

RESEARCH ARTICLE

Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria

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ABSTRACT

Increasing water temperatures owing to anthropogenic climate change are predicted to negatively impact the aerobic metabolic performance of aquatic ectotherms. Specifically, it has been hypothesized that thermal increases result in reductions in aerobic scope (AS), which lead to decreases in energy available for essential fitness and performance functions. Consequences of warming are anticipated to be especially severe for warm-adapted tropical species as they are thought to have narrow thermal windows and limited plasticity for coping with elevated temperatures. In this study we test how predicted warming may affect the aerobic performance of Nile perch (*Lates niloticus*), a commercially harvested fish species in the Lake Victoria basin of East Africa. We measured critical thermal maxima (CT_{max}) and key metabolic variables such as AS and excess post-exercise oxygen consumption (EPOC) across a range of temperatures, and compared responses between acute (3-day) exposures and 3-week acclimations. CT_{max} increased with acclimation temperature; however, 3-week-acclimated fish had higher overall CT_{max} than acutely exposed individuals. Nile perch also showed the capacity to increase or maintain high AS even at temperatures well beyond their current range; however, acclimated Nile perch had lower AS compared with acutely exposed fish. These changes were accompanied by lower EPOC, suggesting that drops in AS may reflect improved energy utilization after acclimation, a finding that is supported by improvements in growth at high temperatures over the acclimation period. Overall, the results challenge predictions that tropical species have limited thermal plasticity, and that high temperatures will be detrimental because of limitations in AS.

KEY WORDS: Aerobic scope, Tropical fisheries, Respirometry, Climate change, Thermal tolerance

INTRODUCTION

Increasing water temperatures owing to anthropogenic climate change have been associated with shifts in the phenology, distribution and abundance of aquatic ectotherms in both marine and freshwater systems (Perry et al., 2005; Ficke et al., 2007; Comte et al., 2013), and are known to have profound effects on the physiology of fish species that can lead to reduced fitness in wild populations (Ficke et al., 2007; Crozier and Hutchings, 2014). To cope with rapidly changing thermal regimes, fish must either relocate to more suitable habitats or adjust to novel conditions through phenotypic plasticity and/or genetic change (Rosset and Oertli, 2011). For fishes inhabiting inland systems, where relocation

to higher latitudes is not always possible, *in situ* responses to rising temperatures are essential. Predicting effects of climate change on inland fishes therefore requires an understanding of how thermal increases affect metabolic performance, fitness traits and the capacity for physiological adjustments to rising water temperatures (Clark et al., 2011, 2013a; Gräns et al., 2014; Norin et al., 2014).

In fishes and other ectothermic animals, temperature tolerance limits (thermal windows) can help to predict responses to changes in temperature. On a simplistic level, a thermal window can be understood as a unimodal performance curve with functional efficiency of a given performance measure maximized under a range of optimal temperatures (T_{opt}) and falling to zero upon approaching critical temperatures at the upper ($T_{crit,max}$) and lower ($T_{crit,min}$) thermal limits (Fry, 1947; Brett, 1971; Huey and Stevenson, 1979; Pörtner, 2010; Schulte, 2015). The exact shape of this curve varies among species, and is determined in part by the temperature range in their natural habitat (adaptive background) and their ability to cope with thermal variability (phenotypic plasticity) (Huey and Stevenson, 1979; Pörtner, 2010; Schulte, 2015). For example, warm-adapted tropical species, having evolved in relatively thermo-stable environments, are predicted to have narrow thermal windows compared with their temperate counterparts, and are thought to be living near the upper edge of their thermal tolerance limit (Tewksbury et al., 2008), making them especially sensitive to temperature increases (Stillman, 2003; Tewksbury et al., 2008). Given the high levels of interspecific variation in this trait, a great deal of species-specific testing is required to reveal whether such overarching patterns exist; however, tropical inland fishes are understudied compared with temperate and marine species (Comte et al., 2013). In this study, we test the thermal plasticity of a tropical freshwater fish [the Nile perch, *Lates niloticus* (Linnaeus 1758)] by examining their metabolic performance in a range of water temperatures and comparing responses across acclimation times.

Aerobic scope (AS), defined as the increase in an animal's oxygen consumption from its standard to its maximal metabolic rate (SMR and MMR, respectively), is a key measure of metabolic performance in fishes. For ectotherms, it has been hypothesized that decreases in AS set temperature limits at the upper and lower end of a species' thermal window through a process known as oxygen and capacity-limited thermal tolerance (OCLTT) (Pörtner, 2010). At the upper edge of a species' thermal tolerance limit (approaching $T_{crit,max}$), OCLTT predicts that SMR will increase at a faster rate than MMR because of limitations in the capacity of the cardiorespiratory system to deliver adequate oxygen to respiring tissues, which ultimately manifests as declines in AS (Pörtner and Farrell, 2008; Pörtner, 2010). OCLTT further predicts that AS is closely linked to fitness-related performance traits such as growth and reproductive success, implying that declines in AS may have negative fitness consequences for aquatic ectotherms (Pörtner and Farrell, 2008; Pörtner and Knust, 2007; Pörtner, 2010).

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List of symbols and abbreviations

AS	aerobic scope
CT _{max}	critical thermal maximum
CT _{min}	critical thermal minimum
EPOC	excess post-exercise oxygen consumption
K _{ratio}	condition ratio
LVB	Lake Victoria basin
MMR	maximum metabolic rate
\dot{M}_{O_2}	oxygen consumption rate
OCLTT	oxygen- and capacity-limited thermal tolerance
RT	recovery time
SGR	standard growth rate
SL	standard length
SMR	standard metabolic rate
T _{crit,max}	critical temperature, upper limit
T _{crit,min}	critical temperature, lower limit
TL	total length
T _{opt}	optimal temperature
T _{optAS}	optimal temperature for AS
T _{optFIT}	optimal temperature for fitness-related traits

OCLTT has provided a useful conceptual framework for predicting how ectotherms may respond to thermal stress (Wang and Overgaard, 2007; Pörtner and Farrell, 2008; Farrell et al., 2008, 2009; Pörtner, 2010); however, studies that have tested links between AS and fitness-related traits suggest that the optimal temperatures for AS (T_{optAS}) may not match optimal temperatures for fitness-related traits (T_{optFIT}) (Healy and Schulte, 2012; Gräns et al., 2014), and for some species, maximum oxygen uptake curves lack meaningful temperature optima, with MMR and AS increasing with temperature to near-lethal limits (Ern et al., 2014; Gräns et al., 2014; Norin et al., 2014). This suggests that low AS may not be a central constraint on performance, and that other traits must be considered when making predictions about effects of elevated water temperature on inland fishes.

In addition, relationships between metabolic rate and temperature in ectotherms are affected by exposure time (acclimation) to higher or lower water temperatures. Thermal acclimation occurs through the alteration of behavioural, physiological and morphological characteristics to better suit an environment (Angilletta, 2009; Sandblom et al., 2014), and is thought to increase $T_{crit,max}$ and adjust T_{opt} by reducing basal energy expenditure allowing for maintenance of high AS at temperatures that would normally cause reductions in available energy (Sandblom et al., 2014). Short-term plastic responses such as these can help fishes to survive temperature shifts, and may allow persistence of populations in the face of long-term environmental change (Ghalambor et al., 2007). As such, there is increasing interest in developing predictions of climate change impacts in tropical species through assessments of metabolic acclimation capacity over different time scales (Sandblom et al., 2014).

The aim of the present study is to establish an understanding of how predicted warming will affect the aerobic metabolic performance of the Nile perch, a fish of high food security importance in the Lake Victoria basin (LVB) of East Africa. The Nile perch is a large, piscivorous fish, which was introduced to the LVB in the mid-1900s from Lake Albert in western Uganda (Pringle, 2005) and became one of the most important commercial species in the region through the 1990s. Although Lake Victoria's Nile perch population is showing signs of decline, the fishery continues to be heavily exploited by a rapidly growing human population that is both economically and nutritionally dependent

on this species (Taabu-Munyaho et al., 2016). Although the impacts of climate change in East Africa are uncertain, water temperature increases of 0.2–1.5°C have been detected in Lake Victoria since 1900 (Sitoki et al., 2010; Marshall et al., 2013), and air temperature increases of 1.3–4.5°C are expected by the end of the 21st century (Ogutu-Ohwayo et al., 2016). The Nile perch provides an excellent model to test thermal tolerance hypotheses for two reasons. First, Nile perch are predicted to be sensitive to climate change as they inhabit relatively thermostable environments in both their historical range in Lake Albert (yearly range: 26–31°C, average maximum temperature 28°C; Balirwa et al., 2010) and their introduced range in Lake Victoria (yearly range: 24–30°C, average maximum temperature 27°C; Fig. S1). Second, Nile perch in Lake Nabugabo (near Lake Victoria) have displayed the ability to respond phenotypically to environmental variation in dissolved oxygen concentration and habitat type (Paterson et al., 2010; Nyboer and Chapman, 2013), indicating that this species has high potential for phenotypic adjustments when faced with environmental challenges.

The specific objectives of this study are to (1) determine the response of Nile perch metabolic traits [SMR, MMR, AS, excess post-exercise oxygen consumption (EPOC) and recovery time (RT)] and upper thermal tolerance limits [estimated by critical thermal maximum (CT_{max}) experiments] upon acute (3-day) exposure to a range of temperatures, (2) test for thermal plasticity of these traits in response to 3 weeks of acclimation and (3) compare CT_{max} and metabolic trait responses across exposure times. Based on predictions derived from the OCLTT framework, we expect to find an increase in SMR across temperature treatments, which may lead to a decrease in AS at higher temperatures. If Nile perch are able to make physiological adjustments with acclimation, we would expect to see a lower SMR in acclimated fish than in acutely exposed fish, and increases in magnitude of AS. However, it is possible that thermal acclimation will be physiologically costly (Nilsson et al., 2009), and we may find trade-offs in other physiological traits (i.e. EPOC) or reductions in growth or body condition over the acclimation period.

MATERIALS AND METHODS**Fish collection and holding**

Juvenile Nile perch (*Lates niloticus*) were collected at night using small-mesh boat seines from Entebbe Bay, Uganda, and transported by road to the Aquaculture Research Development Center in Kajjansi, Uganda, where they were stocked into two large (6×10×1 m) outdoor concrete tanks and maintained at 25°C until the start of acclimation trials.

This research was conducted under McGill University Animal Care Protocol 5029.

Three-week acclimation trials

A subset of Nile perch ($n=108$) was transported to the laboratory and randomly distributed among three holding systems containing fresh well water. Each holding system comprised three 200-litre replicate tanks and one 300-litre head tank (Fig. S2). Water from the head tank was filtered, oxygenated and circulated through the replicate tanks at an average rate of 120 l h⁻¹. Water temperature was regulated from the head tanks with Ecotherm® mt512 temperature controllers (Montréal, Québec, Canada) connected to submersible heating coils (Fig. S2). After 48 h of acclimation to laboratory conditions, temperatures were raised at a rate of 1°C day⁻¹ to generate three experimental temperature treatments of 27, 29 and 31°C. These levels were selected based on data collected from

inshore waters around Entebbe Bay (Fig. S1). The lowest experimental temperature was chosen to represent the average maximum temperature experienced by Nile perch in Lake Victoria (27°C; Fig. S1). While 27°C is at the upper edge of the Nile perch's current thermal environment, this water temperature is commonly recorded in Entebbe Bay, where Nile perch for this study were caught. The upper two temperatures encompass those that are predicted to occur under various emissions scenarios (IPCC, 2013).

Twelve Nile perch were stocked into each replicate tank (36 per temperature treatment) for use in both respirometry and CT_{max} trials. During acclimation, live tilapia fry were supplied for food at a rate of two fry per Nile perch per day. Water quality measures (temperature, oxygen, ammonia and nitrite) were taken every morning and evening. There were no significant differences among replicates in any water quality variable, and no differences among treatments in ammonia and nitrite concentration. In all replicates, water temperatures were maintained within $\pm 0.5^\circ\text{C}$ of the target temperature. For respirometry trials, four fish from each replicate tank (12 per temperature treatment) were used to measure oxygen consumption and four fish (12 per treatment) were used in CT_{max} trials (see below). Sample sizes were selected to ensure adequate power based on known levels of variability in respirometry data.

Acute exposure trials

After completing the acclimation trials, a new subset of Nile perch ($n=108$) was randomly selected from the outdoor holding ponds and distributed in the laboratory among the three holding systems for the acute (3-day) trials. Water temperature was gradually increased to 27, 29 and 31°C over 24 h, and then held at this temperature for 3 days (48 h in the replicate tank and 24 h in the respirometer). Following this, a third group of fish was brought in, and the same protocol was followed for 33 and 35°C to test metabolic parameters at extreme high temperatures.

Critical thermal maximum (CT_{max}) protocol

Upper thermal tolerance limits were estimated using established CT_{max} protocols (Fangue et al., 2006; Chen et al., 2013) for Nile perch from both exposure groups (3-week and acute) and all temperature treatments (27–35°C; see Table 1 for sample sizes and body size ranges). For each trial, four to five fish from a given temperature treatment were transferred in the evening into a water-filled cooler, and kept at the temperature of acclimation overnight (~ 8 h) to recover. In the morning, water temperatures in the coolers were raised at a rate of $0.3^\circ\text{C min}^{-1}$. This heating rate has been established as being low enough to allow the fish's body temperature to adjust, but rapid enough to prevent acclimation during the trial (Beitinger et al., 2000). Temperatures were steadily increased until the fish lost equilibrium for at least 10 s. At this time the temperature (CT_{max}) was recorded, and fish were removed from the cooler, weighed and measured, and placed in a bucket of aerated water for recovery.

Respirometry set-up

Oxygen consumption (\dot{M}_{O_2}) was measured using automated intermittent closed-system respirometry. The experimental set-up comprised four polypropylene respirometers (volume=1.57–2.80 litres) submerged in a 300-litre water bath (Fig. S2). Each respirometer was fitted with two sets of airtight tubing. The first formed a closed circulation loop fitted with a continuously operating water pump and a Firesting® fibre-optic cable focused on a contactless oxygen sensor spot (PyroScience Sensor Technology, Bremen, Germany). The second set of tubing was connected to a flush pump used to refresh water and oxygen levels inside the

respirometers. Flush pumps were automated to flush for 5 min of every 10-min loop. Dissolved oxygen levels were maintained above 80% saturation at all times. Fibre-optic cables and external temperature probes recorded oxygen and temperature every 2 s for the duration of each trial. Temperatures in the water bath were maintained within $\pm 0.2^\circ\text{C}$ of the target temperature. Respirometers were cleaned with bleach and rinsed thoroughly every 3 days.

Respirometry protocol

Time validation for Nile perch

Before beginning experimental trials, two tests were conducted to estimate the time required to obtain SMR for Nile perch, to detect diel cycles in activity and \dot{M}_{O_2} , and to investigate recovery times after handling stress and chasing (Clark et al., 2013a; Chabot et al., 2016). These tests were conducted in part because Nile perch had never previously been tested in a respirometer, and also because logistical constraints with power supply in the Kajjasi laboratory prevented running overnight trials, so we needed to determine whether SMR could be reached within an 8–12 h working day. In the first test, three Nile perch were transferred into the respirometers (without chasing). \dot{M}_{O_2} was measured for 48 h and plotted against time of day. In the second test, recovery times of three fish were compared after transfer to the respirometer (without chasing) and after a standard chase protocol (described below). Each fish was subjected to each treatment sequentially, and metabolic rates were measured for 16 h after entry into the respirometer. \dot{M}_{O_2} was plotted against time and inspected visually to identify the point at which \dot{M}_{O_2} levelled off at its lowest point (SMR). To verify that this visual inspection was accurate, the \dot{M}_{O_2} × time plot was imported into R studio v.0.99.482, where the 'breakpoints' function in the 'strucchange' package was used to compute optimal breakpoints in the \dot{M}_{O_2} × time regression relationship (Zeileis et al., 2015). Chow tests were then used to determine whether the coefficients of slopes before and after the breakpoints differed from one another (Zeileis et al., 2015). The time of the last optimal breakpoint was used for comparison with visual estimates. Recovery times were compared between the two stressors (chase versus transfer) within each fish.

We found that once metabolic rates had stabilized, they did not decrease further during the 48 h test period (Fig. S3A), nor did we detect diel variation over a 24 h cycle (Fig. S3B). \dot{M}_{O_2} measurements decreased rapidly post-transfer and post-chase, and levelled off after 2.4 and 3 h, respectively (Fig. S4). Recovery times (RT) of Nile perch were on average 45 min longer after chasing (average RT: 177 min) as compared with transfer without chasing (average RT: 143 min) (Fig. S4, Table S1). The breakpoint validation confirmed that our visual inspections were reasonably accurate, if conservative, with an average difference among estimates of +30 min (Table S1). From these findings we determined that a 9 h respirometry trial would be sufficient to obtain sequential measurements of MMR and SMR.

Respirometry trials

Metabolic traits (SMR, MMR, AS, EPOC and RT) were measured in every individual from both acclimated and acute trials (see Table 1 for sample sizes and body size ranges). In preparation for each trial, four Nile perch from a given temperature treatment were fasted for 48 h to ensure a post-absorptive state. Before starting a trial, the respirometry system was calibrated, and background oxygen consumption was measured for 20 min. We used a 3-min exhaustive chase protocol to induce MMR in Nile perch following methods described in Roche et al. (2013). In this protocol, fish were

Table 1. Sample sizes and body size [mass, standard length (SL) and total length (TL)] of acutely exposed and 3-week-acclimated Nile perch in each temperature treatment for oxygen consumption (\dot{M}_{O_2}) and critical thermal maximum (CT_{max}) trials

Trait	Temperature (°C)	N	\dot{M}_{O_2}			N	CT _{max}		
			Mean±s.e.m.	Min.	Max.		Mean±s.e.m.	Min.	Max.
3-week-acclimated									
Mass (g)	27	12	24.53±1.77	15.8	36.2	8	17.2±2.24	6.6	25.9
	29	11	29.49±4.72	7.3	54.5	7	18.2±2.22	9.6	28.8
	31	10	33.39±2.59	23.5	52.3	11	19.4±2.42	7.8	35.0
SL (cm)	27	12	11.6±0.33	9.6	13.4	8	10.2±0.50	7.6	12.2
	29	11	11.6±0.69	8.1	15.4	7	10.2±0.45	8.2	12.2
	31	10	12.7±0.35	10.9	14.2	11	10.5±0.43	7.9	12.8
TL (cm)	27	12	13.7±0.40	11.5	16.1	8	12.2±0.55	9.3	14.3
	29	11	13.8±0.82	9.6	18.2	7	11.9±0.58	9.7	14.7
	31	10	15.2±0.42	13.3	17.2	11	12.6±0.53	9.5	15.7
Acutely exposed (3-day)									
Mass (g)	27	10	21.5±2.18	11.1	34.1	7	11.6±1.97	8.0	21.6
	29	12	28.5±2.26	16.5	41.3	13	13.6±1.40	6.7	24.3
	31	10	28.2±2.56	18.1	42.8	11	12.1±1.81	6.1	24.1
	33	12	17.9±2.82	4.6	34.1	5	5.7±0.36	4.5	6.6
	35	9	15.2±3.44	6.2	35.8	5	5.6±0.69	4.6	8.4
SL (cm)	27	10	10.8±0.44	8.7	12.7	7	8.4±0.48	7.0	10.8
	29	12	11.6±0.32	9.7	13.0	13	9.1±0.35	7.3	11.4
	31	10	11.5±0.36	9.7	13.2	11	8.6±0.38	7.1	10.8
	33	12	9.9±0.60	6.5	12.2	5	6.8±0.19	6.3	7.3
	35	9	9.3±0.0	7.1	13.0	5	7.0±0.31	6.5	8.2
TL (cm)	27	10	12.9±0.52	10.4	15.4	7	10.2±0.56	8.7	12.6
	29	12	13.9±0.37	11.6	15.4	13	10.9±0.42	8.7	13.5
	31	10	13.7±0.46	11.6	16.3	11	10.5±0.44	8.8	13.0
	33	12	12.0±0.72	7.7	15.3	5	8.1±0.08	7.8	8.3
	35	9	11.3±0.81	8.7	15.5	5	8.5±0.40	7.9	10.1

transferred from their holding tank into a tub containing 25 litres of aerated water at the trial temperature. Fish were chased by hand within the tub for exactly 3 min; all fish were physically exhausted following the protocol. Each Nile perch was then weighed and measured [total length (TL) and standard length (SL)] and transferred into the respirometer within 20 s of the chase. This brief period of air exposure following the chase protocol can improve accuracy of MMR estimates when swimming respirometry is not possible (Roche et al., 2013). The respirometer was sealed rapidly following the transfer, and \dot{M}_{O_2} measurements were started immediately. Fish were then left to recover in the respirometers for a minimum of 9 h, until they reached their lowest metabolic rate. Fish were then removed and post-trial background respiration was quantified for 20 min.

Calculations of metabolic traits

Raw Firesting \dot{M}_{O_2} data files were processed with LabChart7 software (ADInstruments Inc., Colorado Springs, CO, USA) to calculate linear regressions between oxygen concentration and time for each 5-min closed-loop sample. Metabolic rates ($\text{mg O}_2 \text{ min}^{-1}$) were calculated from these regression slopes after accounting for respirometer volume and fish mass. We did not perform mass corrections ($\text{mg O}_2 \text{ min}^{-1} \text{ kg}^{-1}$) on metabolic data because of allometric effects of body mass on metabolic rate, but accounted for effects of body size in our statistical analysis by including body mass (M) as a covariate in the ANCOVA models (described below) (Packard and Boardman, 1988; García-Berthou, 2001). Background respiration (on average 4.1% of SMR and 1.4% of MMR) was subtracted from the metabolic rates by assuming a linear change between measures taken at the start and end of each trial.

MMR was estimated as the highest \dot{M}_{O_2} measurement recorded over any 3-min period within any 5-min loop. SMR was calculated

as the average of the lowest 10% of all \dot{M}_{O_2} measurements after complete recovery from exhaustive anaerobic exercise. This point was determined for each fish by plotting \dot{M}_{O_2} measurements against time, and visually assessing where the recovery curve levelled off, as described above. Outliers ± 2 s.d. from the mean of the lowest 10% were excluded from the calculation (Clark et al., 2013a). This method of calculating SMR meant that for a small proportion (10%) of fish, only one \dot{M}_{O_2} slope was used to estimate SMR. To ensure that this did not bias our results by underestimating SMR, we recalculated all SMR values with the lowest 30% of slopes. This caused only minimal changes to SMR estimates (average $0.003 \text{ mg O}_2 \text{ l}^{-1}$) and resulted in the same overall patterns. AS was calculated as the difference between MMR and SMR. EPOC, or the amount of oxygen required to recover from MMR, was calculated as the area under the \dot{M}_{O_2} curve estimated by the LOESS (locally weighted smoothing) recovery function until the values of the curve were equal to SMR. LOESS is a non-parametric method often used to fit a smoothed curve to data points in time series (Cleveland and Devlin, 1988), and is suitable for calculations of EPOC because estimates are comparable to summing individual \dot{M}_{O_2} values. Individual RT was calculated as the time (min) required for a fish's \dot{M}_{O_2} to return to SMR.

Growth and condition

To obtain basic estimates of how the process of acclimation impacted growth and body condition, each fish was measured for TL, SL and M prior to the start of the 3-week acclimation period and again just before respirometry. Fish were tagged using visible implant elastomer (Northwest Marine Technology, Shaw Island, WA, USA) during stocking for individual recognition. Daily specific growth rate (SGR) during the 3-week acclimation was

calculated for each individual using the following equation:

$$\text{SGR} = 100 \times \frac{[\log(M_f) - \log(M_i)]}{t}, \quad (1)$$

where M_f is the observed final mass at the time of respirometry, M_i is the observed initial mass at the start of the 3-week acclimation and t is the number of growth days. While the standardized food ration used during acclimation may bias growth data because of inter-individual differences in energetic requirements, M_i did not vary significantly among temperature treatments or replicates, and growth rate and condition ratio were not related to M_i in any of the treatments. We therefore do not expect this to have had a large effect when comparing growth rates among treatments. In addition, when fish are fed a specific ration (as they were in this study), growth rates are usually lower at higher temperatures (Allen and Wootton, 1982; Russell et al., 1996). We interpret our findings in light of this observation.

Condition (K) was calculated at the beginning and end of the 3-week acclimation period using LeCren's equation:

$$K = M/aL^b, \quad (2)$$

where M is the observed body mass, L is the observed total length, and a and b are species-specific constants obtained from the length–mass relationship: $M=aL^b$ (Froese, 2006). This relationship was derived by pooling data for all individuals in the 3-week acclimation experiment, and fitting a linear relationship between $\log TL$ and $\log M$. Change in K was calculated by dividing final by initial K , creating a condition ratio (K_{ratio}) where values >1 represent improved K over the course of the acclimation period.

Statistical analyses

Body mass and metabolic traits were \log_{10} transformed prior to analyses to meet assumptions of statistical tests. Effects of experimental temperature on CT_{max} and all metabolic parameters (SMR, MMR, AS, EPOC and RT) were tested within and between acute and 3-week exposure times. Differences within an exposure time were detected using univariate ANCOVA with experimental temperature as a fixed factor and $\log M_f$ as a covariate. The ANCOVA model accounts for variance in metabolic parameters owing to M by adjusting all rates to a common M based on pooled regression coefficients of the linear $M_{\text{O}_2} \times M$ relationship (García-Berthou, 2001). This method has often been used as an alternative to mass corrections when there are allometric effects of body size on the variable of interest (Packard and Boardman, 1987; Albrecht et al., 1993; García-Berthou, 2001). Differences between exposure times were tested by univariate ANCOVA with exposure time and experimental temperature as fixed factors, and $\log M_f$ as a covariate. Differences in SGR and K_{ratio} among temperatures for acclimated fish were assessed using univariate ANCOVA with $\log M_i$ as a covariate, to account for the influence of initial body size on growth and change in condition (Auer et al., 2015). In all analyses, interaction terms between the covariate and fixed effects were checked and removed from the model if not significant. All analyses were blocked for replicate, and followed by a Sidak multiple comparison procedure. Levene's test was used to confirm equality of error variances, and outliers ± 2 s.d. were removed from analyses.

RESULTS

Critical thermal maxima

Acutely exposed fish showed a significant overall increase in CT_{max} of 2.8°C over an 8°C temperature range (27–35°C; Table 2,

Table 2. Results of a univariate ANCOVA testing for differences in critical thermal maximum (CT_{max}), standard and maximum metabolic rate (SMR and MMR), aerobic scope (AS), excess post-exercise oxygen consumption (EPOC) and recovery time (RT), and fitness-related traits [condition ratio (K_{ratio}) and standard growth rate (SGR)] across experimental temperatures (27, 29 and 31°C) in acutely exposed (3-day-acclimated) and 3-week-acclimated Nile perch

Variable		Acutely exposed			3-week-acclimated			
		Temperature	Replicate	Mass (M_f)	Temperature	Replicate	Mass (M_f)	
CT_{max}	$F_{4,37}$	37.74	0.99	0.01	$F_{2,25}$	9.122	2.805	1.317
	P	<0.001	0.474	<0.001	P	0.022	0.057	0.267
	η^2	0.922	0.255	0.942	η^2	0.788	0.452	0.072
SMR	$F_{4,37}$	5.45	2.35	334.58	$F_{2,24}$	9.12	1.27	55.71
	P	0.011	0.029	<0.001	P	0.015	0.31	<0.001
	η^2	0.665	0.388	0.9	η^2	0.75	0.24	0.699
MMR	$F_{4,37}$	5.75	2.25	266.37	$F_{2,24}$	6.03	0.86	69.6
	P	0.009	0.036	<0.001	P	0.036	0.536	<0.001
	η^2	0.676	0.378	0.878	η^2	0.663	0.177	0.744
AS	$F_{4,37}$	2.9	3.18	135.65	$F_{2,24}$	2.401	0.698	26.02
	P	0.074	0.005	<0.001	P	0.169	0.698	<0.001
	η^2	0.519	0.462	0.786	η^2	0.437	0.138	0.52
EPOC	$F_{4,37}$	0.92	1.86	1.84	$F_{2,24}$	0.595	2.189	7.075
	P	0.485	0.083	0.183	P	0.581	0.08	0.014
	η^2	0.247	0.335	0.047	η^2	0.164	0.354	0.228
RT	$F_{4,37}$	3.57	2.39	<0.001	$F_{2,23}$	0.13	2.962	0.221
	P	0.042	0.027	0.99	P	0.88	0.027	0.643
	η^2	0.566	0.392	<0.001	η^2	0.041	0.436	0.01
CR					$F_{2,20}$	4.737	0.648	3.810
					P	0.067	0.666	0.065
					η^2	0.646	0.139	0.160
SGR					$F_{2,20}$	3.404	1.1250	7.882
					P	0.100	0.320	0.011
					η^2	0.562	0.239	0.068

Bold values are significant.

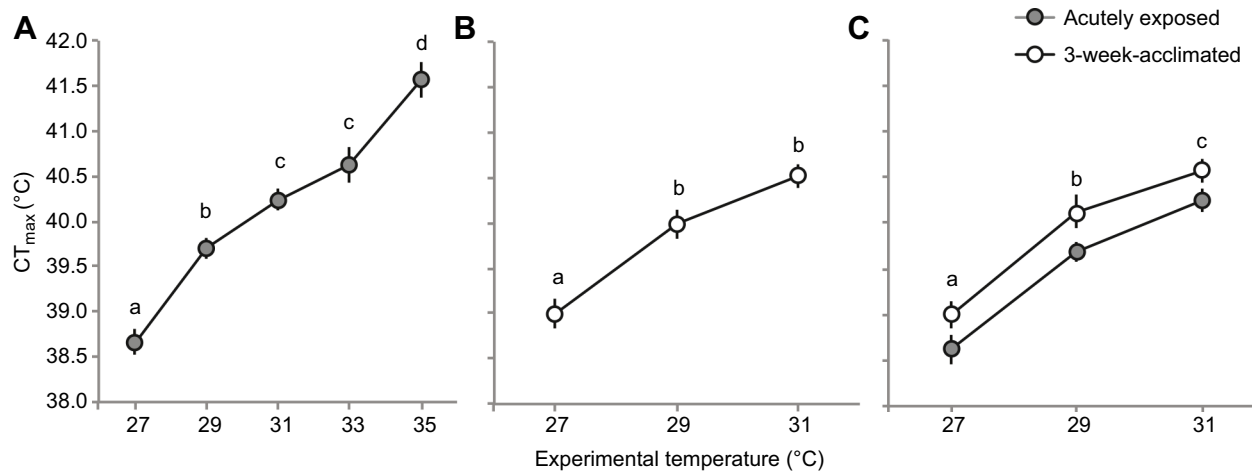


Fig. 1. Critical thermal maxima (CT_{max} ; mean \pm s.e.m.) for Nile perch across acclimation time and experimental temperature. (A,B) Results of ANCOVA testing for acutely exposed (A) and acclimated (B) Nile perch after exposure to a range of experimental temperatures. (C) Results of two-way ANCOVAs comparing CT_{max} across exposure time and experimental temperature. CT_{max} differed significantly among temperatures for both acclimation groups, and also differed among acclimation times (see Tables 2 and 3). Different lowercase letters in all panels indicate differences in CT_{max} among acclimation temperatures. In C, these letters indicate overall differences pooled within acclimation time.

Fig. 1A), with Nile perch exposed to 35°C having CT_{max} values approaching 42°C and a slope relating CT_{max} to acclimation temperature of 0.33 ± 0.024 . *Post hoc* tests revealed differences among all levels except 31 and 33°C (Fig. 1A). CT_{max} in 3-week-acclimated fish also increased significantly with acclimation temperature (Table 2, Fig. 1B), showing an overall increase of 1.5°C over a 4°C acclimation range (27–31°C) with a slope relating CT_{max} to acclimation temperature of 0.40 ± 0.058 . *Post hoc* tests revealed differences between 27 and 29°C and marginal differences between 29 and 31°C ($P=0.068$; Fig. 1B). Comparisons of acutely exposed and 3-week-acclimated fish revealed that longer acclimation led to higher CT_{max} at all temperatures (Table 3, Fig. 1C), with an average 0.5°C increase in CT_{max} in acclimated fish at a given temperature.

Metabolic traits: SMR, MMR, AS, EPOC and RT

SMR, MMR and AS all showed strong positive relationships with body mass for both acutely exposed and 3-week-acclimated fish (Fig. 2), a relationship that was consistent within each treatment \times acclimation time combination (Table 4). \dot{M}_{O_2} stabilized quickly after all trials, with an average RT of 308 min (5 h). This is longer than the 3 h determined in our time validation trials;

however, all fish reached SMR within 7.5 h, providing a minimum of 1.5 h (nine slopes) of \dot{M}_{O_2} measures for SMR calculation.

The metabolic performance of Nile perch was influenced both by experimental temperature and exposure time. For acutely exposed fish, there was a general increase in SMR and MMR with temperature (Table 2, Fig. 3A). The overall effect of temperature on AS in the full model was marginally significant; however, *post hoc* tests revealed a sharp increase between 29 and 31°C (Table 2, Fig. 3B). For acutely exposed fish, there were no differences among temperatures for EPOC, but RT was lowest in fish exposed to 35°C (Table 2, Fig. 3C,D). The effects of replicate were significant for SMR, MMR, AS and RT; however, partial η^2 values indicate a much lower influence than the treatment effect (Table 2). At the two highest temperatures in the acute exposure trial (33 and 35°C), Nile perch experienced high mortality rates, with 29% and 42% loss of all fish stocked into these treatments, respectively.

Three-week-acclimated fish showed a significant increase in SMR and MMR with temperature, but there was no effect of temperature treatment on AS (Table 2, Fig. 3E,F). *Post hoc* tests revealed that for SMR the main difference existed between the lower two levels (27–29°C) and 31°C, and for MMR between 27°C and the upper two levels (Fig. 3E). There were no differences among

Table 3. Results of univariate ANCOVA examining the effects of experimental temperature (27, 29 and 31°C), exposure time (acutely exposed, 3-week-acclimated) and their interaction on critical thermal maximum (CT_{max}), standard and maximum metabolic rate (SMR and MMR), aerobic scope (AS), excess post-exercise oxygen consumption (EPOC) and recovery time (RT) of Nile perch

Variables		Temperature (T)	Replicate	Exposure time (E)	Mass	T \times E
CT_{max}	$F_{1,44}$	36.67	1.85	8.31	0.64	0.1
	<i>P</i>	<0.001	0.111	0.006	0.427	0.903
SMR	$F_{2,53}$	25.557	0.772	31.402	168.454	4.267
	<i>P</i>	0.001	0.596	<0.001	<0.001	0.019
MMR	$F_{2,53}$	12.443	1.288	23.351	194.43	1.61
	<i>P</i>	0.006	0.279	<0.001	<0.001	0.21
AS	$F_{2,53}$	5.726	1.095	8.132	83.046	1.158
	<i>P</i>	0.037	0.378	0.006	<0.001	0.322
EPOC	$F_{2,53}$	1.252	1.374	8.41	3.932	0.821
	<i>P</i>	0.348	0.242	0.005	0.053	0.446
RT	$F_{2,52}$	1.201	2.063	27.671	1.968	0.593
	<i>P</i>	0.362	0.074	<0.001	0.167	0.556

Bold values are significant.

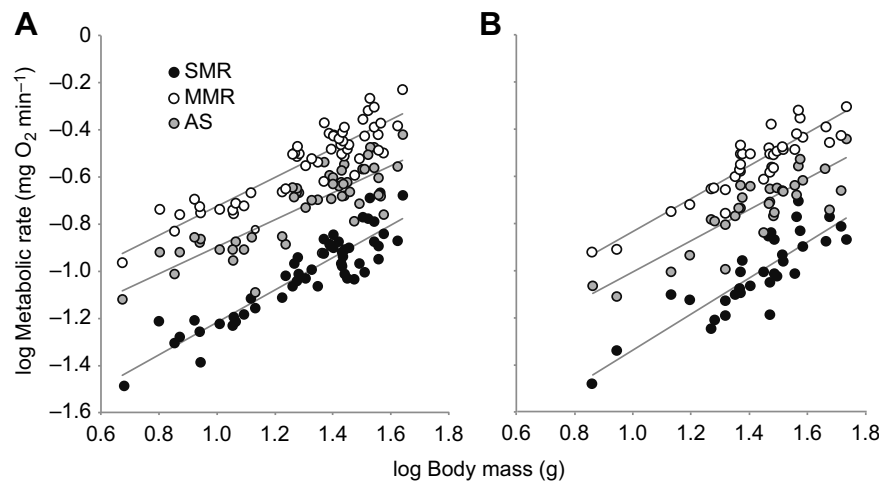


Fig. 2. Linear relationships between log body mass and log standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS).

(A,B) Results for acutely exposed (A; SMR: $R=0.914$, $P<0.001$; MMR: $R=0.896$, $P<0.001$; AS: $R=0.829$, $P<0.001$) and 3-week-acclimated (B; SMR: $R=0.856$, $P<0.001$; MMR: $R=0.908$, $P<0.001$; AS: $R=0.829$, $P<0.001$) Nile perch. Data from all temperatures were pooled for these figures, but trends were similar when separated by temperature treatment (see Table 4).

temperatures for EPOC or RT (Table 2, Fig. 3G,H) in 3-week-acclimated fish. The effects of replicate were non-significant for all models except RT, where again partial η^2 values indicate that they had only minor influence (Table 2).

SMR, MMR and AS were significantly lower in 3-week-acclimated fish compared with acutely exposed fish, especially at 31°C, where they showed 25%, 26% and 28% reductions, respectively (Fig. 4A,B, Table 3). Consistent with previous analyses, there were significant temperature effects on SMR, MMR and AS (Table 3); *post hoc* tests revealed significant differences between the lower two levels and 31°C for all three measures. Acutely exposed fish had a higher EPOC and longer RT than 3-week-acclimated fish (Fig. 4C,D, Table 3). The difference was especially pronounced at 27°C, where 3-week-acclimated fish showed a 46%

lower EPOC and a 23% faster RT. There were no differences among experimental temperatures in EPOC or RT (Fig. 4C,D, Table 3). Apart from SMR, there were no significant interactions between exposure time and experimental temperature, and no effects of replicate in any analyses (Table 3).

Growth and condition

While many experimental fish showed mass loss and a $K_{\text{ratio}} < 1$, temperature had a marginally positive effect on both variables. We proceeded with *post hoc* analyses given the marginal overall effect of temperature ($P=0.067$ for K_{ratio} and $P=0.1$ for SGR). Individuals at higher temperatures showed a greater improvement in body condition than those at cooler temperatures (Table 2, Fig. 5A), with K_{ratio} increasing by 12.0% and 10.8% from 27 to 29°C and from 27 to 31°C, respectively. Temperature also had a marginally positive effect on SGR; fish at higher temperatures had better growth rates (Table 2, Fig. 5B), with a 18% and 24% improvement in growth from 27 to 29°C and from 27 to 31°C, respectively.

Table 4. Pearson's correlations of body mass with metabolic traits [standard and maximum metabolic rate (SMR and MMR) and aerobic scope (AS)] for 3-week-acclimated and acutely exposed (3 days) Nile perch

Relationship	Temperature (°C)	R	P
3-week-acclimated			
SMR×body mass	27	0.697	0.012
	29	0.905	<0.001
	31	0.621	0.041
MMR×body mass	27	0.855	<0.001
	29	0.934	<0.001
	31	0.531	0.051
AS×body mass	27	0.818	0.001
	29	0.824	0.002
	31	0.305	0.363
Acutely exposed			
SMR×body mass	27	0.839	0.002
	29	0.766	0.004
	31	0.932	<0.001
	33	0.988	<0.001
	35	0.987	<0.001
MMR×body mass	27	0.855	0.002
	29	0.71	0.010
	31	0.931	<0.001
	33	0.985	<0.001
	35	0.936	<0.001
AS×body mass	27	0.771	0.009
	29	0.542	0.069
	31	0.909	<0.001
	33	0.979	<0.001
	35	0.785	0.012

Bold values are significant.

DISCUSSION

Results from this study were remarkably consistent, showing that juvenile Nile perch were able to make physiological adjustments that allow them to better tolerate higher temperature conditions. As a first line of evidence, both acutely exposed and 3-week-acclimated Nile perch exhibited plasticity in thermal tolerance as CT_{max} increased with acclimation temperature. Secondly, acutely exposed Nile perch are able to achieve high AS at temperatures above those commonly experienced in their natural habitat, with the optimal temperature for AS in acutely exposed fish registering at ~31°C. Acclimated fish were able to maintain AS across 27–31°C; however, they exhibited reductions in SMR, MMR and AS relative to acutely exposed fish, showing that longer exposure times induced different physiological responses to experimental temperatures. Decreases in \dot{M}_{O_2} in acclimated fish were accompanied by decreases in EPOC and RT across exposure time, indicating that drops in metabolic rate may reflect increased efficiency of cardiorespiratory function after acclimation. This finding is supported by improvements in growth and condition at higher temperatures over the acclimation period despite similar food rationing across temperature treatments.

Critical thermal maximum is affected by temperature and exposure time

Critical thermal tolerance limits have been measured in a wide variety of tropical and temperate fishes (Pörtner and Peck, 2010;

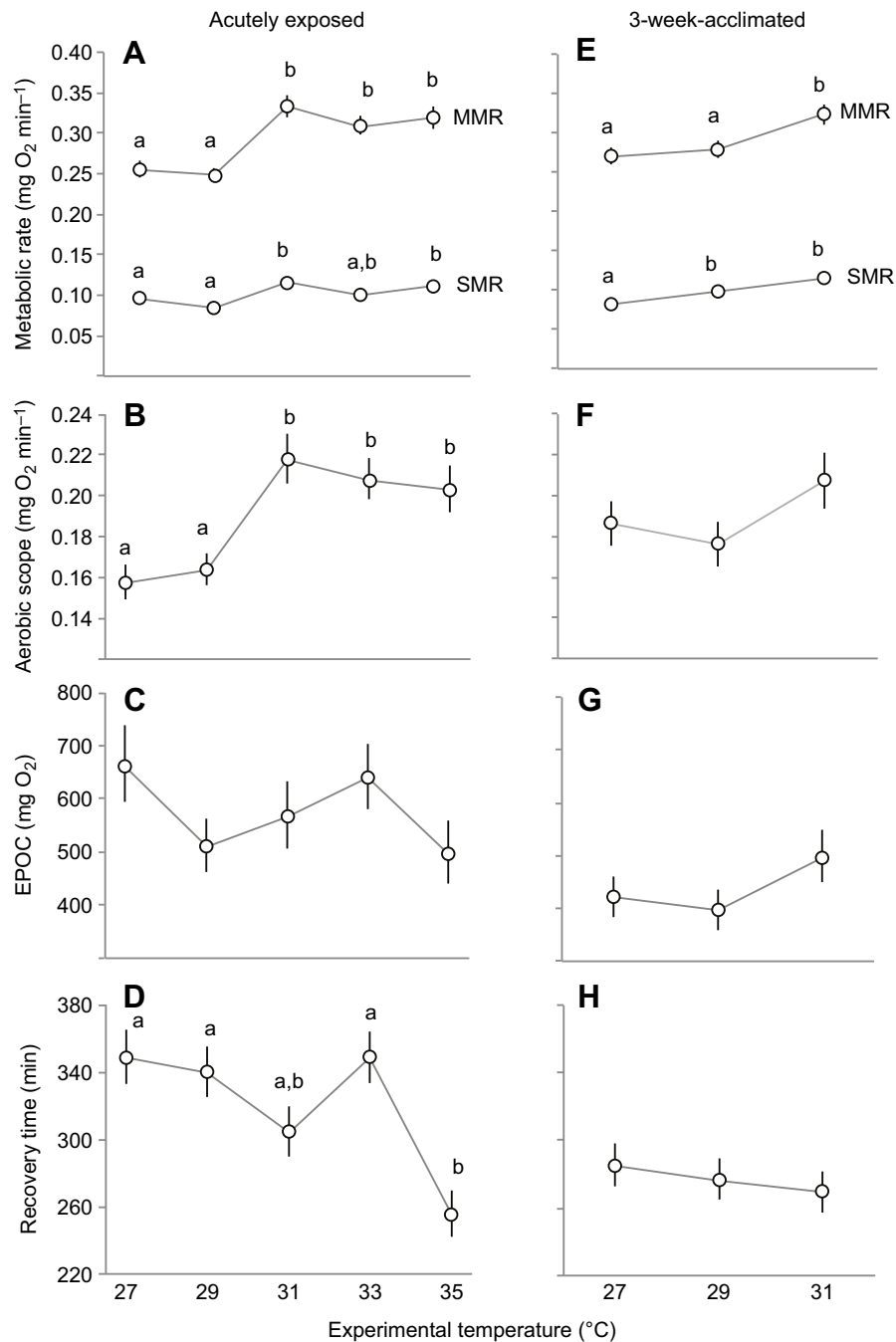


Fig. 3. Results of ANCOVA testing aerobic metabolic performance (means \pm 1 s.e.m.) of juvenile Nile perch over a range of experimental temperatures. (A–D) Acutely exposed fish; (E–H) thermally acclimated fish. From top to bottom: (A,E) standard metabolic rate (SMR) and maximum metabolic rate (MMR); (B,F) aerobic scope; (C,G) excess post-exercise oxygen consumption (EPOC); and (D,H) recovery time (see Table 2). Different lowercase letters indicate significant differences between measurements. Values are estimated marginal means adjusted to a common body mass of 26.7 g with mass exponents for each variable based on pooled regression coefficients within groups. For A and E, error bars are present but fall within the markers.

Beitinger et al., 2000) and provide essential baseline information for evaluating the relative ability of species to persist in the face of climate warming (Madeira et al., 2012; Stillman, 2003). Numerous fish species have demonstrated high acclimation capacity for CT_{max} (Beitinger et al., 2000), and results from the present study indicate that Nile perch follow this trend. Nile perch acutely exposed to 35°C were able to achieve CT_{max} values of nearly 42°C, which are among the highest measured in any fish (reviewed in Beitinger et al., 2000), and both acutely exposed and 3-week-acclimated Nile perch showed a linear increase in CT_{max} with acclimation temperature at a rate comparable to that of eurythermal, temperate species (Beitinger et al., 2000). In addition, longer acclimation allowed Nile perch to push their upper thermal tolerance limit 0.5°C higher than acutely exposed individuals at a given temperature, indicating that existing flexibility in thermal maxima may be further modulated to cope

with persistent temperature increases. This finding challenges the assumption that warm-adapted tropical species have limited capacity for coping with thermal stress as they are able to increase thermal limits at the same rate as species that are predicted to be more plastic. Having a flexible upper thermal tolerance may enhance Nile perch survival in elevated temperatures, particularly when variability in the system is high.

Metabolic variables are affected by temperature

Nile perch showed an increase in SMR, MMR and AS when acutely exposed to increased temperature, with all three variables plateauing after 31°C. In fish, the T_{opt} for metabolism is predicted to correspond to the most frequently encountered environmental temperature in the species' natural range (Asbury and Angilletta, 2010), or closely match historical temperatures encountered by

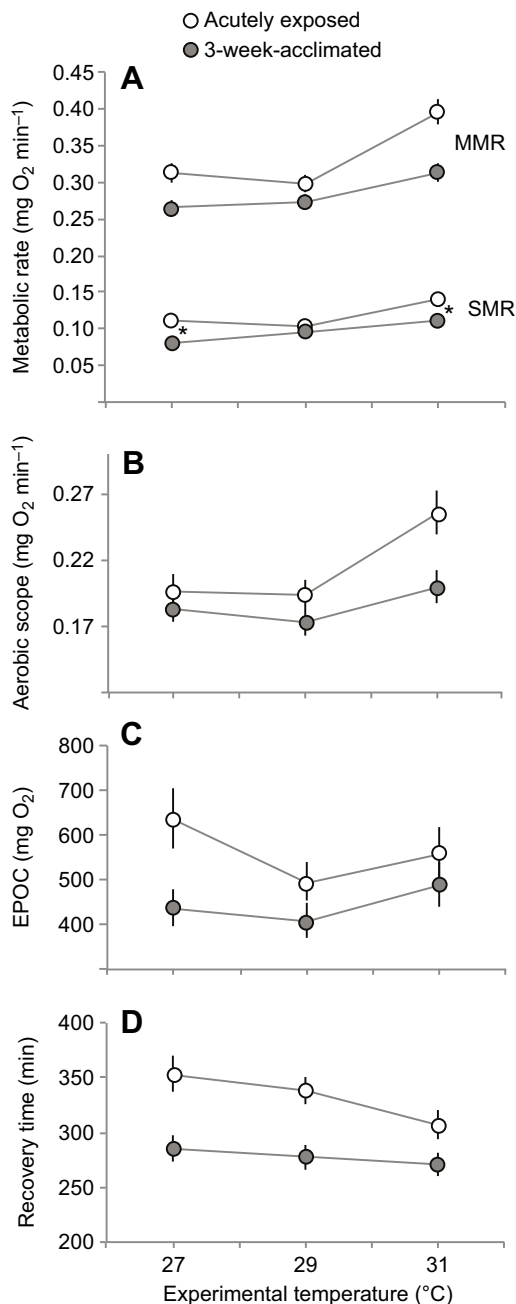


Fig. 4. Results of ANCOVA comparing aerobic metabolic performance (means ± 1 s.e.m.) among acutely exposed (open circles) and thermally acclimated (shaded circles) juvenile Nile perch over a range of temperatures. From top to bottom: (A) standard metabolic rate (SMR) and maximum metabolic rate (MMR); (B) aerobic scope; (C) excess post-exercise oxygen consumption (EPOC); and (D) recovery time (see Table 3). Values in these figures are estimated marginal means adjusted to a common body mass of 25.8 g with mass exponents for each variable based on pooled regression coefficients within groups. For A, error bars are present but fall within the markers. Asterisks in A indicate significant differences among acclimation times within temperature categories for tests where there was a significant interaction among fixed factors.

previous generations (Farrell et al., 2008; Eliason et al., 2011). For acutely exposed Nile perch, AS appears to peak at ~31°C, higher than mean temperatures in their historical and introduced ranges. In addition, acutely exposed fish were able to achieve very high AS even at temperatures that elicited clear signs of physiological stress

(high mortality rates) during the experiment. While the high AS values documented at 33 and 35°C could be due to selection imposed during the experiment, it is still ecologically meaningful that these individuals were able to achieve such responses as selection would have similar effects in natural settings. This finding is comparable to results from a growing number of studies showing that fish are capable of very high AS at temperatures well above their natural range (Clark et al., 2011; Norin et al., 2014), and are comparable to patterns found in Nile perch in a nearby lake (Lake Nabugabo) in the LVB (Chrétien and Chapman, 2016).

Similar to acutely exposed individuals, 3-week-acclimated Nile perch showed overall increases in SMR and MMR with elevated water temperature, but no change in AS. Given their thermo-stable background, it is remarkable that Nile perch are able to achieve the same AS at 29–31°C as at 27°C, especially because exposure to the higher two temperatures would have been extremely rare in their current environment (Lake Victoria) and in their historical range (Lake Albert). The fact that Lake Victoria Nile perch are near the upper edge of their geographic thermal range and are still able to make relatively rapid adjustments to high temperatures provides compelling evidence against the hypothesis that tropical ectotherms will be disproportionately negatively affected by small temperature increases. Much of the evidence for this assertion is derived from studies on terrestrial species (Tewksbury et al., 2008; Deutsch et al., 2008), while data on aquatic species are mixed with variation in acclimation capacity depending on micro-habitat, heating rate and population (Nguyen et al., 2011; Donelson and Munday, 2012). Further research is required to determine the generality of these patterns among tropical aquatic ectotherms.

Metabolic variables are affected by exposure time

Maintenance of AS in acclimated fish can be interpreted as evidence for metabolic compensation (Donelson and Munday, 2012); however, comparing patterns among acclimated and acutely exposed fish is key in evaluating plasticity in AS because metabolic responses can change dramatically with exposure time (Schulte et al., 2011). For example, acclimating Nile perch to 27, 29 and 31°C for 3 weeks resulted in 38%, 7% and 25% respective reductions in SMR compared with acutely exposed fish. Reductions in basal metabolic demands in acclimated fish are expected, and could be beneficial if they lead to lower nutritional requirements to sustain basal metabolism, more efficient metabolic performance and improved physiological functioning (Stillman, 2003; Pörtner, 2010; Donelson et al., 2011). Mechanisms leading to decreases in SMR span multiple levels of biological organization from whole animal to cellular and biochemical adjustments that collectively reduce metabolic expenditures. These include limitations in daily activity, reductions in gonadal development and growth, changes in cardiorespiratory operation, downregulation of mitochondrial function (e.g. change in density), and structural changes to biological membranes allowing for efficient uptake and retention processes in mitochondria and cells (Sinensky, 1974; Pörtner, 2001, 2002; Hazel, 1995; Seebacher et al., 2010). However, adjustments at any of these stages may result in physiological, functional and/or fitness-related trade-offs. For example, sea bass (*Dicentrarchus labrax*) that had higher temperature tolerance had better cardiac function and lower SMR, but also had reduced critical swimming speed compared with those with lower temperature tolerance (Ozolina et al., 2016).

Three-week-acclimated fish also had significantly reduced MMR and AS compared with acutely exposed fish, with the most extreme differences at 31°C, where acclimation resulted in a 26% reduction in

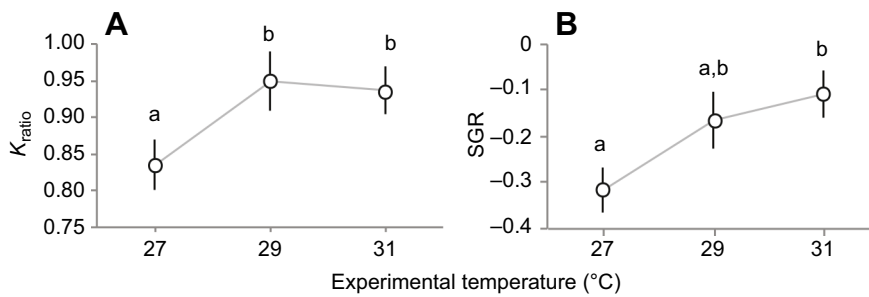


Fig. 5. Changes in condition and growth across experimental temperatures. Results of ANCOVA exploring differences in (A) condition ratio (K_{ratio} ; mean \pm 1 s.e.m.) and (B) specific growth rate (SGR; mean \pm 1 s.e.m.) for Nile perch during 3 weeks of acclimation to 27, 29 and 31°C.

MMR and a 28% reduction in AS. Metabolic theory predicts that acclimation should facilitate maintenance or increase of AS through a reduction in basal metabolic demands, while maximum metabolic rates remain high (Pörtner, 2010). However, in 3-week-acclimated Nile perch, drops in AS were instead due to large reductions in MMR relative to acutely exposed fish. Even though the cardiorespiratory system of Nile perch is capable of higher oxygen uptake, as evidenced by the high MMR and AS of acutely exposed fish, maintenance of this cardiorespiratory performance may be costly, particularly if cellular oxygen demand is lower in acclimated fish (Schulte, 2015). Although mechanisms underlying reductions in oxygen demand are not well known, these processes are certainly temperature dependent, and may be as or more thermally sensitive than processes involved in energy supply (Schulte, 2015). Fish must balance trade-offs between maintaining AS and conserving energy for other physiologically expensive activities. If high MMR is not needed for daily functioning, adequate growth or sufficient reproductive investment, then it may be unnecessary. Nile perch may therefore have the ability to modulate AS to conserve energy if high metabolic performance is not needed for daily activities (Norin et al., 2014).

Other performance measures provide insight

Given these patterns, it is important to integrate multiple interacting physiological and fitness-related traits with various exposure times to achieve a fuller picture of factors setting temperature tolerance limits in ectotherms. For Nile perch, AS in 3-week-acclimated fish never reached its maximum; however, there was evidence for improved growth rate and condition at 29 and 31°C relative to 27°C, suggesting that the lower MMR and AS did not represent loss of physiological capability. This important finding supports the hypothesis that the low SMR, MMR and AS in acclimated fish (relative to acutely exposed individuals) reflect improved energy utilization efficiency (Zeng et al., 2010) and indicate that allocation of energy to maintenance of high MMR may not be beneficial for individual fitness. While these changes in growth could be due to natural variations in energy requirements across temperatures, most studies show that higher temperatures elicit lower growth rates when fish are fed a standard ration (Allen and Wootton, 1982; Russell et al., 1996), so higher growth rates at the upper temperatures further support this conclusion.

In addition to higher growth at elevated temperatures, acclimated Nile perch had lower EPOC and faster RT than acutely exposed fish. A lower EPOC indicates that less oxygen is required to recover from exercise (e.g. rebuilding high-energy phosphates, restoring biochemical balances in metabolites, and replenishing stores of myoglobin and haemoglobin in the blood; Wood, 1991; Wang et al., 1994; Zeng et al., 2010), and a faster RT indicates that less time is needed to deliver sufficient oxygen to depleted tissues. EPOC and RT can have fitness consequences for ectotherms relying on anaerobic activities for survival (e.g. prey capture and predator avoidance), as prolonged recovery times may limit their capacity to perform repeated locomotive functions (Zeng et al., 2010). The

ability to improve EPOC and RT with acclimation may therefore be important to juvenile Nile perch, who are both predators and prey in their natural environment.

Our results suggest that acutely exposed Nile perch have a higher reliance on anaerobic metabolic pathways to support exhaustive chase and lower energy utilization efficiency (i.e. use more energy stores when exercised) than fish that have had time to adjust to high temperatures (Zeng et al., 2010). While this could explain the larger EPOC and a longer RT of acutely exposed fish, we did not establish the level of anaerobic effort by measuring lactate or pH in either muscle or blood, so this remains speculative. Another possible explanation is that 3-week-acclimated fish have developed improved aerobic metabolism, including more efficient oxygen uptake and/or delivery strategies. While improved cardiorespiratory efficiency may not intuitively fit with a reduced MMR, this pattern is not unprecedented (Zeng et al., 2010; Killen et al., 2015), and plastic changes in heart function have been shown to occur on very short time scales (Gamperl and Farrell, 2004; Franklin et al., 2007; Keen et al., 2017). Future studies that assess gill size and RVM in warm-acclimated Nile perch would help to elucidate whether such mechanisms underlie changes in metabolic efficiency.

Implications for climate change

Measurements of AS are often used to predict impacts of climate warming on aquatic ectotherms assuming that the thermal optimum for AS (T_{optAS}) coincides with the optimal temperature for physical condition and other fitness measures (T_{optFIT}). While some studies have confirmed this (Brett, 1971; Del Toro-Silva et al., 2008; Grenchik et al., 2013), there is mounting evidence that temperatures that maximize AS do not always match those that elicit the highest growth rate or reproductive output (Healy and Schulte, 2012; Ern et al., 2014; Gräns et al., 2014; Norin et al., 2014). Likewise, the relative drops in AS in acclimated Nile perch do not necessarily indicate physiological weakening, as these individuals also show lower EPOC, faster TR, and higher growth and condition ratio at high temperatures. Results such as these challenge the assumption that AS is an important determinant of evolutionary fitness in Nile perch, and indicate that limitations in AS may not be the primary physiological restraint acting on fishes' thermal optima, nor are they likely to be the direct cause of fitness declines in these species (Schulte, 2015). In addition, fishes that show the ability to maintain AS well above their most frequently encountered temperatures may provide an argument against the relevance of T_{optAS} for predicting responses of fish species to climate change (Clark et al., 2013b; Schulte, 2015). The increasing trend in SMR and MMR, and the lack of change in AS up to 31°C in acclimated Nile perch could indicate that our experimental temperatures did not push juvenile Nile perch past their T_{optAS} ; however, considering that 31°C is at the upper edge of ecological relevance for this species, this further calls into question the power of T_{optAS} to predict T_{optFIT} in Nile perch (Clark et al., 2013b).

Finally, our study highlights the importance of acclimation when experimentally predicting performance of ectotherms at high temperatures. In the context of climate change, it is important to distinguish short-term from long-term responses to understand how persistent temperature increases may change, because phenotypic plasticity acting over different exposure times has the capacity to differently alter the position of the thermal optimum. Considering the T_{opt} of only acutely exposed or acclimated fish may cause erroneous conclusions when developing predictions of the effects of climate change (Schulte et al., 2011). It is therefore possible that the advanced thermal compensation mechanisms achieved by Nile perch could lead to resilience of Nile perch populations to warming lake temperatures despite apparent declines in acclimated fish in AS relative to acutely exposed fish.

Conclusions

Results from this study challenge assumptions about tropical species' metabolic capabilities, and predictions from the OCLTT hypothesis that higher temperatures will be detrimental owing to limitations in AS. We found that Nile perch have a significant capacity to acclimate to elevated water temperatures, and physiological adjustments made over short time scales could pave the way for reducing the impacts of global warming and improve the chances that Nile perch populations will persist under predicted warming scenarios. This is a significant finding considering the economic and food-security importance of this species in East Africa; however, longer exposure times spanning the lifetime of the organism, studies across life-history stages, and investigations into trans-generational plasticity and genetic adaptation would improve our predictions by elucidating long-term effects of chronic thermal stress, which may have consequences for population fitness and fishery sustainability.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.N., L.C.; Methodology: E.N., L.C.; Validation: E.N.; Formal analysis: E.N., L.C.; Data curation: E.N.; Writing - original draft: E.N.; Writing - review & editing: E.N., L.C.; Supervision: L.C.; Project administration: L.C.; Funding acquisition: E.N., L.C.

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Data availability

Data are available from the Dryad Digital Repository (Nyboer and Chapman, 2017): <http://dx.doi.org/10.5061/dryad.2s590>.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.163022.supplemental>

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