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CORRECTION

Correction: Thermal performance curves for aerobic scope in a tropical fish (*Lates calcarifer*): flexible in amplitude but not breadth

Hanna Scheuffele, Francesc Rubio-Gracia and Timothy D. Clark

There was an error in *J. Exp. Biol.* (2021) **224**, jeb243504 (doi:10.1242/jeb.243504).

The units for oxygen consumption rate ($\dot{M}_{O_2}$) were incorrectly given as mg O_2 h^{-1} 65.72 g^{-1}, whereas they should be listed as mg O_2 min^{-1} 65.72 g^{-1}. This applies to calculations of standard metabolic rate, maximum metabolic rate and absolute aerobic scope throughout most of the paper (including in the Results and figure axes). Both the online full-text and PDF versions of the paper have been corrected. The associated figshare files have also been updated (https://doi.org/10.6084/m9.figshare.16624978).

The authors apologise to readers for any inconvenience caused by this error.

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RESEARCH ARTICLE

Thermal performance curves for aerobic scope in a tropical fish (*Lates calcarifer*): flexible in amplitude but not breadth

Hanna Scheuffele[1,*], Francesc Rubio-Gracia[2] and Timothy D. Clark[1]

ABSTRACT

Aerobic metabolic scope is a popular metric to estimate the capacity for temperature-dependent performance in aquatic animals. Despite this popularity, little is known of the role of temperature acclimation and variability in shaping the breadth and amplitude of the thermal performance curve for aerobic scope. If daily thermal experience can modify the characteristics of the thermal performance curve, interpretations of aerobic scope data from the literature may be misguided. Here, tropical barramundi (*Lates calcarifer*) were acclimated for ∼4 months to cold (23°C), optimal (29°C) or warm (35°C) conditions, or to a daily temperature cycle between 23 and 35°C (with a mean of 29°C). Measurements of aerobic scope were conducted every 3–4 weeks at three temperatures (23, 29 and 35°C), and growth rates were monitored. Acclimation to constant temperatures caused some changes in aerobic scope at the three measurement temperatures via adjustments in standard and maximum metabolic rates, and growth rates were lower in the 23°C-acclimated group than in all other groups. The metabolic parameters and growth rates of the thermally variable group remained similar to those of the 29°C-acclimated group. Thus, acclimation to a variable temperature regime did not broaden the thermal performance curve for aerobic scope. We propose that thermal performance curves for aerobic scope are more plastic in amplitude than in breadth, and that the metabolic phenotype of at least some fish may be more dependent on the mean daily temperature than on the daily temperature range.

KEY WORDS: Metabolic rate, OCLTT, Temperature acclimation, Climate change, Thermal variability, Barramundi, Asian sea bass

INTRODUCTION

All life on Earth, independent of its form, must convert energy into a usable product in order to survive, grow and reproduce. Effective and efficient energy utilization underlies an organism's fitness, and thus understanding the 'cost of living' has intrigued scientists for decades (e.g. Kleiber, 1932). Oxygen plays a major role in the production of adenosine triphosphate (ATP), and hence measurements of oxygen uptake have proven to be invaluable for understanding the energetic requirements of animals. Maintenance metabolism [aka standard metabolic rate (SMR) in ectotherms, or basal metabolic rate (BMR) in endotherms] represents the minimal rate of energy (oxygen) use required to keep the body alive, while the upper limit of aerobic metabolism (typically achieved during maximal exercise) is termed the maximum metabolic rate (MMR). The difference between these two extremes is called aerobic scope and represents an animal's capacity to simultaneously supply oxygen to energy-demanding fitness traits such as activity, reproduction and growth (Clark et al., 2013).

The speed of all biological processes in ectotherms, including metabolic rate, is dependent on environmental temperature. Physiological performance of each species is generally adapted to a temperature window based on the thermal environment inhabited by the species, whereby peak performance occurs at some optimal temperature (T_{opt}) and decreases to either side of this optimum (Tewksbury et al., 2008). It has been proposed that the temperature dependence of aerobic scope can be used as an accurate proxy of temperature-dependent fitness, such that the thermal performance curve for aerobic scope essentially overlays the thermal performance curve for fitness. This concept was first proposed in fish over 70 years ago (Fry, 1947; Fry and Hart, 1948) and has been further developed as the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis (most recently described in Pörtner et al., 2017). This idea has become increasingly attractive to scientists interested in understanding fitness consequences of the thermal environment, as it suggests that relatively simple aerobic scope measurements across an ecologically relevant temperature range can be predictive of lifetime performance and fitness. While the OCLTT hypothesis has been criticized for a lack of testable predictions and empirical support (Clark et al., 2013; Jutfelt et al., 2018), there remain aspects of this debate that have received almost no empirical attention.

Notably, the OCLTT hypothesis presumes a relatively static thermal performance curve for aerobic scope for each life stage of each species, suggesting that there is little capacity for acclimation of the aerobic scope thermal performance curve in amplitude or breadth (Pörtner and Farrell, 2008; Pörtner and Knust, 2007). This idea was opposed by Norin et al. (2014), who instead suggested that the thermal performance curve for aerobic scope within species may be plastic and could acclimate to prevailing thermal conditions, at least in amplitude but possibly also in breadth. If the suggestion by Norin et al. (2014) is true, it may be expected that individuals living at a relatively constant temperature should acclimate their peak aerobic scope to that temperature and suffer clear decrements in aerobic performance when exposed to colder or warmer conditions (i.e. exhibiting a narrow thermal window akin to a thermal specialist; see Fig. 1, cold and warm acclimated). In contrast, individuals from the same species experiencing regular thermal variability may exhibit a broader thermal performance curve and only experience decrements in aerobic performance once more extreme water temperatures are encountered (i.e. a broad thermal window akin to a thermal generalist; Fig. 1, thermally variable). Moreover, according to the concept of Jensen's inequality ('the fallacy of the average'; Denny, 2017), it may be expected that fish experiencing thermal variation will have a lower peak aerobic

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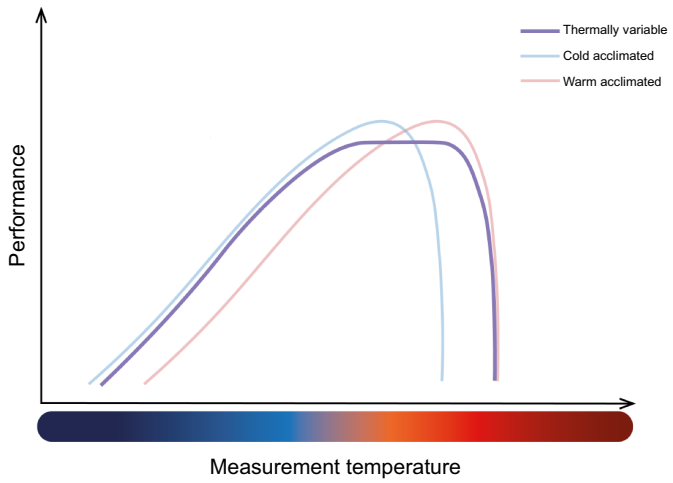

Fig. 1. Schematic representation of thermal performance curves. Curves are shown for cold-acclimated (blue) and warm-acclimated (red) aquatic ectotherms, as well as a third group experiencing a broader temperature spectrum (thermally variable, purple). Aerobic performance is suggested to be maximized at the acclimation temperature and progressively limited at warmer and colder temperatures. Individuals experiencing thermal variability are thought to exhibit a broader thermal performance curve, with a maximum covering a wider temperature range than that for their acclimated conspecifics. According to the concept of Jensen's inequality, thermally variable environments may also lead to a reduction in peak performance (purple curve) compared with constant thermal environments.

scope than conspecifics at a constant temperature, despite both experiencing the same average temperature (see Fig. 1). These important intraspecific interactions between thermal environment and aerobic capacity must be resolved if we are to understand the role of aerobic scope in long-term animal performance and fitness.

This knowledge gap is especially pertinent given that rising water temperatures, caused by anthropogenic greenhouse gas emissions, are thought to be pushing species into thermal ranges that cause performance and fitness declines (Pecl et al., 2017; Tewksbury et al., 2008). Overlaid on the chronic warming trend is an increase in the intensity and frequency of shorter-term extreme thermal events, amplifying the pressure on aquatic ectotherms (Sunday et al., 2012). Thus, the question of how metabolic capacities can be modulated in response to thermal variability is an important one, but it has received scant attention (Beauregard et al., 2013; Lyytikäinen and Jobling, 1998; Morash et al., 2018, 2021; Morissette et al., 2020; Oligny-Hébert et al., 2015). Without an understanding of the plasticity of the thermal performance curve for aerobic scope, it is not possible to gauge the potential role of the OCLTT hypothesis in contributing to performance and fitness decrements with climate warming.

Here, we used the tropical, eurythermal barramundi (*Lates calcarifer*) as a model organism to test the plasticity of aerobic scope in response to contrasting acclimation environments, namely constant daily temperatures of 23, 29 or 35°C, and a variable temperature cycling daily between 23 and 35°C with a mean of 29°C. Based on prevailing theory, we hypothesize that the three constant temperature groups will exhibit a relatively narrow thermal performance curve for aerobic scope that coincides with the respective acclimation conditions. In contrast, we hypothesize that the cycling temperature group will exhibit a broader aerobic scope thermal performance curve that reflects the daily exposure to the ∼12°C temperature range. Equally, we predict that the concept of Jensen's inequality will underlie a reduction in peak aerobic scope of the cycling temperature group. Finally, we expect that the acclimation responses in aerobic scope will translate into predictable differences in performance (measured as growth) across treatment groups. This research answers a call for more ecologically relevant temperature profiles in performance measurements of ectotherms (Morash et al., 2018), and aims to better understand the impacts of climate-induced thermal variability on the performance of fish populations.

MATERIALS AND METHODS

Animals and holding conditions

All experiments were conducted in accordance with the guidelines set by the Deakin University Animal Ethics Committee (#B27-2018), which complies with the Australian Code for the Care and Use of Animals for Scientific Purposes set out by the Australian Federal Government.

Juvenile barramundi, *Lates calcarifer* (Bloch 1790) (n≈450; ∼12 g), were obtained in February 2019 from Mainstream Aquaculture in Werribee, VIC, Australia, and transported to Deakin University's Queenscliff Marine Lab (QML). The fish were the progeny of multiple male/female broodstock and were raised in freshwater at 29°C, which is considered to be within the optimal temperature range for growth (Bermudes et al., 2010). As a eurythermal species, juvenile barramundi are often found in highly fluctuating environments such as estuaries and coastal swamps throughout Northern Australia (Pusey et al., 2004), and therefore can experience drastic daily temperature changes of ±10°C (Collins et al., 2013; Loong et al., 2005; Newton et al., 2010), which are likely to intensify in future climate change scenarios (IPCC, 2021; Loong et al., 2005). Upon arrival at QML, the fish were split across three freshwater holding tanks (200 l each, with vigorous aeration) at 29°C and allowed to adjust for at least 6 h prior to seawater transition. This was achieved by slightly opening a valve and allowing a flow of seawater (0.06 l min^{-1} $tank^{-1}$) to flush through the tanks. Salinity increased by ∼11 ppt day^{-1} and the fish were kept for 3 days upon reaching 35 ppt prior to further handling. The flow-through rate of seawater was increased to 0.4 l min^{-1} once full salinity was achieved, and temperature was maintained at 29°C using commercial heating units. Fish were not fed during the transition to seawater.

After 3 days at 35 ppt, fish were lightly anaesthetized (0.08 ml Aqui-S l^{-1} water) in groups of ∼10 to intraperitoneally inject all individuals with an 8 mm passive integrated transponder (PIT) tag for identification purposes, prior to being placed in the experimental holding facilities. The holding facilities consisted of four independent, recirculating rack systems, each containing 15 holding tanks with individual aeration (tank dimensions 400×300×300 mm L×W×H, water depth 250 mm, water volume 30 l). All tanks in each rack were supplied by a recirculating pump that drew water at 120 l min^{-1} from a sump tank (dimensions 1000×500×400 mm, water volume 200 l), and all water returned to the sump after spilling over a standpipe in each tank. Each sump contained a particle filter, a biofilter, a protein skimmer and a submersible heater (1 or 2 kW), and all water passed through a UV sterilizer in transit to the holding tanks. Each sump was supplied with clean seawater at ≥0.4 l min^{-1} (water exchange maximized but dependent on temperature treatment) to ensure a water exchange of >85% per day (i.e. ≥570 l day^{-1}).

Nine fish were haphazardly placed into each holding tank and given 8 weeks to habituate at 29°C prior to setting treatment temperatures. Three (out of 15) holding tanks per rack were left empty, making up a total of 108 fish per rack system (432 fish in

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total). After the habituation period, one rack was left at 29°C to act as a control treatment, while the temperature was gradually adjusted (1°C day^{-1}) on the three other racks to establish the treatment conditions: 23°C, 35°C, and cycling daily between 23 and 35°C with a mean around 29°C. In the last case, the 2 kW sump heater was controlled by a timer to switch on at 07:30 h once overnight temperatures had reached 23–24°C, and off at 16:30 h once daytime temperatures had reached 34–35°C. Temperature was constantly monitored by sensors (Web ID temperature sensor, IOTMSS, Perth, WA, Australia) and logged using iButtons (Thermochron®, Maxim Integrated Products, Inc., San Jose, CA, USA) throughout the entire experimental period. Respirometry experiments commenced within 14 days of the experimental temperatures being reached and continued every 3–4 weeks for ∼4 months (details below). Furthermore, every 3–4 weeks, all fish from each tank were individually netted and lightly anaesthetized in a bucket containing 0.08 ml of Aqui-S l^{-1} seawater at the respective acclimation temperature. The PIT tag of each fish was read, and total length and body mass measurements were recorded prior to the fish being placed back into its holding tank. This provided biometric measurements at 5 time points throughout the 4 month experiment.

All tanks and sumps were cleaned weekly, and *ad libitum* food (3 mm floating barramundi pellets, Skretting) was supplied 3 times per week for the first 14 weeks of the 25 weeks the fish spent in the holding system, and then 5 times per week thereafter to encourage more rapid growth. To obtain a representative measure of food consumption across treatment groups, food intake of each rack system was measured for 29 days between the period of 27 May to 24 June 2019. Ammonia, nitrite and nitrate concentrations (API Saltwater master test kit, Mars, Inc., Chalfont, PA, USA) were measured 5 times a week at first and twice a week once the system was stable (i.e. biofilters had stabilized). The light cycle was kept at 12 h:12 h light:dark, where a gradual increase in intensity occurred during the first hour starting at 07:00 h (sunrise) and a gradual decrease occurred during the last hour starting at 18:00 h (sunset). Fish were always fasted for 48 h prior to being used in experiments.

Respirometry system

The respirometry system was custom-built using four opaque reservoir tanks (dimensions 900×500×200 mm) each containing four submerged and semi-transparent plastic chambers (dimensions 200×140×80 mm, volume 1.8 l) with clip-down lids, as in Norin et al. (2014). Protocols followed best practice in aquatic respirometry (Clark et al., 2013). Prior to experiments, deoxygenated water was injected down the overflow pipe of each respirometer while they were sealed for ∼1 h to ensure no oxygen exchange with the reservoir tank or atmosphere. Each chamber contained a recirculation loop with an integrated oxygen sensor (Firesting, PyroScience, Aachen, Germany), and oxygen data were recorded from each chamber every 5 s to a laptop computer running Oxygen Logger software (PyroScience). The four chambers within each reservoir tank were supplied by an Eheim flush pump, which drew water from the reservoir tank at 12.8 l min^{-1} (∼3.2 l min^{-1} per respirometer). The flush pump was connected to a timer set to a 10 min:5 min flush:seal cycle at all times except during measurements of MMR, where individual respirometers were manually sealed to measure oxygen consumption ($\dot{M}_{O_2}$) post-exercise and flushed once water O_2 saturation reached ∼80% (details below). All reservoir tanks received continuous flow from a sump tank (dimensions 650×450×650 mm), which itself received a flow-through of clean seawater (35 ppt; flow rate adjusted depending on desired respirometry temperature) and contained a 1 kW submersible heater to regulate temperature.

Respirometry protocol

Starting once fish had thermally acclimated to their treatment conditions for at least 2 weeks, and continuing every 3–4 weeks thereafter (i.e. at five time points throughout the 4 month experiment), respirometry experiments were conducted on fish that were naive to the respirometers. Each respirometry test commenced in the afternoon when a subset of 15 fish (taken from two treatment groups, and at least six tanks per treatment; detailed handling protocols are given in Fig. S1 in the figshare repository: https://doi.org/10.6084/m9.figshare.16624978) were taken from their holding tanks, scanned for their PIT tag to ensure they had not been used previously, and placed individually into respirometers for overnight measurement of $\dot{M}_{O_2}$ at their respective treatment temperature (fish from the cycling treatment were taken from their tanks around mid-morning when temperature was 29°C, held in spare tanks at 29°C until the afternoon, and then handled similarly to the 29°C control fish for subsequent respirometry). Overnight measurements allowed the determination of SMR. One randomly assigned respirometer chamber always remained empty, in order to continuously quantify microbial background respiration. All respirometers were covered with black tarpaulin (leaving some gaps for light penetration) to minimize fish disturbance during measurements. More details on how the treatment groups were handled are given in Fig. S1: https://doi.org/10.6084/m9.figshare.16624978).

The following morning, all 15 fish were individually netted from their respirometer and transferred to a circular exercise arena (diameter 380 mm, water depth 160 mm) at the corresponding temperature in order to undergo an exhaustive exercise protocol, as described previously (Norin et al., 2014). Briefly, each fish underwent a 2 min exercise protocol, where the experimenter first agitated the water near the tail to encourage burst swimming. Once the fish started to become unresponsive to this technique, the experimenter commenced tail-tapping of the fish to continue to encourage maximum exercise. All fish were visibly exhausted at the conclusion of the 2 min protocol, at which point they were taken from the arena, weighed, then immediately placed back into the same respirometer. $\dot{M}_{O_2}$ measurements commenced directly afterwards (within 10 s), and the post-exercise data were measured for ∼2 h in order to capture the MMR (Norin and Clark, 2016). Subsequently, the respirometry system was adjusted to one of the other treatment temperatures (23, 29 or 35°C) at ∼2°C h^{-1}. $\dot{M}_{O_2}$ was again measured overnight to obtain SMR at the second test temperature.

The exercise protocol was repeated the following morning to obtain MMR at the new temperature, and then the same protocol was repeated a third time to provide SMR and MMR data at the final test temperature. Approximately 2 h after the third and final exercise protocol, the respirometry system was adjusted back to the acclimation temperature at ∼2°C h^{-1} and all fish were removed from respirometers and returned to their holding tanks. A transient malfunctioning (and subsequent repair) of one Firesting oxygen-sensing unit meant that sample sizes were reduced on some occasions (sample sizes are given in figure legends).

We validated this experimental protocol prior to commencing the full respirometry trials by measuring SMR and MMR of individuals at 29°C followed by 35°C, 23°C and again at 29°C. We compared the results of the first 29°C measurement and the second 29°C measurement using repeated measures ANOVA and did not find any

differences (see Table S1 in figshare: https://doi.org/10.6084/m9.figshare.16624978). This confirmed that ∼24 h was sufficient time between exercise bouts to ensure that neither SMR nor MMR was progressively altered.

Data analysis and statistics

The text files obtained from the Firesting O_2 software were imported into the program LabChart 7 (ADInstruments Pty Ltd, Bella Vista, NSW, Australia). $\dot{M}_{O_2}$ (mg O_2 min^{-1} $fish^{-1}$) was calculated by determining the slopes from linear regressions of the water oxygen concentration over time during each 5 min measuring (sealed) cycle. An average of ∼107 $\dot{M}_{O_2}$ data points were obtained for each fish. The data were subsequently compiled in Microsoft Excel (version 2106). SMR for each individual and for each temperature was taken as the average of the lowest 10% of values obtained at each temperature. MMR for each individual and temperature was taken as the highest $\dot{M}_{O_2}$ value over a 5 min period post-exercise, usually occurring within 3 min post-exercise (Norin and Clark, 2017). Microbial background respiration was always minor but was accounted for by subtracting the time-matched $\dot{M}_{O_2}$ of the empty respirometer from all $\dot{M}_{O_2}$ measurements from respirometers containing fish. Absolute aerobic scope (AAS) was calculated as MMR−SMR and factorial aerobic scope (FAS) as MMR/SMR. Further analyses were conducted using RStudio (version 1.1.453). The data for all $\dot{M}_{O_2}$ measurements (SMR, MMR, AAS and FAS) and for body mass were log–log transformed and linear regressions were fitted with treatment as an interaction term for each measurement temperature. We visually assessed the log–log transformed data for linearity, normality of the residuals, homoscedasticity and independence of residual error terms. We further combined the $\dot{M}_{O_2}$ measurements of all time points by standardizing the SMR and MMR to the overall average mass of 65.72 g. To do this, we used the regressions obtained from the linear models but retained the residual from the original data points. The AAS and FAS were then calculated using these standardized data. The standardized data were then tested for normality using Shapiro–Wilk tests as well as homogeneity of variances using Levene's tests. Data were then statistically compared using analyses of variance (ANOVA) followed by Tukey's honest significance tests (Tukey HSD) if normally distributed, or by using Kruskal–Wallis by ranks tests followed by Dunn's tests if data were non-normally distributed.

Furthermore, we examined the degree of correlation between SMR and MMR using Pearson's product-moment correlation analyses on the mass-corrected SMR and MMR data for every treatment group and measurement temperature. In order to compare aerobic scope across measurement temperatures, SMR, MMR and AAS data were interrogated using linear mixed-effects models fitted by restricted maximum likelihood (with the lme4 package in R; Bates et al., 2015), in order to control for repeated measures of $\dot{M}_{O_2}$ for individual fish. The respective metabolic parameter (SMR, MMR or AAS) was set to be the response variable, while acclimation condition and measurement temperature were considered fixed effects (including an interaction between the two), and individual was used as a random effect (intercept). Tukey adjusted pairwise comparisons of the estimated marginal means were performed using the emmeans package (R package version 1.5.4; https://CRAN.R-project.org/package=emmeans).

We also combined the mass data collected during respirometry measurements with the mass data collected separately for all fish from holding tanks every 3–4 weeks (see above). As the mass of individual fish was measured repeatedly throughout the experiment, we used linear mixed models fitted by restricted maximum likelihood. We fitted several models on the log–normal transformed or log–log transformed data with body mass as the response variable and chose the model with the highest Akaike information criterion (AIC). Time and acclimation condition were always considered to be fixed effects with an interaction, and individual was set to be a random intercept and/or slope. The model with the highest AIC was found to be on log–normal transformed data with individual set to be a random intercept and slope. Furthermore, Tukey-adjusted pairwise comparison of the estimated marginal means was performed. Specific growth rates (SGR, in % day^{-1}) were calculated using the linear mixed model output for parameter b (±standard error for b) according to the formulas: $SGR=(10^{b}-1)\times100$, and $s.e.=(10^{s.e.(b)}-1)\times100$.

Feed intake data were transformed to % body mass day^{-1}. This was done by calculating the average mass for one fish for each treatment at the last body mass recording prior to starting the feed intake measurement. The daily feed intake was averaged and divided by the total number of fish in each treatment. These values were subsequently used to calculate the feed intake as % body mass day^{-1}. We furthermore visually inspected the data for normal distribution. Data were then statistically interrogated using one-way repeated measures ANOVA with a Greenhouse–Geisser sphericity correction, followed by two paired t-tests. Fig. 1 was generated using Adobe Illustrator© (version 25.4.1) and all other graphic output was generated using ggplot2 for R (Wickham et al., 2016).

RESULTS

Metabolism

The slopes of the regressions between log-$\dot{M}_{O_2}$ and log-body mass were generally similar across acclimation groups when measured at different temperatures, with a few exceptions that reached statistical significance (see Figs S2a–c, S3a–c, S4a–c and S5a–c and Table S2 in figshare: https://doi.org/10.6084/m9.figshare.16624978). Herein, we focus on comparing body mass-standardized $\dot{M}_{O_2}$ data between treatment groups.

SMR

When standardized for body mass (i.e. SMR per 65.72 g of fish), there was an expected increase in SMR in all acclimation groups as measurement temperature increased (cf. Fig. 2A–C). Within measurement temperatures, there were some notable differences between acclimation groups. Within the 23°C measurement temperature (Fig. 2A), 23°C-acclimated fish had a higher SMR than the thermal cycling treatment group (mean±s.d.: 0.1±0.01 versus 0.08±0.01 mg O_2 min^{-1} 65.72 g^{-1}, respectively; P=0.03). Within the 29 and 35°C measurement temperatures (Fig. 2B,C), the 35°C-acclimated group showed a pronounced metabolic acclimation (depression) compared with the other three acclimation groups (e.g. 0.18±0.02 mg O_2 min^{-1} 65.72 g^{-1} for 35°C-acclimated fish measured at 35°C versus ≥0.23 mg O_2 min^{-1} 65.72 g^{-1} for the other three acclimation groups measured at 35°C; Fig. 2C). As per our findings at the 23°C measurement temperature, the 23°C-acclimated group exhibited the highest SMR when measured at 35°C (0.26±0.02 mg O_2 min^{-1} 65.72 g^{-1}; P≤0.009 for all comparisons; Fig. 2C).

MMR and correlations with SMR

MMR tended to track the same patterns as described for SMR above. Specifically, there was a general elevation in MMR as measurement temperature increased (cf. Fig. 2D–F), and 23°C-acclimated fish tended to exhibit an elevated MMR when

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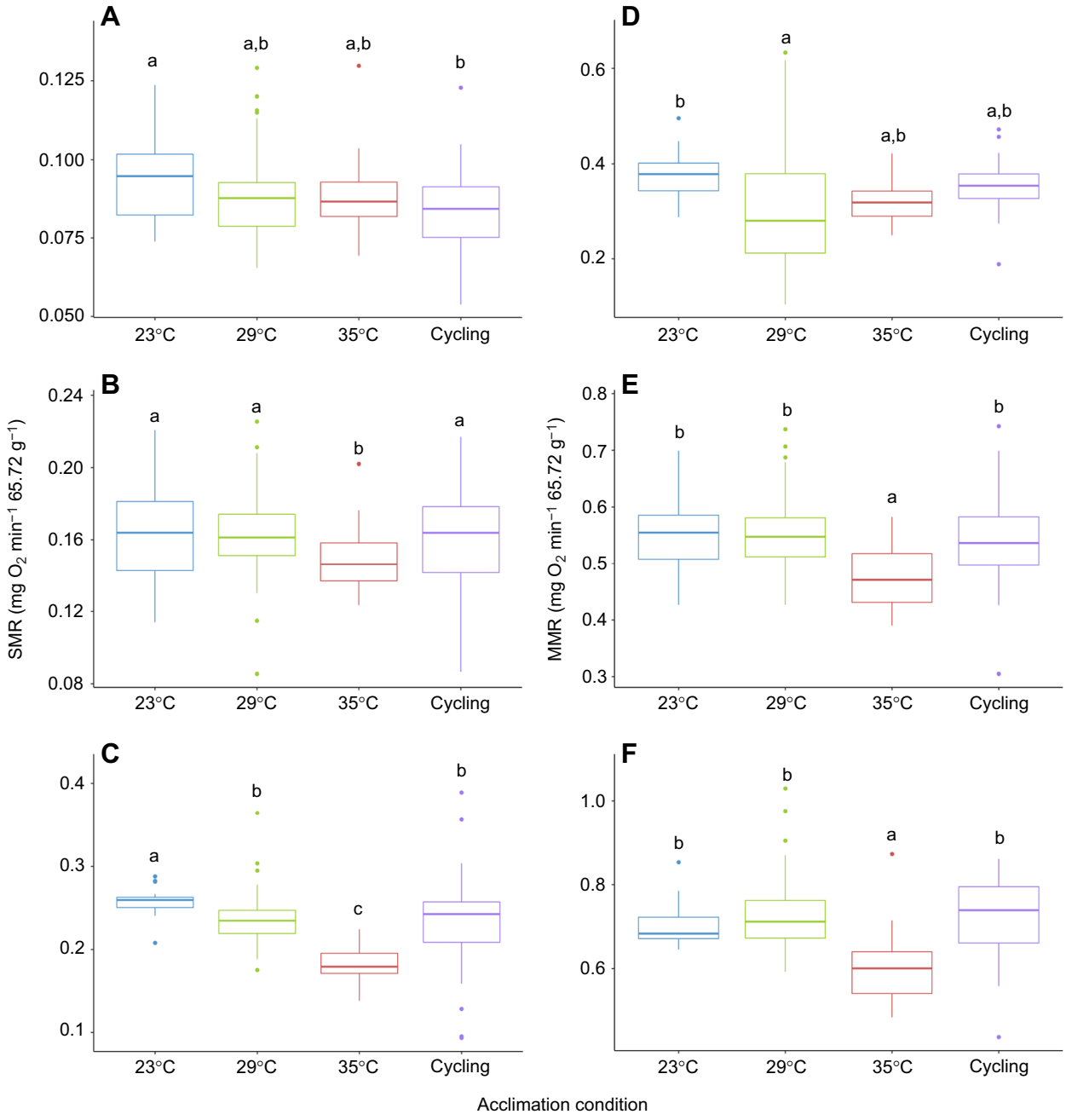

Fig. 2. Standard metabolic rate and maximum metabolic rate of barramundi (*Lates calcarifer*) measured at 23, 29 and 35°C. Fish were reared under cold (23°C, *n*=31), control (29°C, *n*=59), warm (35°C, *n*=29) and thermally variable (cycling, *n*=32) acclimation conditions. The last treatment group experienced daily undulations of temperature between 23 and 35°C, with a mean temperature of 29°C. Standard metabolic rate (SMR; A–C) and maximum metabolic rate (MMR; D–F) were measured at 23°C (A,D), 29°C (B,E) or 35°C (C,F) and data were standardized to the average body mass of 65.72 g. Significant differences ($P \leq 0.05$) between treatments are indicated by different lowercase letters (tested with ANOVA followed by Tukey HSD if data were normally distributed, or using Kruskal–Wallis by ranks tests followed by Dunn's tests if data were non-normally distributed).

measured at 23°C [only significantly different when comparing 23°C-acclimated fish (0.37±0.05 mg O_2 min^{-1} 65.72 g^{-1}) versus 29°C-acclimated fish (0.31±0.13 mg O_2 min^{-1} 65.72 g^{-1}); P=0.01]. Most notably, 35°C-acclimated fish exhibited MMR depression compared with the other three acclimation groups when measured at 29 and 35°C (e.g. 0.61±0.08 mg O_2 min^{-1} 65.72 g^{-1} for 35°C-acclimated fish measured at 35°C versus ≥0.7 mg O_2 min^{-1} 65.72 g^{-1} for the other three acclimation groups measured at 35°C; Fig. 2F).

SMR and MMR were positively correlated in barramundi acclimated to 29°C and to cycling thermal conditions, independent of measurement temperature (Pearson's coefficients between 0.39 and 0.65, P<0.05; Table S3 in figshare: https://doi.org/10.6084/m9.figshare.16624978). Fish acclimated to 23°C also showed a significant positive correlation between SMR and MMR, but only when measured at 29°C (Pearson's coefficient=0.49, P<0.05; Table S3 in figshare: https://doi.org/10.6084/m9.figshare.16624978). No correlation between SMR and

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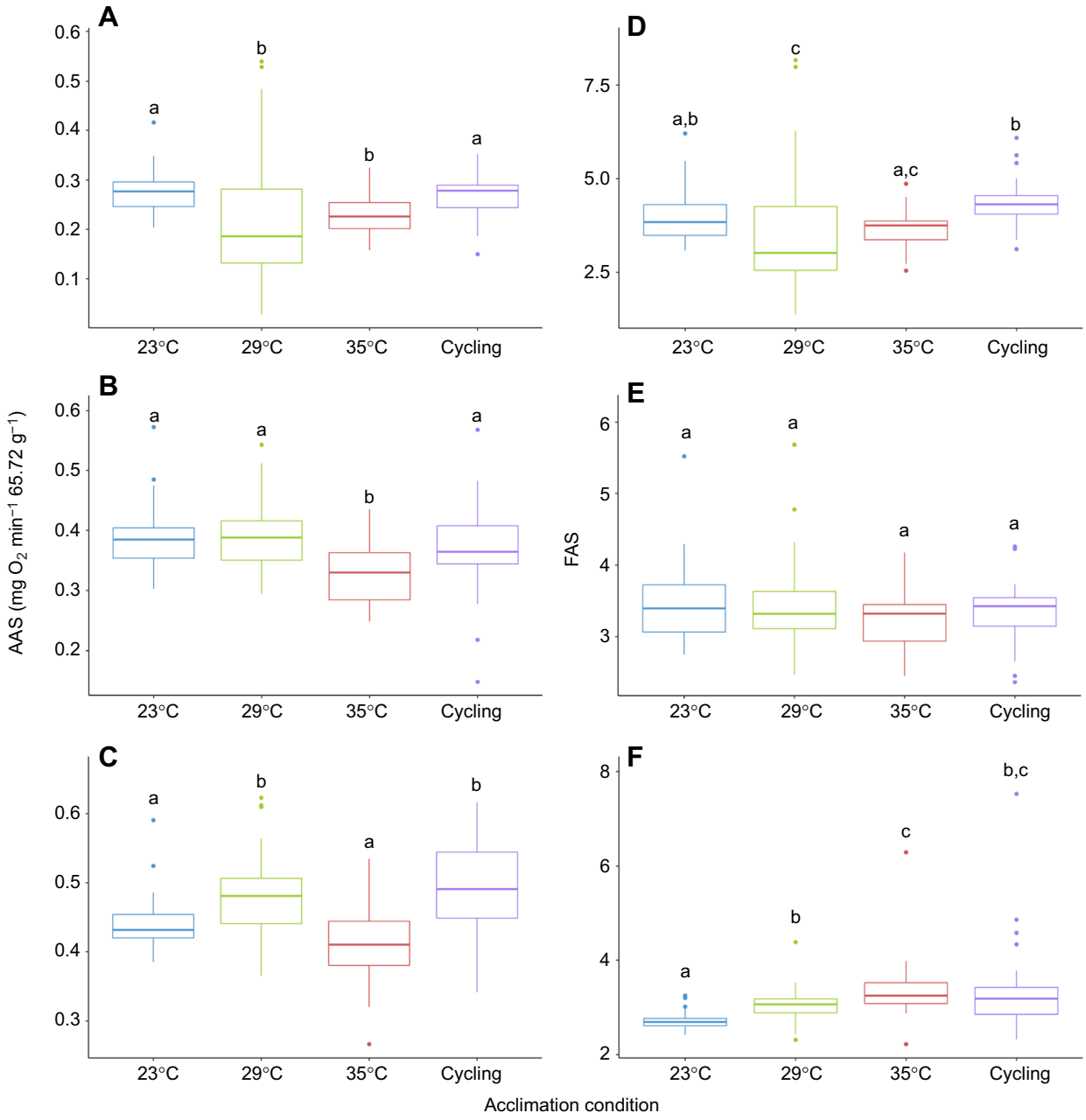

Fig. 3. Absolute aerobic scope and factorial aerobic scope of barramundi measured at 23, 29 and 35°C. Fish were reared under cold (23°C, *n*=31), control (29°C, *n*=59), warm (35°C, *n*=29) and thermally variable (cycling, *n*=32) acclimation conditions. The last treatment group experienced daily undulations of temperature between 23 and 35°C, with a mean temperature of 29°C. Absolute aerobic scope (AAS; A–C) and factorial aerobic scope (FAS; D–F) were calculated from metabolic rates measured at 23°C (A,D), 29°C (B,E) or 35°C (C,F) and data were standardized to the average body mass of 65.72 g. Significant differences ($P \leq 0.05$) between treatments are indicated by different lowercase letters (tested with ANOVA followed by Tukey HSD if data were normally distributed, or using Kruskal–Wallis by ranks tests followed by Dunn's tests if data were non-normally distributed).

MMR was found for any of the measurement temperatures in 35°C-acclimated fish.

AAS and FAS

AAS increased significantly with measurement temperature (for statistics, see Figs 3A–C and 4A; Table S4 in figshare: https://doi.org/10.6084/m9.figshare.16624978). When measured at 23°C, AAS was lower in 29°C-acclimated (0.21±0.12 mg O_2 min^{-1} 65.72 g^{-1}) and 35°C-acclimated (0.23±0.04 mg O_2 min^{-1} 65.72 g^{-1}) fish than in those in the other acclimation groups (≥0.27 mg O_2 min^{-1} 65.72 g^{-1} for 23°C-acclimated and thermally cycling fish, $P \leq 0.02$; Fig. 3A). Fish acclimated to 35°C maintained a lower AAS than the other groups when measured at 29°C (0.33±0.05 mg O_2 min^{-1} 65.72 g^{-1} versus ≥0.37 mg O_2 min^{-1} 65.72 g^{-1}; $P \leq 0.013$; Fig. 3B), and both 35°C- and 23°C-acclimated fish showed a lower AAS than the other two groups when measured at 35°C (≤0.44 mg O_2 min^{-1} 65.72 g^{-1} versus ≥0.48 mg O_2 min^{-1} 65.72 g^{-1}; $P \leq 0.002$; see Fig. 3C).

FAS was not as clearly dependent on body mass as the other metabolic parameters (Fig. S5A–C in figshare: https://doi.org/10.6084/m9.figshare.16624978). While all four acclimation groups had similar FAS when measured at 29°C (Fig. 3E), some differences

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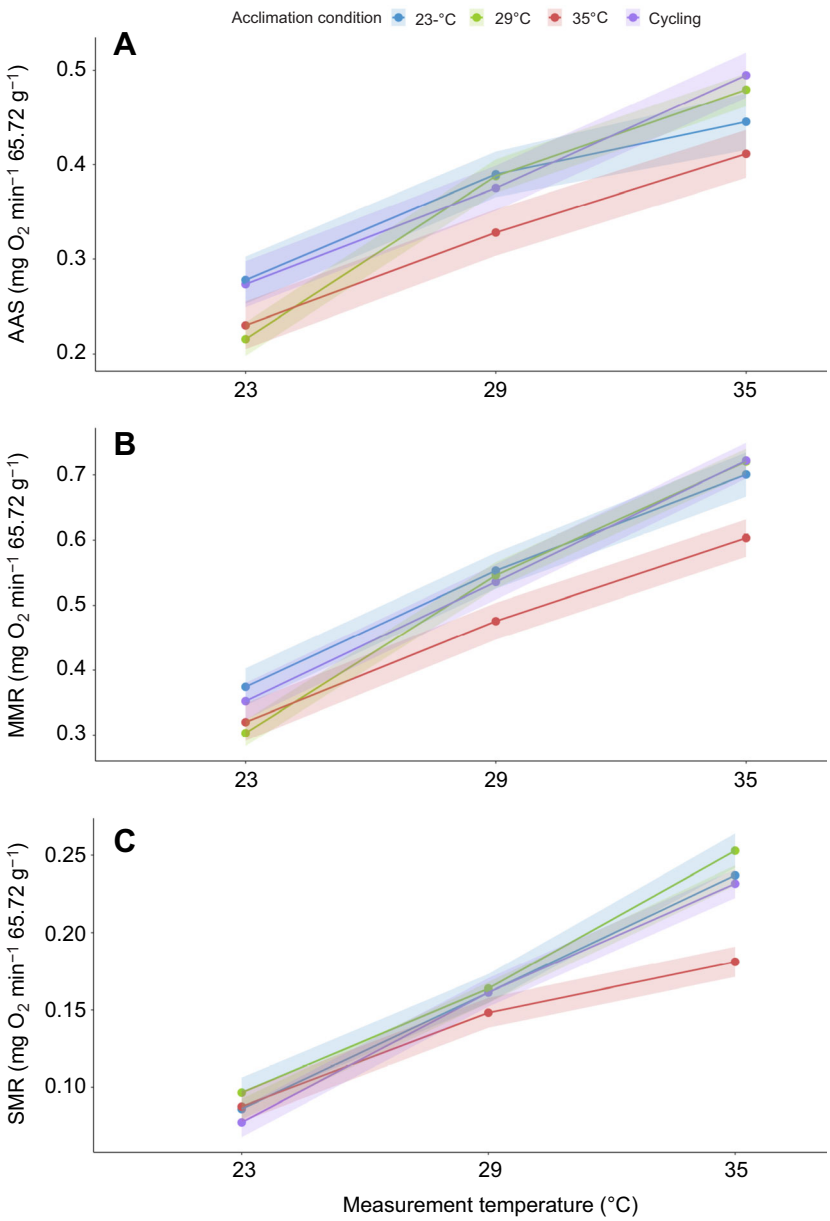

Fig. 4. Mixed effects models (shaded confidence intervals) of AAS, MMR and SMR of barramundi measured at 23, 29 and 35°C. Fish were reared at 23°C (n=31), 29°C (n=59), 35°C (n=29) and under daily cycling temperatures between 23 and 35°C (around a mean of 29°C, n=32). AAS (A) was calculated from MMR (B) and SMR (C), which were measured at 23, 29 and 35°C and data were standardized to a grand mean body mass of 65.72 g. Fixed effects included measurement temperature and treatment (including the interaction between the two) and individual was included as a random effect. Statistics are available from figshare (Table S4; https://doi.org/10.6084/m9.figshare.16624978).

between groups emerged when FAS was measured at 23 and 35°C. Notably, when measured at 23°C, the thermal cycling treatment exhibited a higher FAS than the 29°C- and 35°C-acclimated treatment groups (4.36±0.63 versus ≥3.65, P≤0.003), while 23°C-acclimated fish had a higher FAS (3.98±0.71) than fish acclimated to 29°C (3.51±1.46, P=0.02; Fig. 3D). When measured at 35°C, FAS of 23°C-acclimated fish was lower than that of the other three acclimation groups (2.73±0.23 versus ≥3.06; P≤0.001), and 35°C-acclimated fish also had a higher FAS (3.38±0.68) than 29°C-acclimated fish (3.06±0.32; P=0.007; Fig. 3F).

Growth and feed intake

Fish reared in the thermally cycling treatment group grew at a similar rate to fish in the constant 29 and 35°C treatments (Fig. 5A; SGR 1.25±0.02, 1.23±0.02 and 1.16±0.02% day^{-1}, respectively; for regression details, see Table S5 in figshare: https://doi.org/10.6084/m9.figshare.16624978), despite spending ∼4 h per day below 24°C. Fish acclimated to a constant 23°C had a lower growth rate (0.72±0.02% day^{-1}) and consequently were smaller than fish from the other three treatment groups at the conclusion of the 4 month trial (Fig. 5A).

Fish reared under 23°C showed significantly lower feed intake than all other acclimation conditions (1.37±0.35% body mass day^{-1}, P<0.001 for all comparisons; Fig. 5B). Feed intake was similar for fish acclimated to 35°C (2.05±0.36% body mass day^{-1}) and those in the cycling acclimation treatment (1.99±0.21% body mass day^{-1}, P>0.05), yet the former was significantly higher than that of fish in the 29°C-acclimation treatment (1.83±0.28% body mass day^{-1}, P=0.04).

DISCUSSION

Metabolic performance across acclimation groups

In contrast to our hypothesis, we show that acclimation to daily cyclical temperature (23 to 35°C, mean of 29°C) does not meaningfully broaden the thermal performance curve for aerobic scope compared with fish acclimated to a constant temperature of 29°C (Figs 3 and 4A). There was also no indication of a decline in peak AAS of the cyclical temperature group compared with the

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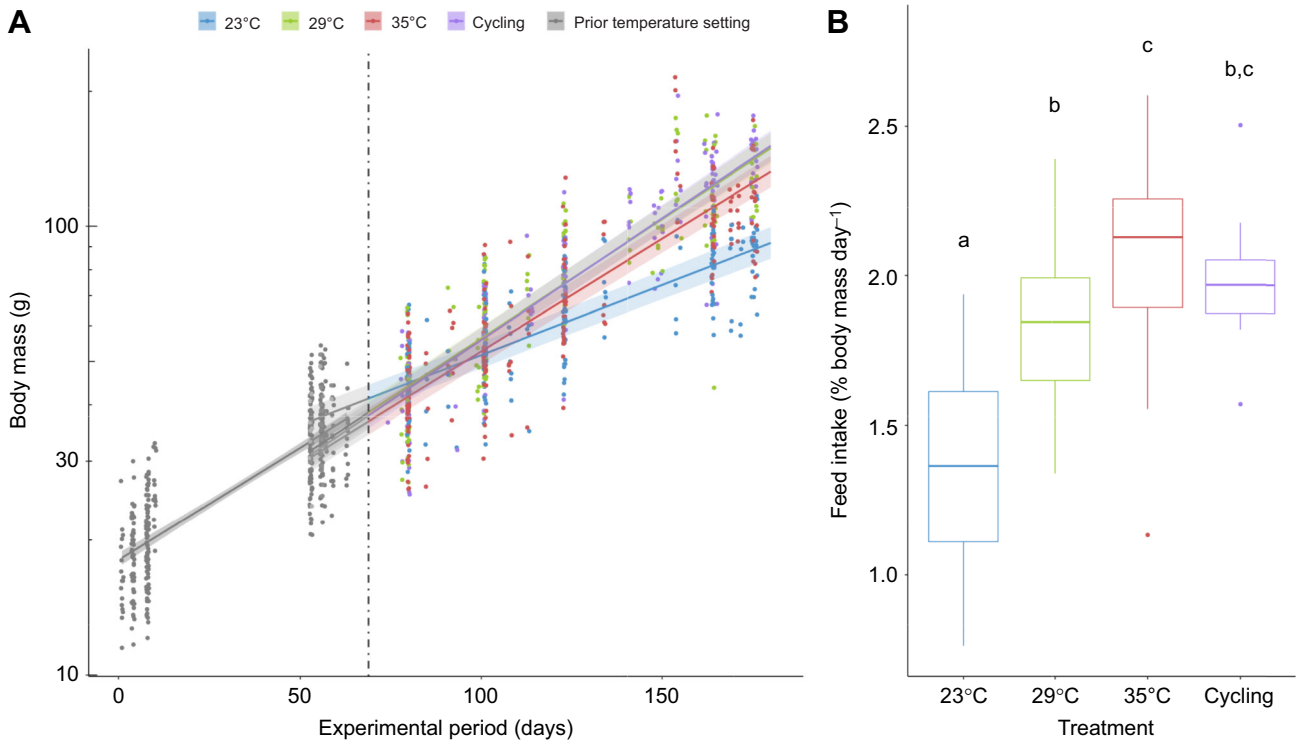

Fig. 5. Increase of body mass over time and daily feed intake of barramundi reared under different temperature regimes. Fish were reared under cold (23°C, *n*=31), control (29°C, *n*=59), warm (35°C, *n*=29) and thermally variable (cycling, *n*=32) treatment conditions. The last treatment group experienced daily undulations of temperature between 23 and 35°C, with a mean temperature of 29°C. The water temperature prior to setting the experimental treatment conditions was 29°C (shown in grey in A; vertical broken line indicates the time point when temperature was changed to treatment conditions). Body mass data (A) were log–normal transformed and linear mixed effect models were fitted for each treatment. Statistics are available from figshare (Table S5; https://doi.org/10.6084/m9.figshare.16624978). Feed intake (B) is given for fish in each treatment group and significant differences (P<0.05) between treatment groups are indicated by different lowercase letters.

constant 29°C group (Fig. 3B), contrasting with the concept of Jensen's inequality (Denny, 2017). Nevertheless, there was some indication that, at the 23°C measurement temperature, fish in the thermally cycling acclimation group benefited from a marginally (yet statistically significant) higher AAS than 29°C-acclimated fish (Fig. 3A). Notably, however, this did little to change the shape of the aerobic scope thermal performance curve (Fig. 4A). In the wild and in captivity, barramundi can encounter summer temperatures of up to 36°C, and experience temperature changes of ±10°C per day (Isaza et al., 2019; Loong et al., 2005). The present findings show that the metabolism of this species is well adapted to a broad temperature range including high thermal variability. While there are few previous studies on other species to compare against, Morissette et al. (2020) observed no differences in aerobic performance between Atlantic salmon (*Salmo salar*) acclimated to cycling temperatures (16 to 21°C) compared with fish acclimated to a constant temperature of 18.5°C, although in that study metabolic rate was only measured at one temperature (18.5°C). Thus, current evidence suggests that mean daily temperatures may be more crucial than the daily temperature range in regulating metabolic phenotype.

Similar to the findings of Norin et al. (2014), we show that AAS of barramundi continually increases with temperature in all acclimation groups (Fig. 4A), instead of levelling off or following a bell-shaped performance curve as proposed within the OCLTT hypothesis (Pörtner et al., 2017). Comparable results were reported for pink salmon (*Oncorhynchus gorbuscha*; Clark et al., 2011), indicating that peak aerobic performance can occur at supra-optimal temperatures and therefore the thermal performance curve for aerobic scope aligns poorly with the thermal performance curves for other fitness-related metrics (Clark et al., 2013).

Fish reared at the highest temperature of 35°C showed a depressed SMR at their acclimation temperature (Figs 2C and 4C), which remained steady after 3–4 weeks of acclimation (Fig. S6C in figshare: https://doi.org/10.6084/m9.figshare.16624978), in line with current understanding of the effects of warm acclimation on baseline metabolism (Seebacher et al., 2015). Lowered oxygen demand in warm-acclimated ectotherms at high temperature could be a consequence of a trade-off between reducing the mitochondrial cost [i.e. proton leakage, which is linked to reactive oxygen species (ROS) production; Cheng et al., 2017] and maximizing aerobic performance at acclimation temperatures (Pörtner, 2012). Metabolic processes, such as mitochondrial energy (ATP) production with a concomitant increase of ROS generation due to proton leakage, are accelerated at higher temperatures. If not sufficiently combatted by antioxidant defence mechanisms, ROS can cause oxidative cell damage in fish (Tripathy, 2016). According to Pörtner (2012), warm-acclimated ectotherms mitigate the adverse effects of an increased ATP production by reducing mitochondrial density and/or efficiency, while paying the price with reduced aerobic capacity. This lowered capacity is reflected by a reduced SMR and MMR (red symbols in Figs 2B,C,E,F and 4B,C) and a reduced AAS (see Figs 3B,C and 4A). The relative decline in AAS with warm acclimation corroborates the findings of Norin et al. (2014). The opposite effect is seen in ectotherms acclimated to lower temperatures, where ROS generation is decelerated and hence mitochondria can proliferate (as described by Johnston and Dunn, 1987). This could explain the generally high SMR in barramundi acclimated to (and measured at) 23°C (Fig. 2A),

and the tendency for a high MMR and AAS (Figs 2D and 3A). When subjected to temperatures above acclimation conditions, these cold-acclimated fish continue to show high metabolic rates (Fig. 2B,C,E,F), providing support for a structural change at the level of the mitochondria.

In contrast to our study, 15°C-acclimated Atlantic killifish (*Fundulus heteroclitus*) were reported to show a negligible change in AAS when acutely exposed to temperatures ranging from 5 to 33°C, yet ∼4 weeks of acclimation to 25 or 30°C resulted in a significant elevation in AAS compared with other acclimation temperatures throughout the 5–33°C range (Healy and Schulte, 2012). The mechanisms underlying contrasting thermal response profiles of acutely exposed and acclimated fish across species have received little attention, but further knowledge in this area will help to forecast the impacts of environmental change on fish communities.

There was evidence of a positive correlation between SMR and MMR in fish acclimated to 29°C and to daily thermal cycling, but not in the 23°C- or 35°C-acclimated fish. That is, individual fish from the 29°C and thermal cycling treatments that exhibited a low SMR also showed a low MMR and vice versa, regardless of measurement temperature (Table S3 in figshare: https://doi.org/10.6084/m9.figshare.16624978). Such a coupling between SMR and MMR has been detected both intra- and inter-specifically (Killen et al., 2016; Norin et al., 2016), and it may be mechanistically linked to the mitochondrial traits described above. The observed correlation of SMR and MMR could be a result of the antagonistic selection for individuals with increased locomotor activity versus individuals with a higher tolerance of resource limitation (as described interspecifically by Killen et al., 2016).

Linking metabolism to growth performance and feed intake

Barramundi exposed to cyclical thermal conditions had essentially the same growth performance as fish acclimated to 29 or 35°C (Fig. 5A). Similar to our study, Morissette et al. (2020) found no effect of daily temperature undulations on growth of Atlantic salmon (*S. salar*) when compared with conspecifics maintained at constant temperature (details above). In contrast, brook trout (*Salvelinus fontinalis*) exposed to daily temperature fluctuations (7 to 13°C) were significantly smaller than their conspecifics reared under constant conditions of 10°C (Pisano et al., 2019). Given the scarcity of studies experimentally testing the effects of thermal variability on growth, understanding these potentially species-specific effects must await further research.

Barramundi acclimated to 35°C showed a similar growth performance to 29°C-acclimated fish (Fig. 5A), despite an 11.5% elevation in SMR when measured at their acclimation temperature (cf. red symbol in Fig. 2C versus green symbol in Fig. 2B). This apparent contradiction was made possible by an elevated feed intake in 35°C-acclimated fish (Fig. 5B), such that the additional energy supply compensated for the additional energy expenditure. Increased feed intake at high temperatures has previously been recorded for barramundi (Bermudes et al., 2010; Katersky and Carter, 2005) and other fish species such as the common carp (*Cyprinus carpio*; Pang et al. (2016)) and the Atlantic salmon (Handeland et al., 2008). Corroborating our growth findings, Bermudes et al. (2010) found that juvenile barramundi grew fastest across a temperature plateau of 29.1–34.9°C, and Katersky and Carter (2005) showed that juvenile barramundi reared at 36°C grew faster than conspecifics reared at 27°C. Lacking clear metabolic compensation in the cold (Figs 2A,D and 3A,D), the 23°C-acclimated fish in the present study were undoubtedly below their thermal optimum, characterized by lower feed intake and slower growth (Fig. 5). Reduced feed intake and growth have previously been reported for cold-acclimated barramundi (Bermudes et al., 2010), common carp (Pang et al., 2016) and Atlantic salmon (Handeland et al., 2008). This decrease in feed intake (appetite) in cold-acclimated fish may be linked to lowered digestive enzyme activity in suboptimal temperatures (Pelletier et al., 1993), consequently lowering digestive efficiency (similar to the findings of Bermudes et al., 2010, and Luo and Xie, 2008).

In sum, this study shows that barramundi acclimated to a ∼12°C daily temperature cycle show similar metabolic thermal performance and growth rates to conspecifics reared under constant thermal conditions with the same daily mean (29°C). Thus, it seems that the breadth of the thermal performance curve for aerobic capacity may be set by average thermal conditions rather than daily thermal extremes. Nevertheless, there were clear metabolic responses in fish acclimated to stable warm or cold conditions (e.g. Fig. 2C), suggesting that the thermal performance curve for aerobic scope may be more flexible in amplitude than in breadth (see Norin et al., 2014). Equally, it is possible that aerobic scope is not a good proxy for whole-animal performance, and instead it may be more informative to focus on acclimation responses in SMR and MMR. Indeed, clear acclimation responses in SMR and MMR of 35°C-acclimated fish measured at 35°C (Fig. 2C,F) are less apparent when examining aerobic scope (Fig. 3C). While there remain many questions regarding the utility of the OCLTT hypothesis and the aerobic scope thermal performance curve (Clark et al., 2013; Jutfelt et al., 2018), there is little doubt that understanding SMR, MMR and field metabolic rate of fishes will help to improve our capacity to forecast and manage the impacts of environmental change on fish populations.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: H.S., T.D.C.; Methodology: H.S., T.D.C.; Validation: H.S., T.D.C.; Formal analysis: H.S.; Investigation: H.S., F.R.-G., T.D.C.; Resources: T.D.C.; Data curation: H.S., T.D.C.; Writing - original draft: H.S., T.D.C.; Writing - review & editing: H.S., F.R.-G., T.D.C.; Visualization: H.S.; Supervision: T.D.C.; Project administration: H.S.; Funding acquisition: T.D.C.

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Data availability

Raw data and supplementary information are publicly available from figshare: https://doi.org/10.6084/m9.figshare.16624978

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