45
% Protein	13.40 ± 1.09	13.91 ± 1.21	12.61 ± 1.45	10.76 ± 0.85
% Lipid	3.88 ± 0.41	3.08 ± 0.25	3.70 ± 0.63	3.93 ± 0.16
% Ash	5.45 ± 0.84	4.31 ± 0.50	4.60 ± 0.60	5.26 ± 0.60

Represented are means and standard error values for dietary proximate composition in *Ulva* sp., *Artemia* sp., and proximate body composition from whole opaleye from experiments 1 and 2. Proximate body composition were statistically analyzed using 2-way ANOVA and no significant differences were found between treatment groups. When sample size <3 standard error was not calculated and is listed as NA.

Table S2.2 AIC Outputs for Polynomial Curves

AIC outputs for warm ABT test f_{hmax} polynomial curves				
Model	**Formula**	**df**	**AIC**	**ΔAIC**
Model 1	poly(acute_temp, 3) * diet * temp + (1 | fish_id)	18	5282.61153	0
Model 2	poly(acute_temp, 3) * temp + diet + (1 | fish_id)	11	5295.12706	12.515531
Model 3	poly(acute_temp, 3) * temp + (1 | fish_id)	10	5297.84122	15.2296905
Model 4	poly(acute_temp, 2) * diet * temp + (1 | fish_id)	14	5331.73874	49.1272062
Model 5	poly(acute_temp, 3) * diet + temp + (1 | fish_id)	11	5425.26489	142.653365
Model 6	poly(acute_temp, 4) + temp + diet + (1 | fish_id)	9	5427.25882	144.64729
Model 7	poly(acute_temp, 4) + temp * diet + (1 | fish_id)	10	5428.15216	145.540635
Model 8	poly(acute_temp, 3) + temp + diet + (1 | fish_id)	8	5429.2756	146.664069
Model 9	poly(acute_temp, 3) + temp * diet + (1 | fish_id)	9	5430.17554	147.564011
Model 10	poly(acute_temp, 3) + temp + (1 | fish_id)	7	5432.27141	149.659875
Model 11	poly(acute_temp, 3) + diet + (1 | fish_id)	7	5433.3873	150.775766
Model 12	poly(acute_temp, 3) + (1 | fish_id)	6	5435.91176	153.300235
Model 13	poly(acute_temp, 2) + temp + diet + (1 | fish_id)	7	5491.9684	209.356872
Model 14	poly(acute_temp, 2) + temp * diet + (1 | fish_id)	8	5492.77923	210.167701
Model 15	acute_temp + temp + diet + (1 | fish_id)	6	5742.45159	459.840058
Model 16	acute_temp + temp * diet + (1 | fish_id)	7	5743.27212	460.660589
Model 17	acute_temp + temp + (1 | fish_id)	5	5745.43438	462.822848
Model 18	acute_temp + diet + (1 | fish_id)	5	5749.62894	467.017406
Model 19	acute_temp + (1 | fish_id)	4	5751.89944	469.287913
AIC outputs for cold test f_{hmax} polynomial curves				
Model	**Formula**	**df**	**AIC**	**ΔAIC**
Model 1	poly(acute_temp, 4) + diet + (1 | fish_id)	8	1620.06402	0
Model 2	poly(acute_temp, 4) * diet + (1 | fish_id)	12	1623.74238	3.67835332
Model 3	poly(acute_temp, 3) + diet + (1 | fish_id)	7	1636.74603	16.6820023
Model 4	poly(acute_temp, 3) * diet + (1 | fish_id)	10	1639.36915	19.3051258
Model 5	poly(acute_temp, 3) + (1 | fish_id)	6	1640.02832	19.9642973
Model 6	poly(acute_temp, 2) * diet + (1 | fish_id)	8	1640.27173	20.2077023
Model 7	poly(acute_temp, 2) + diet + (1 | fish_id)	6	1641.6658	21.6017758
Model 8	acute_temp + diet + (1 | fish_id)	5	1992.02421	371.960185
Model 9	acute_temp + (1 | fish_id)	4	1994.25531	374.191287

Represented are model formulas as input into R and AIC output results. df = degrees of freedom, AIC = Akaike Information Criterion ΔAIC = AIC(model)—AIC(min AIC value), acute_temp = acute temperature, fish_id = individual fish.

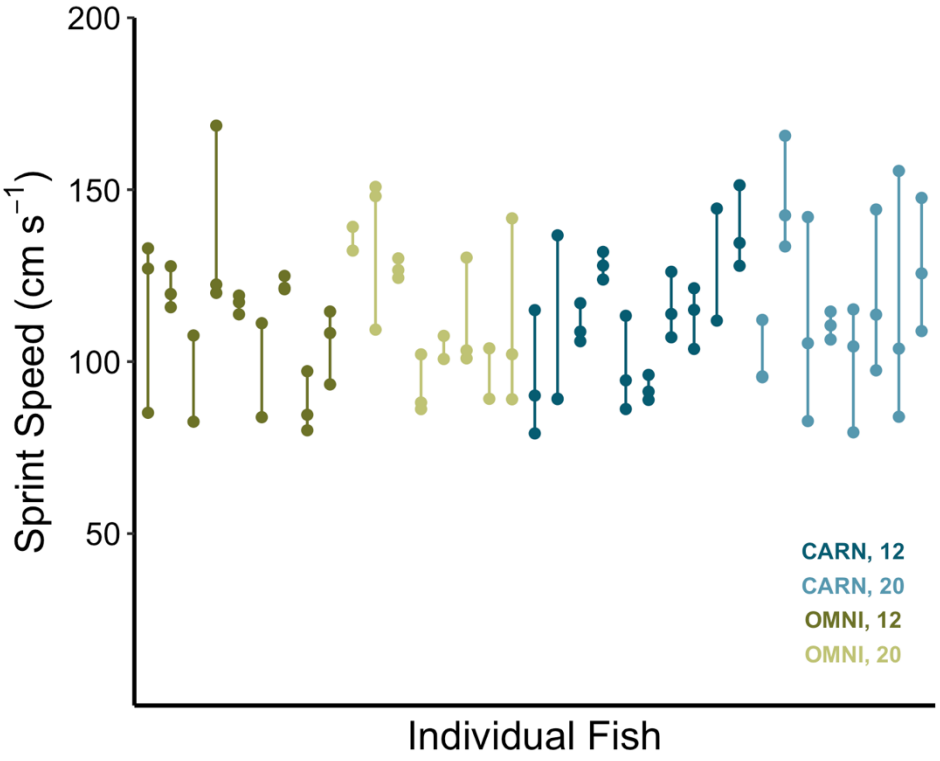

Figure S2.1 Figure illustrating repeatability of sprint performance across individuals. Each dot indicates a max sprint performance (cm s^{-1}) calculated from an individual sprint trial. Colors indicate treatments with dark blue (carnivorous diet at 12°C), dark green (omnivorous diet at 12°C), light blue (carnivorous diet at 20°C), light green (omnivorous diet at 20°C).

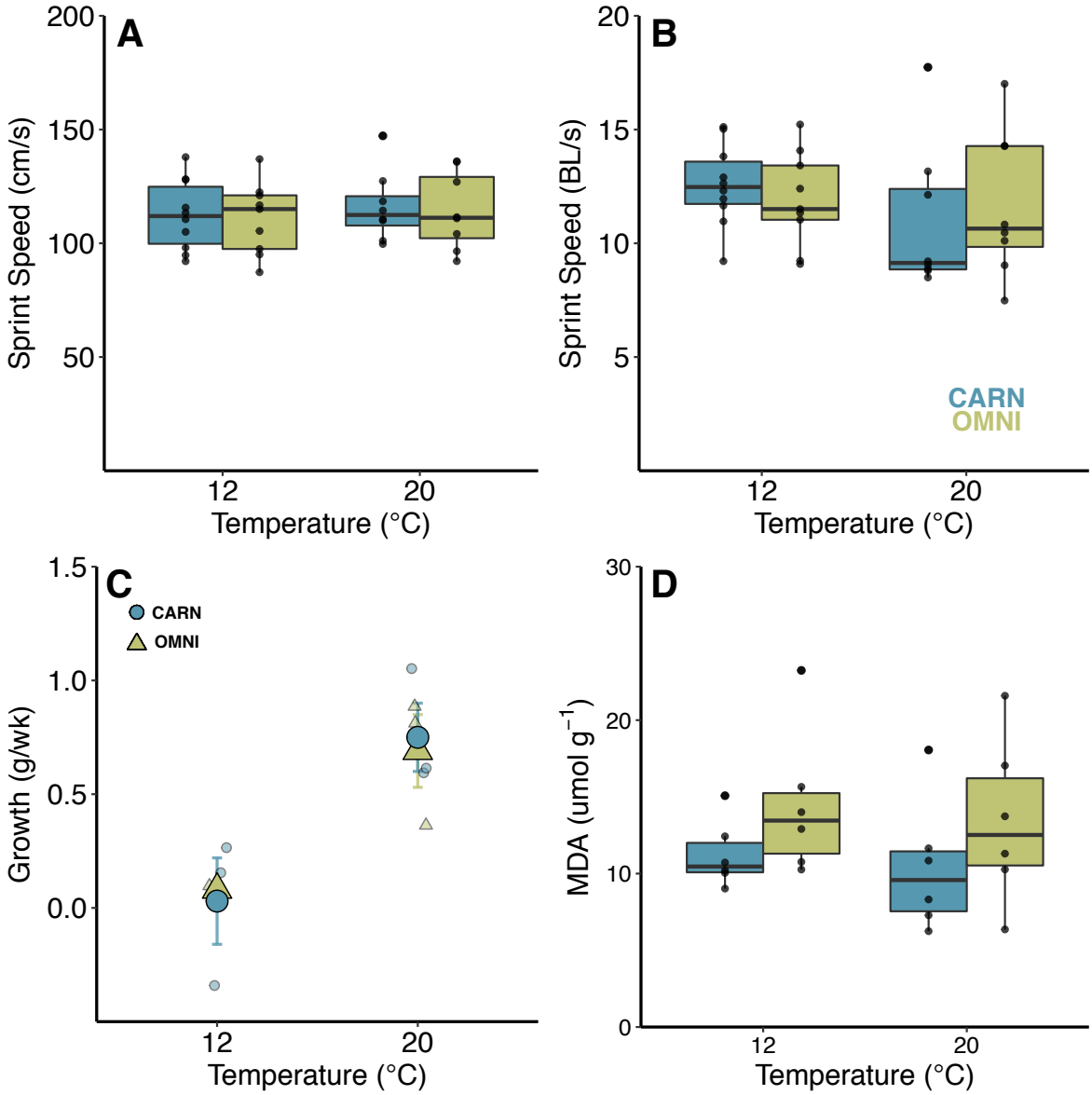

Figure S2.2 Performance in opaleye acclimated to 12°C or 20°C and fed either a carnivorous (blue) or omnivorous (green) diet. Presented are **A)** sprints measured as speed in cm s^{-1} and **B)** sprints measured as speed in BL s^{-1}, **C)** Growth rate (average fish mass (g) gained per week per tank) **D)** Lipid Peroxidation (LPO) in liver tissue measured as malondialdehyde concentration (MDA) in µmol $gram^{-1}$ of liver tissue. In panel A, B, D box plots represent interquartile ranges (boxes and whiskers), median values (solid lines) and outliers (> 1.5 beyond interquartile range) are plotted as data points outside the whiskers. In panel C, large circles and triangles indicate mean (± SEM) values for the carnivorous (*Artemia* sp.) and omnivorous diet treatments (*Artemia* sp. and *Ulva* sp.), respectively.

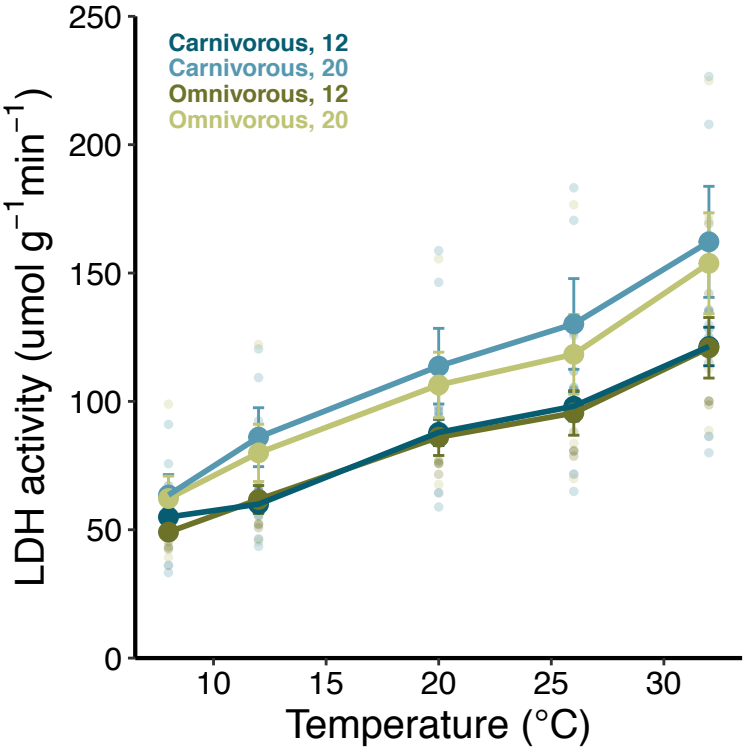

Figure S2.3 Lactate dehydrogenase (LDH) activity in µmol per gram wet white muscle tissue weight in opaleye acclimated to 12°C (dark colors) or 20°C (light colors) and fed either a carnivorous (*Artemia* sp., represented as blues) or omnivorous diet (*Artemia* sp. and *Ulva* sp., represented as greens). Circles represent mean values and error bars indicate SEM**.** For each sample, LDH activity was measured at 5 different temperatures (8, 12, 20, 26, 32°C). Lactate dehydrogenase activity was higher at 20°C compared to 12°C but did not differ across diets. Lactate dehydrogenase activity also increased with acute temperature exposure. Acute temp: df = 4, χ^2 = 1061.711, p<0.001; acclimation temp: df = 1, χ^2 = 5.132, p = 0.023; diet: df = 1, χ^2 = 0.172, p = 0.679; acute temp × acclimation temp: df = 4, χ^2 = 22.526, p < 0.001.

Table S3.1 Ibutton data from temperature acclimations

Replicate	12°C			20°C		
	Carn	Herb	Omni	Carn	Herb	Omni
1	12.1 ± 0.7	11.5 ± 0.5	12.6 ± 0.5	20.1 ± 0.7	19.6 ± 0.8	20.4 ± 0.5
2*	12.3 ± 0.4	12.1 ± 0.6	11.7 ± 0.5	20.0 ± 0.5	20.6 ± 0.6	20.4 ± 0.5
3	12.0 ± 0.3	12.5 ± 0.3	12.1 ± 0.3	19.5 ± 0.3	19.9 ± 0.4	19.6 ± 0.5
4	11.6 ± 0.2	11.9 ± 0.2	12.5 ± 0.3	20.1 ± 0.2	20.0 ± 0.3	20.8 ± 0.3
5	12.2 ± 0.4	12.6 ± 0.2	12.3 ± 0.4	20.2 ± 0.3	20.8 ± 0.2	20.7 ± 0.3

Represented are mean (±SD) for Ibutton (temperature logger, Maxim Integrated) data from each experimental replicate. *indicates some Ibutton data missing in middle, however daily checks were consistently within the temperature range.

Table S3.2 Stomach content information for omnivorous treatment

Timepoint	# Fish with ogo detected	# Fish with brine shrimp detected	Ogo stomach contents (% body mass)	Brine shrimp stomach contents (% body mass)	Ogo:total stomach contents (%)
Before change (12°C)	0/11	4/11	0.0000	0.0631	0.0000
Day 1 at 20°C	0/12	2/12	0.0000	0.1529	0.0000
Day 3 at 20°C	2/12*	3/12	0.0005	0.1576	0.3163
Day 7 at 20°C	1/8**	5/8	0.0000	0.2133	0.0000
Day 14 at 20°C	11/22***	14/22	0.1005	0.3344	23.1088

Represented are the number of fish with ogo and brine shrimp detected in their stomach contents at the time of sampling. The amount of each diet item found in the stomach is calculated as the percent body mass of the individual fish and is presented as the average across all fish at that timepoint. *1 fish regurgitated ogo during test and no brine shrimp was detected in the tank. Therefore, not included in % body mass calculations. ** Ogo detected in gut, but stomach was empty. Therefore, not included in % body mass calculations. ***For 2 fish there was no brine shrimp left in tank at the time of sampling. These are included in % body mass calculations.

Table S3.3 Dietary and whole-body proximate composition (% wet weight)

Dietary Proximate Composition (% dry weight)			
	Gracilaria pacifica	*Artemia* sp.	
% Protein	20.73 ± 2.86	28.34 ± 1.41	
% Lipid	3.70 ± 0.72	11.03 ± 0.30	
% Ash	16.69 ± 3.84	12.38 ± 0.62	
Whole Body Proximate Composition (% dry weight)			
	Carn	Herb	Omni
% Protein	37.41 ± 4.27[a]	35.57 ± 2.02[a]	43.02 ± 2.29[a]
% Lipid	18.57 ± 0.97[a]	7.24 ± 0.91[b]	20.74 ± 0.67[a]
% Ash	14.76 ± 1.51[a]	23.40 ± 2.45[b]	11.91 ± 0.52[a]

Represented are means and standard error values for dietary proximate composition in *Gracilaria pacifica* (n = 6 per measure), *Artemia* sp. (n = 3 per measure), and proximate body composition from whole opaleye (minus some dissected out organs; n = 7-8 per diet treatment) sampled on day 14 post temperature change to 20°C. Proximate body composition for opaleye were statistically analyzed using 1-way ANOVA and post-hoc Tukey HSD when appropriate. Letters indicate significant differences between treatment groups.

Table S3.4 Dietary fatty acid composition

Summary Statistics for diet samples (mean ± SEM)		
Fatty Acid	**Brine (pmol/mg dry tissue mass)**	**Ogo (pmol/mg dry tissue mass)**
12:0	128.53 ± 12.73	63.93 ± 14.92
14:0	1657.06 ± 159.76	859.68 ± 186.96
15:0	210.11 ± 15.99	43.41 ± 12.12
16:0	4294.66 ± 544.33	5989.52 ± 1318.51
16:1	6075.68 ± 557.75	254.34 ± 106.97
17:0	146.19 ± 12.99	14.35 ± 3.68
17:1	267.59 ± 21.37	10.76 ± 4.14
18:0	1805.23 ± 307.00	407.58 ± 84.66
18:1	12260.74 ± 1554.57	952.27 ± 232.51
18:2	7902.38 ± 798.00	100.43 ± 39.05
18:3:n3	21160.92 ± 2028.76	151.75 ± 125.90
18:3:n6	417.99 ± 63.66	12.63 ± 3.27
18:4	10300.76 ± 795.33	60.88 ± 41.12
20:0	18.12 ± 2.53	0.36 ± 0.36
20:1	184.85 ± 19.19	10.21 ± 2.01
20:2	241.25 ± 24.54	42.26 ± 8.72
20:3:n3	630.41 ± 58.71	5.06 ± 3.70
20:3:n6	124.87 ± 14.10	43.28 ± 8.44
20:3:n9	80.91 ± 11.19	18.07 ± 3.41
20:4	1681.76 ± 203.53	439.00 ± 85.99
20:5	4746.66 ± 366.01	81.71 ± 36.17
22:0	11.37 ± 1.42	4.78 ± 1.31
22:1	6.20 ± 1.41	9.01 ± 1.14
22:2	0.93 ± 0.08	2.28 ± 0.68
22:3	6.89 ± 1.25	0.14 ± 0.14
22:4	18.01 ± 1.85	4.00 ± 0.63
22:5:n3	63.48 ± 6.59	1.16 ± 0.82
22:5:n6	117.7 ± 15.13	2.43 ± 1.27
22:6	837.4 ± 59.68	9.46 ± 7.94
23:0	1.45 ± 0.17	0.09 ± 0.09
24:0	3.89 ± 0.46	3.17 ± 1.10
24:1	0.48 ± 0.16	23.13 ± 6.23
26:0	1.67 ± 0.31	0 ± 0
Chain Length	17.88 ± 0.01	16.46 ± 0.04
DBI	1.83 ± 0.02	0.46 ± 0.03
HUFA	8308.10 ± 658.63	604.29 ± 95.47
MUFA	18795.54 ± 1974.30	1259.72 ± 344.41
PUFA	40024.24 ± 3217.62	370.22 ± 208.94
SFA	8278.27 ± 959.33	7386.85 ± 1609.52
total	75406.44 ± 6579.36	9621.30 ± 2077.09

Means ± SEM for each fatty acid from diet samples. Fatty acids are also summarized by average chain length, number of double bonds (DBI), and amount of saturated fatty acids (SFA; no double bonds), monounsaturated fatty acids (MUFA; one double bond), polyunsaturated fatty acids (PUFA; 2 double bonds at any chain length or greater than 2

double bonds and less than 20 carbons in the chain), and highly unsaturated fatty acids (HUFA; greater than or equal to 3 double bonds and 20 or more carbons in the chain length). Samples are standardized to dry weight and presented as pmol/mg of dry tissue mass. Fatty acids are reported as chain length: number of double bonds.

Table S3.5 Ventricle fatty acid composition

Summary Statistics for ventricle fatty acids (mean ± SEM)						
Fatty Acid	**Carnivorous (pmol/mg tissue)**		**Omnivorous (pmol/mg tissue)**		**Herbivorous (pmol/mg tissue)**	
	12	**20**	**12**	**20**	**12**	**20**
12:0	15.59 ± 2.27	18.41 ± 1.91	12.31 ± 1.79	15.34 ± 1.40	16.00 ± 2.09	16.39 ± 1.99
14:0	136.07 ± 18.47	141.63 ± 19.32	133.40 ± 17.64	145.18 ± 14.85	92.32 ± 3.95	106.24 ± 5.71
15:0	52.13 ± 6.00	44.32 ± 3.04	49.72 ± 6.73	46.66 ± 2.97	45.43 ± 4.71	47.96 ± 4.11
16:0	4578.38 ± 480.57	4683.44 ± 343.79	4329.96 ± 450.18	4674.41 ± 380.33	4645.31 ± 550.05	3973.04 ± 365.53
16:1	544.14 ± 109.41	434.74 ± 56.81	588.10 ± 117.04	437.28 ± 50.4	314.76 ± 34.22	267.19 ± 15.88
17:0	92.60 ± 11.92	80.37 ± 5.26	84.84 ± 9.88	89.62 ± 7.18	85.42 ± 11.87	75.72 ± 9.07
17:1	61.11 ± 8.88	55.40 ± 2.98	60.32 ± 11.48	62.98 ± 7.15	37.99 ± 4.7	30.08 ± 2.87
18:0	2539.88 ± 361.60	2335.91 ± 155.54	2131.24 ± 244.57	2457.98 ± 220.51	2389.31 ± 289.46	2352.46 ± 228.23
18:1	4529.38 ± 751.19	4372.17 ± 570.61	4612.46 ± 702.39	4961.47 ± 865.48	1782.38 ± 241.04	1289.18 ± 123.98
18:2	1010.21 ± 155.47	1088.89 ± 108.89	970.44 ± 128.08	1146.77 ± 147.78	409.98 ± 48.51	242.10 ± 18.15
18:3:n3	1442.08 ± 332.62	1470.50 ± 220.14	1734.98 ± 313.34	1616.48 ± 258.19	256.34 ± 28.55	154.56 ± 21.71
18:3:n6	21.82 ± 3.60	19.93 ± 2.60	23.71 ± 2.36	20.41 ± 2.04	9.50 ± 0.96	7.76 ± 0.57
18:4	108.43 ± 24.04	89.41 ± 10.65	133.33 ± 19.25	96.04 ± 16.34	27.93 ± 2.65	21.78 ± 4.16
20:0	22.13 ± 3.94	14.14 ± 2.65	20.71 ± 3.90	17.57 ± 2.03	15.00 ± 2.18	13.86 ± 2.09
20:1	204.10 ± 35.68	138.43 ± 14.88	200.21 ± 30.87	149.32 ± 16.86	98.97 ± 11.37	72.90 ± 6.96
20:2	138.10 ± 15.79	123.64 ± 5.41	130.59 ± 16.24	129.89 ± 10.26	90.48 ± 7.85	63.54 ± 4.8
20:3:n3	211.89 ± 28.03	245.16 ± 15.21	230.06 ± 31.46	248.74 ± 24.89	94.04 ± 7.87	61.83 ± 8.35
20:3:n6	212.26 ± 20.51	190.42 ± 13.90	221.90 ± 19.06	186.93 ± 13.17	137.73 ± 13.76	96.56 ± 6.11

20:3:n9	233.14 ± 35.10	226.50 ± 34.14	288.76 ± 30.02	269.04 ± 31.03	190.77 ± 31.78	254.56 ± 27.85
20:4	4554.23 ± 497.59	4440.62 ± 499.04	5296.24 ± 417.69	5014.89 ± 419.83	4015.09 ± 563.10	5078.91 ± 394.71
20:5	1918.87 ± 230.56	2073.28 ± 155.61	2281.73 ± 274.3	1891.19 ± 157.76	1399.57 ± 187.62	785.19 ± 79.69
22:0	7.29 ± 1.77	4.78 ± 1.24	5.73 ± 1.36	5.70 ± 1.11	3.16 ± 0.62	1.69 ± 0.44
22:1	27.78 ± 6.43	15.24 ± 2.24	22.79 ± 4.6	16.59 ± 3.13	9.12 ± 1.36	6.11 ± 0.89
22:2	9.88 ± 1.52	8.49 ± 0.80	8.38 ± 1.45	9.90 ± 1.55	4.30 ± 0.60	2.71 ± 0.36
22:3	9.74 ± 1.62	9.10 ± 0.79	9.29 ± 1.78	10.13 ± 1.63	4.10 ± 0.59	2.16 ± 0.40
22:4	283.62 ± 20.30	307.36 ± 28.93	296.83 ± 41.22	295.28 ± 16.64	271.49 ± 22.31	288.57 ± 21.99
22:5:n3	1865.78 ± 202.43	1877.32 ± 174.26	1942.69 ± 240.84	1839.16 ± 74.25	1578.26 ± 159.58	1477.07 ± 168.74
22:5:n6	225.27 ± 10.94	292.53 ± 20.34	233.42 ± 12.50	289.44 ± 9.26	224.41 ± 27.72	216.40 ± 15.51
22:6	8576.93 ± 916.91	8820.27 ± 999.92	10192.78 ± 818.83	8706.84 ± 996.29	9112.59 ± 1073.22	12088.71 ± 1536.26
23:0	2.13 ± 0.38	1.27 ± 0.32	1.52 ± 0.30	1.86 ± 0.38	1.43 ± 0.36	1.22 ± 0.27
24:0	3.66 ± 0.55	2.22 ± 0.49	2.67 ± 0.58	3.23 ± 0.76	3.64 ± 1.22	2.31 ± 0.68
24:1	12.43 ± 1.82	11.26 ± 1.15	8.87 ± 1.31	13.83 ± 1.98	10.67 ± 1.88	9.20 ± 0.94
26:0	1.21 ± 0.41	0.80 ± 0.46	1.06 ± 0.56	1.51 ± 0.97	2.91 ± 1.85	1.19 ± 0.81
Chain Length	19.44 ± 0.10	19.44 ± 0.14	19.61 ± 0.11	19.44 ± 0.14	19.7 ± 0.12	20.04 ± 0.11
DBI	3.15 ± 0.12	3.17 ± 0.16	3.38 ± 0.12	3.15 ± 0.16	3.41 ± 0.14	3.77 ± 0.13
SFA	7451.07 ± 802.35	7327.30 ± 465.78	6773.17 ± 709.04	7459.06 ± 604.58	7299.94 ± 841.02	6592.08 ± 600.46
MUFA	5378.94 ± 903.34	5027.24 ± 634.32	5492.74 ± 846.69	5641.47 ± 934.43	2253.88 ± 277.05	1674.66 ± 144.16
PUFA	2730.52 ± 516.94	2800.87 ± 343.35	3001.43 ± 468.02	3019.49 ± 417.67	798.53 ± 72.62	492.44 ± 39.43
HUFA	18091.73 ± 1442.71	18482.56 ± 1541.71	20993.7 ± 1178.24	18751.66 ± 1210.11	17028.04 ± 1401.78	20349.94 ± 1756.79
total	33652.29 ± 3054.71	33637.99 ± 1264.95	36261.02 ± 2414.87	34871.69 ± 1209.08	27380.48 ± 2107.91	29109.08 ± 2045.39

Means ± SEM for each fatty acid from ventricle samples taken after 2 weeks at 12°C and 2 weeks at 20°C. Fatty acids are also summarized by average chain length, number of double bonds (DBI), and amount of saturated fatty acids (SFA; no double bonds), monounsaturated fatty acids (MUFA; one double bond), polyunsaturated fatty acids (PUFA; 2 double bonds at any chain length or greater than 2 double bonds and less than 20 carbons in the chain), and highly unsaturated fatty acids (HUFA; greater than or equal to 3 double bonds and 20 or more carbons in the chain length). Samples are presented as pmol/mg wet tissue mass. Fatty

acids are reported as chain length: number of double bonds. Carnivorous = brine diet, omnivorous = ogo + brine diet, herbivorous = ogo diet.

Table S3.6 Summary Statistics for all metrics from the ABT test.

Timepoint	Diet	n	Mean ± SEM			
			Peak f_{Hmax}	T_{AB}	T_{PEAK}	T_{ARR}
Before change (12°C)	Carn	10	163.82 ± 6.03	21.2 ± 0.6	26.3 ± 0.5	28.2 ± 0.6
	Herb	10	165.74 ± 7.81	20.4 ± 0.8	26.9 ± 0.6	28.0 ± 0.5
	Omni	11	164.78 ± 7.21	21.0 ± 0.5	27.1 ± 0.7	28.5 ± 0.9
Day 1 at 20°C	Carn	9	181.36 ± 6.72	22.5 ± 0.7	27.1 ± 0.7	28.6 ± 0.7
	Herb	11	174.56 ± 5.71	23.2 ± 0.3	28.6 ± 0.8	29.7 ± 0.9
	Omni	10	177.34 ± 6.79	23.6 ± 0.5	27.4 ± 0.4	28.3 ± 0.6
Day 3 at 20°C	Carn	11	193.94 ± 7.18	23.5 ± 0.4	28.4 ± 0.5	30.2 ± 0.5
	Herb	9	184.56 ± 5.25	23.3 ± 0.7	28.5 ± 0.3	30.2 ± 0.3
	Omni	11	189.83 ± 5.17	23.8 ± 0.4	29.5 ± 0.6	30.6 ± 0.7
Day 7 at 20°C	Carn	8	195.00 ± 4.49	24.1 ± 0.2	28.2 ± 0.4	29.9 ± 0.5
	Herb	8	186.03 ± 7.87	24.1 ± 0.2	28.5 ± 0.6	32.1 ± 0.3
	Omni	7	205.73 ± 10.58	24.2 ± 0.6	28.8 ± 0.6	31.3 ± 1.1
Day 14 at 20°C	Carn	12	207.78 ± 6.04	24.5 ± 0.4	30.0 ± 0.4	31.4 ± 0.3
	Herb	11	178.36 ± 5.47	24.4 ± 0.3	30.1 ± 1.0	32.1 ± 0.9
	Omni	15	219.88 ± 5.65	25.4 ± 0.4	30.2 ± 0.4	31.9 ± 0.4

Means ± SEM for each test are presented. T_{AB}, breakpoint temperature of the heart; T_{PEAK}, temperature corresponding to maximum heart rate; T_{ARR}, temperature at the onset of cardiac arrhythmia; Peak f_{Hmax}, maximum heart rate achieved during the warm ABT test.

Table S3.7

SIC outputs for warm ABT test acclimation capacity polynomial curves				
Model #	**df**	**SIC**	**ΔSIC**	**Formula**
Model 1	27	8848.30	0.00	poly(temp, 3) * acc temp * diet + (1 | fish_id) + (1 | rep)
Model 2	33	8874.08	25.78	poly(temp, 4) * acc temp * diet + (1 | fish_id) + (1 | rep)
Model 3	15	8966.85	118.55	poly(temp, 4) * acc temp + diet + (1 | fish_id) + (1 | rep)
Model 4	13	8968.92	120.62	poly(temp, 4) * acc temp + (1 | fish_id) + (1 | rep)
Model 5	13	8980.37	132.07	poly(temp, 3) * acc temp + diet + (1 | fish_id) + (1 | rep)
Model 6	11	8982.16	133.86	poly(temp, 3) * acc temp + (1 | fish_id) + (1 | rep)
Model 7	15	9172.63	324.33	poly(temp, 3) * diet + (1 | fish_id) + (1 | rep)
Model 8	10	9175.00	326.70	poly(temp, 3) + acc temp + diet + (1 | fish_id) + (1 | rep)
Model 9	11	9181.22	332.92	poly(temp, 4) + acc temp + diet + (1 | fish_id) + (1 | rep)
Model 10	13	9185.91	337.61	poly(temp, 4) + acc temp * diet + (1 | fish_id) + (1 | rep)
Model 11	7	9195.89	347.59	poly(temp, 3) + (1 | fish_id) + (1 | rep)
Model 12	15	9602.05	753.75	temp * acc temp * diet + (1 | fish_id) + (1 | rep)
Model 13	8	9633.44	785.14	temp + acc temp + diet + (1 | fish_id) + (1 | rep)
Model 14	10	9634.98	786.68	temp + acc temp * diet + (1 | fish_id) + (1 | rep)
Model 15	6	9636.42	788.11	temp + acc temp + (1 | fish_id) + (1 | rep)
Model 16	5	9656.92	808.61	temp + (1 | fish_id) + (1 | rep)
Model 17	7	9658.25	809.95	temp + diet + (1 | fish_id) + (1 | rep)

Represented are model formulas as input into R and SIC output results. df = degrees of freedom, SIC = Schwarz Information Criterion ΔSIC = SIC(model)—SIC(min SIC value), temp = acute temperature, acc temp = acclimation temperature (12 or 20°C), fish_id = individual fish., rep = replicate number of experiment.

Table S3.8 Statistics outputs from ANOVA for all metrics from the ABT test.

Parameter	Factor	Chisq	Df	Pr(>Chisq)
Peak f_{Hmax}	Timepoint	71.6087	4	<0.001
	Diet	14.3710	2	<0.001
	Diet x Timepoint	19.4204	8	0.0128
T_{AB}	Timepoint	120.9319	4	<0.001
	Diet	3.6067	2	0.1647
T_{PEAK}	Timepoint	57.8216	4	<0.001
	Diet	2.9669	2	0.2269
T_{ARR}	Timepoint	69.3436	4	<0.001
	Diet	3.2205	2	0.1998

Table S3.9 % Acclimated for thermal limits across timepoints

	T_{AB} (% acclimated)			T_{PEAK} (% acclimated)			T_{ARR} (% acclimated)		
Diet	**Day 1**	**Day 3**	**Day 7**	**Day 1**	**Day 3**	**Day 7**	**Day 1**	**Day 3**	**Day 7**
Carn	39.4	69.7	90.9	21.6	56.8	51.4	12.5	62.5	53.1
Herb	68.3	70.7	80.5	53.1	50.0	50.0	41.5	53.7	100.0
Omni	59.6	63.8	74.5	9.7	77.4	54.8	-5.9	61.8	82.4

Represented are the percent (%) of the total acclimation response for cardiac thermal limits (day 14 at 20°C - day 14 at 12°C) that was achieved on day 1, 3, 7 during acclimation to 20°C. Values are calculated as 100 x (thermal limit on day X at 20°C - thermal limit on day 14 at 12°C) / (thermal limit on day 14 at 20°C - thermal limit on day 14 at 12°C), where X =

the day of testing. T_{AB} = breakpoint temperature of the heart; T_{PEAK} = temperature corresponding to maximum heart rate; T_{ARR} = temperature at the onset of cardiac arrhythmia.

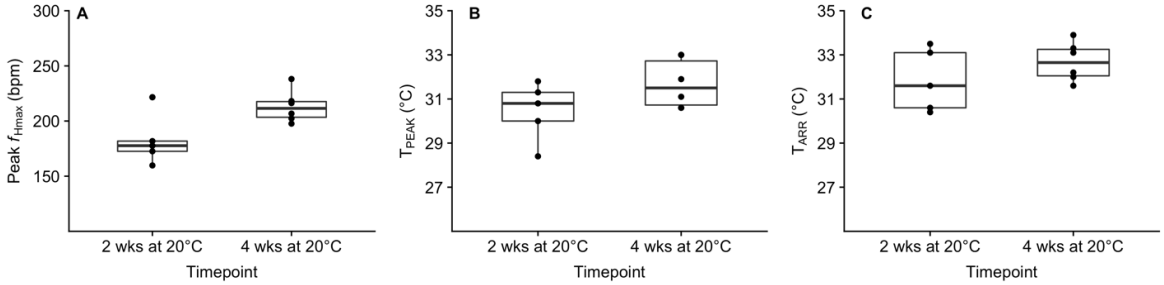

Figure S3.1 Cardiac performance in opaleye acclimated to 20°C for 2 or 4 weeks and fed a carnivorous diet. Presented are box plots of A) Peak f_{Hmax} (bpm), B) T_{PEAK} (°C) and C) T_{ARR} (°C). Box plots represent interquartile ranges (boxes and whiskers), median values (solid lines) and outliers (> 1.5 beyond interquartile range) are plotted as data points outside the whiskers. Groups were compared using a two-tailed t-test. Peak f_{Hmax} was found to be significantly different after 4 weeks compared to 2 weeks ($p = 0.026$). However, T_{PEAK} and T_{ARR} were not significantly different between timepoints ($p = 0.126$ and $p = 0.257$ respectively).

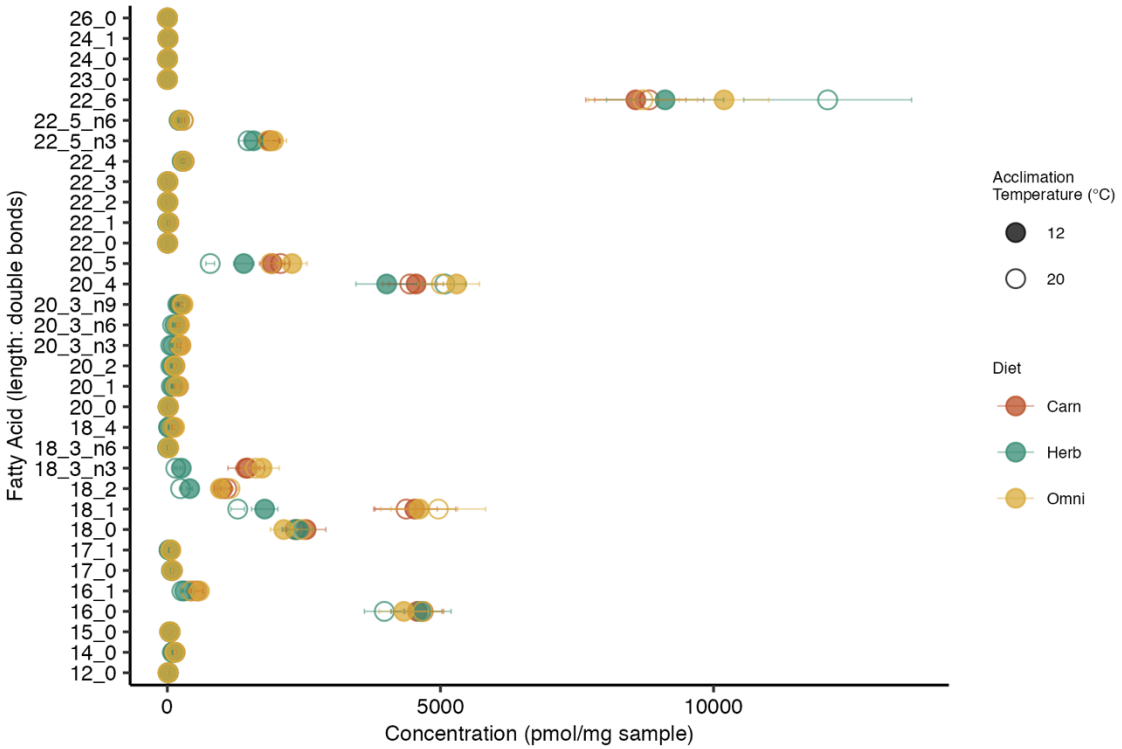

Figure S3.2 Mean ± SEM for various fatty acids in opaleye ventricles acclimated to 12°C (closed circles) and 20°C (open circles). Colors indicate diet treatment, with carnivorous = red, herbivorous = green, omnivorous = yellow.

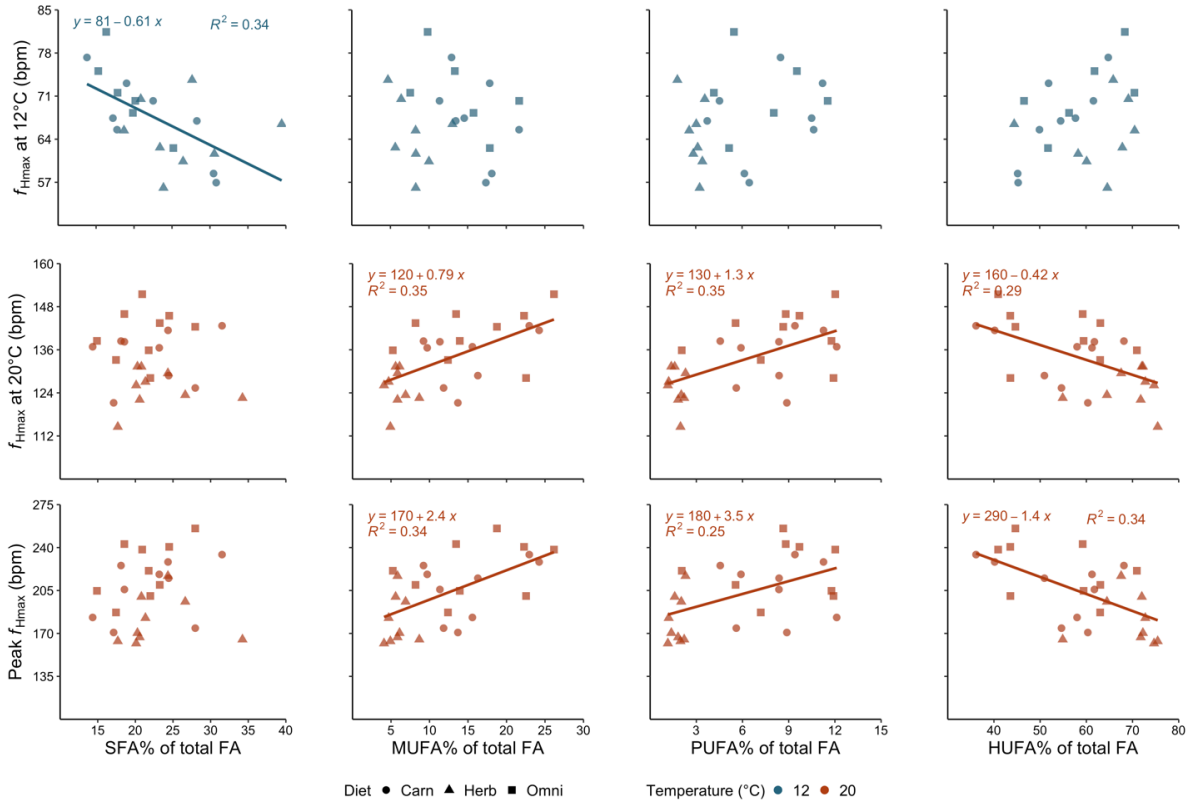

Figure S3.3 Linear regressions between various measures of max heart rate (f_{Hmax}) in 12 and 20°C acclimated opaleye and fatty acids. Each column is a different category of fatty acid presented as the proportion of the ventricle, with saturated fatty acids (SFA; no double bonds), monounsaturated fatty acids (MUFA; one double bond), polyunsaturated fatty acids (PUFA; 2 double bonds at any chain length or greater than 2 double bonds and less than 20 carbons in the chain), and highly unsaturated fatty acids (HUFA; greater than or equal to 3 double bonds and 20 or more carbons in the chain length). Lines are overlayed on plots where there was a significant linear regression between parameters (alpha <0.05). Equations and R^2 values are provided in each significant plot. Colors indicate acclimation temperature (red = 20°C, blue = 12°C). Symbols indicate diets, with herbivorous = triangle, omnivorous = square, carnivorous = circle.

Table S4.1: Ibutton data

Temp (°C)	Ration size	Diet type	Replicate tank		
			rep1	rep2	rep3
15	low	omega-enriched	15.1 ± 0.5	15.3 ± 0.5	15.1 ± 0.6
		omega-poor	15.0 ± 0.5	14.8 ± 0.6	15.7 ± 0.5
	high	omega-enriched	15.6 ± 0.4	14.9 ± 0.5	15.3 ± 0.5
		omega-poor	15.5 ± 0.7	15.3 ± 0.4	14.9 ± 0.6
22	low	omega-enriched	21.8 ± 0.4	22.3 ± 0.4	21.6 ± 0.5
		omega-poor	21.9 ± 0.4	21.9 ± 0.3	21.7 ± 0.3
	high	omega-enriched	21.7 ± 0.3	21.9 ± 0.4	21.9 ± 0.4
		omega-poor	22.1 ± 0.4	22.0 ± 0.4	22.2 ± 0.3

Represented are mean (±SD) for Ibutton (temperature logger; iButtonLink LLC, Whitewater, WI, USA) data from each experimental replicate tank.