The Impact of Acclimation on Standard and Maximum Metabolic Rate in a Small Freshwater Fish

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ABSTRACT

The ability of freshwater fish to acclimate quickly to water temperature variation is imperative when living in shallow changeable environments. However, while it has often been assumed that maximum metabolic rate is constant and therefore that metabolic scope (the difference between maximum and standard metabolic rates) decreases with ambient temperature, this assumption is weakly supported and remains controversial. We investigated acclimation in a temperate, shallow-dwelling Australian freshwater fish, the Pacific blue-eye (*Pseudomugil signifer*), to rising water temperatures. We placed wild-caught fish into three acclimation treatments (24°C, 28°C, and 30°C) and measured metabolic rate at three test temperatures (24°C, 28°C, and 30°C). We found that fish acclimated (recovered standard metabolic rate) to housing temperatures before the first measurement at 10 d. Moreover, we found that regardless of acclimation temperature, standard metabolic rate, maximum metabolic rate, and aerobic scope all increased with test temperature. Our findings suggest that maximum metabolic rate and metabolic scope can adjust rapidly to ambient temperature. More research is needed to understand the generality of these effects, as well as their consequences for fitness.

Keywords: acclimation, thermal compensation, oxygen consumption rate, freshwater fish.

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Introduction

Animals live in dynamic ever-changing environments that fluctuate over space and time. One environmental feature, temperature, plays a particularly large role in many aspects of ectotherm biology, including metabolic rate, reproductive success, sex determination, and performance (Fry 1947; Pauly 1979; Behrens and Lafferty 2007; Ospina-Alvarez and Piferrer 2008; Pankhurst and King 2010). Typically, as environmental and body temperatures increase, performance increases to a peak at the optimum temperature and then decreases sharply as temperatures continue to rise (thermal performance curves; Angilletta 2009). Thus, variability in the thermal environment can impact individual performance and fitness, as well as population persistence. In response to changes in the thermal environment, thermal performance curves may change via physiological acclimation. Physiological acclimation is described as reversible phenotypic plasticity that occurs through exposure to an environment over time periods ranging from hours to months (Angilletta et al. 2002; Angilletta 2009; Schulte et al. 2011). When thermal acclimation occurs, it may provide complete compensation, partial compensation, or overcompensation (Huey and Berrigan 1996; Havird et al. 2020). Often, partial or complete thermal acclimation will result in increased tolerance and performance similar to, or more extreme than, that resulting from the acclimation temperature, which could increase population resilience under climate change scenarios (Sandblom et al. 2014; Seebacher et al. 2014; Colinet et al. 2015). Whether physiology changes at a rate and magnitude that matches environmental change might depend on pace of life and thermal habitat and could therefore vary considerably among species (Angilletta et al. 2002; Seebacher et al. 2015).

The dependence of performance on ambient temperature is thought to be linked to metabolic rate, particularly aerobic scope (Pörtner and Knust 2007). Aerobic scope is the difference between maximum oxygen consumption ($\dot{V}O_2$) and resting $\dot{V}O_2$ (Fry 1947) or, more simply, the capacity for aerobic activity once basic metabolic demands (standard metabolic rate [SMR]; i.e., resting state) are met (Pörtner and Knust 2007). Aerobic scope may follow a thermal performance curve whereby scope declines as environmental temperatures increase from temperatures within the optimal range to extremely warm temperatures, thereby constraining the performance of animals (the oxygen and capacity limited thermal tolerance [OCLTT] hypothesis; Pörtner and Knust 2007; see also Farrell et al. 2009; Pörtner 2010; Sandblom et al. 2016; Pörtner et al. 2017). This decline in scope with increasing

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temperatures could arise if maximum metabolic rate (MMR; i.e., exercise state) remains constant (or declines at stressful temperatures) whereas SMR continues to increase with increasing temperature. Such a pattern has long been assumed, and it is the basis for widely accepted models of metabolic plasticity (Pörtner et al. 2001; Pörtner and Knust 2007). However, some empirical studies have found that MMR can be just as sensitive as SMR to increasing temperatures, often to the point of causing an increase in aerobic scope at extreme high temperatures (Fry 1947; Clark et al. 2011; Ern et al. 2014; Gräns et al. 2014; Norin et al. 2014). Increased knowledge across ecologically and taxonomically diverse fish species could help to interpret interspecific variation and reveal general patterns.

Prior physiological acclimation to warm temperatures can allow recovery (partial or full) of aerobic scope (MMR−SMR), owing to an acclimatory reduction of SMR (table 1). For many fish, recovery of SMR can occur over short time spans of weeks or days. For example, rainbow trout (*Oncorhynchus mykiss*) acclimate to both cooling and warming temperature treatments after only 4 d of exposure (Evans 1990). The ability to acclimate quickly to changes in temperatures may increase environmental resilience, enabling fish to buffer extreme or novel temperatures anticipated under anthropogenic habitat modification or climate change (Seebacher et al. 2010, 2015). Indeed, the rate of anthropogenic temperature change is often rapid, meaning that the speed at which individuals acclimate to temperature fluctuations will become crucial for species persistence (Havird et al. 2020). Despite this, studies investigating the rate of acclimation (taking repeated measures) are relatively rare (table 1).

In addition, much of the research on acclimation has been conducted on select model species, leaving large geographic and phylogenetic areas unstudied (Seebacher et al. 2015). Despite the concern over oceanic species' abilities to acclimate to future climate scenarios, marine species are predicted to be less affected by temperature changes than freshwater species because of the relatively smaller magnitude of temperature change predicted in oceans than in freshwater habitats (Seebacher et al. 2015). However, we know little about how small freshwater fish respond to warming waters. Freshwater environments are isolated and fragmented within a terrestrial landscape, making them highly dependent on rainfall and more susceptible than oceanic environments to larger swings in water temperature (Morrongiello et al. 2011).

We aimed to investigate the acclimation ability of a temperate, shallow-dwelling Australian freshwater fish, the Pacific blue-eye (*Pseudomugil signifer*), to rising water temperatures and to test the prediction that as environmental (i.e., test) temperature increases, SMR would increase and MMR would remain stable (Fry 1947; Pörtner and Knust 2007), thus reducing aerobic scope at higher test temperatures (fig. 1). While this hypothesis is unlikely to capture the complexity and diversity of metabolic responses across fish species, testing predictions from this straightforward hypothesis can nonetheless provide insights that will help develop a more nuanced model. The Pacific blue-eye is a shoaling fish that forms large schools (100–150 fish) in coastal drainage systems on the east coast of Australia (Allen et al. 2002). It is euryhaline (i.e., able to tolerate a wide range of salinity), is found in completely fresh waters as well as estuaries, and has been found in waters as cool as 10°C and as warm as 30°C (ANGFA Aquatic Survey Database 2022). We captured wild Pacific blue-eyes from a freshwater stream, subjected them to one of three acclimation temperature treatments (24°C, 28°C, and 30°C) for either a 10-d duration or a 30-d duration, and then assayed them at three test temperatures. The 24°C treatment represents a normal summer temperature for this population, while the 28°C and 30°C treatments represent warming scenarios while still being within the natural temperature range of this species (ANGFA Aquatic Survey Database 2022; J. Ruszczyk, personal communication). While this species has been surveyed at 30°C, this is a daytime summer temperature and not representative of what this species would experience continuously in their habitat. Based on previous work investigating fish acclimation (table 1), we predicted that thermal compensation would be evident as similar SMRs in fish from all acclimation temperature treatments when tested at the same temperature as their acclimation temperature (fig. 1). This could occur in two ways. First, SMR retains its sensitivity to test temperature, but fish acclimated to a warmer temperature exhibit reduced SMR at any given ambient temperature (i.e., a reduction in the intercept; fig. 1). Second, fish acclimated to a warmer temperature could exhibit reduced sensitivity to temperature, such that the slope between SMR and ambient temperature is steepest for fish acclimated to the coolest temperature treatment (24°C). In addition, we expected the rate of acclimation to take up to 30 d based on evidence that ectotherm acclimation usually occurs within 3–4 wk of a chronic temperature change (Bouchard and Guderley 2003).

Methods

Wild Capture and Husbandry

Fish were captured in groups of 20–22 at four time steps between December 2020 and March 2021 at Deep Creek Reserve, Narrabeen, New South Wales, Australia (−33.70956, 151.27535), a freshwater tributary that flows into a large estuary. A hand net was placed in the water, and frozen pea and prawn puree was thrown into the net as bait until fish entered the net. Fish were then put into plastic fish bags with creek water in groups of six. Fish were transported to an animal facility at the University of New South Wales (Sydney) and randomly assigned to acclimation temperature treatments. Fish were acclimated to the lab aquariums over the course of 2 h by placing fish bags on top of the water in the aquarium and adding a cup of tank water to the fish bag every half hour. Fish were kept in groups of three or four individuals (one male and two or three females) in aquariums (10 L), and water was maintained at the treatment temperature by keeping the tanks inside temperature-controlled rooms. Aquariums included enrichment items (such as driftwood, aquarium ornaments, and spawning substrate made of wool, taking up 25% of aquarium space) as well as a sponge filter to maintain water quality. Fish were fed daily with frozen pea and prawn puree. All tanks were cleaned with a siphon gravel cleaner, and 30% of the

Table 1: Studies that have investigated timescales of standard metabolic rate (SMR) recovery in fish by taking repeated measurements at different time points

Species	Reported size (cm)	Average adult size (cm)	Location	Repeated measures	*N*	Experimental temperatures (°C)	Time for SMR recovery	Reference
Pagothenia borchgrevinki	28	28	Antarctic Ocean	Days 1, 5, 9, 13, 17, 21, 25	16	−1, 4	25 d	Robinson and Davison (2008)
Pagothenia borchgrevinki	28	28	Antarctic Ocean	Days 7, 28, 42, 56	5	−1, 4	42 d	Enzor et al. (2017)
Trematomus bernacchii	35	35	Antarctic Ocean	Days 7, 28, 42, 56	5	−1, 4	56 d	Enzor et al. (2017)
Myoxocephalus scorpius	15–30	24–60	Arctic Ocean	Days 7, 28, 56	12	10, 16	56 d	Sandblom et al. (2014)
Oncorhynchus mykiss	NA	60	Pacific Ocean	Day 5 (hourly)	11	10, 15, 20	Warm and cold acclimation: 5 d	Evans (1990)
Oncorhynchus mykiss	NA	60	Pacific Ocean	Day 5 (hourly)	NA	5, 10, 15	Warm acclimation: 25 h; cold acclimation: 50 h	Schlieper (1950)
Oreochromis niloticus	60	60	African lakes	Day 14 (daily)	10	20, 30, 35	Warm acclimation: 4–5 d; cold acclimation: 7–14 d	Fernandes and Rantin (1986)
Prochilodus scrofa	45	54	South American rivers	Day 15 (daily)	20	15, 25, 35	Warm and cold acclimation: 1–3 d	Barrionuevo and Fernandes (1998)
Hoplias malabaricus	NA	65	Brazilian lakes	Day 7 (continously)	30	15, 20, 25, 30, 35	Warm acclimation: 2–7 d; cool acclimation: 11–13 d	Rantin et al. (1985)
Salmo salar	NA	38	...	Day 12 (hourly)	36	6–30	Warm acclimation: 4 d; cold acclimation: 12 d	Peterson and Anderson (1969)
Trematomus bernacchii (juveniles)	4.1	35	Antarctic Ocean	Days 2, 7, 14, 28	756	−1, 2	28 d	Davis et al. (2018)

Note. Average adult size is included to inform whether fully developed fish were used in each study, as body size and developmental stage can influence oxygen consumption. *N* denotes the overall sample size used in each study. NA = not available.

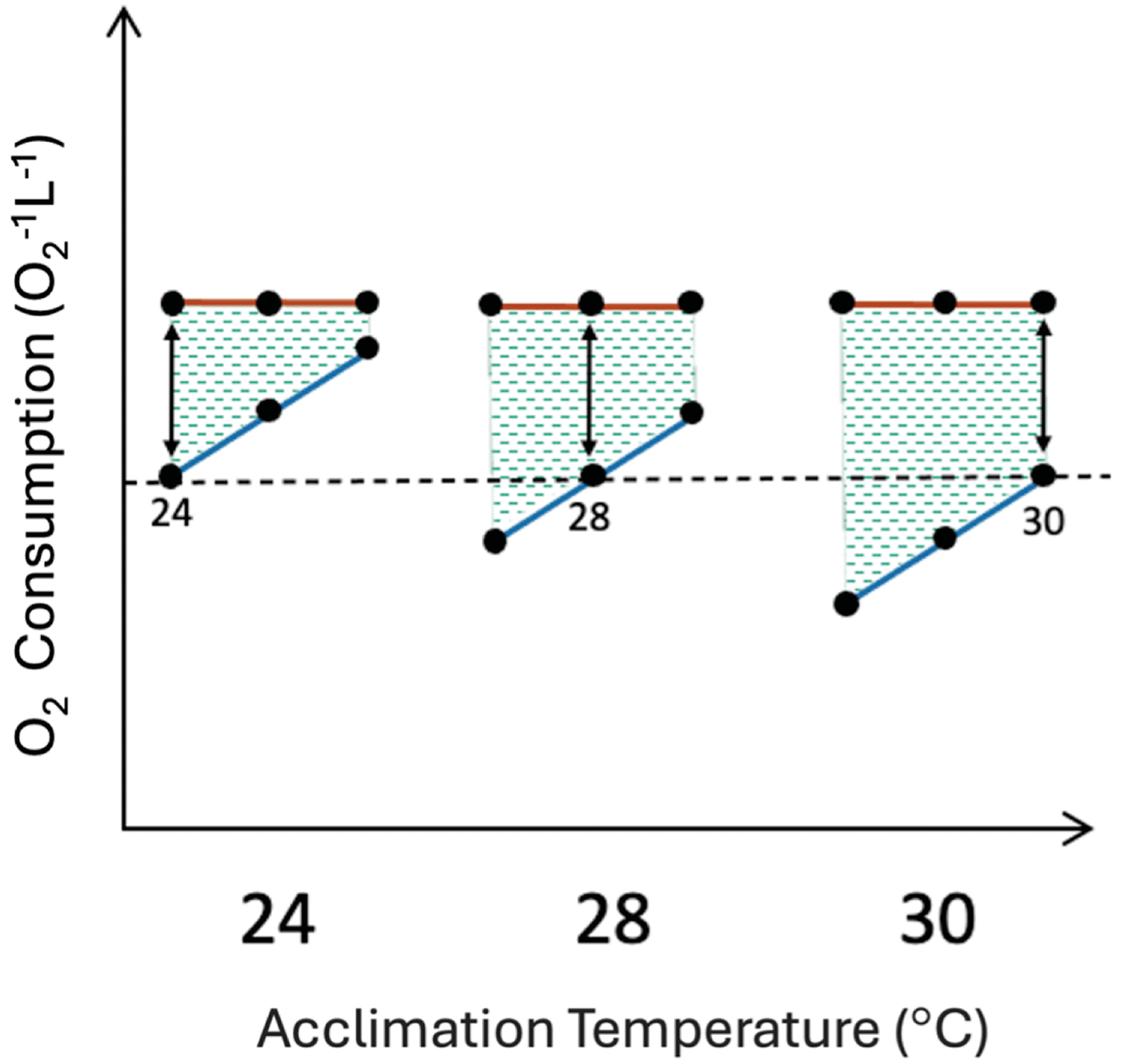

Figure 1. Predicted metabolic responses to test temperatures for fish from each of three acclimation temperatures. Standard metabolic rate is represented by the blue line, maximum metabolic rate is represented by the red line, and aerobic scope is represented by the shaded space between the two. The *x*-axis is acclimation temperature, and the three dots for each acclimation temperature represent three matching test temperatures (continuous scale). The dashed line represents baseline standard metabolic rate (e.g., standard metabolic rate at 24°C when acclimated to 24°C). Here, acclimation occurs as a reduction in the intercept of the standard metabolic rate curve, but it could also arise as a reduction in sensitivity to test temperature (shallower slope). While the relationship of metabolism and temperature is nonlinear over large temperatures, we focus on ecologically relevant temperatures where a linear relationship is a reasonable approximation.

water (salinity: 2 ppt; pH: 8.0; KH: 120 ppm; GH: 400 ppm) was changed once per week.

Experiment

Upon arrival to the lab, fish were placed into one of three temperature acclimation treatments: 24°C, 28°C, or 30°C ($N = 30$ fish per treatment, $N = 9$ aquariums per treatment, $N = 90$ total fish). The experiment was run in six collection batches ($N = 15$ individuals per batch) to allow for measurements to take place within a 3-d time frame after the acclimation period. Fish were acclimated to these treatment conditions for 10 or 30 (± 2) d. At 10 (± 2) d, half the fish were measured for SMR and MMR over a 3-d period using closed respirometry ($N = 15$ fish per each

temperature treatment). Each fish participated in one SMR and one MMR trial at one test temperature per day, for a total of 3 d of testing. Fish were fasted for 24 h before $\dot{V}O_2$ measurements. SMR measurements were carried out first, and MMR measurements were carried out second. SMR and MMR were measured at three test temperatures that matched the acclimation treatments (fig. 2). Test temperature sequence for each batch of fish was chosen randomly from a list composed of each sequence possibility, sampled without replacement across batches.

SMR and MMR were measured with a closed respirometry system. An Oxy-10 mini oxygen meter (PreSens) was used to measure oxygen content (mg/L) in a 100-mL glass respirometry chamber (jar). The Oxy-10 mini uses fiber-optic cables that read the reflectance of a PSt3 oxygen sensor spot (detection limit: 15 ppb; 0%–100% oxygen) glued on the inside of the sealed container with silicon (Kwik-Sil silicone elastomer, World Precision Instruments). To measure SMR, fish were held in the open respirometry chambers (open lid and airline tubing maintaining high

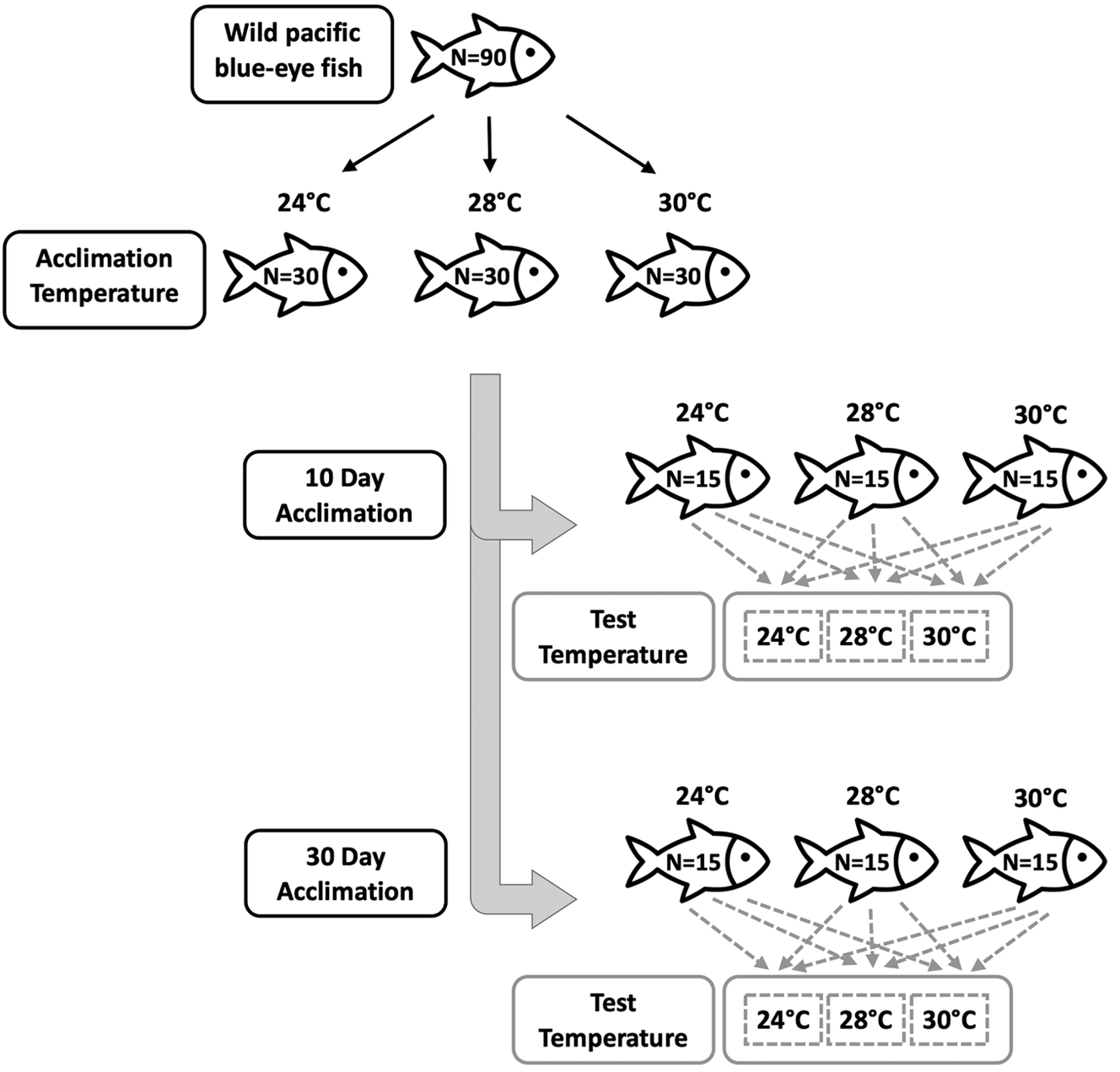

Figure 2. Experimental design. Fish were caught from the wild and then placed into acclimation treatments in the lab ($N = 90$ total fish measured). At 10 d, fish ($N = 15$) from each acclimation treatment in the 10-d group were tested for standard metabolic rate and then maximum metabolic rate at each test temperature. Standard metabolic rate and maximum metabolic rate measurements were also carried out on assay-naive fish ($N = 15$) after 30 d of exposure to the acclimation temperature treatment.

oxygen levels) at the test temperature (24°C, 28°C, or 30°C) for 1 h to adjust to the changed conditions before measurement. Respirometers were then closed, and measurements of water oxygen content (mg/L) were taken every 15 s for 2 h. This SMR sampling regime was chosen after validation trials ($N = 6$ fish) to determine the period of time needed for fish to reach a resting state. These fish were held in aquariums at 24°C and used only in validation trials. For the validation trials, airline tubing was placed inside the chambers to maintain oxygen levels. Respirometers were closed, and $\dot{V}O_2$ (mg/L) was measured over varying time spans (1–3 h). This was repeated across the three temperature treatments (24°C, 28°C, and 30°C; with 24 h of rest between temperatures; Clark et al. 2011; Norin et al. 2014), as time to reach SMR may vary across temperatures. It was determined that approximately 1.5 h were needed for fish to reach a resting state. However, we also determined that the process of closing the respirometry chambers led to elevated $\dot{V}O_2$ regardless of the duration of the initial rest phase. Thus, the final SMR measurement methods involved 1 h of open-chamber acclimation, followed by 2 h of closed-chamber monitoring. Of these 2 h of monitoring, we excluded the first 1 h 40 min of measurements, using only the last 20 min for SMR calculations (see below).

After measurements of SMR were taken, the respirometers were opened, and airline tubing was used to reoxygenate the water. After 30 min of oxygenation, MMR trials began. To measure MMR, fish were moved to a respirometry chamber (100 mL) that contained a stir bar with mesh covering. We used the critical swimming speed protocol (as opposed to the exhaustive chase protocol often more suitable for benthic ambush predators) described in Clark et al. (2013) and Norin and Clark (2016), as it was the most suitable for the Pacific blue-eye, a fast-swimming pelagic fish. The chamber was placed on a magnetic stir plate. Initially, each fish was allowed to swim at a flow rate of ~5 cm/s for 2 min (average swimming speed of Pacific blue-eyes: 10 cm/s; Booth et al. 1985). After 2 min, the flow was increased by ~3 cm/s at 30-s intervals. This continued until the fish was unable to keep up with the current. The speed was then turned down by 3 cm/s to allow the fish to keep up with the current. This speed was considered the fastest swimming speed for the individual fish. Then $\dot{V}O_2$ was recorded for 7–12 min, until there was a steady decline in oxygen concentration. If at any stage the fish could not keep up with the current, the speed was turned down ~3 cm/s until the fish was able to maintain itself in the current. If the fish stopped swimming altogether, it was removed from the respirometry chamber and placed in a small (~10-L) holding tank on its own to recover. At the end of the MMR assay, all fish were weighed to the nearest milligram and then placed in individual tanks (10 L) to ensure individual identification across the three measuring days.

Calculating Metabolic Rates and Aerobic Scope

$\dot{V}O_2$ (mg/L) for SMR was calculated using the slope of the regression of $\dot{V}O_2$ (mg/L) on time (min), which used the $\dot{V}O_2$ data from the last 20 min of $\dot{V}O_2$ measurements recorded during the SMR assay multiplied by the volume of the respirometry chamber (L). This can be expressed by the following equation:

$$\dot{V}O_2 = \text{Ma} \times V \times \beta O_2,$$

where Ma is the rate of change in O_2 saturation, V is the volume of the respirometer, and βO_2 is the oxygen capacitance of air-saturated water at each treatment temperature. These values were obtained for each treatment temperature (24°C: $\dot{V}O_2 = 8.418$ mg/L; 28°C: $\dot{V}O_2 = 7.827$ mg/L; 30°C: $\dot{V}O_2 = 7.558$ mg/L).

$\dot{V}O_2$ for MMR was calculated using the steepest 3-min slope of $\dot{V}O_2$ during the MMR swimming speed assay, multiplied by the volume of the respirometry chamber. All slopes were calculated from the input data using Prism 9 (ver. 9.0.2). Aerobic scope was then calculated as the difference between MMR and SMR.

Statistical Analysis

Using the lme4 (ver. 1.1.27.1; Bates et al. 2014) and lmerTest (ver. 3.1-3; Kuznetsova et al. 2017) packages in R (ver. 4.3.1), we ran separate liner mixed effect models using a restricted maximum likelihood to examine whether aerobic scope, SMR, and MMR (response variables) were significantly affected by the duration of the acclimation period ("acclimation time"), the acclimation temperature treatment, and the test temperature. We additionally included the interactions between acclimation and test temperatures and individual mass as predictor variables. We ran two separate analyses for each response variable. The first analysis tests how acclimation temperature impacts the slope of SMR and MMR to test temperature. For this analysis, test temperature was included as a continuous predictor variable, and acclimation temperature was included as a categorical predictor variable. Including test temperature as a continuous variable enabled comparisons of slopes across acclimation treatments. The second analysis tests how acclimation to different temperatures impacts SMR and MMR at the same test temperature. To do this, both test temperature and acclimation temperature were included as categorical variables. The response variables and mass as linear terms returned fan-shaped residuals, so the response variables and mass were log transformed (natural log) to ensure that residuals were normally distributed. Despite log transforming, there was still a single data point skewing the data; this outlier was removed from the dataset. Fish ID was included as a random effect to account for repeated measures of the same fish across test temperatures. Initially, a nested random effect term was included to account for batches of fish measured; however, we encountered singularity errors across multiple analyses owing to few fish IDs per batch and treatment temperature. Therefore, the batch random effect was excluded from the models. We found that acclimation time did not have a significant effect on any of our response variables. However, we have included separate figures by acclimation time in the appendix (available online). The sequence of test temperatures had no effect on SMR, MMR, or aerobic scope (results not reported), so test sequence order was not included in analyses. The anova function from the lmerTest package was used to determine which moderators from the linear mixed effect models were significant. The

anova function within the lmerTest package corrects denominator degrees of freedom using the Satterthwaite method (Kuznetsova et al. 2017). The emmeans and emtrends functions from the lsmeans package (ver. 2.30-0; Lenth 2018) were used to run Tukey post hoc comparisons of treatment groups for the significant moderators.

Results

Acclimation of the Slope of SMR and MMR across Test Temperatures

For SMR, there was a significant interaction between acclimation and test temperatures (table 2). Tukey post hoc comparisons suggest that the slope for fish acclimated to 24°C (slope = 0.000935) is steeper than the slope for fish acclimated to 28°C (slope = 0.000431; Tukey, $t = 2.20$, $P = 0.074$) and 30°C (slope = 0.000138; Tukey, $t = 3.44$, $P = 0.0021$; fig. 3), but the slope did not differ significantly between the 28°C and 30°C acclimation treatment groups (Tukey, $t = 1.26$, $P = 0.419$). This indicates that acclimation to warm temperatures involves reducing SMR mostly in warm temperatures (reducing the overall slope between SMR and test temperature) rather than lowering SMR in all temperatures (reducing the intercept). However, acclimation temperature also had a significant main effect on SMR, with lower SMR values recorded at a given test temperature as acclimation temperatures increase (emmeans: for 24°C, SMR = 0.000935; for 28°C, SMR = 0.000431; for 30°C, SMR = 0.000138; table 2). Those acclimated to 28°C ($P = 0.0740$) and 30°C ($P = 0.0021$) had a significantly lower SMR on average, at the same test temperature, than those acclimated to 24°C. There was no difference in SMR at the same test temperature between those acclimated to 28°C and those acclimated to 30°C. Unsurprisingly, SMR increased with test temperature and individual mass. The number of days spent in the acclimation treatments was not an important factor for SMR (table 2).

Contrary to the prediction that MMR would remain relatively stable across test temperatures, MMR significantly increased with test temperature (table 2). As a result, aerobic scope also increased with test temperature (table 2). Heavier fish had higher MMR and aerobic scope values (table 2). Neither MMR nor aerobic scope was affected by acclimation temperature or the interaction between acclimation and test temperatures (table 2). The number of days spent in the acclimation treatments was not an important factor for MMR or aerobic scope (table 2).

Acclimation as Thermal Compensation for Changing Temperatures

When treating test temperature as a categorical variable to examine thermal compensation, the results were similar to those from the previous analyses (table 3). There was a significant interaction between test and acclimation temperatures (table 3). Tukey post hoc comparisons show that for fish acclimated to 24°C, there was a significant difference in SMR if tested at 28°C or 30°C compared to at 24°C (fig. 4; table S1, available online). Similarly, for those acclimated to 28°C, there was a significant decrease in SMR when tested at 24°C compared to at 28°C (fig. 4; table S1). SMR did not vary significantly among the groups tested at their matching acclimation temperature (24°C-24°C, 28°C-28°C, 30°C-30°C), indicating that individuals had experienced thermal acclimation (fig. 4; table S1).

Table 2: Two-way ANOVA results for standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope of Pacific blue-eyes maintained across three acclimation treatments

	Estimate (SE)	*F* (df)	*P*
SMR (natural log):			
Acclimation temperature	...	**4.38 (2, 174.7)**	**.014**
Test temperature	**.0009 (.0002)**	**28.8 (1, 168.7)**	**<.001**
Test temperature × acclimation temperature	...	**6.21 (2, 168.9)**	**.003**
Mass (log)	**.0018 (.0009)**	**4.62 (1, 91.4)**	**.034**
Acclimation time	...	.058 (1, 89.8)	.810
MMR (natural log):			
Acclimation temperature	...	.713 (2, 173.9)	.248
Test temperature	**.0031 (.001)**	**20.74 (1, 169.8)**	**<.001**
Test temperature × acclimation temperature	...	.906 (2, 169.7)	.406
Mass (log)	**.015 (.04)**	**11.88 (1, 87.2)**	**<.001**
Acclimation time	...	1.32 (1, 85.2)	.254
Aerobic scope (natural log):			
Acclimation temperature	...	.468 (2, 175.4)	.627
Test temperature	**.0038 (.0018)**	**13.46 (1, 171.5)**	**<.001**
Test temperature × acclimation temperature	...	.555 (2, 171.4)	.575
Mass (log)	**.013 (.004)**	**10.87 (1, 88.1)**	**.0014**
Acclimation time	...	1.53 (1, 86.3)	.219

Note. Acclimation treatment was categorical; test treatment was continuous. Fish ID was included as a random effect. Significant results are shown in bold. $N = 248$ observations, $N = 90$ individuals. df = degrees of freedom.

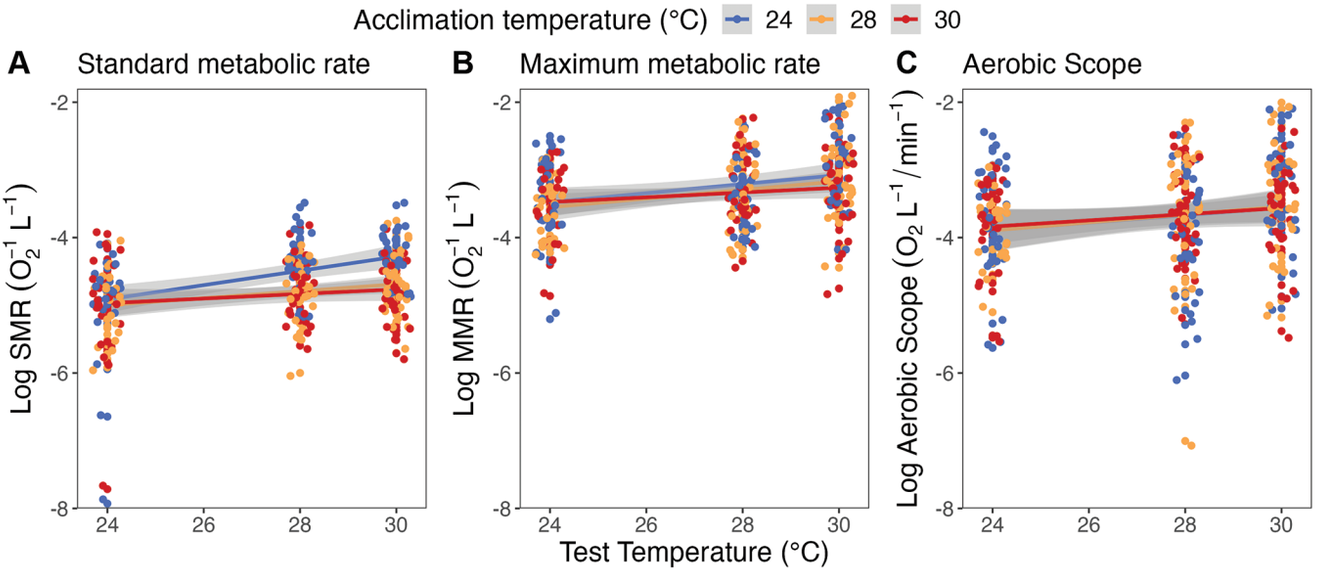

Figure 3. Standard metabolic rate (SMR; *A*), maximum metabolic rate (MMR; *B*), and aerobic scope (measured as oxygen consumption; *C*) across three test temperatures.

SMR varied with the main effect of acclimation treatment (table 3), being higher overall in 24°C-acclimated fish (emmeans, SMR = 0.0119) than in 28°C-acclimated fish (emmeans, SMR = 0.009; $P = 0.001$) and 30°C-acclimated fish (emmeans, SMR = 0.00885; $P = 0.0006$) but not significantly different between 28°C- and 30°C-acclimated fish ($P = 0.981$). SMR also varied with the main effect of test temperature, being lower overall when tested at 24°C (emmeans, SMR = 0.0081) than at 28°C (emmeans, SMR = 0.0108; $P < 0.0001$) and 30°C (emmeans, SMR = 0.0109; $P < 0.0001$); no differences were found between 28°C- and 30°C-tested fish ($P = 0.988$). Test temperature retained its significant influence on MMR and aerobic scope (table 3). MMR was higher at 30°C test temperatures (emmeans, MMR = 0.0516) than at 24°C (emmeans, MMR = 0.0355;

Table 3: Two-way ANOVA results for standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope of Pacific blue-eyes maintained across three acclimation treatments

	Estimate (SE)	*F* (df)	*P*
SMR (natural log):			
Acclimation temperature	...	**10.18 (2, 89.9)**	**<.001**
Test temperature	...	**16.28 (2, 166.2)**	**<.001**
Test × acclimation temperature	...	**3.53 (4, 166.3)**	**.0086**
Mass (log)	**.0019 (.0009)**	**4.88 (1, 91.8)**	**.0297**
Acclimation time	...	.0341 (1, 90.2)	.781
MMR (natural log):			
Acclimation temperature	...	1.90 (2, 84.8)	.155
Test temperature	...	**11.58 (2, 163.2)**	**<.001**
Test × acclimation temperature	...	1.40 (4, 163.3)	.236
Mass (log)	**.0143 (.009)**	**11.42 (1, 86.8)**	**.0011**
Acclimation time	...	1.36 (1, 85.2)	.246
Aerobic scope (natural log):			
Acclimation temperature	...	.742 (2, 85.9)	.479
Test temperature	...	**8.468 (2, 164.8)**	**<.001**
Test × acclimation temperature	...	1.423 (4, 164.9)	.229
Mass (log)	**.0124 (.004)**	**9.34 (1, 87.9)**	**.003**
Acclimation time	...	1.423 (1, 86.4)	.208

Note. Acclimation treatment was categorical; test treatment was continuous. Fish ID was included as a random effect. Significant results are shown in bold. $N = 248$ observations, $N = 90$ individuals. df = degrees of freedom.

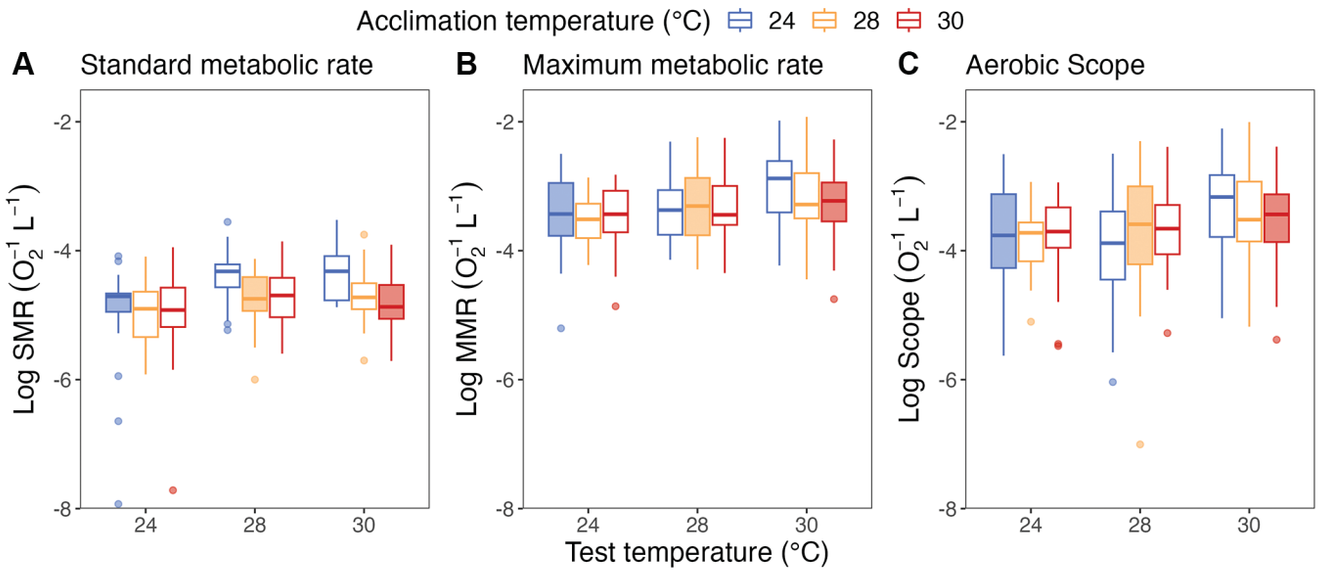

Figure 4. Standard metabolic rate (SMR; *A*), maximum metabolic rate (MMR; *B*), and aerobic scope (measured as oxygen consumption; *C*) across three test temperatures. Filled boxes represent the treatments where acclimation and test temperatures matched in order to show the effect of thermal compensation.

$P < 0.0001$) and 28°C (emmeans, MMR = 0.042; $P = 0.01$) test temperatures; no differences were found between test temperatures of 24°C and 28°C. Similarly, aerobic scope was higher at 30°C test temperatures (emmeans, aerobic scope = 0.041) than at 28°C (emmeans, aerobic scope = 0.031; $P = 0.011$) and 24°C (em-means, aerobic scope = 0.0274; $P = 0.0004$) test temperatures; no differences were found between test temperatures of 24°C and 28°C.

Discussion

We investigated the acclimation ability of a temperate Australian freshwater fish, the Pacific blue-eye (*Pseudomugil signifer*), to rising water temperatures. Within 10 d of exposure to a water temperature treatment, fish showed complete thermal compensation, exhibiting similar SMRs when each was tested at their acclimation temperature. This was accomplished not by lowering SMR overall but by reducing thermal sensitivity to temperature; in particular, the slope of SMR with test temperature was shallower in the warm-acclimated treatments (28°C and 30°C) than in the 24°C treatment.

In contrast, MMR and aerobic scope were not significantly affected by acclimation temperature or the interaction between acclimation and test temperatures. These results add support for the "plastic floors and concrete ceilings" hypothesis, showing that while SMR can be influenced by periods of warm acclimation, MMR is not (Sandblom et al. 2016). In this study, MMR increased with warmer test temperatures to such an extent that aerobic scope also increased (broadened) at warmer test temperatures regardless of acclimation temperature. Thus, contrary to predictions, increases in SMR at warmer test temperatures did not lead to a narrowing of aerobic scope as SMR approached the MMR limit, even when considering only the cool-acclimated treatment (24°C). In an ecological context, this suggests that the heightened thermal sensitivity of cool-acclimated fish has energetic costs but not performance-related costs when confronted with an acute rise in temperature, such as a heat wave.

The OCLTT hypothesis predicts that aerobic scope will narrow as ambient temperature increases because of SMR approaching a fixed MMR (Pörtner and Knust 2007). Studies using model species such as eelpout (*Zoarces viviparus*), Atlantic cod (*Gadus morhua*), and spider crab (*Maja squinado*) support this hypothesis (Pörtner et al. 2001; Mark et al. 2002; Pörtner and Knust 2007), finding that MMR declines with increasingly extreme temperatures, causing an overall decline in aerobic scope. Our contrasting results of increasing MMR and aerobic scope with test temperature mirror those results found in other studies in fish (Fry 1947; Claireaux et al. 2000; Clark et al. 2005, 2011; Eliason et al. 2013; Ern et al. 2014; Gräns et al. 2014; Norin et al. 2014; Raby et al. 2016; Lapointe et al. 2018). For example, in juvenile barramundi (*Lates calcarifer*) acclimated for 5 wk, both SMR and MMR increased with ambient temperature, but as in the present study, the increase was greater for MMR than for SMR, and aerobic scope was broadest at the hottest temperature (38°C; Norin et al. 2014). Similarly, in brown bullhead (*Ameiurus nebulosus*), aerobic scope increased up to the upper incipient lethal temperature of ~37°C (Fry 1947), and in pink salmon (*Oncorhynchus gorbuscha*), maximum aerobic scope was reached at the hottest temperature experienced by the species at any point in its life cycle (21°C; Clark et al. 2011). A possible explanation for the varying results is that aerobic scope (i.e., excess metabolic capacity) is used for different energetic demands across species (Clark et al. 2013). For example, an active pelagic fish like the Pacific blue-eye may prioritize aerobic scope for locomotion and foraging, as it is continuously moving and eating small prey

items. By contrast, a benthic ambush predator, like the southern catfish (*Silurus meridionalis*), might prioritize its energy budget for digesting big prey items while remaining relatively still (Fu et al. 2009).

The results from this study, along with the others mentioned above, suggest that peak aerobic scope (capacity to supply oxygen to tissues) is often aligned with the top end of ecologically relevant temperatures (Fry 1947; Ern et al. 2014; Norin et al. 2014; Raby et al. 2016), raising the question as to why animals do not live at hotter temperatures. Likely, the simplest explanation is that there are many negative impacts of living at hot temperatures for long periods of time. For example, studies show that growth rate may be reduced at high temperatures compared to at cooler temperatures (Ern et al. 2014; Gräns et al. 2014; Enzor et al. 2017). Additionally, extended exposure to temperatures in the higher thermal range has been shown to reduce lifespan, which could potentially reduce net reproductive output (Dembski et al. 2006). This could be a result of the increased threat of oxidative stress from reactive oxygen species causing damage to membranes and proteins, which has been shown to increase as individuals reach their thermal maximum (Abele et al. 2002; Seebacher et al. 2010; Blier et al. 2014; Schulte 2015). Furthermore, animals commonly exhibit stress responses as temperatures near their thermal maximum and generally have a preference to live in lower temperatures (González et al. 2010; Clark et al. 2011; García-Guerrero et al. 2022). Thus, while some fish may have increased capacity to supply oxygen to tissues at high temperatures for short-term benefit (i.e., escaping predators), this may not equate to higher fitness over a long exposure because of the costs of living in hot temperatures long term. While aerobic scope is one useful measure of physiological performance, it needs to be measured along with other indicators of performance at ecologically relevant temperatures to gain a complete picture and predict which temperatures species can persist in under future climate scenarios.

In our study, we found that Pacific blue-eyes acclimated SMR to treatment temperatures before the first measurement at 10 d. The length of time it takes individuals to acclimate to the point of recovering original SMR has received little attention in the literature. Studies investigating the length of acclimation time required to recover SMR find highly variable timescales, ranging from a few days to 14 wk depending on species across different habitats (Arctic/Antarctic, temperate, and tropical; table 1). Additionally, we found that there was no significant difference in SMR, MMR, or aerobic scope between the 10- and 30-d acclimation times. The results suggest that Pacific blue-eyes can acclimate rapidly in comparison with most other fish species that have been examined (table 1). This may be a result of living in shallow environments that fluctuate in temperature often. Clearly, more research is needed comparing fish that live in shallow thermally fluctuating environments with those living at deeper or thermally stable environments. A timescale of 3 wk is often assumed to be a reasonable acclimation period for ectothermic animals, but it may be useful for future research on small eurythermal fish to investigate shorter acclimation experiment timescales, particularly if speed of acclimation is a focus of the study (Bouchard and Guderley 2003; Dupont et al. 2023; Einum et al. 2023).

Understanding variation in the speed of acclimation could provide insight into evolutionary adaptation and predicting species-specific responses to environmental change. Evolutionary theory predicts that animals experiencing greater within-generation variability in temperature should also have greater capacity for thermal acclimation (Gabriel et al. 2005). However, a meta-analysis investigating thermal acclimation across animals found that species from thermally stable environments were better able to acclimate and buffer against temperature changes than species from thermally variable environments (Seebacher et al. 2015). Although acclimation to warming temperatures has been well studied, much of this research is conducted on model species, leaving many species and large geographical areas unstudied (Seebacher et al. 2015). Thus, while some clear patterns of acclimation ability have emerged for a small number of species, it is becoming clear that these findings cannot be extrapolated across species. Future research should investigate why different fish have different metabolic responses to temperature and why some fish respond in ways that challenge the assumptions of the OCLTT hypothesis. Life history, or pace of life syndromes, is likely important in shaping metabolic responses to temperature (Dammhahn et al. 2018). For example, having a broad aerobic scope at high temperatures would be advantageous to a pelagic fish that has to continually move and feed, whereas this would not benefit a benthic fish that spends more time stationary and feeds only every few days (Clark et al. 2013). The way to move forward in the field of fish metabolism is to look at fish that are phylogenetically similar but live in different ecological niches with different paces of life.

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Literature Cited

Abele D., K. Heise, H.-O. Pörtner, and S. Puntarulo. 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J Exp Biol 205:1831–1841. https://doi.org/10.1242/jeb.205.13.1831.

Allen G.R., S.H. Midgley, and M. Allen. 2002. Field guide to the freshwater fishes of Australia. Western Australian Museum, Perth.

ANGFA Aquatic Survey Database. 2022. ANGFA Aquatic Survey Database. https://db.angfa.org.au/display.php?tbl=fish&id=1.

Angilletta M.J., Jr. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford.

Angilletta M.J., Jr., P.H. Niewiarowski, and C.A. Navas. 2002. The evolution of thermal physiology in ectotherms. J Therm Biol 27:249–268.

Barrionuevo W.R. and M.N. Fernandes. 1998. Time-course of respiratory metabolic adjustments of a South American fish, *Prochilodus scrofa*, exposed to low and high temperatures. J Appl Ichthyol 14:37–41. https://doi.org/10.1111/j.1439-0426.1998.tb00611.x.

Bates D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48.

Behrens M.D. and K.D. Lafferty. 2007. Temperature and diet effects on omnivorous fish performance: implications for the latitudinal diversity gradient in herbivorous fishes. Can J Fish Aquat Sci 64:867–873.

Blier P.U., H. Lemieux, and N. Pichaud. 2014. Holding our breath in our modern world: will mitochondria keep the pace with climate changes? Can J Zool 92:591–601.

Booth D.J., G.H. Pyke, and W.J.R. Lanzing. 1985. Prey detection by the blue-eye *Pseudomugil signifer* Kner (Atherinidae): analysis of field behaviour by controlled laboratory experiments. Mar Freshw Res 36:691–699. https://doi.org/10.1071/mf9850691.

Bouchard P. and H. Guderley. 2003. Time course of the response of mitochondria from oxidative muscle during thermal acclimation of rainbow trout, *Oncorhynchus mykiss*. J Exp Biol 206:3455–3465. https://doi.org/10.1242/jeb.00578.

Claireaux G., D.M. Webber, J.-P. Lagardère, and S.R. Kerr. 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). J Sea Res 44:257–265. https://doi.org/10.1016/S1385-1101(00)00053-8.

Clark T.D., K.M. Jeffries, S.G. Hinch, and A.P. Farrell. 2011. Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. J Exp Biol 214:3074–3081. https://doi.org/10.1242/jeb.060517.

Clark T.D., T. Ryan, B.A. Ingram, A.J. Woakes, P.J. Butler, and P.B. Frappell. 2005. Factorial aerobic scope is independent of temperature and primarily modulated by heart rate in exercising Murray cod (*Maccullochella peelii peelii*). Physiol Biochem Zool 78:347–355. https://doi.org/10.1086/430034.

Clark T.D., E. Sandblom, and F. Jutfelt. 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J Exp Biol 216:2771–2782. https://doi.org/10.1242/jeb.084251.

Colinet H., B.J. Sinclair, P. Vernon, and D. Renault. 2015. Insects in fluctuating thermal environments. Annu Rev Entomol 60:123–140. https://doi.org/10.1146/annurev-ento-010814-021017.

Dammhahn M., N.J. Dingemanse, P.T. Niemelä, and D. Réale. 2018. Pace-of-life syndromes: a framework for the adaptive integration of behaviour, physiology and life history. Behav Ecol Sociobiol 72:62.

Davis B.E., E.E. Flynn, N.A. Miller, F.A. Nelson, N.A. Fangue, and A.E. Todgham. 2018. Antarctic emerald rockcod have the capacity to compensate for warming when uncoupled from CO_2-acidification. Glob Change Biol 24:e655–e670. https://doi.org/10.1111/gcb.13987.

Dembski S., G. Masson, D. Monnier, P. Wagner, and J.C. Pihan. 2006. Consequences of elevated temperatures on life-history traits of an introduced fish, pumpkinseed *Lepomis gibbosus*. J Fish Biol 69:331–346. https://doi.org/10.1111/j.1095-8649.2006.01087.x.

Dupont L., M. Thierry, L. Zinger, D. Legrand, and S. Jacob. 2023. Beyond reaction norms: the temporal dynamics of phenotypic plasticity. Trends Ecol Evol 39:41–51.

Einum S. and T. Burton. 2023. Divergence in rates of phenotypic plasticity among ectotherms. Ecol Lett 26:147–156.

Eliason E.J., S.M. Wilson, A.P. Farrell, S.J. Cooke, and S.G. Hinch. 2013. Low cardiac and aerobic scope in a coastal population of sockeye salmon *Oncorhynchus nerka* with a short upriver migration. J Fish Biol 82:2104–2112. https://doi.org/10.1111/jfb.12120.

Enzor L.A., E.M. Hunter, and S.P. Place. 2017. The effects of elevated temperature and ocean acidification on the metabolic pathways of notothenioid fish. Conserv Physiol 5:cox019.

Ern R., D.T.T. Huong, N.T. Phuong, T. Wang, and M. Bayley. 2014. Oxygen delivery does not limit thermal tolerance in a tropical eurythermal crustacean. J Exp Biol 217:809–814. https://doi.org/10.1242/jeb.094169.

Evans D.O. 1990. Metabolic thermal compensation by rainbow trout: effects on standard metabolic rate and potential usable power. Trans Am Fish Soc 119:585–600.

Farrell A.P., E.J. Eliason, E. Sandblom, and T.D. Clark. 2009. Fish cardiorespiratory physiology in an era of climate change. Can J Zool 87:835–851. https://doi.org/10.1139/Z09-092.

Fernandes M. and F. Rantin. 1986. Thermal acclimation of teleost *Oreochromis niloticus* (Pisces, Ciclidae). Rev Hydrobiol Trop 19:163–168.

Fry F.E.J. 1947. Effects of the environment on animal activity. Biological Series 55. Publication of the Ontario Fisheries Laboratory 68. University of Toronto Press, Toronto.

Fu S.-J., L.-Q. Zeng, X.-M. Li, X. Pang, Z.-D. Cao, J.-L. Peng, and Y.-X. Wang. 2009. The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. J Exp Biol 212:2296–2302. https://doi.org/10.1242/jeb.027102.

Gabriel W., B. Luttbeg, A. Sih, and R. Tollrian. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. Am Nat 166:339–353.

García-Guerrero M., N. Avilés-Espinoza, G. Lizarraga-Sanchez, G. Herrera-Rodríguez, D. Valdez-Martínez, P. Hernández-Sandoval, M. García-Guerrero, et al. 2022. Maximum critical temperature and oxygen consumption during thermoregulation in *Macrobrachium americanum* (Bate, 1868) adult prawns. Lat Am J Aquat Res 50:301–309. https://doi.org/10.3856/vol50-issue2-fulltext-2824.

González R.A., F. Díaz, A. Licea, A. Denisse Re, L. Noemí Sánchez, and Z. García-Esquivel. 2010. Thermal preference, tolerance and oxygen consumption of adult white shrimp *Litopenaeus vannamei* (Boone) exposed to different acclimation temperatures. J Therm Biol 35:218–224. https://doi.org/10.1016/j.jtherbio.2010.05.004.

Gräns A., F. Jutfelt, E. Sandblom, E. Jönsson, K. Wiklander, H. Seth, C. Olsson, et al. 2014. Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. J Exp Biol 217:711–717. https://doi.org/10.1242/jeb.096743.

Havird J.C., J.L. Neuwald, A.A. Shah, A. Mauro, C.A. Marshall, and C.K. Ghalambor. 2020. Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q_{10} effects: why methodology matters. Funct Ecol 34:1015–1028.

Huey R.B., D. Berrigan, G.W. Gilchrist, and J.C. Herron. 1996. Testing the adaptive significance of acclimation: a strong inference approach. Am Zool 39:323–336.

Kuznetsova A., P.B. Brockhoff, and R.H. Christensen. 2017. lmerTest package: tests in linear mixed effects models. J Stat Softw 82:1–26.

Lapointe D., M.S. Cooperman, L.J. Chapman, T.D. Clark, A.L. Val, M.S. Ferreira, J.S. Balirwa, D. Mbabazi, M. Mwanja, and L. Chhom. 2018. Predicted impacts of climate warming on aerobic performance and upper thermal tolerance of six tropical freshwater fishes spanning three continents. Conserv Physiol 6:coy056.

Lenth R. 2018. Package "lsmeans." https://cran.r-project.org/web/packages/lsmeans/lsmeans.pdf.

Mark F.C., C. Bock, and H.-O. Pörtner. 2002. Oxygen-limited thermal tolerance in Antarctic fish investigated by MRI and ^{31}P-MRS. Am J Physiol 283:R1254–R1262. https://doi.org/10.1152/ajpregu.00167.2002.

Morrongiello J.R., S.J. Beatty, J.C. Bennett, D.A. Crook, D.N.E.N. Ikedife, M.J. Kennard, A. Kerezsy, et al. 2011. Climate change and its implications for Australia's freshwater fish. Mar Freshw Res 62:1082. https://doi.org/10.1071/MF10308.

Norin T. and T.D. Clark. 2016. Measurement and relevance of maximum metabolic rate in fishes. J Fish Biol 88:122–151.

Norin T., H. Malte, and T.D. Clark. 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. J Exp Biol 217:244–251. https://doi.org/10.1242/jeb.089755.

Ospina-Alvarez N. and F. Piferrer. 2008. Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. PLoS ONE 3:e2837.

Pankhurst N.W. and H.R. King. 2010. Temperature and salmonid reproduction: implications for aquaculture. J Fish Biol 76:69–85.

Pauly D. 1979. Gill size and temperature as governing factors in fish growth: a generalization of von Bertalanffy's growth formula. Berichte aus dem Institut für Meereskunde an der Christian-Albrechts-Universität Kiel 63. Institute of Oceanography, University of Kiel, Kiel, Germany.

Peterson R.H. and J.M. Anderson. 1969. Influence of temperature change on spontaneous locomotor activity and oxygen consumption of Atlantic salmon, *Salmo salar*, acclimated to two temperatures. J Fish Res Board Can 26:93–109.

Pörtner H.-O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J Exp Biol 213:881–893. https://doi.org/10.1242/jeb.037523.

Pörtner H.-O., B. Berdal, R. Blust, O. Brix, A. Colosimo, B. De Wachter, A. Giuliani, et al. 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). Cont Shelf Res 21:1975–1997. https://doi.org/10.1016/S0278-4343(01)00038-3.

Pörtner H.-O., C. Bock, and F.C. Mark. 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J Exp Biol 220:2685–2696.

Pörtner H.-O. and R. Knust. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95–97.

Raby G.D., M.T. Casselman, S.J. Cooke, S.G. Hinch, A.P. Farrell, and T.D. Clark. 2016. Aerobic scope increases throughout an ecologically relevant temperature range in coho salmon. J Exp Biol 219:1922–1931.

Rantin F.T., M.N. Fernandes, M.C.H. Furegato, and J.R. Sanches. 1985. Thermal acclimation in the teleost *Hoplias malabaricus* (Pisces-Erythrinidae). Bol Fisiol Anim 9:103–109.

Robinson E. and W. Davison. 2008. The Antarctic notothenioid fish *Pagothenia borchgrevinki* is thermally flexible: acclimation changes oxygen consumption. Polar Biol 31:317–326. https://doi.org/10.1007/s00300-007-0361-4.

Sandblom E., T.D. Clark, A. Gräns, A. Ekström, J. Brijs, L.F. Sundström, A. Odelström, A. Adill, T. Aho, and F. Jutfelt. 2016. Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. Nat Commun 7:11447. https://doi.org/10.1038/ncomms11447.

Sandblom E., A. Gräns, M. Axelsson, and H. Seth. 2014. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. Proc R Soc B 281:20141490. https://doi.org/10.1098/rspb.2014.1490.

Schlieper C. 1950. Temperaturbezogene Regulationen des Grundumsatzes bei wechselwarmen Tieren. Biol Zentralbl 69:216–226.

Schulte P.M. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. J Exp Biol 218:1856–1866. https://doi.org/10.1242/jeb.118851.

Schulte P.M., T.M. Healy, and N.A. Fangue. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr Comp Biol 51:691–702.

Seebacher F., J. Beaman, and A.G. Little. 2014. Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. Funct Ecol 28:137–148. https://doi.org/10.1111/1365-2435.12156.

Seebacher F., M.D. Brand, P.L. Else, H. Guderley, A.J. Hulbert, and C.D. Moyes. 2010. Plasticity of oxidative metabolism in variable climates: molecular mechanisms. Physiol Biochem Zool 83:721–732. https://doi.org/10.1086/649964.

Seebacher F., C.R. White, and C.E. Franklin. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nat Clim Change 5:61–66. https://doi.org/10.1038/nclimate24.