Science of the Total Environment 922 (2024) 171057

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Warming-induced "plastic floors" improve hypoxia vulnerability, not aerobic scope, in red drum (*Sciaenops ocellatus*)

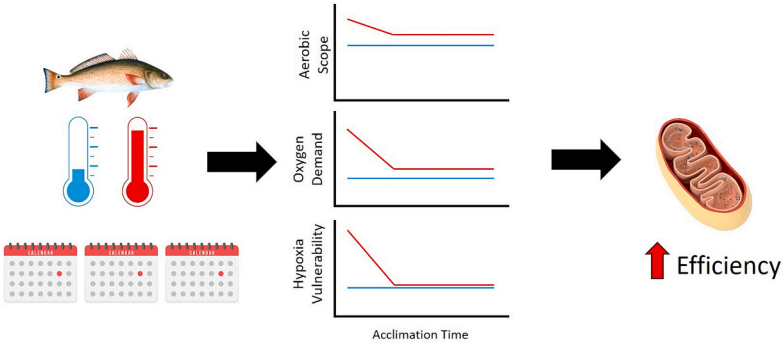
Adam D. Zambie [a,b], Kerri Lynn Ackerly [a], Benjamin Negrete Jr [a,c], Andrew J. Esbaugh [a,*]

[a] Department of Marine Science, University of Texas at Austin, Port Aransas, TX 78373, United States
[b] Department of Biological Sciences, Idaho State University, Pocatello, ID 83209, United States
[c] Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

HIGHLIGHTS

- A 12-week warming acclimation significantly reduced baseline oxygen demand.
- Warming acclimation did not improve aerobic scope.
- Acclimation-induced reduction in oxygen demand reduced critical oxygen threshold.
- Warming acclimation improved mitochondrial efficiency in the liver but not the heart.
- Acclimation significantly increased the range of metabolically available habitats.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Daniel Wunderlin

Keywords:
Standard metabolic rate
Maximum metabolic rate
Critical oxygen threshold
Mitochondrial proton leak
OXPHOS
Fish
Heat waves
Metabolic index

ABSTRACT

Ocean warming is a prevailing threat to marine ectotherms. Recently the "plastic floors, concrete ceilings" hypothesis was proposed, which suggests that a warmed fish will acclimate to higher temperatures by reducing standard metabolic rate (SMR) while keeping maximum metabolic rate (MMR) stable, therefore improving aerobic scope (AS). Here we evaluated this hypothesis on red drum (*Sciaenops ocellatus*) while incorporating measures of hypoxia vulnerability (critical oxygen threshold; P_{crit}) and mitochondrial performance. Fish were subjected to a 12-week acclimation to 20 °C or 28 °C. Respirometry was performed every 4 weeks to obtain metabolic rate and P_{crit}; mitochondrial respirometry was performed on liver and heart samples at the end of the acclimation. 28 °C fish had a significantly higher SMR, MMR, and P_{crit} than 20 °C controls at time 0, but SMR declined by 36.2 % over the 12-week acclimation. No change in SMR was observed in the control treatment. Contrary to expectations, SMR suppression did not improve AS relative to time 0 owing to a progressive decline in MMR over acclimation time. P_{crit} decreased by 27.2 % in the warm-acclimated fishes, which resulted in temperature treatments having statistically similar values by 12-weeks. No differences in mitochondrial traits were observed in the heart – despite a Δ8 °C assay temperature – while liver respiratory and coupling control ratios were significantly improved, suggesting that mitochondrial plasticity may contribute to the reduced SMR with warming. Overall, this work suggests that warming induced metabolic suppression offsets the deleterious

* Corresponding author.
E-mail address: a.esbaugh@austin.utexas.edu (A.J. Esbaugh).

https://doi.org/10.1016/j.scitotenv.2024.171057
Received 27 July 2023; Received in revised form 19 January 2024; Accepted 15 February 2024
Available online 18 February 2024

consequences of high oxygen demand on hypoxia vulnerability, and in so doing greatly expands the theoretical range of metabolically available habitats for red drum.

1. Introduction

Warming water temperatures are an imminent stressor to marine ectotherms that cause a suite of physiological challenges including increased energetic demands, compromised oxygen supply capacity, and decreased growth (Jutfelt et al., 2021; Portner, 2010; Salvatteci et al., 2022; Sandblom et al., 2014). Pioneering work by Fry and Hart (1948) outlined the effect of temperature on the metabolic rates of fishes and brought forth the concept of aerobic scope (AS) – the difference between standard (SMR) and maximum metabolic rate (MMR) – which has since become an important metric for predicting the short and long-term effects of thermal stress (Clark et al., 2013; Deutsch et al., 2015; Deutsch et al., 2020; Pörtner and Farrell, 2008). During acute warming the SMR of aquatic ectotherms will increase according to thermodynamic constants, which effectively raises the baseline cost of living (e.g. Deutsch et al., 2015; Deutsch et al., 2020; Fry and Hart, 1948). MMR will similarly increase with acute warming, with the exception that many species exhibit a thermal plateau, or even decline, in maximum oxygen consumption rates associated with cardiorespiratory failure at high temperatures (e.g. Ackerly and Esbaugh, 2021; Eliason et al., 2013; Norin et al., 2014). The effects of warming on SMR and MMR may result in a decline in AS, which is commonly interpreted as a decline in available energy for important functions such as exercise, digestion, growth and reproduction. As such, constraints of AS due to warming represent a crucial issue for many species.

While the dynamic responses of AS to changes in water temperature have rightly drawn a great deal of research attention, it is important to note that AS can also be impacted by other climate change stressors — such as hypoxia (Ackerly and Esbaugh, 2020; Claireaux and Chabot, 2016; Negrete Jr. et al., 2022; Pan et al., 2017) or ocean acidification (Couturier et al., 2013; Esbaugh, 2018; Lefevre, 2016). Ocean deoxygenation, in particular, is now recognized as a serious consequence of climate change owing to the increased prevalence of marine hypoxic zones (Breitburg et al., 2018; Gallo and Levin, 2016; Levin, 2018). The effects of hypoxia on AS are straightforward, as it has long been established that reductions in the ambient partial pressure of oxygen (PO_2) result in reduced MMR (Claireaux and Chabot, 2016; Fry, 1947; Fry, 1971). While the exact relationship between declining PO_2 and MMR has been the topic of recent debate (Esbaugh et al., 2021; Farrell et al., 2021; Seibel et al., 2021b; Seibel and Deutsch, 2020), it is clear that at some PO_2 an animal's MMR will intersect with SMR, driving AS to zero. This point is known as the critical oxygen threshold (P_{crit}) (Claireaux and Chabot, 2016; Negrete Jr. and Esbaugh, 2019; Seibel et al., 2021a; Ultsch and Regan, 2019), and while other measures of hypoxia tolerance exist (Wood, 2018), the P_{crit} is a particularly valuable measure of hypoxia vulnerability because it denotes the PO_2 at which an organism can no longer meet the demands of SMR without relying on unsustainable anaerobic metabolism. Recent work has attempted to use the combined effects of temperature and hypoxia on animal metabolism (i.e. factorial AS; MMR/SMR) to identify metabolically available habitat for species. This framework is known as the metabolic index (ϕ) and provides a useful mechanistic tool to predict the potential effects of climate change on species range distributions as a consequence of dynamic shifts in oxygen and temperature (Deutsch et al., 2015; Deutsch et al., 2020). Importantly, the thermal sensitivity of hypoxia vulnerability is a crucial component when calculating ϕ.

A commonly used approach to study the effects of thermal, or hypoxic, stress on fishes is to use acute exposures whereby animals are exposed to relatively rapid changes in environmental condition followed by physiological measurement (e.g. Eliason et al., 2011; Eliason et al., 2013; Eliason and Farrell, 2016; Ern et al., 2017; Ern et al., 2016; Norin et al., 2014). While this approach is valid for many ecologically relevant thermal stress scenarios, it is also important to consider long-term thermal acclimation (i.e. phenotypic plasticity). This is particularly important in the context of climate change, and any predictive modeling associated with climate change, as phenotypic plasticity has been specifically highlighted as a plausible route for population and species resilience (Bell, 2013; Gonzalez et al., 2013). In fact, climate change related stressors, including warming (Norin et al., 2014; Robinson and Davison, 2008; Sandblom et al., 2014; Scheuffele et al., 2021), hypoxia (Negrete Jr. et al., 2022; Pan et al., 2017) and ocean acidification (Esbaugh, 2018; Esbaugh et al., 2016) have previously been shown to impact respiratory traits in fishes via phenotypic plasticity. Work by Sandblom et al. (2016) has brought forth the "plastic floors, concrete ceilings" hypothesis; proposing that certain fish exhibit metabolic plasticity with the intent of increasing aerobic scope over the course of warming acclimation. More specifically, this hypothesis suggests that fish actively reduce SMR in warm environments with no matched reduction in MMR, thus increasing AS and the energy available for activity, digestion and growth.

The "plastic floors, concrete ceilings" hypothesis provides a useful guidepost for incorporating acclimation into mechanistic models that aim to predict the effects of climate change on marine fishes, however, several important questions remain. First and foremost are the interactive effects of warming on SMR, MMR and P_{crit}, which are the three principle metabolic traits used to predict species biogeography on the basis of temperature and oxygen (e.g. Deutsch et al., 2015; Deutsch et al., 2020; Franco et al., 2022; Parouffe et al., 2023). A second important consideration is whether warming induced reductions in SMR represent improvements in efficiency (Sandblom et al., 2016) or physiological suppression of important pathways. Finally, we must assess whether the magnitude of any physiological acclimation is ecologically meaningful, particularly in the context of performance and metabolically available habitat. To this end, the current study sought to explore the effects of warming acclimation over a 12-week period on baseline metabolism, maximum respiratory performance and hypoxia vulnerability in red drum (*Sciaenops ocellatus*). This species is commonly found in the subtropical and tropical coastlines of eastern North and Central America, and is known to exhibit physiological plasticity to a wide array of environmental stressors (Allmon and Esbaugh, 2017; Dichiera et al., 2022; Esbaugh and Cutler, 2016; Esbaugh et al., 2016; Martin and Esbaugh, 2021; Watson et al., 2014). In accordance with the "plastic floors, concrete ceilings" hypothesis, we hypothesize that red drum will exhibit a progressive decline in SMR over acclimation time that will coincide with a significant improvement in AS and reduction in P_{crit}. We further hypothesize that the elevated AS will coincide with significant improvements in growth rate and mitochondrial efficiency of two aerobically active tissues, the heart and liver. Finally, we hypothesize that any observed improvements in metabolic traits following warm acclimation will expand the scope of metabolically available habitat, as predicted through the metabolic index.

2. Methods

All experimental procedures and protocols used in this study were approved by The University of Texas at Austin Institutional Animal Care and Use Committee (AUP-2018-00231; AUP-2021-00204).

2.1. Animal husbandry and experimental design

Juvenile red drum were obtained from Ekstrom Aquaculture (Palacios, TX). Fish were transported to The University of Texas at Austin

Marine Science Institute (UTMSI) by truck under constant aeration. At UTMSI, fish were maintained at 20 ± 0.1 °C and 38 ± 0.4 ppt with constant aeration on a 14:10 light dark cycle in 545 L recirculating tanks outfitted with a biofilter for 4 weeks prior to experimentation. Fish were fed ad libitum daily on a diet of commercial fish pellets (Purina Animal Nutrition), unless otherwise noted. Salinity, O_2 and pH were measured daily using handheld meters and ammonia was tested using a commercial aquarium test kit every other day.

To initiate the experiment, fish were randomly assigned to either a control group (3 replicate 545 L tanks; N = 28 fish; 12.0 ± 2.1 g) that remained at 20 ± 0.1 °C, or a warm-acclimation group (3 replicate 545 L tanks; N = 28 fish; 14.0 ± 1.0 g) maintained at 28 ± 0.1 °C. Replicate tanks for the respective treatments were connected to a common biofiltration system, and each system had a digitally controlled temperature control unit. Water quality was measured as described above. Temperatures for the warm-acclimation group were raised to 28 °C in daily 2 °C increments, with the acclimation start date defined as the day at which the target temperature was reached. A second random subset of fish were subjected to an acute temperature transfer to 28 °C (n = 12, 15.3 ± 1.4 g) or 20 °C (n = 11, 16.1 ± 1.6 g), which is defined herein as week 0. Acute-acclimation was performed as described in Dichiera et al. (2021) where temperature was raised 2 °C hourly until acclimation temperature was reached, after which the fish were maintained overnight followed by respirometry measurements (see below). The average fish mass of unfed animals was taken for each replicate tank of both temperature treatments every 4 weeks.

2.2. Intermittent flow respirometry

Intermittent-flow respirometry was used to assess SMR, MMR and P_{crit}. This system included fiberoptic oxygen electrodes connected to an associated meter (mini 4-channel, PreSens; Witrox 4-channel, Loligo Systems) and laptop. The O_2 data were fed into software (AutoResp, Loligo Systems) that recorded O_2 consumption rates while controlling flush and circulation pumps via a power relay data acquisition unit (Loligo Systems). The protocol was modeled on previous work in red drum (Ackerly and Esbaugh, 2020; Ackerly and Esbaugh, 2021; Dichiera et al., 2021; Khursigara et al., 2018), and began by manually chasing the fish to exhaustion (approximately 3 min) followed by a 1-min air exposure period after which fish were placed in respirometry chambers. Note that chase protocols have generally been found to provide comparable results to exhaustive swim trials (Killen et al., 2017). The first 3-h of oxygen consumption data for each trial were omitted from SMR analysis on the basis of elevated post-exercise oxygen consumption (EPOC). Note that prior work in our lab has shown EPOC and the biochemical recovery from exhaustive exercise in red drum is complete in <3 h (Ackerly and Esbaugh, 2020; Ackerly and Esbaugh, 2021; Dichiera et al., 2021; Johansen and Esbaugh, 2017; Martin et al., 2023). Fish stayed in the chambers for an additional 24 h, which was used for SMR determination. At the end of the SMR period fish were subjected to a closed-system P_{crit} trial wherein the flush circuit was closed allowing the fish respiration to draw down oxygen in the chamber until loss of equilibrium was reached, or oxygen in the chamber reached 5 % air saturation. Note that previous work in red drum has demonstrated no difference between closed-circuit P_{crit} determinations and those in which N_2 is used to lower PO_2 (Negrete Jr. and Esbaugh, 2019). At the conclusion of P_{crit} trials fish were removed from the chambers and moved to non-experimental holding tanks. All respirometry was conducted at the treatment temperature and salinity, and on fish starved for 48 h.

The approximate total volume of the respirometry chambers (acrylic) and tubing (vinyl) was 680 mL, with water volume to body mass ratios ranging from 85 to 21. Fish were measured in groups of 4 over 3 consecutive days with thorough cleaning of the chambers and holding system between trials. Two separate water bath systems were established (20 °C and 28 °C), which consisted of an approximately 95 L holding bath that contained 4 respirometry chambers, and a 140 L sump bath where temperature was regulated using WILLHI digital temperature relay control units connected to titanium aquarium heating elements. Each respirometry chamber was partially wrapped in black plastic to limit visual cues. Respirometry cycles were 3-min flush, 1-min wait, and 3-min measure. Chamber mixing during measurement was provided by a recirculation pump circuit attached to each chamber (maximum pump flow of 120 L/h). Measurements of mass-specific O_2 consumption (MO_2, mg O_2 kg^{-1} h^{-1}) were calculated using AutoResp software (Loligo, Denmark) with manually entered temperature values. O_2 measurements were taken every second using a Presens fiber optic system placed within the circulation circuit. Measurements were omitted when an $R^2 < 0.95$ was obtained for the MO_2 slope. SMR was calculated as the 20th percentile of all MO_2 measurements (Chabot et al., 2016; Negrete Jr. and Esbaugh, 2019). Analysis of MMR was performed in R statistical software (R version 4.0.2) (R Core Team, 2021) using the *rollregress* package following the rolling regression method described by Prinzing et al. (2021). Briefly, over a designated time interval (i.e., 3-min measurement interval) a regression of declining oxygen in a chamber is applied to a designated time window (60 s) using the raw data extracted from AutoResp. This method generated ~121 slopes of oxygen decline for each 180 s measurement period, and the highest of these slopes was defined as the MMR for that interval. The first 3 intervals post-exercise were analyzed for each fish and the highest single slope was used to define MMR. Absolute aerobic scope (AAS) was calculated as the difference between MMR and SMR. P_{crit} was calculated as the point where SMR intersected with the regression line of the oxyconformation phase (i.e. when MO_2 declined linearly with declining PO_2) of the P_{crit} trial (Negrete Jr. and Esbaugh, 2019).

Measurements of background respiration were taken before and after fish were placed in the respirometry chambers using respirometry cycles of 3-min flush, 1-min wait, and 26-min measure. Individual MO_2 were corrected assuming a linear increase in background respiration.

2.3. Mitochondrial respirometry

After 12 weeks, a subset of individuals (warm-acclimated: n = 8, mass: 45.0 ± 4.7 g; control-acclimated: n = 6, mass: 30.6 ± 3.1 g) from each acclimation were used to assess mitochondrial performance of heart and liver tissue. These tissues were chosen because of their important contributions to baseline aerobic metabolism. Individuals were euthanized with an overdose of buffered MS-222 (500 mg L^{-1} tricaine methanesulfonate, 1 g L^{-1} $NaHCO_3$) until operculum movement stopped after which a spinal transection was performed. Individuals had the whole heart and liver dissected and placed immediately on ice. The ventricle was subsequently isolated and both liver and ventricle tissue were gently blotted on tissue paper and weighed (heart: 10 ± 0.6 mg; liver: 31.2 ± 1.3 mg). Each tissue was then placed in 500 μL ice cold respiration buffer (0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM lactobionic acid, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM D-sucrose, buffered to 7.0 using 5 M KOH) and gently homogenized in separate 7 mL glass Dounce homogenizers (Wheaton, USA) on ice. Homogenates were then transferred to an Oroboros Oxygraph-2 k respirometer system (Oroboros Instruments, Innsbruck, Austria) test chamber (2.2 mL total volume of respiration buffer + homogenate) to quantify mitochondrial performance. For each individual, the ventricle and liver samples were run simultaneously. Chamber temperatures reflected the acclimation temperature (warm-acclimated: 28 °C; control-acclimated: 20 °C). Oxygen consumption in the chambers was measured as mass-specific O_2 flux (pmol s^{-1} mg^{-1}) in real time using Datlab (Oroboros Instruments Innsbruck, Austria).

Mitochondrial performance and efficiency were measured using a substrate inhibitor titration as previously described for red drum (Ackerly et al., 2023; Johansen and Esbaugh, 2019). The following titration sequence was used: a) 280 U/mL catalase + 3 μL 3 % H_2O_2 (bringing

chamber to 200 % air saturation to prevent oxygen limitations on mitochondrial performance; b) 2 mM malate + 5 mM pyruvate + 10 mM glutamate (to provide substrates for Complex I); c) 1 mM steps of ADP to saturation (to induce CI-OXPHOS); d) 10 μM cytochrome *c* (to assess mitochondrial membrane integrity); e) 10 mM succinate (substrate for Complex II) + 1 mM ADP (to induce maximal respiration; OXPHOS); f) 5 nM oligomycin (to inhibit ATP synthase and measure LEAK); and g) 2.5 μM antimycin a (to assess residual oxygen consumption (ROX) through inhibition of OXPHOS). All mitochondrial performance was assessed and reported here as ROX-corrected values. Oxygen consumption of each step is reported as mass-specific O_2 flux (pmol s^{-1} mg^{-1}). The respiratory control ratio (RCR) was calculated as OXPHOS divided by LEAK, and the coupling control ratio (CCR) was calculated as LEAK divided by OXPHOS. OXPHOS Capacity was calculated as OXPHOS minus LEAK, and OXPHOS Control Efficiency was calculated as OXPHOS Capacity divided by OXPHOS. Mitochondrial membrane integrity was assessed by calculating the percent (%) change in respiration between CCI-OXPHOS and the addition of cytochrome *c*. There was no significant change to respiration rates in either cardiac or liver tissues, which on average was <15 %.

2.4. Statistical analysis

All statistical analysis was conducted in Sigma Plot version 13. To account for the differential growth rates, and thus sizes, of fish in the warm and control acclimations, all data were scaled to a common mass (the average mass of all fish used at the time of testing) using red drum specific scaling exponents for SMR (Pan et al., 2016) and MMR (Ackerly and Esbaugh, 2020). This was preferred to incorporating mass as a covariate owing to the fact that the previously established scaling exponents were developed using a more robust data set (i.e. much larger range in mass). A two-way analysis of variance (ANOVA) with temperature and time point as factors was then used to test for differences in SMR, MMR and P_{crit}, with a Holm-Sidak post-hoc test used when significant interactions between temperature and time were present. An analysis of covariance (ANCOVA) was also used to assess the effect of temperature on P_{crit} with SMR as a covariant. The SMR and P_{crit} data sets did not conform to normality and equal variance – as tested using a Shapiro-Wilk and Brown-Forsythe test, respectively – so they were log transformed. Mitochondrial traits were analyzed using a *t*-test; in the event of non-normal data a Mann-Whitney Rank Sum test was performed. OXPHOS and CI control ratios and OSPHOS control efficiency (i.e. all percentage data sets) were subjected to a Logit transformation prior to statistical testing. Specific growth rate was calculated as described in Crane et al. (2020) and tested using a repeated measures 2-way ANOVA with temperature and time as factors.

2.5. Metabolic index modeling of habitat availability

The metabolic index (Deutsch et al., 2015; Deutsch et al., 2020) was used as a prevailing mechanistic model to explore the significance of the observed acclimation responses on habitat availability with respect to temperature and PO_2. Modeling of ϕ was performed according to the following formula:

$$\phi = \frac{\alpha_s}{\alpha_D}\beta^{\varepsilon} p_{O2} exp\left\{\frac{E_o}{k_B}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right\},$$

as described by Deutsch et al. (2020). α_D is oxygen demand (i.e. SMR) per unit body mass (β) at a reference temperature (T_{ref}), and ε is the allometric scaling of the oxygen supply to demand ratio. ε is assumed to be zero on the basis of known scaling exponents for SMR (Pan et al., 2016) and MMR (Ackerly and Esbaugh, 2020) in red drum. α_s is oxygen supply capacity, which denotes how much oxygen is physiologically available at a given PO_2. E_o is the sensitivity of hypoxia vulnerability (i.e. P_{crit}) to temperature, as described by an Arrhenius function (Boltzman constant, k_B). T_{ref} is the temperature of the control treatment (20 °C), while *T* is the prediction temperature. All values used in modeling are presented in Table 4. The reference SMR was taken as the average of all 20 °C values throughout the study. E_o for non-acclimated estimates was based on the difference in P_{crit} between 20 °C and 28 °C treatments at the earliest time point, while E_o for acclimated fishes was taken at the final time point. α_s was calculated as SMR divided by P_{crit} for all 20 °C data. The critical oxygen partial pressure for maximum metabolic rate (P_{cmax}) was also calculated MMR divided by α_s. This value denotes the PO_2 above which MMR will not increase with increasing oxygen, and thus serves as a hard ceiling for prediction of MMR (Esbaugh et al., 2021; Seibel and Deutsch, 2020). Importantly, P_{cmax} was sufficiently high across the thermal range – as modeled using E_{pcmax} (see Supplemental Fig. S1) – that it was not necessary to incorporate it into the presented model outputs.

3. Results

3.1. Metabolic responses to warming

Mass normalized SMR was significantly affected by both acclimation temperature ($F_{1,72} = 145.49$, $p < 0.001$) and acclimation time ($F_{3,72} = 6.125$, $p < 0.001$) with a significant interaction between temperature and time being present ($F_{3,72} = 15.695$, $p < 0.001$) (Fig. 1). After acute transfer to 28 °C, red drum increased SMR by 95 % from 155.1 ± 8.5 to 302.3 ± 15.4 mg O_2 kg^{-1} h^{-1}. Control fish showed no within treatment change in SMR over the course of the acclimation period, whereas warm-acclimated fish showed a significant decline over the 12-week time course. The significant decline in SMR in warm-acclimated fish was observed at the 4-, 8- and 12-week time points. At the conclusion of the acclimation period warm-acclimated fish retained a significantly higher SMR than control fish ($t = 2.054$; $p = 0.044$); however, the difference was only 15 % with warm and control fish averaging 200.7 ± 5.4 and 174.9 ± 5.8 mg O_2 kg^{-1} h^{-1}, respectively.

Mass-normalized MMR (Fig. 2) and AAS (Fig. 3) showed similar trends to those of SMR, with a significant effect of temperature (MMR: $F_{1,71} = 114.99$, $p < 0.001$; AAS: $F_{1,71} = 54.03$, $p < 0.001$) and acclimation time (MMR: $F_{3,71} = 14.97$, $p < 0.001$; AAS: $F_{3,71} = 9.705$, $p < 0.001$), as well as a significant interaction between temperature and time (MMR: $F_{3,71} = 8.78$, $p < 0.001$; AAS: $F_{3,71} = 5.157$, $p < 0.001$). The warm-acclimated fish had a higher MMR and AAS at all tested time points, but the warm-acclimated values declined over time with

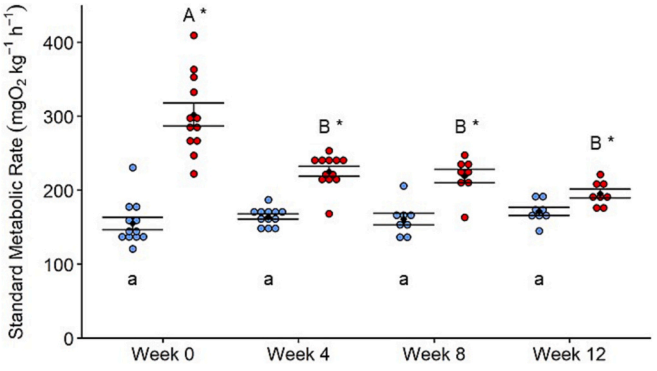


Fig. 1. Effects of temperature and acclimation time on standard metabolic rate in red drum. Fish were housed at either a control temperature of 20 °C (blue) or 28 °C (red) for 12-weeks. Standard metabolic rate was measured immediately after transfer (week 0) and every 4 weeks thereafter (N = 12, 12, 8 and 8 for weeks 0, 4, 8 and 12, respectively). Data were log transformed prior to statistical analysis. Different letters denote statistically different groups within a temperature treatment, and asterisks denote a significant difference between temperatures within a time point based on 2-way ANOVA analysis and Holm-Sidak post-hoc testing ($P \leq 0.05$).

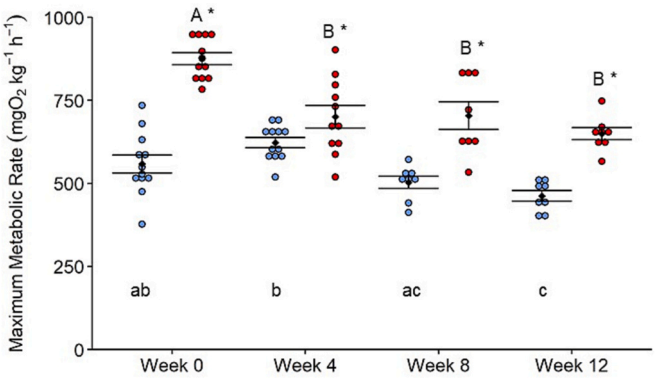


Fig. 2. Effects of temperature and acclimation time on maximum metabolic rate in red drum. Fish were housed at either a control temperature of 20 °C (blue) or 28 °C (red) for 12 weeks. Maximum metabolic rate was measured immediately after transfer (week 0) and every 4 weeks thereafter (20 °C: N = 12, 12, 8 and 8 for weeks 0, 4, 8 and 12, respectively. 28 °C: N = 12, 11, 8 and 8 for weeks 0, 4, 8 and 12, respectively). Different letters denote statistically different groups within a temperature treatment, and asterisks denote a significant difference between temperatures within a time point based on 2-way ANOVA analysis and Holm-Sidak post-hoc testing ($P \leq 0.05$).

significant differences from control observed at 4-weeks (MMR: $t = 5.243$, $p < 0.001$; AAS: $t = 3.141$, $p = 0.012$), 8-weeks (MMR: $t = 4.709$, $p < 0.001$; AAS: $t = 2.626$, $p = 0.042$) and 12-weeks (MMR: $t = 6.214$, $p < 0.001$; AAS: $t = 3.564$, $p = 0.004$). Interestingly, a significant decline from time 0 was also observed in the control acclimation, but only at 12-weeks (MMR: $t = 2.644$, $p = 0.04$; AAS: 3.360, $p = 0.005$). The MMR and AAS of warm-acclimated fish showed a 25.6 % and 20.6 % decrease, respectively, from time 0 to week-12. In both cases, measurements stabilized after the 4-week timepoint. The 12-week warm-acclimated and control fish had an average MMR of 690.7 ± 21.2 and 488.5 ± 17.1 mg O_2 kg^{-1} h^{-1}, respectively. The AAS for 12-week warm-acclimated and control fish was 453.8 ± 16.8 and 291 ± 15.4 mg O_2 kg^{-1} h^{-1}, respectively.

3.2. The effect of warming on hypoxia vulnerability

P_{crit} was significantly affected by temperature acclimation (Fig. 4; $F_{1,66} = 42.045$, $p < 0.001$), but not acclimation time ($F_{3,66} = 2.113$, $p = 0.107$), with a significant interaction between temperature and time ($F_{3,66} = 9.327$, $p < 0.001$). Control fish had no significant change in hypoxia vulnerability over the course of acclimation with an average P_{crit} of 23.8 ± 0.7 % air saturation (i.e. 5.0 kPa). Warm-acclimated fish showed improvement in hypoxia tolerance over acclimation with P_{crit} decreasing 26.7 % from time 0 ($P_{crit} = 36.6 \pm 1.4$) to week-12 (26.7 ± 1.2). Notably, there was no significant difference in P_{crit} values between the control fish and warm-acclimated fish for either the 4-week ($t = 1.939$, $p = 0.057$) or 12-week time points ($t = 0.539$, $p = 0.592$), although the 4-week time point was only just outside of statistical significance.

An ANCOVA was used to explore the mechanistic basis for the declining P_{crit} in warm-acclimated red drum, with SMR identified as a significant model covariate ($F_{1,70} = 11.273$, $p = 0.001$) (Fig. 5a). The relationship between SMR and P_{crit} was the same for both treatment temperatures ($F_{1,70} = 0.002$, $p = 0.967$). As such, the decline in P_{crit} during warm-acclimation can be explained by the decline in SMR, which is demonstrated by similarity in variance adjusted mean P_{crit} values across treatment ($F_{1,66} = 1.032$, $p = 0.313$), time ($F_{3,66} = 0.432$, $p = 0.730$) and their interaction ($F_{3,66} = p = 0.250$) (Table 1). A combined linear regression of non-transformed data is provided in Fig. 5b ($p < 0.001$; $R^2 = 0.54$).

3.3. The effect of warming on cardiac mitochondrial efficiency

The mitochondrial performance of ventricle and liver tissue homogenates were assessed in control and warm-acclimated fish following the 12-week acclimation period (Table 2). Note that all tests were performed at the animal's acclimation temperature. In the heart, warm-acclimation had no effect on OXPHOS, LEAK, OXPHOS Capacity (i.e. OXPHOS minus LEAK) or OXPHOS Control Efficiency (i.e. OXPHOS Capacity/OXPHOS). There was also no effect of warming on RCR or CCR. In contrast, warm-acclimation doubled OXPHOS in the liver, although this increase was just outside of significance (one-tailed *t*-test, $p = 0.059$). Warming also significantly reduced the CCR and improved RCR, OXPHOS Capacity and OXPHOS Control Efficiency (one-tailed *t*-

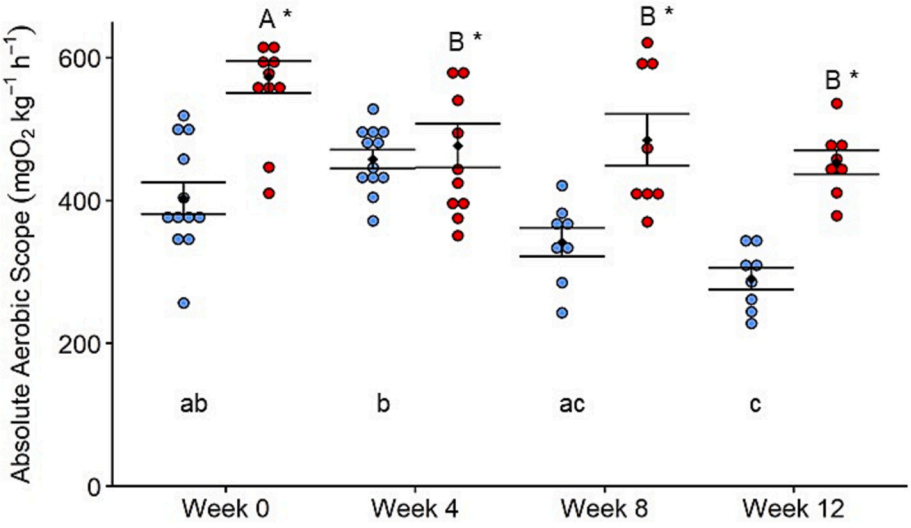


Fig. 3. Effects of temperature and acclimation time on absolute aerobic scope in red drum. Fish were housed at either a control temperature of 20 °C (blue) or 28 °C (red) for 12 weeks. Absolute aerobic scope was measured immediately after transfer (week 0) and every 4 weeks thereafter (20 °C: N = 12, 12, 8 and 8 for weeks 0, 4, 8 and 12, respectively. 28 °C: N = 12, 11, 8 and 8 for weeks 0, 4, 8 and 12, respectively). Different letters denote statistically different groups within a temperature treatment, and asterisks denote a significant difference between temperatures within a time point based on 2-way ANOVA analysis and Holm-Sidak post-hoc testing ($P \leq 0.05$).

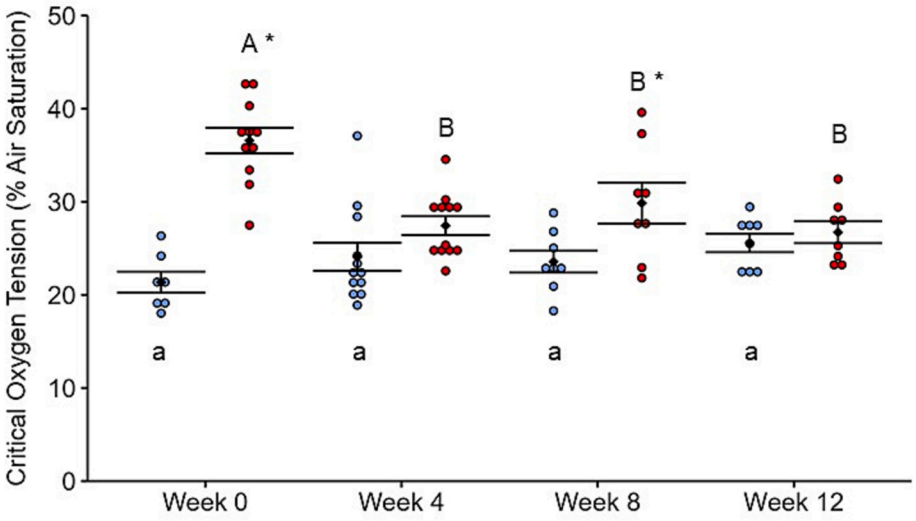


Fig. 4. Effects of temperature and acclimation time on critical oxygen threshold in red drum. Fish were housed at either a control temperature of 20 °C (blue) or 28 °C (red) for 12-weeks. Critical oxygen threshold was measured immediately after transfer (week 0) and every 4 weeks thereafter (20 °C: N = 7, 12, 8 and 8 for weeks 0, 4, 8 and 12, respectively. 28 °C: N = 11, 12, 8 and 8 for weeks 0, 4, 8 and 12, respectively). Different letters denote statistically different groups within a temperature treatment, and asterisks denote a significant difference between temperatures within a time point based on 2-way ANOVA analysis and Holm-Sidak post-hoc testing ($P \leq 0.05$).

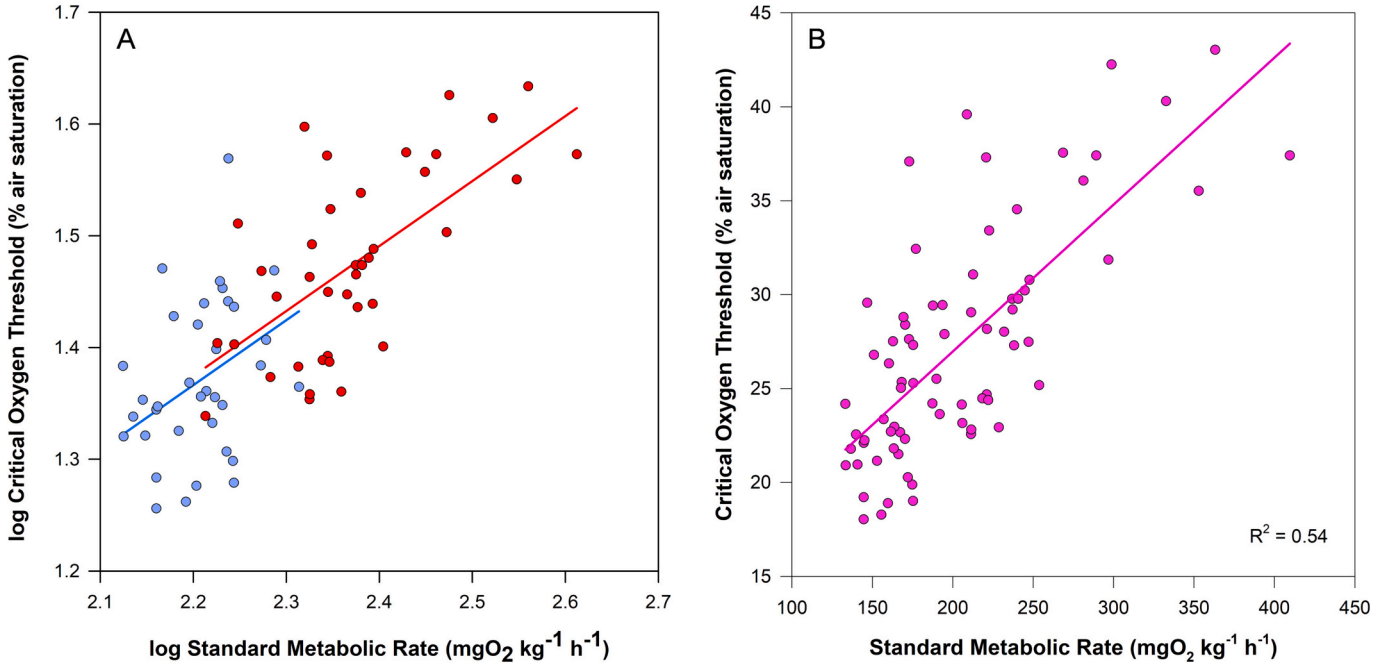


Fig. 5. The relationship between standard metabolic rate and critical oxygen threshold in red drum as an effect of acclimation temperature. Fish were housed at either a control temperature of 20 °C (blue, N = 35) or 28 °C (red, N = 39) for 12-weeks. Note that data were log transformed for statistical analysis (Panel A) and the acclimation treatment compiles across all time points. Both acclimation treatments exhibited statistically similar slopes between standard metabolic rate and critical oxygen threshold (equal slopes test, $P = 0.628$), and critical oxygen threshold was not different between treatments after accounting for standard metabolic rate (ANCOVA, $P = 0.721$). Panel B shows the linear regression of all non-log transformed data from both acclimation treatments ($R^2 = 0.54$, $P < 0.001$, N = 74).

test, $p \leq 0.05$). There was no significant effect of warming on LEAK.

3.4. The effect of warming on growth

Average fish mass in the warm-acclimated and control-acclimated replicate tanks was monitored over the course of the 12-week experiment, which was used to calculate specific growth rates (Table 3). Both acclimations began the experiment with the same average mass (*t*-test, $p = 0.454$). The average mass specific growth rate of the warm-acclimation group was significantly higher than control throughout the duration of the experiment (2-way repeated measures ANOVA, $F_{1,17} = 13.556$, $p = 0.021$), and there was a significant effect of time in both acclimation groups with specific growth rate increasing in both treatments as the experiment progressed ($F_{2,17} = 88.511$, $p < 0.001$). While no interaction between time and treatment was detectable ($F_{2,17} = 2.895$, $p = 0.113$), it is interesting that the mean specific growth rate at 12 weeks was nearly identical between temperature treatments.

Table 1
Standard metabolic rate adjusted[1] critical oxygen thresholds (% air saturation) for warm and control acclimated red drum over a 12-week time course.

	Week 0	Week 4	Week 8	Week 12
20 °C	22.7 ± 1.3	24.0 ± 1.5	23.8 ± 1.2	24.8 ± 0.8
28 °C	25.5 ± 0.9	22.7 ± 0.7	25.2 ± 1.8	24.1 ± 1.3

[1] All values were adjusted to the average control standard metabolic rate of 162 $mgO_2\ kg^{-1}\ h^{-1}$ using the ANCOVA log-log model slopes (0.582), intercepts (20° = 0.0867; 28° = 0.0950) and individual sample residuals. No significant differences were observed between treatments, time points or the interaction of the two (2-way ANOVA; $P > 0.05$).

Table 2
Indices of mitochondrial performance from liver and ventricle tissue homogenates of red drum acclimated to control or warm temperatures for 12 weeks.

	Heart		Liver	
	Control (n = 5)	Warm (n = 8)	Control (n = 6)	Warm (n = 8)
OXPHOS[1]	114.5 ± 11.3	99.7 ± 9.7	10.9 ± 2.2	23.3 ± 5.8
LEAK[1]	23.1 ± 9.2	13.1 ± 3.0	2.8 ± 1.0	2.5 ± 0.4
Respiratory CR	8.8 ± 2.8	11.3 ± 2.9	5.1 ± 1.0	9.1 ± 1.6*
Coupling CR	0.18 ± 0.06	0.15 ± 0.04	0.24 ± 0.05	0.14 ± 0.03*
OXPHOS capacity[1]	91.4 ± 3.3	85.1 ± 10.9	8.0 ± 1.3	20.8 ± 5.5*
OXPHOS efficiency[1]	0.82 ± 0.06	0.85 ± 0.04	0.76 ± 0.05	0.86 ± 0.03*

* Denotes a significant difference between temperatures. One-tailed *t*-test; $P < 0.05$.
[1] pmol O_2/s/mg tissue.

Table 3
Average fish mass per exposure replicate by time point and acclimation group. Values shown are averages ± S.E.M. (N = 3).

Timepoint	Acclimation†	Fish mass (g)	Growth rate (%/day)
Initial	Control	12.0 ± 2.1	–
	Warm	14.0 ± 1.0	–
Week 4[a]	Control	13.2 ± 2.3	0.29 ± 0.10
	Warm	16.8 ± 0.9	0.68 ± 0.05
Week 8[b]	Control	15.6 ± 2.9	0.60 ± 0.05
	Warm	22.5 ± 1.3	1.09 ± 0.08
Week 12[c]	Control	24.2 ± 4.7	1.52 ± 0.06
	Warm	34.9 ± 3.5	1.63 ± 0.16

Letters denotes significantly different main effect groupings with time (repeated measures 2-way ANOVA, Holm-Sidak $P < 0.05$).
† Denotes a significant main effect of temperature ($P = 0.021$).

Table 4
Input parameters for metabolic index-based predictions of habitat availability for control and acclimated fish.

Metabolic trait	Non-acclimated	Acclimated
α_s ($mgO_2\ g^{-1}\ h^{-1}\ kPa^{-1}$)[a]	33.01	33.01
α_D ($mgO_2\ g^{-1}\ h^{-1}$)[a]	164.7	164.7
E_o (eV)	0.4097	0.1115
P_{cmax} (kPa)[a]	16.85	16.85
E_{pcmax} (eV)	0.2598	0.1038

[a] Values calculated from the combined data collected at 20 °C, which serves as the reference point for warming based predictions using both acclimated and non-acclimated thermal sensitivity constants.

3.5. The effect of acclimation on predictions of metabolic habitat availability

The metabolic index model was used to theoretically assess how the observed changes in thermal sensitivity to hypoxia vulnerability as a consequence of acclimation would impact prediction of metabolically available habitat. Contour plots for 20 °C control and 12-week 28 °C acclimated fish are presented in Fig. 6 (also see Fig. S1). If we assume that red drum require a $\phi = 3$ to sustain a population (see Discussion section) then control fish would require an average PO_2 of approximately 15 kPa at the reference temperature, but these fish would require hyperoxia for habitats to be metabolically available above 26.2 °C. In other words, even small declines in PO_2 can constrain metabolically available habitat at higher temperatures. In contrast, when incorporating the much lower thermal sensitivity of hypoxia vulnerability observed following acclimation, the model outputs suggest that red drum are more capable of inhabiting warmer waters. In fact, red drum would, in theory, have the metabolic capacity to maintain populations in excess of 40 °C assuming fully oxygenated water is available.

4. Discussion

The current study sought to expand our understanding of the effects of acclimation on the thermal sensitivities of metabolic traits in marine fish. We specifically sought to explore whether red drum – a marine teleost native to the Gulf of Mexico and surrounding areas – conform to the "plastic floors, concrete ceilings" hypothesis whereby warm acclimation would stimulate a reduction in SMR relative to acutely transferred individuals, with a coinciding increase in AAS. Furthermore, we sought to provide insight on possible mechanisms of warming induced metabolic suppression by exploring differences in mitochondrial performance in the heart and liver of warm-acclimated and control individuals. Interestingly, while red drum did exhibit the anticipated decline in SMR with acclimation time – as well as the hypothesized changes in mitochondrial efficiency in at least one tissue – there was no improvement in AAS because of reductions in MMR over acclimation time. Instead, the metabolic suppression seems to primarily benefit hypoxia vulnerability, which becomes nearly insensitive to warming.

Several studies have previously documented warm-acclimation induced reductions of SMR relative to warmed but unacclimated (i.e. acute transfer) individuals, including studies on European perch (*Perca fluviatilis*) (Sandblom et al., 2016), barramundi (*Lates calcarifer*) (Norin et al., 2014; Scheuffele et al., 2021), shorthorn sculpin (*Myoxocephalus scorpius*) (Sandblom et al., 2014) and the marbled rockcod (*Notothenia rossii*) (Strobel et al., 2012). This was also observed at the upper and lower thermal range of a southern killifish (*Fundulus heteroclitus*) population, although the northern population showed no effects of acclimation to the same temperatures (Healy and Schulte, 2012). Across these same studies the effects of acclimation on MMR are diverse, with the European perch and southern killifish showing no acclimation-induced decline in MMR relative to warmed but unacclimated fish (i.e. concrete ceiling), while sculpin and barramundi both showed significant declines in MMR with acclimation time at warm temperatures. MMR was not measured in the marbled rockcod study. It should be noted that MMR is notoriously challenging to estimate (Killen et al., 2017), as we can never know for certain why a fish ceases to exercise, but if we take all of these data at face value two distinct patterns emerge. In one case, found in the perch and killifish, the warming-induced SMR suppression results in a significant elevation in AAS. In the second case, warm acclimation also causes a decline in MMR after which AAS is comparable in control and warming conditions (Sandblom et al., 2014; Scheuffele et al., 2021). Of course, the exact temperature range used for a given experiment will affect these patterns, as exemplified by additional work in barramundi whereby a 39 °C acclimation resulted in collapse in MMR and AAS despite the reduced SMR (Norin et al., 2014). The red drum generally conforms to the latter pattern as they show a significant decline in SMR over the acclimation time course, but this is paired with a decline in MMR. While red drum exhibited higher the AAS at the conclusion of the acclimation period in the warmer temperature, it is important to note that this is somewhat due to an unexplained decline in control MMR and AAS over the course of the study. If the AAS of 12-

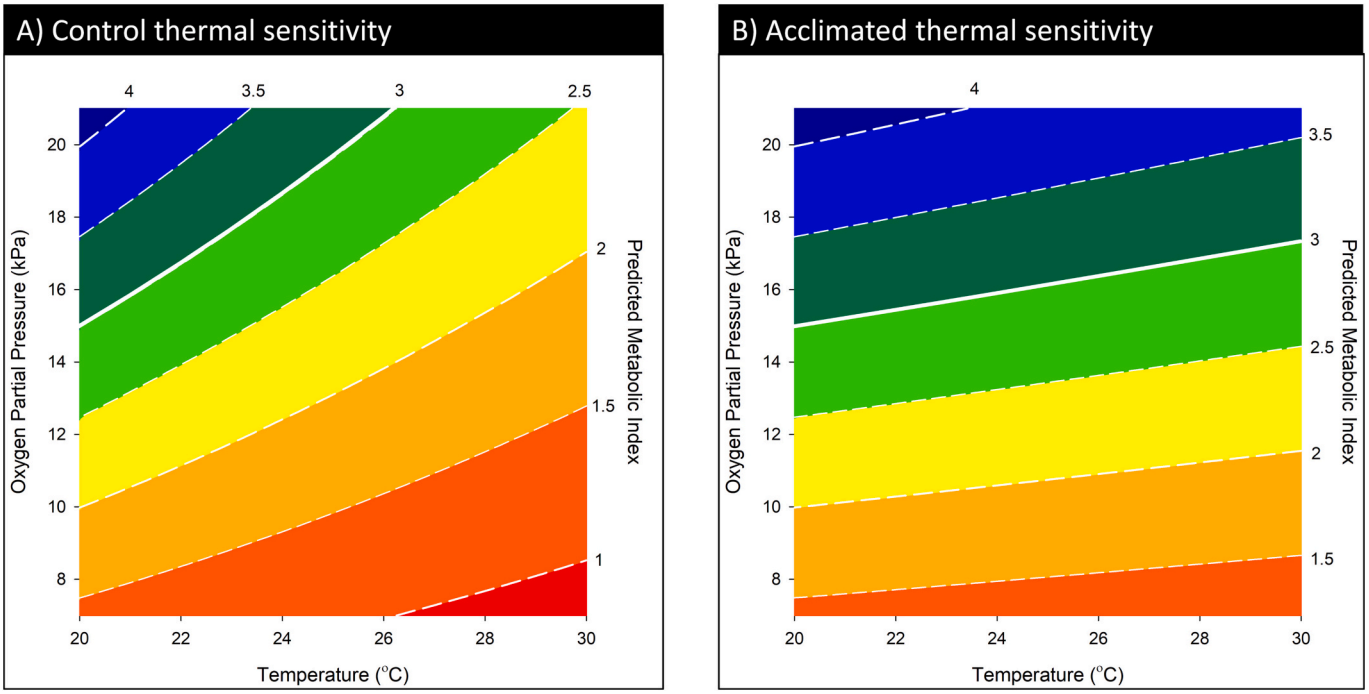


Fig. 6. The effects of acclimation on metabolic index predictions across a range of temperatures and oxygen partial pressures. Panel A shows metabolic index estimates incorporating a thermal sensitivity of hypoxia vulnerability as observed with no acclimation, while Panel B shows estimates of a less thermally sensitive hypoxia vulnerability as calculated at week 12. The solid white line represents a metabolic index of 3, which is presented as a theoretical critical metabolic habitat threshold. All formula values are presented in Table 4.

week warm-acclimated fish is compared to the time 0 control fish, there are no significant differences in AAS (*t*-test, $p = 0.119$); however, it is notable that within the warm-acclimated fish alone the AAS was highest immediately post-warming.

The thermal acclimation of respiration and baseline metabolism are generally framed in the context of AAS or FAS, whereby animals acclimate to offset acute AAS collapses (e.g. Eliason et al., 2013; Eliason and Farrell, 2016) thereby maximizing AAS. This improves the energy available for exercise, growth and digestion. An excellent example of this is the time course for warming in European perch that shows a dramatic increase in SMR that reduces AAS by almost half after 1 week, but after SMR suppression at 8 weeks the AAS has recovered to almost 80 % of control (Sandblom et al., 2016). However, red drum does not conform to this pattern as AAS was actually highest at the earliest tested time point and declined over the course of the acclimation. This is not an altogether uncommon response. For example, work on notothenioid species showed similar declines in AAS following a 4-week 5 °C warming acclimation, albeit in this case the AAS in the control and warmed fish were identical at the end of the acclimation period (Robinson and Davison, 2008). Similarly, barramundi acclimated to 39 °C also significantly reduced AAS so it aligned to that of control conditions (Norin et al., 2014). With this in mind, it is possible that red drum are attempting to maintain AAS at an "ideal" level, though it is hard to hypothesize why a high AAS would be actively reduced considering the competitive ecological benefits it is thought to impart. Instead, we offer an alternative hypothesis: that warming-induced reductions in SMR are not undertaken to maximum AAS in normoxia, but to reduce hypoxia vulnerability (i.e P_{crit}).

The P_{crit} is defined as the oxygen tension below which oxygen supply can no longer meet baseline demand. As such, P_{crit} can be lowered either by improving respiratory performance, or by reducing oxygen demand. For example, when red drum are exposed to prolonged hypoxia, they lower P_{crit} by altering the hemoglobin (Hb)-O_2 affinity through dynamic regulation of particular Hb isoforms (Negrete Jr. et al., 2022; Pan et al., 2017). These respiratory changes also improve hypoxia-constrained MMR (Negrete Jr. et al., 2022) and swimming performance (Dichiera et al., 2022), and are coincident with improvements in red muscle mitochondrial efficiency (Ackerly et al., 2023). Here, red drum take the opposite approach by actively lowering SMR (i.e. oxygen demand), thus returning P_{crit} to control levels by 12 weeks. Importantly, the changes in P_{crit} were solely due to changes in SMR as demonstrated by the consistent covariance of SMR and P_{crit} between temperature treatments, and by the SMR adjusted P_{crit} values provided in Table 1. It might seem curious that hypoxia and warming induce different strategies of plasticity with respect to reducing P_{crit}; however, this is likely due to the fact that hypoxia-induced changes in Hb-O_2 are driven in part by a reduced Root effect and pH sensitivity. The Root effect has been implicated in mediating oxygen supply during periods of exercise (Alderman et al., 2016; Dichiera et al., 2023; Dichiera and Esbaugh, 2020; Dichiera et al., 2021; Harter et al., 2019; Randall et al., 2014; Rummer and Brauner, 2015; Rummer et al., 2013), and as such, reductions in Root effect and Hb-O_2 may be counter-productive in the context of warming.

The mechanisms by which warm-acclimation can reduce SMR have largely focused on cardiac physiology. Acute warming causes a swift increase in heart rate that drives an increase in cardiac output (Farrell, 2002; Farrell et al., 2009), but acclimation has been shown to reduce heart rate at high temperatures to control rates (Sandblom et al., 2016). Here we provide an additional mechanism by which fish can reduce oxygen demand, which is by reducing the amount of respired oxygen that does not lead to ATP production (i.e. LEAK respiration). When the liver mitochondria of 12-week acclimated fish of the two treatments were compared, the warm-acclimated fish had a significantly reduced CCR and a significantly improved RCR. This means that under OXPHOS conditions, the liver mitochondria are generating more ATP per mole of oxygen. This is more significant when considering that the overall OXPHOS and OXPHOS Capacity in the liver are also elevated at higher temperatures, which leads to a significant improvement in OXPHOS Control Efficiency. It is also noteworthy that the observed warming-induced improvements in mitochondrial efficiency occurred in the liver, which has previously been linked to SMR via individual (Salin

et al., 2016b) and population level (Chung et al., 2018) variation. Put simply, the available evidence suggests that fish with higher SMR have higher OXPHOS and LEAK respiration rates in their liver, which has been hypothesized to impact growth efficiency (Salin et al., 2016a; Salin et al., 2019) and behavioral phenotypes (Chung et al., 2018; Le Roy et al., 2021).

Interestingly, no changes in mitochondrial performance were noted in the heart, despite the fact that assays were performed at different temperatures. It is important to note that the general expectations, based solely on the known influence on biochemical reaction rates, was that assay temperature should be sufficient to drive increases in raw mitochondrial traits, such as OXPHOS and LEAK. The fact that there was no difference in traits may indicate that red drum actively reduce mitochondrial density with the intent of maintaining a consistent OXPHOS capacity. This would be in-line with observed suppression of basal heart rate in other species (Sandblom et al., 2016). One important caveat is that assay temperature alone may not have significant effects on OXPHOS in cardiac tissue, as exemplified by the European perch where a 9 °C difference in assay temperature did not cause significant increases in OXPHOS in any treatment — although changes in LEAK respiration were noted (Leo et al., 2017; Pichaud et al., 2019). Nonetheless, the data provided here suggest that mitochondrial plasticity likely contribute to reductions in baseline metabolism following warm-acclimation although further work is needed to extend this conclusion to the heart.

While the experiment described here was not specifically designed to assess growth as a consequence of warming acclimation – an N of 3 replicate tanks is not ideal – the collected data nonetheless provide some interesting insight that should provide fodder for future studies. As expected, the fish in warmer water grew at a significantly faster rate across all time points; however, it is most interesting to note that the growth rate during the final month was generally similar between the two groups. A difference in mass did emerge as the experiment progressed, and it is not uncommon for specific growth rate to decline as fish increase in size (e.g. Khursigara et al., 2021), although that was not observed here as fish grew better over time. Also, if the growth rates of similar size ranges are compared (i.e. 20 °C week-12 vs 28 °C week-8), the warm-acclimated fish showed a lower growth rate. This data may point to a possible cost of acclimation, namely that metabolic suppression might coincide with reduced growth rates. Interestingly, it is now well known that fish size at age declines with warming and is likely to have significant impacts on fish populations and fisheries health in the context of climate change. Current mechanistic explanations, while strongly debated (Lefevre et al., 2018; Pauly, 2021; Pauly and Cheung, 2018a; Pauly and Cheung, 2018b), propose that gill oxygen limitations are the cause for the reduced growth (Cheung et al., 2013); however, the data presented here may suggest that warming induced suppression of oxygen demand may be the cause reduce growth rates independent of oxygen supply capacity or MMR.

The data presented here contribute to a developing literature base exploring the effects of thermal acclimation on metabolic traits in fishes, while also serving to highlight implications for hypoxia vulnerability that have as yet been overlooked. Importantly, metabolic traits are now being used as a mechanistic basis for the development and implementation of species spatial distribution models, such as the metabolic index model (Deutsch et al., 2015; Deutsch et al., 2020; Franco et al., 2022; Parouffe et al., 2023; Seibel and Birk, 2022). The thermal sensitivity of hypoxia vulnerability is central linchpin of the metabolic index model, as when combined with α_s it effectively defines the cumulative effects of temperature and oxygen on MMR. To provide a theoretical examination of the significance of our data to predictions of metabolically available habitat, particularly with respect to the observation that acclimation essentially renders hypoxia vulnerability (i.e. P_{crit}) thermally insensitive, we have performed ϕ modeling across a range of temperatures and PO_2 values using E_o values from acclimated and non-acclimated animals. We have anchored these efforts to a ϕ_{crit} of 3, which is a general average that defines whether a habitat is metabolically available for marine species (Deutsch et al., 2015; Deutsch et al., 2020). The take home message from this analysis is simply that thermally-insensitive hypoxia vulnerability would significantly expand the characteristics of metabolically available habitat for red drum. For example, our analysis explored environments with predominant temperatures between 20 and 30 °C and PO_2 values ranging from 7 kPa to fully saturated. Non-acclimated fish E_o values result in only 17 % of these habitats to be qualified as metabolically available. When using the E_o value from acclimated fish, the metabolically available habitat nearly doubles to 33 %, and this trend would only be exacerbated if higher temperature habitats were added. We stress that this analysis should not be used as a true representation of metabolically available habitats for red drum, as these outputs will change with a species specific ϕ_{crit}, but instead as a demonstration of the significant implications of the thermal acclimation of hypoxia vulnerability.

Overall, the work described here shows that red drum conform to the general pattern of warm-acclimated suppression of SMR; however, this does not appear to be directly related to improving AAS. It is true that any drop in SMR will improve AAS, relative to that same non-suppressed individual, but the simple fact that acclimation did not improve AAS and resulted in a progressive decline in AAS over time may suggest an alternative driving force. We propose that warming-induced suppression of SMR should be viewed in the context of oxygen demand. Acute increases in SMR often respond with a Q_{10} of approximately 2 (2.3 in the current study), which can increase the risk on individuals in two important ways. First is the increased need for resource acquisition (i.e. food) to support a higher metabolic rate. The second is the risk associated with oxygen demand itself, as animals with higher baseline SMR values will – all other things being equal – also have higher P_{crit} and thus increased hypoxia vulnerability. Hypoxia in aquatic environments is a common stressor that is exacerbated with warming, and is expected to increase in prevalence with climate change (Gallo and Levin, 2016; Levin, 2018) and is a major driver that defines the biogeographical range limitations of species (Deutsch et al., 2015; Deutsch et al., 2020; Seibel and Birk, 2022). This work contributes to a growing body of literature that suggests fishes have the capacity to suppress SMR with warming, with the presumed benefit of aiding respiratory performance and expanding metabolically available habitat. However, it is important to note that the trade-offs associated with warming-induced metabolic suppression have yet to be thoroughly explored.

Funding

Funding for this work was provided by a National Science Foundation research grant to AJE (#2002549). Further support for BNJ was provided through a National Science Foundation Graduate Research Fellowship (#1610403).

CRediT authorship contribution statement

Adam D. Zambie: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Kerri Lynn Ackerly:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. **Benjamin Negrete:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Andrew J. Esbaugh:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available in Dryad upon publication.

Acknowledgments

We would like to thank Leigh Walsh for her help with animal husbandry.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171057.

References

Ackerly, K.L., Esbaugh, A.J., 2020. The additive effects of oil exposure and hypoxia on aerobic performance in red drum (*Sciaenops ocellatus*). Sci. Total Environ. 737, 140174.
Ackerly, K.L., Esbaugh, A.J., 2021. The effects of temperature on oil-induced respiratory impairment in red drum (*Sciaenops ocellatus*). Aquat. Toxicol. 233, 105773.
Ackerly, K.L., Negrete Jr., B., Dichiera, A.M., Esbaugh, A.J., 2023. Hypoxia acclimation improves mitochondrial efficiency in the aerobic swimming muscle of red drum (*Sciaenops ocellatus*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 282, 111443.
Alderman, S.L., Harter, T.S., Wilson, J.M., Supuran, C.T., Farrell, A.P., Brauner, C.J., 2016. Evidence for a plasma-accessible carbonic anhydrase in the lumen of salmon heart that may enhance oxygen delivery to the myocardium. J. Exp. Biol. 219, 719–724.
Allmon, E.B., Esbaugh, A.J., 2017. Carbon dioxide induced plasticity of branchial acid-base pathways in an estuarine teleost. Sci. Rep. 7, 45680.
Bell, G., 2013. Evolutionary rescue and the limits of adaptation. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20120080.
Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., et al., 2018. Declining oxygen in the global ocean and coastal waters. Science 359, eaam7240.
Chabot, D., Steffensen, J.F., Farrell, A.P., 2016. The determination of standard metabolic rate in fishes. J. Fish Biol. 88, 81–121.
Cheung, W.W.L., Sarmiento, J.L., Dunne, J., Frölicher, T.L., Lam, V.W.Y., Deng Palomares, M.L., et al., 2013. Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. Nat. Clim. Chang. 3, 254–258.
Chung, D.J., Healy, T.M., McKenzie, J.L., Chicco, A.J., Sparagna, G.C., Schulte, P.M., 2018. Mitochondria, temperature, and the pace of life. Integr. Comp. Biol. 58, 578–590.
Claireaux, G., Chabot, D., 2016. Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. J. Fish Biol. 88, 232–251.
Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782.
Couturier, C.S., Stecyk, J.A., Rummer, J.L., Munday, P.L., Nilsson, G.E., 2013. Species-specific effects of near-future CO_2 on the respiratory performance of two tropical prey fish and their predator. Compar. Biochem. Physiol. A Mol. Integr. Physiol. 166, 482–489.
Crane, D.P., Ogle, D.H., Shoup, D.E., 2020. Use and misuse of a common growth metric: guidance for appropriately calculating and reporting specific growth rate. Rev. Aquac. 12, 1542–1547.
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., Penn, J.L., Seibel, B., 2020. Metabolic trait diversity shapes marine biogeography. Nature 585, 557–562.
Dichiera, A.M., Esbaugh, A.J., 2020. Red blood cell carbonic anhydrase mediates oxygen delivery via the root effect in red drum. J. Exp. Biol. 223.
Dichiera, A.M., Khursigara, A.J., Esbaugh, A.J., 2021. The effects of warming on red blood cell carbonic anhydrase activity and respiratory performance in a marine fish. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 260, 111033.
Dichiera, A.M., Negrete Jr., B., Ackerly, K.L., Esbaugh, A.J., 2022. The role of carbonic anhydrase-mediated tissue oxygen extraction in a marine teleost acclimated to hypoxia. J. Exp. Biol. 225.
Dichiera, A.M., De Anda, V., Gilmour, K.M., Baker, B.J., Esbaugh, A.J., 2023. Functional divergence of teleost carbonic anhydrase 4. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 277, 111368.
Eliason, E.J., Farrell, A.P., 2016. Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. J. Fish Biol. 88, 359–388.
Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., et al., 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332, 109–112.
Eliason, E.J., Clark, T.D., Hinch, S.G., Farrell, A.P., 2013. Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. Conserv. Physiol. 1, cot008.
Ern, R., Norin, T., Gamperl, A.K., Esbaugh, A.J., 2016. Oxygen-dependence of upper thermal limits in fishes. J. Exp. Biol. 219, 3376–3383.
Ern, R., Johansen, J.L., Rummer, J.L., Esbaugh, A.J., 2017. Effects of hypoxia and ocean acidification on the upper thermal niche boundaries of coral reef fishes. Biol. Lett. 13.
Esbaugh, A.J., 2018. Physiological implications of ocean acidification for marine fish: emerging patterns and new insights. J. Comp. Physiol. B 188, 1–13.
Esbaugh, A.J., Cutler, B., 2016. Intestinal Na^+, K^+, $2Cl^-$ cotransporter 2 plays a crucial role in hyperosmotic transitions of a euryhaline teleost. Physiol. Rep. 4.
Esbaugh, A.J., Ern, R., Nordi, W.M., Johnson, A.S., 2016. Respiratory plasticity is insufficient to alleviate blood acid-base disturbances after acclimation to ocean acidification in the estuarine red drum, *Sciaenops ocellatus*. J. Comp. Physiol. B 186, 97–109.
Esbaugh, A.J., Ackerly, K.L., Dichiera, A.M., Negrete Jr., B., 2021. Is hypoxia vulnerability in fishes a by-product of maximum metabolic rate? J. Exp. Biol. 224.
Farrell, A.P., 2002. Cardiorespiratory performance in salmonids during exercise at high temperature: insights into cardiovascular design limitations in fishes. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 132, 797–810.
Farrell, A.P., Eliason, E.J., Sandblom, E., Clark, T.D., 2009. Fish cardiorespiratory physiology in an era of climate change. Can. J. Zool. 87, 835–851.
Farrell, A.P., Mueller, C.A., Seymour, R.S., 2021. Coming up for air. J. Exp. Biol. 224.
Franco, A.C., Kim, H., Frenzel, H., Deutsch, C., Ianson, D., Sumaila, U.R., et al., 2022. Impact of warming and deoxygenation on the habitat distribution of Pacific halibut in the Northeast Pacific. Fish. Oceanogr. 31, 601–614.
Fry, F.E., Hart, J.S., 1948. The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. 94, 66–77.
Fry, F.E.J., 1947. Effects of the environment on animal activity. Ontario Fish. Res. Lab. Publ. Biol. Ser. 55, 1–62.
Fry, F.E.J., 1971. 1 — The effect of environmental factors on the physiology of fish. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. 6. Academic Press, pp. 1–98.
Gallo, N.D., Levin, L.A., 2016. Fish ecology and evolution in the world's oxygen minimum zones and implications of ocean deoxygenation. Adv. Mar. Biol. 74, 117–198.
Gonzalez, A., Ronce, O., Ferriere, R., Hochberg, M.E., 2013. Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20120404.
Harter, T.S., Zanuzzo, F.S., Supuran, C.T., Gamperl, A.K., Brauner, C.J., 2019. Functional support for a novel mechanism that enhances tissue oxygen extraction in a teleost fish. Proc. Biol. Sci. 286, 20190339.
Healy, T.M., Schulte, P.M., 2012. Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (*Fundulus heteroclitus*). Physiol. Biochem. Zool. 85, 107–119.
Johansen, J.L., Esbaugh, A.J., 2017. Sustained impairment of respiratory function and swim performance following acute oil exposure in a coastal marine fish. Aquat. Toxicol. 187, 82–89.
Johansen, J.L., Esbaugh, A.J., 2019. Oil-induced responses of cardiac and red muscle mitochondria in red drum (*Sciaenops ocellatus*). Comp. Biochem. Physiol. C Toxicol. Pharmacol. 219, 35–41.
Jutfelt, F., Norin, T., Åsheim, E.R., Rowsey, L.E., Andreassen, A.H., Morgan, R., et al., 2021. 'Aerobic scope protection' reduces ectotherm growth under warming. Funct. Ecol. 35, 1397–1407.
Khursigara, A.J., Johansen, J.L., Esbaugh, A.J., 2018. Social competition in red drum (*Sciaenops ocellatus*) is influenced by crude oil exposure. Aquat. Toxicol. 203, 194–201.
Khursigara, A.J., Johansen, J.L., Esbaugh, A.J., 2021. The effects of acute crude oil exposure on growth and competition in red drum, *Sciaenops ocellatus*. Sci. Total Environ. 751, 141804.
Killen, S.S., Norin, T., Halsey, L.G., 2017. Do method and species lifestyle affect measures of maximum metabolic rate in fishes? J. Fish Biol. 90, 1037–1046.
Le Roy, A., Mazué, G.P.F., Metcalfe, N.B., Seebacher, F., 2021. Diet and temperature modify the relationship between energy use and ATP production to influence behavior in zebrafish (*Danio rerio*). Ecol. Evol. 11, 9791–9803.
Lefevre, S., 2016. Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction. Conserv. Physiol. 4, cow009.
Lefevre, S., McKenzie, D.J., Nilsson, G.E., 2018. In modelling effects of global warming, invalid assumptions lead to unrealistic projections. Glob. Chang. Biol. 24, 553–556.
Leo, E., Kunz, K.L., Schmidt, M., Storch, D., Pörtner, H.O., Mark, F.C., 2017. Mitochondrial acclimation potential to ocean acidification and warming of polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*). Front. Zool. 14, 21.
Levin, L.A., 2018. Manifestation, drivers, and emergence of open ocean deoxygenation. Ann. Rev. Mar. Sci. 10, 229–260.
Martin, L., Esbaugh, A.J., 2021. Osmoregulatory plasticity during hypersaline acclimation in red drum, *Sciaenops ocellatus*. J. Comp. Physiol. B 191, 731–740.
Martin, L., Negrete Jr., B., Esbaugh, A.J., 2023. The effects of size on exhaustive exercise and recovery in a marine sportfish, the red drum (*Sciaenops ocellatus*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 266, 110844.
Negrete Jr., B., Esbaugh, A.J., 2019. A methodological evaluation of the determination of critical oxygen threshold in an estuarine teleost. Biol. Open 8.
Negrete Jr., B., Ackerly, K.L., Dichiera, A.M., Esbaugh, A.J., 2022. Respiratory plasticity improves aerobic performance in hypoxia in a marine teleost. Sci. Total Environ. 849, 157880.
Norin, T., Malte, H., Clark, T.D., 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. J. Exp. Biol. 217, 244–251.
Pan, Y.K., Ern, R., Esbaugh, A.J., 2016. Hypoxia tolerance decreases with body size in red drum *Sciaenops ocellatus*. J. Fish Biol. 89 (2), 1488–1493.

Pan, Y.K., Ern, R., Morrison, P.R., Brauner, C.J., Esbaugh, A.J., 2017. Acclimation to prolonged hypoxia alters hemoglobin isoform expression and increases hemoglobin oxygen affinity and aerobic performance in a marine fish. Sci. Rep. 7, 7834.
Parouffe, A., Garçon, V., Dewitte, B., Paulmier, A., Montes, I., Parada, C., et al., 2023. Evaluating future climate change exposure of marine habitat in the South East Pacific based on metabolic constraints. Front. Mar. Sci. 9.
Pauly, D., 2021. The gill-oxygen limitation theory (GOLT) and its critics. Sci. Adv. 7, eabc6050.
Pauly, D., Cheung, W.W.L., 2018a. On confusing cause and effect in the oxygen limitation of fish. Glob. Chang. Biol. 24, e743–e744.
Pauly, D., Cheung, W.W.L., 2018b. Sound physiological knowledge and principles in modeling shrinking of fishes under climate change. Glob. Chang. Biol. 24, e15–e26.
Pichaud, N., Ekström, A., Breton, S., Sundström, F., Rowinski, P., Blier, P.U., et al., 2019. Cardiac mitochondrial plasticity and thermal sensitivity in a fish inhabiting an artificially heated ecosystem. Sci. Rep. 9, 17832.
Portner, H.O., 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213, 881–893.
Pörtner, H.O., Farrell, A.P., 2008. Physiology and climate change. Science 322, 690–692.
Prinzing, T.S., Zhang, Y., Wegner, N.C., Dulvy, N.K., 2021. Analytical methods matter too: establishing a framework for estimating maximum metabolic rate for fishes. Ecol. Evol. 11, 9987–10003.
R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Randall, D.J., Rummer, J.L., Wilson, J.M., Wang, S., Brauner, C.J., 2014. A unique mode of tissue oxygenation and the adaptive radiation of teleost fishes. J. Exp. Biol. 217, 1205–1214.
Robinson, E., Davison, W., 2008. The Antarctic notothenioid fish *Pagothenia borchgrevinki* is thermally flexible: acclimation changes oxygen consumption. Polar Biol. 31, 317–326.
Rummer, J.L., Brauner, C.J., 2015. Root effect haemoglobins in fish may greatly enhance general oxygen delivery relative to other vertebrates. PloS One 10, e0139477.
Rummer, J.L., McKenzie, D.J., Innocenti, A., Supuran, C.T., Brauner, C.J., 2013. Root effect hemoglobin may have evolved to enhance general tissue oxygen delivery. Science 340, 1327–1329.
Salin, K., Auer, S.K., Anderson, G.J., Selman, C., Metcalfe, N.B., 2016a. Inadequate food intake at high temperatures is related to depressed mitochondrial respiratory capacity. J. Exp. Biol. 219, 1356–1362.
Salin, K., Auer, S.K., Rudolf, A.M., Anderson, G.J., Selman, C., Metcalfe, N.B., 2016b. Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration. Physiol. Biochem. Zool. 89, 511–523.
Salin, K., Villasevil, E.M., Anderson, G.J., Lamarre, S.G., Melanson, C.A., McCarthy, I., et al., 2019. Differences in mitochondrial efficiency explain individual variation in growth performance. Proc. Biol. Sci. 286, 20191466.
Salvatteci, R., Schneider, R.R., Galbraith, E., Field, D., Blanz, T., Bauersachs, T., et al., 2022. Smaller fish species in a warm and oxygen-poor Humboldt current system. Science 375, 101–104.
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. Proc. Biol. Sci. 281, 20141490.
Sandblom, E., Clark, T.D., Gräns, A., Ekström, A., Brijs, J., Sundström, L.F., et al., 2016. Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nat. Commun. 7, 11447.
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. J. Exp. Biol. 224.
Seibel, B.A., Birk, M.A., 2022. Unique thermal sensitivity imposes a cold-water energetic barrier for vertical migrators. Nat. Clim. Change 11, 1052–1058.
Seibel, B.A., Deutsch, C., 2020. Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature. J. Exp. Biol. 223.
Seibel, B.A., Andres, A., Birk, M.A., Burns, A.L., Shaw, C.T., Timpe, A.W., et al., 2021a. Oxygen supply capacity breathes new life into critical oxygen partial pressure (Pcrit). J. Exp. Biol. 224.
Seibel, B.A., Andres, A., Birk, M.A., Shaw, T., Timpe, A., Welsh, C., 2021b. Response to 'Coming up for air'. J. Exp. Biol. 224.
Strobel, A., Bennecke, S., Leo, E., Mintenbeck, K., Portner, H.O., Mark, F.C., 2012. Metabolic shifts in the Antarctic fish *Notothenia rossii* in response to rising temperature and PCO_2. Front. Zool. 9, 28.
Ultsch, G.R., Regan, M.D., 2019. The utility and determination of Pcrit in fishes. J. Exp. Biol. 222.
Watson, C.J., Nordi, W.M., Esbaugh, A.J., 2014. Osmoregulation and branchial plasticity after acute freshwater transfer in red drum, *Sciaenops ocellatus*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 178, 82–89.
Wood, C.M., 2018. The fallacy of the P_{crit} — are there more useful alternatives? J. Exp. Biol. 221, jeb163717.