© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

RESEARCH ARTICLE

Cardiac plasticity influences aerobic performance and thermal tolerance in a tropical, freshwater fish at elevated temperatures

Elizabeth A. Nyboer* and Lauren J. Chapman

ABSTRACT

Fishes faced with novel thermal conditions often modify physiological functioning to compensate for elevated temperatures. This physiological plasticity (thermal acclimation) has been shown to improve metabolic performance and extend thermal limits in many species. Adjustments in cardiorespiratory function are often invoked as mechanisms underlying thermal plasticity because limitations in oxygen supply have been predicted to define thermal optima in fishes; however, few studies have explicitly linked cardiorespiratory plasticity to metabolic compensation. Here, we quantified thermal acclimation capacity in the commercially harvested Nile perch (*Lates niloticus*) of East Africa, and investigated mechanisms underlying observed changes. We reared juvenile Nile perch for 3 months under two temperature regimes, and then measured a series of metabolic traits (e.g. aerobic scope) and critical thermal maximum (CT_{max}) upon acute exposure to a range of experimental temperatures. We also measured morphological traits of heart ventricles, gills and brains to identify potential mechanisms for compensation. We found that long-term (3 month) exposure to elevated temperature induced compensation in upper thermal tolerance (CT_{max}) and metabolic performance (standard and maximum metabolic rate, and aerobic scope), and induced cardiac remodeling in Nile perch. Furthermore, variation in heart morphology influenced variations in metabolic function and thermal tolerance. These results indicate that plastic changes enacted over longer exposures lead to differences in metabolic flexibility when organisms are acutely exposed to temperature variation. Furthermore, we established functional links between cardiac plasticity, metabolic performance and thermal tolerance, providing evidence that plasticity in cardiac capacity may be one mechanism for coping with climate change.

KEY WORDS: Climate change, Thermal plasticity, Nile perch, Tropical fisheries, Heart remodeling, Acclimation capacity

INTRODUCTION

Climate change models forecast persistent, global temperature increases of 1–4°C, and increases in the frequency of extreme climatic events over the next century (IPCC, 2014; Seneviratne et al., 2014). Such drastic changes will directly affect aquatic ecosystems, and threaten the ecological and physiological stability of fish species in freshwater habitats (Ficke et al., 2007). As ectotherms, fish depend on their thermal environment to regulate metabolic functions, and when water temperatures exceed specific optima they face performance limitations at all levels of biological organization. To cope, fish must either relocate to more suitable habitats or adapt to novel conditions through genetic change and/or environmentally induced phenotypic plasticity (Schulte et al., 2011; Seebacher et al., 2014). Fish species often modify physiological functioning through thermal acclimation (Angilletta, 2009; Seebacher et al., 2014), a type of phenotypic plasticity that can alter thermal tolerance limits and optimize performance under novel temperature regimes (Schulte et al., 2011). Capacity for thermal acclimation varies dramatically among species, and is hypothesized to be lower in tropical fish that experience little seasonal variation in their thermal environment (Tewksbury et al., 2008). Given the significance of many inland tropical fishes to local and regional food security (FAO, 2014; Lynch et al., 2016), it is of increasing importance to understand the capacity for and mechanisms underlying modifications of thermal tolerance in these species.

For ectotherms, metabolic rate sets the pace for many physiological functions, and can have important fitness implications (Brown et al., 2004). Measurements of metabolic performance can therefore serve as comprehensive indicators of physiological condition (Sibly et al., 2012), and can be used to assess ability to cope with various environmental stressors (Brown et al., 2004). Key metabolic parameters often measured in this context include standard and maximum metabolic rate (SMR and MMR, respectively), which represent the lower and upper limits of oxygen uptake for fishes. SMR corresponds to basal oxygen demand, or the lowest possible metabolic rate in a resting, post-absorptive fish (Fry, 1971; Brett and Groves, 1979), and MMR corresponds to the maximum rate of oxygen consumption, usually measured after exhaustive exercise (Fry, 1971; Clark et al., 2011; Roche et al., 2013; Norin and Clark, 2016). Aerobic scope (AS), which is calculated as the difference between SMR and MMR, determines the range of oxygen-demanding processes that can be performed simultaneously by a fish, and is thought to be a key mechanism determining energy allocation, fitness and biogeography in ectotherms (Pörtner and Farrell, 2008; Pörtner, 2010; Clark et al., 2013).

As first observed by Fry (1947), and quantified in several recent studies (Healy and Schulte, 2012; Gräns et al., 2014; Norin et al., 2014), SMR increases exponentially with temperature, whereas MMR is predicted to increase initially, and then decline at the highest temperatures, ultimately bringing about a decline in AS at high temperatures. According to the oxygen- and capacity-limited thermal tolerance (OCLTT) concept, declines in performance (i.e. AS) at high temperatures are predicted because the cardiorespiratory system cannot keep pace with oxygen demands in respiring tissues as increasing temperatures elevate metabolic demands (Pörtner and Knust, 2007; Pörtner et al., 2017). Mismatches between oxygen supply and demand are therefore thought to define thermal limits in teleost fishes, and may have negative fitness consequences (e.g. reductions in body mass or condition) if, as predicted, drops in AS are

Department of Biology, McGill University, Office N3/11, Stewart Biology Building, 1205 Avenue Docteur Penfield, Montreal, Quebec, Canada, H3A 1B1.

*Author for correspondence (elizabeth.nyboer@mail.mcgill.ca)

E.A.N., 0000-0003-3004-009X

Received 30 January 2018; Accepted 4 June 2018

Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

List of symbols and abbreviations

AGFL	average gill filament length
AS	aerobic scope
AS_{Q10}	Q_{10}-corrected aerobic scope
CT_{max}	critical thermal maximum
$CT_{max,adj}$	adjusted critical thermal maximum
DO	dissolved oxygen
FBL	filament base length
FD	filament density
HSI	hepatosomatic index
K	LeCren's condition factor
M_b	body mass
MMR	maximum metabolic rate
MMR_{Q10}	Q_{10}-corrected maximum metabolic rate
$\dot{M}_{O_2}$	oxygen consumption rate
OCLTT	oxygen- and capacity-limited thermal tolerance
RVM	relative ventricular mass
SL	standard length
SMR	standard metabolic rate
SMR_{Q10}	Q_{10}-corrected standard metabolic rate
T_{avg}	average rearing temperature (~25°C)
TFN	total filament number
TGFL	total gill filament length
THA	total hemibranch area
TL	total length
T_{warm}	warm rearing temperature (~29°C)
V_b	total cardiac output (volume of blood pumped per minute)
%CM	percent of compact myocardium

causally linked to key processes such as growth (Pörtner and Knust, 2007). A corollary of this hypothesis is that alterations to the cardiorespiratory system that increase efficiency of oxygen uptake and delivery can improve thermal tolerance and aerobic performance in fishes exposed to elevated water temperatures. Although the OCLTT hypothesis proposes a clear mechanistic explanation for thermal limitation, numerous recent empirical studies have shown that declines in performance at high temperatures may be driven by mechanisms other than a mismatch between oxygen supply and demand, and have provided convincing evidence that optimal temperatures for AS do not necessarily elicit maximal performance in all fitness-related measures (Clark et al., 2013; Gräns et al., 2014; Norin et al., 2014; Wang et al., 2014; Brijs et al., 2015; Ern et al., 2016), raising questions about the broad applicability of this framework (Jutfelt et al., 2018). In addition, there are many pathways for fish to respond to oxygen limitation, scaling from whole-body to cellular levels (Pörtner, 2002; Angilletta, 2009; Biro and Stamps, 2010), and alterations to any of these pathways can lead to improvements in metabolic performance (e.g. reduced basal oxygen demand) and maintenance of fitness-related traits.

Nevertheless, cardiorespiratory function may still play an important role in metabolic compensation, and investigating relationships between cardiorespiratory traits and thermal tolerance and/or metabolic performance can allow a straightforward test of at least one prediction generated by OCLTT. If cardiorespiratory function can sustain performance at high temperatures, plastic changes in heart and gill morphology that improve oxygen uptake and delivery may be key to acclimation capacity (Sollid et al., 2005; Farrell et al., 2009; Dalziel et al., 2012; Anttila et al., 2013; Jayasundara and Somero, 2013). Both heart and gill morphology have been shown to change with exposure to increased temperatures (Sollid et al., 2005; Klaiman et al., 2011; McBryan et al., 2016; Keen et al., 2017). The heart is the primary power generator for the circulatory system, and is often a central focus in investigations of physiological plasticity in fishes (Farrell et al., 2009; Jayasundara and Somero, 2013). Changes in temperature have a significant effect on heart rate in teleosts, which influences total cardiac output (V_b, volume of blood pumped per minute). However, morphological changes induced by long-term exposure to different water temperatures can facilitate maintenance of V_b across a wide thermal range (Gamperl and Farrell, 2004; Klaiman et al., 2011; Keen et al., 2017). For example, fishes exposed to warmer temperatures generally have decreased relative ventricular mass (RVM), leading to reduced stroke volume (Keen et al., 2017); however, V_b can be maintained through improved force of contraction (Gamperl and Farrell, 2004), which is often enabled by adjustments to the proportions of the myocardial layers (i.e. spongy versus compact) in the ventricle. Gills, in contrast, function primarily in oxygen uptake. Changes in the environment that lead to new respiratory or osmoregulatory demands can result in alterations to gill structure over varying time scales (Sollid et al., 2003, 2005; Langerhans et al., 2007; Chapman et al., 2008). For example, fish that are exposed to low dissolved oxygen (DO) concentrations can increase gill size, which presumably improves efficiency of oxygen uptake (Chapman et al., 2008). In addition, elevated temperature may affect other physiologically demanding organs if the bulk of energetic resources are allocated to basal functions. For example, brain size has been observed to decrease under stressful conditions (Crispo and Chapman, 2010; Toli et al., 2016). Given the high metabolic cost associated with the development of large brains, the ability to reduce brain size through plasticity when a large brain is not critical for fitness is likely to be favored (Crispo and Chapman, 2010).

Alterations in cardiorespiratory function and morphology have been shown to improve thermal tolerance and performance in a number of fish species (Eliason et al., 2011, 2017; Anttila et al., 2013; Keen et al., 2017). For example, increases in cardiac scope correlate positively with aerobic scope within populations of sockeye salmon (Eliason et al., 2011), and larger RVM correlates positively with upper thermal tolerance limits (i.e. critical thermal maximum, CT_{max}) in some populations of Atlantic salmon (Anttila et al., 2013). Likewise, gills can undergo surface area increases that improve oxygen uptake upon exposure to warmer temperatures (McBryan et al., 2016); however, few studies have explicitly linked plasticity in heart or gill morphology to metabolic compensation, and these patterns have, to our knowledge, never been tested in tropical fishes. In addition, little is known about how developmental plastic responses will benefit fishes faced with rapidly changing thermal environments (i.e. acute exposure to novel temperatures), which is relevant when predicting climate change effects where both long-term increases and rapid changes in temperature are expected.

In this study, we investigated these patterns in a tropical, freshwater teleost, the Nile perch [*Lates niloticus* (Linnaeus 1758)], a large, primarily piscivorous fish that was introduced to the Lake Victoria basin in East Africa in the mid-20th century, and currently forms the basis of a lucrative 250–310 million USD yr^{-1} export fishery (Indian Ocean Commission, 2015). Nile perch regularly experience temperatures in the range of 23–27°C in Lake Victoria, and similarly stable temperatures in their ancestral habitat in Lake Albert (range: 26–31°C; Nyboer and Chapman, 2017). Despite these narrow thermal ranges, recent studies have demonstrated high levels of acclimation capacity in metabolic rate and upper thermal tolerance of Nile perch under both short (3 day) and longer-term (3 week) exposures (Chrétien and Chapman, 2016; Nyboer and Chapman, 2017), making it an interesting subject for testing responses over longer acclimation times (i.e. time enough for developmental differences to take place) and for evaluating

Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

mechanisms underlying thermal flexibility. The objectives of this study were to: (1) determine the acclimation capacity of upper thermal tolerance limits (CT_{max}) and metabolic rates of juvenile Nile perch reared for 3 months under elevated temperature, (2) examine links between variation in metabolic function, body mass and condition, and plastic responses in oxygen uptake and delivery capacities of cardiorespiratory organs and (3) assess whether plastic changes associated with rearing are beneficial for coping with rapid temperature changes (i.e. acute exposures to a range of temperatures).

We predicted that we would detect plasticity in metabolic traits and CT_{max} leading to metabolic compensation in fish reared under warmer conditions. We also anticipated finding differences in heart and/or gill morphology among rearing regimes; specifically, we expected smaller RVM, thicker compact myocardium and larger gill sizes in warm-reared fish, as these are possible mechanisms for coping with the increased oxygen demand likely to be experienced under elevated temperatures. If these morphological changes improve thermal tolerance and metabolic performance, we would expect to find relationships among these traits within rearing groups.

MATERIALS AND METHODS

Overview

Wild-caught juvenile Nile perch were captured in July and reared for 3 months under two temperature regimes, one resembling annual average temperatures in Lake Victoria (∼25°C maximum; control) and one resembling temperatures predicted under climate change (∼29°C maximum). Fish from these rearing temperatures were then acutely exposed in the laboratory (3 days) to a range of experimental temperatures encompassing both rearing temperatures and a higher temperature (33°C) not previously experienced in either regime, thereby testing their ability to cope with rapid changes in temperature. SMR, MMR, AS and CT_{max} were measured, and organs (livers, hearts, gills, brains) were examined to identify potential mechanisms for metabolic compensation. This research was conducted under McGill University Animal Care Protocol 5029.

Fish collection and rearing

Young (<2 months old) juvenile Nile perch [12.4±0.67 cm total length (TL), mean±s.e.m., n=600] were collected by fishers in July 2015 with small-mesh beach seines at Entebbe Bay, Uganda. Specimens were transported to the Aquaculture Research and Development Center in Kajjansi, Uganda, where they were stocked into six large (6×10×1 m) outdoor concrete aquaculture ponds and reared for 3 months (2 July to 5 October 2015). This rearing period was chosen as we anticipated this time frame would allow for some morphological and developmental responses to occur based on Nile perch growth rates (Nkalubo, 2012), and because it spanned a portion of one dry and one rainy season, capturing some natural seasonal variation. The ponds were organized into three blocks, each with one replicate of the two thermal regimes described above (Fig. S1A). These rearing temperatures shall be referred to as the average rearing temperature, or T_{avg} (∼25°C), and the warm rearing temperature, or T_{warm} (∼29°C). To create these divergent thermal regimes, wooden frames were constructed over each pond and covered with either greenhouse plastic (TeraMax®, Teris, Montréal, QC, Canada) or agricultural shade-cloth (GreenTek Shade-Rite®, Teris, Montréal, Québec; Fig. S1B). Prior to stocking, pond water quality parameters including temperature (°C), luminosity (i.e. lux, expressed in lm m^{-2}), DO, turbidity (Secchi depth, expressed in cm) and pH were carefully monitored for 4 months to ensure that water temperatures and physicochemical conditions were stable. Each pond was equipped with flow-through inlet and outlet mechanisms so that water inside the ponds could be flushed directly with ∼25°C local river water (see Fig. S1B for a schematic diagram of the pond set-up).

During stocking, all ponds were filled with river water and maintained at the natural ∼25°C. Experimental fish (n=100 per rearing pond) were weighed to the nearest 0.01 g, and measured for TL and standard length (SL) to the nearest 0.1 cm to ensure uniformity of initial stocking size (Table S1). After 3 days, T_{warm} ponds were heated to ∼29°C over 48 h. This was accomplished by temporarily cutting inflow of cool river water to the T_{warm} ponds, which allowed the water in the ponds to warm under the greenhouse

Table 1. Results of two-way ANOVA on water quality variables

Variable		Rearing temperature	Block	Rearing temperature×Block
Maximum temperature (°C)	$F_{1,511}$	1832.797	5.866	9.337
	P	**<0.001**	**0.003**	**<0.001**
	η^2	0.782	0.022	0.035
Minimum temperature (°C)	$F_{1,511}$	1766.408	18.962	10.882
	P	**<0.001**	**<0.001**	**<0.001**
	η^2	0.776	0.069	0.041
Lux (lm m^{-2})	$F_{1,511}$	29.049	0.848	9.249
	P	**<0.001**	0.429	**<0.001**
	η^2	0.048	0.003	0.031
Average DO (mg l^{-1})	$F_{1,377}$	0.428	0.694	
	P	0.513	0.500	
	η^2	0.001	0.004	
pH	$F_{1,232}$	1.819	0.59	
	P	0.179	0.555	
	η^2	0.008	0.005	
Secchi	$F_{1,372}$	1.081	1.279	
	P	0.299	0.28	
	η^2	0.003	0.007	

Variables include maximum and minimum water temperature, lux (luminosity), dissolved oxygen (DO), pH and turbidity (Secchi depth), across rearing temperatures (T_{avg}, T_{warm}) and among blocks. Bold P-values indicate significance at α<0.05.

covers. During this time, DO concentrations were monitored to ensure maintenance of adequate oxygen levels. After T_{warm} ponds had warmed to the desired temperature (~29°C), all ponds (T_{avg} and T_{warm}) were flushed each day for the same amount of time with fresh river water. The large volume (60 m^3) of the ponds ensured that the T_{warm} ponds stayed within 3°C of the desired temperature, even during periods of flushing. Throughout the experiment, water quality parameters (temperature, luminosity, DO, turbidity and pH) were recorded twice daily, once in early morning and once in mid-afternoon to capture daily fluctuations. Twenty-four-hour HOBO™ light and temperature loggers (Onset®, Bourne, MA, USA) took hourly temperature and luminosity readings for the duration of the rearing period (see Table S2, Fig. S2 for a summary of all water quality parameters). To determine how water quality parameters varied between rearing temperatures (T_{avg} and T_{warm}) and among rearing pond replicates (blocks 1, 2 and 3), data from the HOBO loggers (temperature and lux) and from daily water sampling (DO, turbidity and pH) were plotted over time for visual assessment (Fig. S2A–E) and analyzed with two-way ANOVA, with rearing temperature and block as fixed effects. Water temperatures in the T_{warm} rearing ponds had higher maximum (28.6°C) and minimum (27.1°C) daily temperature than those in the T_{avg} regime (maximum=24.5°C; minimum=23°C; Table 1; Table S2, Fig. S2A). There were significant effects of block and block×rearing temperature; however, the effect size (η^2) of these factors was small compared with rearing temperature alone (Table 1). *Post hoc* tests revealed that differences among blocks were only apparent in the T_{warm} rearing tanks, with replicate 1 of T_{warm} significantly warmer than replicates 2 (P=0.002) and 3 (P<0.001). Average lux (lm m^{-2}) also differed among rearing temperatures, with T_{warm} having higher luminosity than T_{avg}; however, the effect size was small, and differences were apparent only in blocks 1 and 3 (Table 1). There were no differences in any other water quality variables (DO, turbidity, pH) between rearing temperatures or among blocks (Table 1, Table S2, Fig. S2B–E).

During rearing, Nile perch were fed daily on live, aquaculture-reared Nile tilapia fry, which were graded to ensure appropriate prey sizes. Daily feeding rations were calculated to equal ~15% of the biomass of Nile perch stocked in each pond to ensure adequate food supply at rates similar to natural conditions as estimated by Nile perch bioenergetic analyses (Kitchell et al., 1997) and from previous trials of Nile perch rearing. The levels were adjusted based on estimates of mortalities and estimated growth rates of Nile perch in the ponds.

Laboratory experiments

After completing 3 months in the rearing ponds, Nile perch were transported from ponds to the laboratory, where they were placed in temperature-controlled tanks (n=4 per tank) containing filtered, oxygenated well water (see Nyboer and Chapman, 2017 for a description of the laboratory system). All fish were individually marked using Visible Implant Elastomer (Northwest Marine Technology Inc., Shaw Island, WA, USA). We employed a fully crossed experimental design for laboratory tests so that individuals from each rearing temperature (T_{avg} and T_{warm}) and block (3 replicates) were subjected to each experimental laboratory temperature for metabolic rate experiments (Fig. S1A, Table 2). Laboratory tanks were initially held at the average maximum rearing temperature (29 or 25°C) and then slowly raised or lowered to the desired experimental temperature (25, 29 or 33°C) over a 12 h period, and then held for 3 days. Fish were brought into the laboratory in a staggered fashion so that each batch was held for the same 12 h+3 day period before experimental trials. On the first day of acclimation, Nile perch were fed two tilapia fry each, and

Table 2. Sample sizes (N) and means±s.e.m. of body mass (M_b), standard length (SL), total length (TL) and LeCren's condition factor (K) of juvenile Nile perch used in respirometry experiments

Rearing temperature	Experimental temperature and block	N	M_b (g)	TL (cm)	SL (cm)	K
T_{avg}	25	7	49.11±4.45	16.98±0.17	14.2±0.24	1.01±0.015
	1	2	34.12±2.01	15.55±0.04	13±0	0.97±0.023
	2	2	51.4±2.18	17.3±0.02	14.35±0.09	1.03±0.007
	3	3	57.57±4.83	17.73±0.14	14.9±0.21	1.01±0.017
	29	9	30.95±3.26	14.66±0.24	12.26±0.3	1.04±0.026
	1	2	25.56±3.34	13.95±0.1	11.65±0.06	1.06±0.04
	2	3	34.19±1.87	15.1±0.11	12.6±0.15	1.11±0.027
	3	4	30.68±7.16	14.7±0.35	12.32±0.44	0.99±0.011
	33	9	23.44±2.27	13.94±0.24	11.67±0.26	0.95±0.013
	1	2	18.77±1.43	12.75±0.24	10.75±0.22	1.04±0.046
	2	3	27.45±3.6	14.7±0.13	12.4±0.18	0.91±0.037
	3	4	22.77±4.04	13.97±0.24	11.6±0.26	0.93±0.019
T_{warm}	25	9	42.9±5.26	15.91±0.27	13.31±0.36	1.09±0.023
	1	3	36.52±3.26	15.46±0.15	12.96±0.15	1.04±0.018
	2	3	56.24±12.77	17.26±0.33	14.33±0.5	1.11±0.035
	3	3	35.95±5.02	15±0.17	12.63±0.25	1.11±0.01
	29	10	33.33±4.8	15.31±0.32	12.69±0.41	0.98±0.038
	1	3	20.69±0.28	13.6±0.19	11.13±0.16	1.02±0.054
	2	4	48.16±6.38	17.1±0.21	14.17±0.3	1±0.04
	3	3	26.22±3.45	14.63±0.14	12.26±0.17	0.9±0.012
	33	7	30.9±4.9	15.17±0.27	12.65±0.36	0.91±0.023
	1	2	19.94±0.67	14.1±0.03	11.55±0.06	0.86±0.028
	2	3	42.67±5.19	16.6±0.16	13.93±0.24	0.94±0.011
	3	2	24.2±7.02	14.1±0.2	11.85±0.24	0.92±0.04

Each rearing temperature×experimental temperature×block combination is represented. Sample sizes were selected to ensure adequate power based on known levels of variability in respirometry data, and accounting for availability of specimens in ponds.

thereafter (48 h) food was withheld to ensure a post-absorptive state during trials. In total, the respirometry experiments were completed over a 3-week period (5–26 October 2015), so fish brought into the laboratory last had an extra 17 days in the rearing ponds.

Metabolic traits

Metabolic traits (SMR, MMR, AS) were estimated for Nile perch from each rearing temperature×block×experimental temperature combination (Table 2) by measuring oxygen consumption rate ($\dot{M}_{O_2}$) using intermittent-flow respirometry. The experimental set-up is described in detail in Nyboer and Chapman (2017), but briefly, four polypropylene respirometers (volume=1.57–2.80 l), each fitted with a Firesting® temperature probe and fiber optic cable focused on a contactless oxygen sensor spot (PyroScience Sensor Technology, Bremen, Germany), were submerged in a temperature-controlled water bath held at the desired experimental temperature. Oxygen and temperature were recorded every 2 s, and outputs were monitored and recorded by Firesting® Profix software (PyroScience) for the duration of each trial. Respirometers were set on automated 10-min loops comprising a 5-min closed portion during which DO levels were measured, and a 5-min open portion during which fresh water was flushed through the respirometry chambers.

For each trial, MMR was measured first using an established 3-min exhaustive chase protocol (Roche et al., 2013). In short, this protocol involved hand-chasing individual Nile perch to exhaustion and then transferring them to the respirometer, where they were held for an average of 10 h after the chase, allowing ample time for recovery and to derive estimates of SMR (Nyboer and Chapman, 2017), with oxygen consumption measured throughout the 10-h period. Twenty-minute 'empty' runs were conducted before and after each trial to quantify background respiration. Prior to this study, a number of exploratory trials were conducted on recovery times of Nile perch to validate this protocol. Results from these experiments showed that reliable SMR measures could be obtained <10 h after employing exhaustive chase (Nyboer and Chapman, 2017). Fish were weighed for body mass (M_b) and measured for SL and TL after the trials.

Critical thermal maximum

After respirometry trials, fish were returned to their rearing ponds for ∼10 days to recover, after which a subset were returned to the laboratory and re-acclimated to the same experimental temperature for 3 days to measure CT_{max} as an estimate of thermal tolerance. Owing to stresses incurred during this transfer process, a smaller sample size was used for CT_{max} trials (Table S3); however, both this current work and previous studies showed low variance in CT_{max} values, thus even with low sample sizes these findings are likely to be accurate. CT_{max} was measured following Chen et al. (2013). In these trials, groups of two to four Nile perch from each rearing temperature×experimental temperature combination were transferred into a water-filled cooler, held overnight (∼8 h) and then subjected to a constant temperature increase of 0.3°C min^{-1}. This heating rate does not allow for acclimation during the trial, but also does not lethally shock the animal (Beitinger et al., 2000). CT_{max} was determined as the temperature at which each fish lost equilibrium for 10 s.

Calculations of metabolic traits

Metabolic rates (SMR and MMR) were estimated by calculating linear regressions between oxygen concentration and time for the closed period of each loop, omitting the first and last 30 s of each closed portion to ensure the water in the chamber had fully mixed. Oxygen concentrations were converted to metabolic rate (mg O_2 min^{-1}) after accounting for respirometer volume and fish mass. Background respiration (on average 9.9% of SMR and 3.6% of MMR) was subtracted from the metabolic rates by assuming a linear change between the two 20-min 'empty' runs. MMR is the highest $\dot{M}_{O_2}$ measurement recorded over any 3-min period throughout the trial. To calculate SMR, $\dot{M}_{O_2}$ measurements were plotted against time to detect the point where the recovery curve leveled off (on average 4.4±1.6 h, mean±s.d.). SMR was then calculated as the mean of the lowest 10% of all $\dot{M}_{O_2}$ measurements after this point. Outliers ±2 s.d. from the mean were excluded from this calculation (Clark et al., 2013). We followed methods outlined in Nyboer and Chapman (2017) to ensure that calculations of SMR did not over- or under-estimate this trait. We detected allometric effects of body mass on metabolic rate, and therefore used mass adjustments (following Ultsch, 1995) instead of classic mass corrections (mg min^{-1} kg^{-1}) to account for the effects of body size on metabolic rate. Mass-adjusted AS was calculated as the difference between mass-adjusted MMR and SMR (note that from here on, mass-adjusted metabolic traits will be referred to as MMR, SMR and AS).

Body size, condition and organ measurements

To estimate fish condition (K), fish were weighed for M_b to the nearest 0.01 g and measured for TL and SL to the nearest 0.1 cm before respirometry trials. K was calculated using LeCren's equation as detailed in Froese (2006). Because there was homogeneity of slopes in the bi-logarithmic M_b×SL relationship among rearing temperatures and blocks, coefficients were derived by pooling data for all individuals.

At the end of all experimental trials, fish were euthanized with a sharp blow to the head, and livers, hearts, gills and brains were removed. Livers were weighed fresh to the nearest 0.001 g, and hepatosomatic index (HSI) was calculated as a percentage of M_b. All other tissues were preserved in 10% formalin until processing.

Preserved heart ventricles for a subset of fish (Table S3) were isolated from the rest of the heart tissues (Fig. 1A), blotted to remove excess liquid, and weighed five times to the nearest 0.01 g. The mean was used to calculate RVM as a percentage of M_b. Ventricles were then embedded horizontally in paraffin, sectioned (4 µm thickness) along the dorso-ventral axis (Fig. 1A,B), stained with hematoxylin and eosin, and photographed at 4.5× magnification with a Leica 6SD zoom microscope with a Lumenera Scientific Infinity camera (Ottawa, ON, Canada). ImageJ v. 1.50i (https://imagej.nih.gov) was used to quantify the areas of the spongy and compact myocardial layers (Fig. 1B). The area of compact myocardium was divided by the total area (compact+spongy) of the heart section to calculate the percent of compact myocardium (%CM).

To quantify gill size, four gill arches from the left side of the branchial basket of 9–10 fish (Table S3) from each rearing temperature were extracted, laid flat and photographed at 6.3× magnification on both hemibranchs using the same microscope setup described above. A series of six gill traits were selected to represent the overall size and area of the hemibranchs. These measures were taken to the nearest 0.1 mm or 1 mm^2 and included: total filament number (TFN), total hemibranch area (THA), total gill filament length (TGFL), average gill filament length (AGFL), filament base length (FBL) and filament density (FD) (Chapman et al., 2008; Crispo and Chapman, 2010). TFN was calculated as the number of filaments along the filament base on both hemibranchs. THA was the sum of the area of all filaments on both hemibranchs.

Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

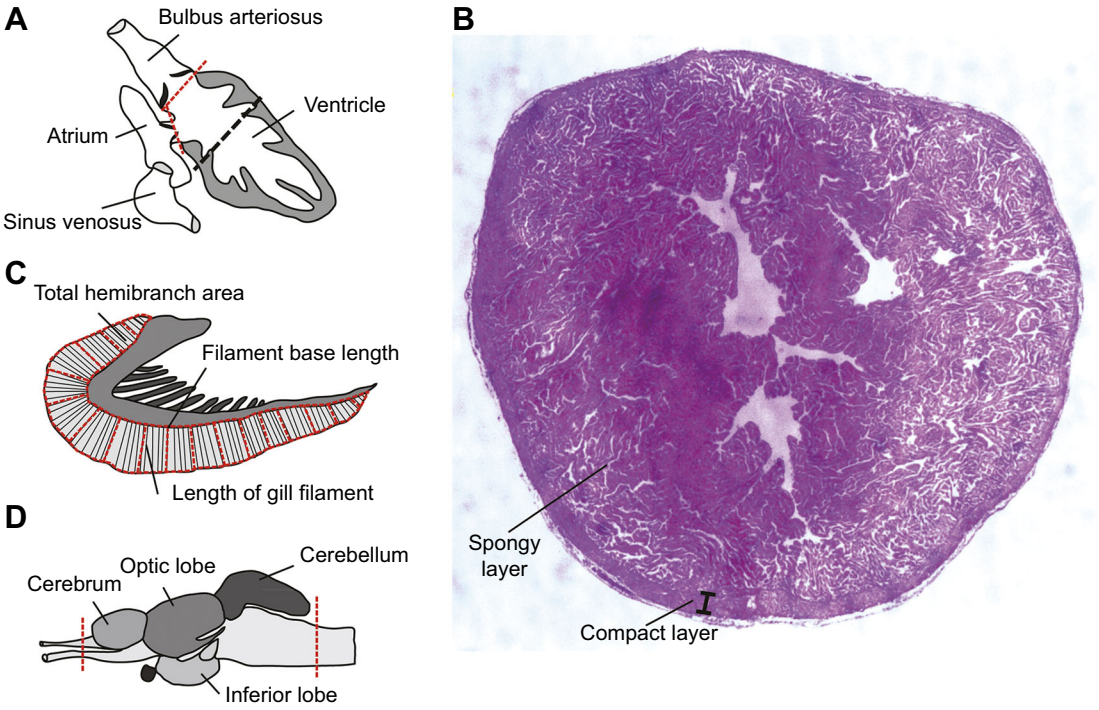

Fig. 1. Illustrations depicting morphology and dissection points for Nile perch organs. (A) Dissection points (dashed red lines) and the location of the cross-section (dashed black line) for the heart ventricles. (B) A low-magnification image of an H&E-stained cross-section of a heart ventricle with the spongy and compact myocardium layers indicated. (C) One side of a gill arch with measurements used for analysis of gill size (dashed red lines). (D) Dissection points for brains (dashed red lines).

TGFL was quantified by measuring the length of every 10th filament, and using the average of the two outer measures to estimate the lengths of the nine filaments in between (Fig. 1C). AGFL was calculated as the TGFL/TFN ratio on each hemibranch. FBL was calculated as the length of the filament base of each hemibranch (Fig. 1C). FD was calculated as the FBL/TFN ratio on each hemibranch. To obtain estimates for the whole gill, values for TFN, THA, TGFL and FBL were summed across the four arches, and then multiplied by 2 to account for both sides of the branchial basket. Values for FD and AGFL were averaged across the four arches (Chapman et al., 2008; Crispo and Chapman, 2010). We did not measure total gill surface area; however, previous inter-population and rearing studies have found that populations or treatments of fish with a larger TGFL are characterized by a larger TGSA (Chapman and Hulen, 2001; Chapman, 2007; Chapman et al., 2008). We therefore assume that a larger TGFL per gram of Nile perch reflects greater oxygen uptake capacity.

Preserved brains for a subset of fish (Table S3) were processed by trimming excess tissue and ensuring all brains were severed from the spinal cord 1 mm past the cerebellum (Fig. 1D). Blotted brains were weighed five times to the nearest 0.001 g, and the mean was used to calculate relative brain mass (RBM) as a percentage of M_b.

Statistical analysis

Effects of rearing and experimental temperature on SMR, MMR and AS were tested with three-way ANOVA with rearing temperature, block and experimental temperature as fixed factors. Mass-adjusted metabolic traits were $\log_{10}$-transformed to meet assumptions of statistical tests. Interactions among fixed effects were removed from the model if not significant. Effects of rearing and experimental temperature on CT_{max} were tested with a two-way ANOVA with rearing and experimental temperature as fixed factors. For CT_{max}, not all blocks were represented in all rearing temperature×experimental temperature combinations; block was therefore not included as a fixed effect. For tests where the rearing temperature×experimental temperature interaction term was significant, and overall trends across experimental temperature were visually similar, *post hoc* tests for the effect of experimental temperature within rearing temperature and rearing temperature within experimental temperature were conducted to detect differences among levels. Equality of variances and normality of residuals were assessed with diagnostic residual plots and normal Q–Q plots.

Effects of rearing temperature on K, M_b, SL, TL, RVM, %CM, RBM and HSI were analyzed with two-way ANOVA, with rearing temperature and block as fixed effects. Non-significant interaction terms were removed from models. Potential interactive effects of M_b and rearing temperature on organ masses were tested using ANCOVA with raw organ masses as response variables, rearing temperature and block as fixed effects, and M_b as a covariate. Raw liver mass was $\log_{10}$-transformed to meet assumptions of statistical tests. Results of ANCOVAs revealed similar outcomes as the two-way ANOVAs (except for a significant M_b×rearing temperature interaction for ventricle mass), so only ANOVA results are reported (but see Table S4 for comparison with Table 3). Gill traits were first analyzed with each metric separately using ANCOVA with rearing temperature and block as fixed effects, and M_b as a covariate. Second, principal components analyses (PCA) were used to reduce gill metrics to major axes, and PCA scores were used as response variables in ANOVA models with rearing temperature and block as fixed effects. To perform PCA, we first standardized gill traits to a common body mass following Crispo and Chapman (2010).

ANCOVA was used to test the hypothesis that variation in RVM, % CM and gill size had an effect on metabolic performance (SMR, MMR and AS) and thermal tolerance (CT_{max}). Before these tests, Q_{10}

Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

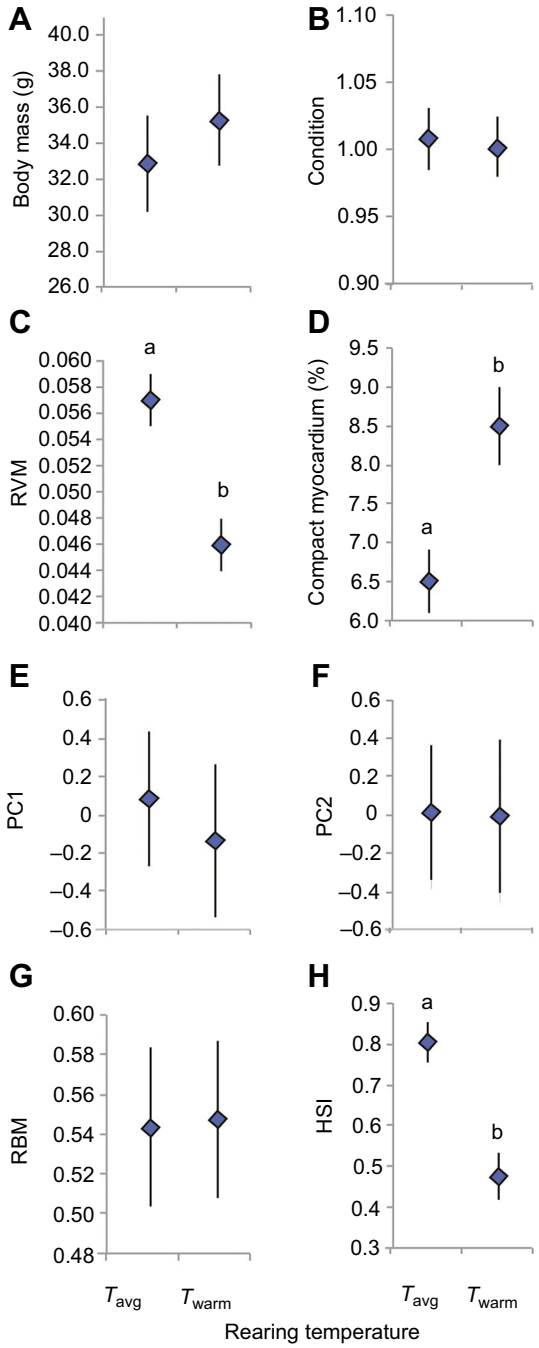

Fig. 2. Results of ANOVA examining the effects of rearing temperature on fitness-related traits and organ traits. Fitness-related traits include (A) body mass and (B) LeCren's condition factor. Organ traits include (C) relative ventricular mass (RVM), (D) percent compact myocardium, (E) PC1 of gills representing gill area, (F) PC2 of gills representing gill width, (G) relative brain mass (RBM) and (H) hepatosomatic index (HSI). Values are presented as means±s.e.m. Different letters indicate significant differences (α=0.05) among rearing temperatures [average rearing temperature (T_{avg}), ~25°C; warm rearing temperature (T_{warm}), ~29°C]. Block was included as a fixed factor in ANOVA models.

corrections were applied to SMR, MMR and AS to adjust for the effects of experimental temperature on metabolic traits. Q_{10} temperature coefficients were calculated using the van 't Hoff equation (McNab, 2002); these values were subsequently used to adjust metabolic rates at a given experimental temperature to the rearing temperature of the individual (e.g. adjusted to 29°C for T_{warm} fish). Q_{10}-corrected metabolic traits are denoted as SMR_{Q10}, MMR_{Q10} and AS_{Q10}. CT_{max} was adjusted using the slope for the linear equation of CT_{max}×experimental temperature within each rearing temperature. Adjusted CT_{max} is denoted as $CT_{max,adj}$. ANCOVAs with $CT_{max,adj}$ or SMR_{Q10} and MMR_{Q10} as response variables, rearing temperature and block as fixed effects, and organ traits (e.g. RVM, %CM, gill PCs) as covariates were applied to detect whether these organ traits had an overall effect on metabolic traits or CT_{max}. Significant effects were confirmed with Pearson's correlation of $CT_{max,adj}$ or SMR_{Q10} and MMR_{Q10} with organ traits. Alpha values were set to 0.05 to confirm significance for all statistical tests. All analyses were performed in IBM SPSS Statistics, version 22.0 (IBM Corp., Armonk, NY, USA).

Table 3. Results of two-way ANVOA testing for differences in body size and organ traits across rearing temperatures and blocks

Trait	Factor	*F*	d.f.	*P*	η^2
M_b (g)	Rearing temperature	0.442	1, 47	0.509	0.009
	Block	6.953		**0.002**	0.228
TL (cm)	Rearing temperature	0.842	1, 47	0.363	0.018
	Block	7.589		**0.001**	0.244
SL (cm)	Rearing temperature	0.606	1, 47	0.44	0.013
	Block	7.473		**0.002**	0.241
K	Rearing temperature	0.031	1, 47	0.861	0.001
	Block	0.536		0.589	0.022
RBM (%M_b)	Rearing temperature	0.013	1, 20	0.911	0.001
	Block	4.459		0.025	0.308
RVM (%M_b)	Rearing temperature	9.219	1, 33	**0.005**	0.218
	Block	2.227		0.124	0.119
%CM	Rearing temperature	11.966	1, 32	**0.002**	0.272
	Block	0.404		0.671	0.025
HSI (%M_b)	Rearing temperature	17.825	1, 28	**<0.001**	0.389
	Block	0.076		0.927	0.005
PC1 gills	Rearing temperature	0.116	1, 15	0.738	0.008
	Block	0.984		0.398	0.116
PC2 gills	Rearing temperature	0.002	1, 15	0.969	0.000
	Block	0.043		0.958	0.006

Traits include body mass (M_b), total and standard length (TL and SL), LeCren's condition factor (*K*), relative brain mass (RBM), relative ventricular mass (RVM), percent compact myocardium (%CM), hepatosomatic index (HSI) and two principal components for gill traits (PC1 and PC2). Rearing temperatures include T_{avg} and T_{warm}, and each regime was represented by three blocks. Bold *P*-values indicate significance at α<0.05.

RESULTS

Body size and condition

Nile perch from both rearing conditions achieved the same M_b, SL, TL and *K* over the 3-month rearing period (Fig. 2A,B). Because the stocking size and feeding regime were the same among rearing temperatures, fish were assumed to have similar growth rates between rearing conditions, although we could not quantify precise feeding rates or activity levels. There were, however, strong effects of block for all body size traits (Table 3). *Post hoc* pairwise comparisons revealed that fish from block 2 were significantly larger for all measures than those from block 1 (M_b: *P*=0.002, TL: *P*=0.001 and SL: *P*=0.001) and significantly or marginally larger than those from block 3 (M_b: *P*=0.064, TL: *P*=0.043 and SL: *P*=0.0064). *K* was stable across rearing temperature and block (Table 3). Mortality rates of Nile perch in the rearing ponds averaged 65%, and were similar across thermal regimes (64.3% in T_{avg}, 66% in T_{warm}), with most (73%) of the mortality occurring in the first 3 weeks of the rearing treatments. Although the source of mortalities could not be determined, we believe that the initial stress of capture and transfer was key, in addition to competition for food

Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

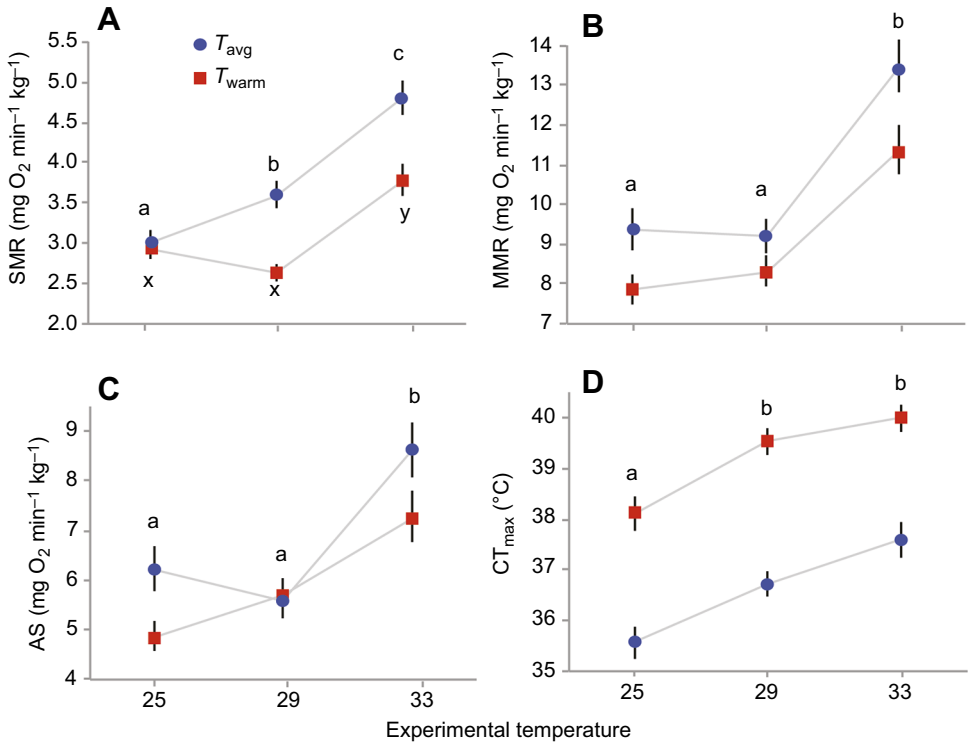

Fig. 3. Results of ANOVAS comparing metabolic traits and thermal tolerance limits in Nile perch among experimental temperatures and between rearing temperatures. (A–C) Results of three-way ANOVA comparing means±s.e.m. for (A) standard metabolic rate (SMR), (B) maximum metabolic rate (MMR) and (C) aerobic scope (AS) between rearing temperatures across a range of experimental temperatures. (D) Results of two-way ANOVA comparing means±s.e.m. of critical thermal maximum (CT_{max}) between rearing temperatures and across experimental temperatures. Different letters in A indicate significant differences among experimental temperatures within a rearing temperature, and asterisks indicate significant differences between rearing temperatures within an experimental temperature. Different letters in B–D indicate overall differences in metabolic traits among experimental temperatures. Significance of results are presented in Table 4. Sample sizes are presented in Table 1.

because of the emaciated appearance of non-surviving fish and the excellent condition of survivors. Although selection imposed by these mortality rates may bias results, similar rates across rearing temperatures validate comparisons.

Metabolic traits and critical thermal maximum

Nile perch reared under warm conditions had low SMRs compared with T_{avg} fish (Fig. 3A, Table 4), a difference that was especially pronounced at 29°C and 33°C, where T_{warm} fish showed a 37% and 27% reduction in $\dot{M}_{O_2}$, respectively. At 25°C, SMR values did not differ between fish from the two rearing temperatures (Fig. 3A). There was a significant rearing temperature×experimental temperature interaction, and *post hoc* tests revealed that T_{warm} fish performed equally well at 29°C as at 25°C, but that SMR increased at 33°C, whereas T_{avg} fish showed significant increases in SMR at each experimental temperature (Fig. 3A). Overall, SMR of T_{avg} fish increased 37% across experimental temperatures compared with a lower 22% increase in T_{warm} fish. Rearing and experimental temperature both had significant effects on MMR (Table 4). T_{warm} Nile perch had a 10–19% lower MMR than T_{avg} fish at all experimental temperatures (Table 4, Fig. 3B). Patterns of increase across experimental temperatures were remarkably similar between rearing temperatures, with MMR remaining constant from 25 to 29°C, and showing an exponential 31% increase at 33°C (Table 4, Fig. 3B). T_{warm} Nile perch showed reduced AS overall, with lower values at both the coolest and warmest experimental temperatures (Table 4, Fig. 3C). At 29°C, however, AS of T_{warm} fish equaled that of the T_{avg} fish. AS showed a general increase with experimental temperature in fish from both rearing temperatures (Table 4, Fig. 3C).

Table 4. Results of two- and three-way ANOVAs examining the effects of rearing temperature and experimental temperature on critical thermal maximum (CT_{max}) and metabolic traits

Variable		Rearing temperature	Experimental temperature	Block	Rearing temperature× Experimental temperature
CT_{max} (°C)	$F_{2,13}$	87.974	14.02		0.199
	P	**<0.001**	**0.001**		0.822
	η^2	0.871	0.683		0.03
SMR (mg O_2 kg^{-1} min^{-1})	$F_{2,43}$	23.623	33.148	0.171	4.929
	P	**<0.001**	**<0.001**	0.843	0.012
	η^2	0.355	0.607	0.008	0.187
MMR (mg O_2 kg^{-1} min^{-1})	$F_{2,43}$	12.914	32.288	0.174	0.366
	P	**0.001**	**<0.001**	0.841	0.696
	η^2	0.231	0.6	0.008	0.017
AS (mg O_2 kg^{-1} min^{-1})	$F_{2,43}$	4.749	16.545	0.13	1.949
	P	**0.035**	**<0.001**	0.878	0.155
	η^2	0.099	0.435	0.006	0.083

The two-way ANOVA tested the effects of rearing temperature (T_{avg}, T_{warm}), experimental temperature (25, 29 and 33°C) and their interaction (R×E) on CT_{max}. The three-way ANOVA tested the effects of rearing temperature, experimental temperature, block and R×E on standard and maximum metabolic rate (SMR and MMR) and aerobic scope (AS). Bold *P*-values indicate significance at α<0.05.

Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

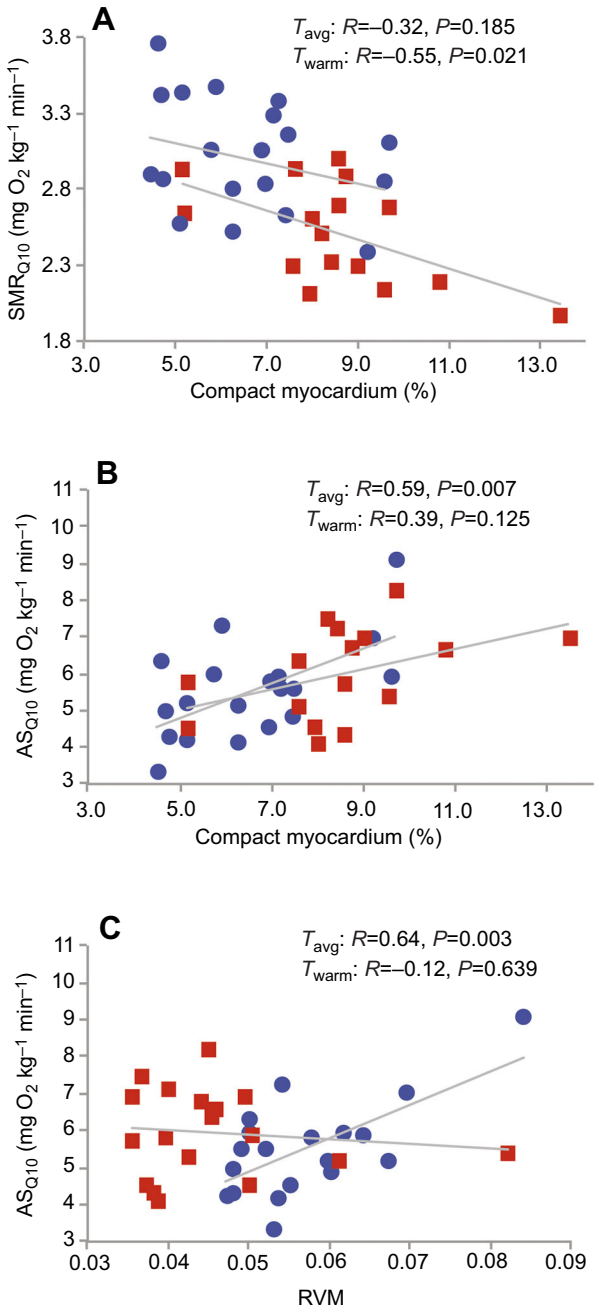

Fig. 4. Linear relationships between metabolic traits and heart traits. (A–C) Q_{10}-corrected metabolic traits (SMR_{Q10} and AS_{Q10}) and heart traits including relative ventricular mass (RVM) and percent compact myocardium. Colors indicate different rearing temperatures (T_{avg}, blue; T_{warm}, red). Correlations for CT_{max} with RVM (not shown) had similar patterns as AS_{Q10} (C), with a marginally significant positive correlation in the T_{avg} group (R=0.756, P=0.082).

Nile perch from both rearing temperatures showed a significant increase of 1.89°C (T_{warm}) and 1.95°C (T_{avg}) in CT_{max} over an experimental temperature range of 7°C (Table 4, Fig. 3D), with a slope relating CT_{max} to temperature of 0.218±0.060 for the T_{warm} fish and 0.244±0.067 for the T_{avg} fish. T_{warm} fish had higher overall CT_{max} (Table 4, Fig. 3D), with values 2.4–2.8°C higher at all experimental temperatures. These individuals were able to achieve CT_{max} of nearly 40°C after only 3 days of acclimation to 33°C, whereas T_{avg} fish reached only 37.5°C.

Organ development and plasticity

Cardiac remodeling was apparent in Nile perch, with T_{warm} fish having reductions in RVM and increases in %CM (Fig. 2CD, Table 3), as predicted. The average RVM of T_{warm} fish (0.046± 0.002 g, mean±s.e.m.) was approximately 20% smaller than that of T_{avg} fish (0.057±0.002 g). Linear regressions of M_b with ventricle mass showed that ventricle size diverged at larger body sizes, indicating possible growth-related changes in the onset of cardiac remodeling (Fig. S3A). We tested this interaction with ANCOVAs with ventricle mass as a response variable and M_b as a covariate, and these tests revealed a significant M_b×rearing temperature interaction for raw ventricle mass. However, differences between rearing temperature were similar to the ANOVA results (Table S4, Table 4). For simplicity, only ANOVA results were retained in the main text (but see Table S4 for comparison). For %CM, ANOVA revealed that T_{warm} fish had a higher %CM compared with T_{avg} fish (Fig. 2D, Table 3). For tests of how variability in ventricle morphology affects Q_{10}-corrected metabolic rates and $CT_{max,adj}$, ANCOVAS with RVM or %CM as covariates reveal that variation in %CM had a negative effect on SMR_{Q10} and a positive effect on AS_{Q10}, but did not influence MMR_{Q10} or $CT_{max,adj}$ (Fig. 4, Table 5), and that variation in RVM had a positive effect on $CT_{max,adj}$ (Table 5). Pearson's correlations confirmed these patterns, and revealed a positive relationship between RVM and AS in T_{avg} fish (Fig. 4).

Gill traits did not differ between rearing temperatures (Table 6). PCA on mass-standardized gill traits extracted two components with eigenvalues >1, with PC1 explaining 66.5% and PC2 explaining 26.9% of the variance. Gill metrics related to filament and hemibranch size (AGFL, TGFL and THA) loaded on PC1, and those related to gill width (AFN and FBL) loaded on PC2 (Table S5). FD was not included in the PCA as it did not correlate with the other traits. Results of ANOVA using PC scores as response variables confirmed the lack of divergence in gill size among rearing temperatures (Fig. 2E,F, Table 3). Rearing temperature had a significant effect on HSI (Fig. 2G, Table 3), with T_{warm} fish having 41% smaller HSI values (0.47±0.03, mean±s.e.m.) than T_{avg} fish (0.81±0.05). Rearing temperature had no effect on RBM (Fig. 2H, Table 3), with fish from both T_{avg} and T_{warm} regimes having remarkably similar values (0.54±0.034 and 0.531±0.045, respectively). None of the gill traits, HSI or RBM had an effect on any Q_{10}-adjusted metabolic traits or $CT_{max,adj}$.

DISCUSSION

The three most important findings of this study were that: (1) Nile perch reared under elevated temperatures showed evidence for compensation in upper thermal tolerance and metabolic rate, and attained similar body size and condition between rearing treatments; (2) Nile perch are capable of cardiac remodeling, and variation in heart morphology correlated with metabolic function in fish from both rearing treatments; and (3) plastic changes enacted over longer exposures effected differences in metabolic flexibility when acutely exposed to temperature change. This work has important implications for climate change resilience in this species, which is of major economic importance to inland fisheries in East Africa.

Developmental plasticity in upper thermal tolerance and metabolic traits

Rearing Nile perch under different temperature regimes for 3 months led to adjustments in upper thermal tolerance (CT_{max}). Given the stenothermal evolutionary history of the Nile perch used in this study, one might expect low thermal plasticity in this population (Tewksbury et al., 2008). However, Nile perch

Journal of Experimental Biology (2018) 221, jeb178087. doi:10.1242/jeb.178087

Table 5. Results of two-way ANCOVA testing for the influence of cardiac traits on thermal tolerance and metabolic performance of Nile perch from different rearing temperatures (T_{avg}, T_{warm})

Variable						
Outcome	Covariate		Rearing temperature	Block	Covariate	Covariate×Rearing temperature
CT_{max}	SMR_{Q10}	$F_{1,11}$	139.898		9.737	
		P	**<0.001**		**0.01**	
		η^2	0.927		0.47	
	RVM	$F_{1,11}$	22.644		10.728	7.233
		P	**0.001**		**0.008**	**0.023**
		η^2	0.694		0.518	0.42
SMR_{Q10}	%CM	$F_{1,31}$	8.031	0.456	6.184	
		P	**0.008**	0.638	**0.018**	
		η^2	0.206	0.029	0.166	
	RVM	$F_{1,31}$	13.89	0.17	0.724	
		P	**0.001**	0.844	0.401	
		η^2	0.303	0.011	0.022	
MMR_{Q10}	%CM	$F_{1,31}$	8.597	0.412	2.464	
		P	**0.006**	0.666	0.127	
		η^2	0.217	0.026	0.074	
	RVM	$F_{1,31}$	3.484	0.336	0.236	
		P	**0.071**	0.717	0.63	
		η^2	0.098	0.021	0.007	
AS_{Q10}	%CM	$F_{1,31}$	0.168	1.009	9.471	
		P	0.684	0.376	**0.004**	
		η^2	0.005	0.061	0.234	
	RVM	$F_{1,31}$	14.244	2.584	1.935	11.958
		P	**0.001**	0.092	0.174	**0.002**
		η^2	0.315	0.143	0.059	0.278

Outcome variables included adjusted critical thermal maximum ($CT_{max,adj}$), and Q_{10}-corrected standard and maximum metabolic rates and aerobic scope (SMR_{Q10}, MMR_{Q10} and AS_{Q10}). Cardiac trait covariates included relative ventricular mass (RVM), and percent compact myocardium (%CM). Block and the covariate×rearing temperature interaction were included as factors in the model. Bold *P*-values indicate significance at α<0.05.

experiencing chronically elevated temperatures were able to increase thermal tolerance without having restrictions on their ability to further modulate thermal limits upon acute temperature increases. This is evidenced by the similar linear increases in CT_{max} with experimental temperature, which indicates comparable levels of plasticity in thermal tolerance between rearing temperatures. These results confirm previously documented trends in Nile perch thermal plasticity over shorter exposures (Nyboer and Chapman, 2017), and are in line with findings in recent meta-analyses that do not find evidence for predicted latitudinal trends in ectotherm thermal plasticity (Seebacher et al., 2014; Gunderson and Stillman, 2015).

Table 6. Results of two-way ANOVA testing the effects of rearing temperature (T_{avg}, T_{warm}) on a series of gill traits

Gill trait		Rearing temperature	Block	M_b
TFN	$F_{1,14}$	0.199	0.154	10.385
	P	0.662	0.858	**0.006**
	η^2	0.014	0.022	0.426
THA (mm^2)	$F_{1,14}$	0.094	0.234	30.933
	P	0.764	0.794	**<0.001**
	η^2	0.007	0.032	0.688
TGFL (mm)	$F_{1,14}$	0.57	0.719	30.065
	P	0.463	0.504	**<0.001**
	η^2	0.039	0.093	0.682
FBL (mm)	$F_{1,14}$	0.000	0.307	23.030
	P	0.986	0.741	**<0.001**
	η^2	0.000	0.042	0.622
AGFL (mm)	$F_{1,14}$	0.202	0.483	17.26
	P	0.66	0.627	**0.001**
	η^2	0.014	0.065	0.552
FD (TFN/FBL)	$F_{1,14}$	0.402	1.762	6.634
	P	0.536	0.208	**0.022**
	η^2	0.028	0.201	0.322

Gill traits include total filament number (TFN), total hemibranch area (THA), total gill filament length (TGFL), filament base length (FBL), average gill filament length (AGFL) and filament density (FD). Body mass (M_b) was included as a covariate, and block as a fixed factor in the model. Bold *P*-values indicate significance at α<0.05.

Rearing temperature affected metabolic performance, with warm-reared fish showing overall reductions in SMR, MMR and AS relative to T_{avg} fish at most experimental temperatures, indicating that compensatory mechanisms underlying basal metabolic reductions are effective during rapid transfer to temperatures both above and below their rearing temperature (as opposed to incurring costs of thermal specialization). Decreases in SMR are very likely indicative of thermal compensation because lower self-maintenance costs presumably allow more energy allocation to growth and reproduction (Priede, 1985; Sandblom et al., 2014). Reductions in SMR in warm-reared fish were accompanied by even greater reductions in MMR, which ultimately resulted in reduced AS relative to the T_{avg}-reared fish. Although decreases in MMR and AS are not necessarily predicted outcomes of thermal compensation, such patterns have been reported in a previous study on acclimated Nile perch (Nyboer and Chapman, 2017), and may indicate that long-term exposure invokes mechanisms that reduce SMR but also lead to reductions in peak performance (MMR). Similar trends have been found in a congener of Nile perch, the tropical barramundi (Norin et al., 2014), and in northern populations of shorthorn sculpin (Sandblom et al., 2014), and suggest that there may be energetic costs associated with maintenance of high MMR.

Reducing basal oxygen demand at the expense of a very high MMR may be the most efficient strategy for coping with elevated temperature conditions over longer time periods (Norin et al., 2014; Sandblom et al., 2014; Nyboer and Chapman, 2017), particularly if total aerobic capacity is rarely (if ever) used in the wild (Norin and Clark, 2016). Interestingly, Nile perch from both rearing temperatures showed similar patterns of increase in MMR across experimental temperatures, with a sharp increase at 33°C, indicating similar metabolic flexibility between rearing temperatures. Rapid increases in MMR upon acute exposure to extreme high temperatures have been documented in previous studies on Nile perch (Nyboer and Chapman, 2017) and in other fish species (Claireaux et al., 2006; Gräns et al., 2014; Norin et al., 2014). Such patterns may reflect stress responses and mobilization of functional reserves (hormone-activated performance capacity) that stimulate cardiovascular activity (Pörtner et al., 2017), and may not be stable over the long term, as shown by the diminished MMR sometimes evident after acclimation (Norin et al., 2014; Sandblom et al., 2014; Nyboer and Chapman, 2017).

Reductions in AS in warm-acclimated fish (at 25 and 33°C) relative to T_{avg} fish may indicate that mechanisms of oxygen uptake and delivery are not able to adjust fast enough to keep pace with increased cellular oxygen demand during aerobic exercise (Pörtner and Knust, 2007; Pörtner et al., 2017). However, these findings are at odds with the increases in AS across experimental temperatures. Fish achieved high AS upon acute exposure to extreme high temperatures, suggesting that, at least for acute exposures, oxygen transport capacity does not immediately decline at high temperatures (and therefore does not limit AS), as previously reported for Atlantic halibut (Gräns et al., 2014), barramundi (Norin et al., 2014) and turbot (Claireaux et al., 2006), among others. These findings challenge one of the fundamental assumptions of the OCLTT concept: that reduction in AS owing to oxygen limitation is a key physiological restraint acting on fishes' thermal optima. Although it is possible that our experimental temperatures were not high enough to observe the predicted reductions in AS, this calls into question the relevance of AS in predicting effects of climate change on aerobic performance, given that 33°C (our highest experimental temperature) is at the upper end of what would be experienced in nature, even in the most extreme climate change predictions.

In addition, the relative drops in AS in the warm-reared Nile perch do not necessarily indicate reductions in fitness, as the lower overall AS in warm-reared Nile perch did not correspond to decreases in body size or condition in this treatment (Clark et al., 2013; Gräns et al., 2014; Norin et al., 2014; Nyboer and Chapman, 2017). However, one must keep in mind that measurements of oxygen consumption are not perfect proxies for energy use (Nelson, 2016), and it is possible that there were differences in activity level, food consumption rates and/or costs of food assimilation (specific dynamic action) among rearing ponds, all of which are important for understanding growth and energy allocation (Chabot et al., 2016a,b). Although we did not measure these aspects of bioenergetics in this study, we did find reductions in HSI in warm-reared fish, indicating possible energetic costs to chronic exposure to elevated temperatures. The liver is a major energy store in fishes, and HSI is an indicator of energetic reserves in fish. HSI is often used as an alternative predictor of condition (Chellappa et al., 1995), and has been shown to relate to reproductive potential (Donelson et al., 2011). HSI can be affected by many factors, including food availability, season and reproductive cycle (Chellappa et al., 1995); however, given the similar age structure, condition and food supply between the two rearing temperatures, these are unlikely to be sources of differentiation in the present study. The reduced HSI in warm-reared fish could indicate that physiological adjustments made upon chronic exposure to warm temperatures are energetically costly given this reduction in energetic reserves. Given that fish in both rearing temperatures were able to achieve the same body size and condition, the lower HSI in T_{warm} fish may indicate that they had to allocate more energy to growth at the higher rearing temperature.

Responses of organ traits

There were no differences in RBM among Nile perch from divergent rearing temperatures. Large brains can enhance individual fitness through improved cognitive ability (Kotrschal et al., 2013), but are highly metabolically demanding, and reductions may be advantageous under physiologically stressful conditions to reduce basal maintenance costs (Poulson, 2001; Crispo and Chapman, 2010; Toli et al., 2016). Although some studies have shown high levels of plasticity in brain traits (Gonda et al., 2011; Kotrschal et al., 2012), the absence of a response in Nile perch brain size may suggest genetic limitations (lack of ability to change brain size via plasticity), but may also indicate that mechanisms employed to compensate for increased temperature are adequate to maintain brain size at both rearing temperatures.

Nile perch also displayed a striking lack of plasticity in gill size and shape among rearing temperatures. This was somewhat surprising given previous work that showed variation in gill size in wild Nile perch occupying divergent oxygen habitats (Paterson et al., 2010), and evidence from other species that exposure to increased temperature can induce alterations in gill morphology to compensate for higher basal metabolic rate and lower oxygen availability (Sollid et al., 2005; McBryan et al., 2016; Phuong et al., 2017). We had predicted a larger gill size in warm-reared fish to enhance oxygen uptake in an environment where oxygen demand would be greater, at least initially. It is of course possible that gill traits not measured in this study (e.g. lamellar area, interlamellar cell mass) or other mechanisms such as changes in blood hemoglobin levels compensate for increased oxygen demand (Weber et al., 1976; Farrell et al., 2009; McBryan et al., 2016). However, because warm-reared Nile perch actually show reductions in oxygen demand (lower SMR) after 3 months of acclimation, it is possible that improvements in oxygen uptake were not necessary. Maintaining small gills may decrease energy required to maintain osmotic balance in the blood, and may be beneficial when larger gills are not needed (Crispo and Chapman, 2010). This finding may indicate that capacity for oxygen uptake is not the limiting factor in the maintenance of aerobic performance, and that Nile perch cope with elevated temperatures through some other, more efficient means (e.g. improvements in efficiency of oxygen delivery). The finding that gill traits do not correlate with any metabolic traits supports this conclusion.

Despite finding no evidence for plastic changes in gill metrics or brain size, results from this study showed clear evidence for cardiac remodeling. To our knowledge, this is the first study to measure heart plasticity in a tropical species exposed to elevated temperatures, and it is interesting that trends documented here are very similar to those found in temperate and arctic fishes: warm acclimation induced smaller RVM and higher %CM, with the opposite effect in fish reared under average temperatures (Klaiman et al., 2011; Gräns et al., 2014; Anttila et al., 2015; Keen et al., 2017). Fish exposed to higher water temperature must increase cardiac output (V_b) to meet increased oxygen demand of tissues. This can be accomplished by quickening heart rate (common during acute thermal stress), developing larger ventricles (increased

volume) and developing stronger contractile muscles (increased pressure), among others (Farrell et al., 2009; Farrell, 2009; Klaiman et al., 2011; Keen et al., 2017; Pörtner et al., 2017). In most cases, RVM correlates tightly with V_b (Dalziel et al., 2012); however, this relationship may be altered based on changes in proportions of the myocardial layers (Klaiman et al., 2011; Keen et al., 2017). For example, increases in spongy myocardium (often corresponding to increased RVM) enhance stroke volume at cooler temperatures, allowing the heart to maintain high V_b at a lower heart rate, thereby reducing heart oxygen demand (Keen et al., 2017). In the present study, T_{avg} Nile perch had a larger RVM, so this may be the most efficient strategy to maximize cardiac output at cooler temperatures. Generally, when fishes are acutely exposed to higher temperatures, V_b is decreased because of loss of pressure generating ability (Klaiman et al., 2011). Given time, however, fish often develop stronger compact myocardial layers (at the expense of RVM) so that the greater force generated by this layer can offset the otherwise negative effects of warmer temperatures (Klaiman et al., 2011). For Nile perch, increasing %CM may be a way of increasing V_b without requiring high heart rates and extra energy reserves. If the changes to the heart result in more efficient oxygen delivery strategies, this could lower basal oxygen demand and contribute to maintenance of a lower SMR (possibly partly explaining the lack of change in gill size). Evidence that warm-reared fish have improved cardiorespiratory function is corroborated by the CT_{max} results insofar as warm-reared fish had higher CT_{max} at all experimental temperatures.

Cardiac plasticity affects thermal tolerance and metabolic function

Changes in cardiorespiratory capacity are often invoked as explanations for alterations in thermal tolerance and metabolic performance in ectotherms (Gamperl and Farrell, 2004; Pörtner and Farrell, 2008; Farrell et al., 2009; Donelson et al., 2011; Jayasundara and Somero, 2013). For fish, much of the evidence for this is derived from studies on salmonid species that link cardiorespiratory function to swim performance and thermal tolerance (Farrell et al., 2009; Anttila et al., 2013, 2014; Eliason et al., 2011, 2017). However, studies that demonstrate functional links between heart morphology and metabolic traits are rare, and the generality of these trends has not been extended to tropical fishes.

Morphological changes in Nile perch ventricles were related to variability in SMR, AS and CT_{max}. For example, larger RVM corresponded to higher CT_{max} and higher AS, especially in T_{avg} fish. Because T_{avg} Nile perch have larger RVM, this may indicate that T_{avg} fish rely on stroke volume (over long-term exposures) to maintain high cardiac output and support better oxygen supply to tissues. This suggests that heart traits are important in determining thermal tolerance and metabolic performance at the level of the whole organism, and supports findings of studies on salmonid species that demonstrated positive relationships among different aspects of cardiorespiratory function and aerobic metabolic performance. For example, Anttila et al. (2013) showed positive correlations of RVM with CT_{max} among families of Atlantic salmon, and Eliason et al. (2011) showed positive relationships between AS and cardiac scope within several families of Pacific sockeye salmon. For T_{avg} Nile perch, the direction of plasticity for both RVM and AS was the same as the direction of covariation among these traits, leading to the conclusion that Nile perch in this group rely on a larger RVM to maintain aerobic performance.

Per cent CM correlated negatively with SMR, especially among T_{warm} individuals; so again, plastic variation among individuals supported the between-treatment effects. In the warm-acclimated fish, it is likely that adjustments in heart rate, stroke volume and pressure generation that accompany increases in %CM all play a role in ensuring adequate oxygen delivery to respiring tissues (which have likely made their own adjustments to reduce basal oxygen demand; Schulte, 2015), while using fewer energy reserves when exposed to higher temperatures, thus maintaining a lower SMR in T_{warm} fish. Our results also showed a positive relationship of AS with %CM (especially in T_{avg} fish). Because AS is calculated as the difference between MMR and SMR, the fact that SMR changes with cardiac morphology, and MMR does not, may be why AS is correlated with heart traits, and indicates that mechanisms controlling MMR may not necessarily be directly related to cardiac morphology. Whatever the case, the finding that temperature-related developmental cardiac plasticity can influence aerobic function provides insight into one of the possible mechanisms (of many) that may underlie resilience to climate warming.

Cardiorespiratory function is likely to be a key mechanism underlying physiological plasticity in metabolic performance, aerobic capacity and thermal tolerance in Nile perch. These data are consistent with one of the predictions generated by the OCLTT hypothesis, namely that cardiorespiratory function is a key mechanism underlying thermal plasticity in fishes and can be useful to compensate for increased oxygen demand (Anttila et al., 2013). These relationships are complex, however, and are not always in the direction predicted by the OCLTT hypothesis (i.e. lower MMR and AS in warm-reared fish, high AS and MMR in individuals acutely exposed to extreme temperatures), highlighting how physiological benefits of heart plasticity are context dependent and likely to change based on interacting stressors, strategies and physiological challenges. The fact that Nile perch from Lake Victoria are near the upper edge of their thermal range and are still able to make plastic adjustments to high temperatures on multiple time scales provides compelling evidence against the prediction that tropical ectotherms will be disproportionately negatively affected by climate warming. This knowledge is especially important because the ability of tropical species to cope with unstable thermal environments is largely unknown, and because tropical fish species, including the Nile perch, provide vital sources of protein in developing nations and regions, such as the Lake Victoria basin in East Africa.

Acknowledgements

We would like to thank Steven Kimera for his work rearing and maintaining Nile perch and his help on the respirometry experiments, Luisa Sarmiento-Trujillo for her work dissecting and measuring organs, and staff and colleagues at the Aquaculture Research and Development Center in Kajjansi, Uganda, for providing infrastructure and intellectual and technical support during this project.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.A.N., L.J.C.; Methodology: E.A.N., L.J.C.; Software: E.A.N.; Formal analysis: E.A.N., L.J.C.; Investigation: E.A.N.; Resources: L.J.C.; Writing - original draft: E.A.N.; Writing - review & editing: E.A.N., L.J.C.; Visualization: E.A.N.; Supervision: L.J.C.; Funding acquisition: L.J.C.

Funding

This research was funded by the Natural Sciences and Engineering Research Council of Canada (PGS-D3-426309 to E.A.N. and by Natural Sciences and Engineering Research Council of Canada Discovery Grant RGPPIN/06675-2015, Canada Research Chair funds for L.J.C.).

Data availability

Data are available from the Dryad Digital Repository (Nyboer and Chapman, 2018): https://doi.org/10.5061/dryad.2k527b8.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.178087.supplemental

References

Angilletta, M. (2009). *Thermal Adaptation. A Theoretical And Empirical Synthesis*. Oxford, UK: Oxford University Press.

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., Wolters, W. R. and Schulte, P. M. (2013). Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size, and myoglobin level. *J. Exp. Biol.* **216**, 1183-1190.

Anttila, K., Jørgensen, S. M., Casselman, M. T., Timmerhaus, G., Farrell, A. P. and Takle, H. (2014). Association between swimming performance, cardiorespiratory morphometry, and thermal tolerance in Atlantic salmon (*Salmo salar* L.). *Front. Mar. Sci.* **1**, 1-10.

Anttila, K., Lewis, M., Prokkola, J. M., Kanerva, M., Seppänen, E., Kolari, I. and Nikinmaa, M. (2015). Warm acclimation and oxygen depletion induce species-specific responses in salmonids. *J. Exp. Biol.* **218**, 1471-1477.

Beitinger, T. L., Bennett, W. A. and McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ. Biol. Fishes* **58**, 237-275.

Biro, P. A. and Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol. Evol.* **25**, 653-659.

Brett, J. R. and Groves, T. D. D. (1979). Physiological energetics. In *Fish Physiology*, Vol. 8. (ed. W. S. Hoar and D. J. Randall), pp. 279-352. New York: Academic Press.

Brijs, J., Jutfelt, F., Clark, T. D., Gräns, A., Ekstrom, A. and Sandblom, E. (2015). Experimental manipulations of tissue oxygen supply do not affect warming tolerance of European perch. *J. Exp. Biol.* **218**, 2448-2454.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771-1789.

Chabot, D., McKenzie, D. J. and Craig, J. F. (2016a). Metabolic rate in fishes: definitions, methods and significance for conservation physiology. *J. Fish Biol.* **88**, 1-9.

Chabot, D., Koenker, R. and Farrell, A. P. (2016b). The measurement of specific dynamic action in fishes. *J. Fish Biol.* **88**, 152-172.

Chapman, L. J. (2007). Morpho-physiological divergence across oxygen gradients in fishes. In *Fish Respiration and the Environment* (ed. M. N. Fernandes, F. T. Rantin, M. L. Glass and B. G. Kapoor), pp. 14-29. Enfield, NH: Science Publishers, Inc.

Chapman, L. J. and Hulen, K. G. (2001). Implications of hypoxia for the brain size and gill surface area of mormyrid fishes. *J. Zool.* **254**, 461-472.

Chapman, L. J., Albert, J. and Galis, F. (2008). Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. *Open Evol. J.* **2**, 75-88.

Chellappa, S., Huntingford, F. A., Strang, R. H. C. and Thomson, R. Y. (1995). Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *J. Fish Biol.* **47**, 775-787.

Chen, Z., Anttila, K., Wu, J., Whitney, C. K., Hinch, S. G. and Farrell, A. P. (2013). Optimum and maximum temperatures of sockeye salmon (*Oncorhynchus nerka*) populations hatched at different temperatures. *Can. J. Zool.* **91**, 265-274.

Chrétien, E. and Chapman, L. J. (2016). Tropical fish in a warming world: thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. *Conserv. Physiol.* **4**, 1-14.

Claireaux, G., Couturier, C. and Groison, A. L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). *J. Exp. Biol.* **209**, 3420-3428.

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

Clark, T. D., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771-2782.

Crispo, E. and Chapman, L. J. (2010). Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. *J. Evol. Biol.* **23**, 2091-2103.

Dalziel, A. C., Ou, M. and Schulte, P. M. (2012). Mechanisms underlying parallel reductions in aerobic capacity in non-migratory threespine stickleback (*Gasterosteus aculeatus*) populations. *J. Exp. Biol.* **215**, 746-759.

Donelson, J. M., Munday, P. L., McCormick, M. I. and Nilsson, G. E. (2011). Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. *Glob. Change Biol.* **17**, 1712-1719.

Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., Gale, M. K., Patterson, D. A., Hinch, S. G. and Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science* **332**, 109-112.

Eliason, E. J., Gale, M. K., Whitney, A. L. and Hinch, S. G. (2017). Intraspecific differences in endurance swim performance and cardiac size in sockeye salmon (*Oncorhynchus nerka*) parr tested at three temperatures. *Can. J. Zool.* **95**, 425-432.

Ern, R., Norin, T., Gamperl, A. K. and Esbaugh, A. J. (2016). Oxygen dependence of upper thermal limits in fishes. *J. Exp. Biol.* **219**, 3376-3383.

FAO (2014). *The State of World Fisheries and Aquaculture-2014 (SOFIA)*. Rome: FAO.

Farrell, A. P. (2009). Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *J. Exp. Biol.* **212**, 3771-3780.

Farrell, A. P., Eliason, E. J., Sandblom, E. and Clark, T. D. (2009). Fish cardiorespiratory physiology in an era of climate change. *Can. J. Zool.* **87**, 835-851.

Ficke, A. D., Myrick, C. A. and Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Rev. Fish Biol. Fish.* **17**, 581-613.

Froese, R. (2006). Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *J. Appl. Ichthyol.* **22**, 241-225.

Fry, F. E. J. (1947). Effects of the environment on animal activity. *Publ. Ont. Fish. Res. Lab.* **68**, 1-52.

Fry, F. E. J. (1971). The effects of environmental factors on the physiology of fish. In *Fish Physiology*, Vol. 6 (ed. W. S. Hoar and D. J. Randall). pp. 1-98. New York, NY: Academic Press.

Gamperl, K. and Farrell, A. P. (2004). Cardiac plasticity in fishes: environmental influences and intraspecific differences. *J. Exp. Biol.* **207**, 2539-2550.

Gonda, A., Herczeg, G. and Merilä, J. (2011). Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*)–local adaptation or environmentally induced variation? *BMC Evol. Biol.* **11**, 75.

Gräns, A., Jutfelt, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I. et al. (2014). Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated CO_2 in Atlantic halibut. *J. Exp. Biol.* **217**, 711-717.

Gunderson, A. R. and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B.* **282**, 20150401.

Healy, T. M. and Schulte, P. M. (2012). Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (*Fundulus heteroclitus*). *Physiol. Biochem. Zool.* **85**, 107-119.

Indian Ocean Commission (2015). *Nile Perch Fishery Management Plan for Lake Victoria, 2015-2019*. Ebene, Mauritus. http://www.fao.org/3/a-bl763e.pdf.

IPCC (2014). *Climate change 2014*: Synthesis Report. In *Contribution of Working Groups I, II, and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. **The Core Writing Team**, R. K. Pachauri and L. A. Meyer), pp. 978-995. Geneva: IPCC.

Jayasundara, N. and Somero, G. N. (2013). Physiological plasticity of cardiorespiratory function in a eurythermal marine teleost, the longjaw mudsucker, *Gillichthys mirabilis*. *J. Exp. Biol.* **216**, 2111-2121.

Jutfelt, R., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., Lefevre, S., Nilsson, G., Metcalfe, G., Metcalfe, N. B., Hickey, A. J. R., (2018). Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *J. Exp. Biol.* **211**, 1-4.

Keen, A. N., Klaiman, J. M., Shiels, H. A. and Gillis, T. E. (2017). Temperature-induced cardiac remodelling in fish. *J. Exp. Biol.* **220**, 147-160.

Kitchell, J. F., Schindler, D. E., Ogutu-Ohwayo, R. and Reinthal, P. N. (1997). The Nile perch in Lake Victoria: interactions between predation and fisheries. *Ecol. Appl.* **7**, 653-664.

Klaiman, J. M., Fenna, A. J., Shiels, H. A., Macri, J. and Gillis, T. E. (2011). Cardiac remodeling in fish: strategies to maintain heart function during temperature change. *PLoS ONE* **6**, e24464.

Kotrschal, A., Rogell, B., Maklakov, A. A. and Kolm, N. (2012). Sex-specific plasticity in brain morphology depends on social environment of the guppy, *Poecilia reticulata*. *Behav. Ecol. Sociobiol.* **66**, 1485-1492.

Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., Immler, S., Maklakov, A. and Kolm, N. (2013). The benefit of evolving a larger brain: big-brained guppies perform better in a cognitive task. *Anim. Behav.* **86**, e4-e6.

Langerhans, R. B., Chapman, L. J. and DeWitt, T. J. (2007). Complex phenotype-environment associations revealed in an East African cyprinid. *J. Evol. Biol.* **20**, 1171-1181.

Lynch, A. J., Cooke, S. J., Deines, A. M., Bower, S. D., Bunnell, D. B., Cowx, I. G., Nguyen, V. M., Nohner, J., Phouthavong, K., Riley, B. et al. (2016). The social, economic, and environmental importance of inland fish and fisheries. *Environ. Rev.* **24**, 1-7.

McBryan, T. L., Healy, T. M., Haakons, K. L. and Schulte, P. M. (2016). Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *J. Exp. Biol.* **219**, 474-484.

McNab, B. K. (2002). *The Physiological Ecology of Vertebrates: a View from Energetics*. Ithaca, New York: Cornell University Press.

Nelson, J. (2016). Oxygen consumption rate *v.* rate of energy utilization of fishes: a comparison and brief history of the two measurements. *J. Fish Biol.* **88**, 10-25.

Nkalubo, W. (2012). Life history traits and growth of Nile perch, *Lates niloticus* (L.), in Lake Victoria, Uganda: Implications for management of the fishery. PhD thesis, Makerere University, Kampala, Uganda.
Norin, T. and Clark, T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *J. Fish Biol.* **88**, 122-151.
Norin, T., Malte, H. and Clark, T. D. (2014). Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *J. Exp. Biol.* **217**, 244-251.
Nyboer, E. A. and Chapman, L. J. (2017). Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria. *J. Exp. Biol.* **220**, 3782-3793.
Nyboer, E. A. and Chapman, L. J. (2018). Data from: Cardiac plasticity influences aerobic performance and thermal tolerance in a tropical, freshwater fish at elevated temperatures. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.2k527b8
Paterson, J. A., Chapman, L. J. and Schofield, P. J. (2010). Intraspecific variation in gill morphology of juvenile Nile perch, *Lates niloticus*, in Lake Nabugabo, Uganda. *Environ. Biol. Fishes* **88**, 97-104.
Phuong, L. M., Huong, D. T. T., Nyengaard, J. R. and Bayley, M. (2017). Gill remodeling and growth rate of striped catfish *Pangasianodon hypophthalmus* under impacts of hypoxia and temperature. *Comp. Biochem. Physiol. Part A* **203**, 288-296.
Pörtner, H.-O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol. Part A.* **132**, 739-761.
Pörtner, H.-O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* **213**, 881-893.
Pörtner, H.-O. and Farrell, A. P. (2008). Physiology and climate change. *Ecology* **322**, 690-692.
Pörtner, H.-O. and Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **5**, 95-97.
Pörtner, H.-O., Bock, C. and Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *J. Exp. Biol.* **220**, 2685-2696.
Poulson, T. L. (2001). Morphological and physiological correlates of evolutionary reduction of metabolic rate among amblyopsid cave fishes. *Environ. Biol. Fishes* **62**, 239-249.
Priede, I. G. (1985). Metabolic scope in fishes. In *Fish Energetics, New Perspectives* (ed. P. Tytler and P. Calow). pp. 33-64. London: Croom Helm.
Roche, D. G., Binning, S. A., Bosiger, Y., Johansen, J. L. and Rummer, J. L. (2013). Finding the best estimates of metabolic rates in a coral reef fish. *J. Exp. Biol.* **216**, 2103-2110.
Sandblom, E., Gräns, A., Axelsson, M. and Seth, H. (2014). Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. *Proc. R. Soc. B* **281**, 20141490.
Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* **218**, 1856-1866.
Schulte, P. M., Healy, T. M. and Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* **5**, 691-702.
Seebacher, F., White, C. R. and Franklin, C. E. (2014). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change* **5**, 61-66.
Seneviratne, S. I., Donat, M. G., Mueller, B. and Alexander, L. V. (2014). No pause in the increase of hot temperature extremes. *Nat. Clim. Change* **4**, 161-163.
Sibly, R. M., Brown, J. H. and Kodric-Brown, A. (2012). *Metabolic Ecology: A Scaling Approach*, pp. 1-392. West Sussex: John Wiley and Sons.
Sollid, J., De Angelis, P., Gundersen, K. and Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *J. Exp. Biol.* **206**, 3667-3673.
Sollid, J., Weber, R. and Nilsson, G. (2005). Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. *J. Exp. Biol.* **208**, 1109-1116.
Tewksbury, J. J., Huey, R. B. and Deutsch, C. A. (2008). Putting the heat on tropical animals. *Science* **320**, 1296.
Toli, E. A., Noreikiene, K., DeFaveri, J. and Merilä, J. (2016). Environmental enrichment, sexual dimorphism, and brain size in sticklebacks. *Ecol. Evol.* **7**, 1691-1698.
Ultsch, G. R. (1995). On adjusting metabolic rates for body size. *Fla. Sci.* **58**, 270-273.
Wang, T., Lefevre, S., Iverson, N. K., Findorf, I., Buchanan, R. and McKenzie, D. J. (2014). Anemia only causes a small reduction in the upper critical temperature of sea bass: is oxygen delivery the limiting factor of acute warming in fishes? *J. Exp. Biol.* **217**, 4275-4278.
Weber, R. E., Wood, S. C. and Lomholt, J. P. (1976). Temperature acclimation and oxygen-binding properties of blood and multiple haemoglobins of rainbow trout. *J. Exp. Biol.* **65**, 333-345.

SUPPLEMENTARY INFORMATION

Table S1. Sample sizes (N) and means ± s.e.m. of body mass (M*b*), standard length (SL) and total length (TL) of wild juvenile Nile perch stocked into 3 replicate blocks of containing among 2 rearing regimes: T_{avg} (~25°C) representing current average temperatures in Lake Victoria and T_{warm} (~29°C) representing conditions predicted under climate change. The average age of Nile perch stocked in the rearing ponds was estimated to be ~2 months based on the von Bertalanffy length-at-age growth equation calculated using previously published growth parameters for Lake Victoria Nile perch (Nkalubo et al., 2012).

Rearing Regime	Block	N	M*b* (g)	SL (cm)	TL (cm)
T_{avg}	1	100	17.76 ± 0.60	10.16 ± 0.29	12.33 ± 0.46
	2	100	19.41 ± 0.74	10.30 ± 0.29	12.42 ± 0.47
	3	100	21.64 ± 0.74	10.24 ± 0.25	12.31 ± 0.44
T_{warm}	1	100	18.45 ± 0.65	10.28 ± 0.26	12.46 ± 0.42
	2	100	19.83 ± 0.65	10.37 ± 0.26	12.55 ± 0.43
	3	100	19.31 ± 0.56	10.28 ± 0.24	12.57 ± 0.39
TOTAL		**600**	**19.41 ± 6.69**	**10.27 ± 1.16**	**12.42 ± 1.39**

Table S2. Means ± s.e.m. for water quality variables: maximum and minimum temperature, lux (luminosity), dissolved oxygen (DO), pH, and turbidity (Secchi depth), across rearing regimes (T_{avg} and T_{warm}) and among replicates (blocks) within a rearing regime.

Rearing regime	Block	Max. temp. (°C)	Min. temp. (°C)	Lux (lm $m^{2\,-1}$)	Avg. DO (mg L^{-1})	pH	Secchi (cm)
T_{avg}	**mean**	**24.5 ± 0.70**	**23.0 ± 0.62**	**85.8 ± 55.0**	**4.253 ± 1.56**	**6.965 ± 0.62**	**51.9 ± 9.92**
	1	24.4 ± 0.66	22.9 ± 0.63	82.5 ± 56.3	4.081 ± 1.49	7.018 ± 0.63	51.75 ± 10.69
	2	24.5 ± 0.74	22.9 ± 0.62	96.5 ± 57.2	4.352 ± 1.52	6.95 ± 0.64	51.25 ± 9.80
	3	24.5 ± 0.71	23.0 ± 0.57	78.1 ± 53.3	4.325 ± 1.68	6.933 ± 0.60	52.71 ± 9.32
T_{warm}	**mean**	**28.6 ± 1.41**	**27.1 ± 1.53**	**112.587 ± 63.4**	**4.358 ± 1.60**	**6.86 ± 0.60**	**50.81 ± 10.59**
	1	29.1 ± 1.56	27.7 ± 1.79	114.222 ± 65.1	4.261 ± 1.82	6.928 ± 0.65	49.57 ± 10.52
	2	28.6 ± 1.38	27.2 ± 1.34	94.592 ± 54.1	4.411 ± 1.55	6.793 ± 0.55	50.47 ± 10.53
	3	28.2 ± 1.04	26.4 ± 1.02	128.039 ± 66.0	4.403 ± 1.43	6.857 ± 0.60	52.37 ± 10.70

Table S3. Sample sizes (N) and means ± s.e.m. of body mass (M*b*), standard length (SL), and total length (TL) for juvenile Nile perch in each rearing regime used in analyses of critical thermal maximum (CT_{max}), relative ventricular mass (RVM), percent compact myocardium (%CM), gill metrics, relative brain mass (RBM), and hepatosomatic index (HSI). Sample sizes were selected to ensure adequate power based on known levels of variability in the traits measured.

Trait	Rearing regime	N	Mb (g)	TL (cm)	SL (cm)
CT_{max}	Tavg	9	29.14 ± 2.00	15.34 ± 0.31	12.76 ± 0.28
	Twarm	10	31.40 ± 3.64	15.99 ± 0.56	13.25 ± 0.48
RVM	Tavg	19	34.73 ± 3.19	15.03 ± 0.37	12.79 ± 0.31
	Twarm	18	39.11 ± 3.89	15.78 ± 0.45	13.13 ± 0.39
%CM	Tavg	19	34.73 ± 3.19	15.3 ± 0.37	12.79 ± 0.31
	Twarm	17	40.39 ± 3.89	15.92 ± 0.46	13.25 ± 0.39
Gills	Tavg	9	37.21 ± 3.23	15.58 ± 0.41	13.03 ± 0.33
	Twarm	10	36.96 ± 3.47	15.65 ± 0.42	12.96 ± 0.35
Brains	Tavg	11	39.35 ± 4.41	15.83 ± 0.52	13.23 ± 0.43
	Twarm	12	41.31 ± 3.35	16.13 ± 0.42	13.44 ± 0.36
HSI	Tavg	19	34.73 ± 3.19	15.3 ± 0.370	12.79 ± 0.31
	Twarm	13	38.21 ± 4.65	15.68 ± 0.49	13.08 ± 0.42

Table S4. Results of 2-way ANCOVA on raw organ masses and percent of complex myocardium among two rearing temperatures (T_{avg} vs. T_{warm}) and among replicates (block) with body mass (M*b*) as a covariate.

Variables		Rearing regime	Block	M*b*	M*b* x RR
Ventricle mass (g)	$F_{(1, 31)}$	11.933	0.475	203.411	31.831
	P	**0.002**	**0.627**	**<0.001**	**<0.001**
	η^2	0.278	0.030	0.868	0.507
Liver mass (mg)	$F_{(1, 27)}$	24.828	0.303	85.008	
	P	**<0.001**	0.741	**<0.001**	
	η^2	0.479	0.022	0.759	
% Compact myocardium	$F_{(1, 31)}$	10.306	0.888	3.114	
	P	**0.003**	0.442	0.087	
	η^2	0.250	0.054	0.091	
Brain mass (mg)	$F_{(1, 19)}$	0.146	0.012	14.380	
	P	0.706	0.988	**0.001**	
	η^2	0.008	0.001	0.431	

Table S5. Eigenvalues, percent variance explained, and correlation of each gill metric on the components extracted in the PCA analysis. Gill traits are total hemibranch area (THA), total gill filament length (TGFL), average gill filament length (AGFL), total filament number (TFN), and filament base length (FBL). Filament density (D) was not included in the PCA analysis because it did not correlate with the other traits.

	PC1	PC2
Eigenvalue	3.327	1.346
% Variance	66.5	26.9
THA (mm^2)	**0.890**	0.428
TGFL (mm)	**0.881**	0.440
AGFL (mm)	**0.991**	-0.108
TFN (mm)	0.203	**0.915**
FBL (mm)	0.115	**0.918**

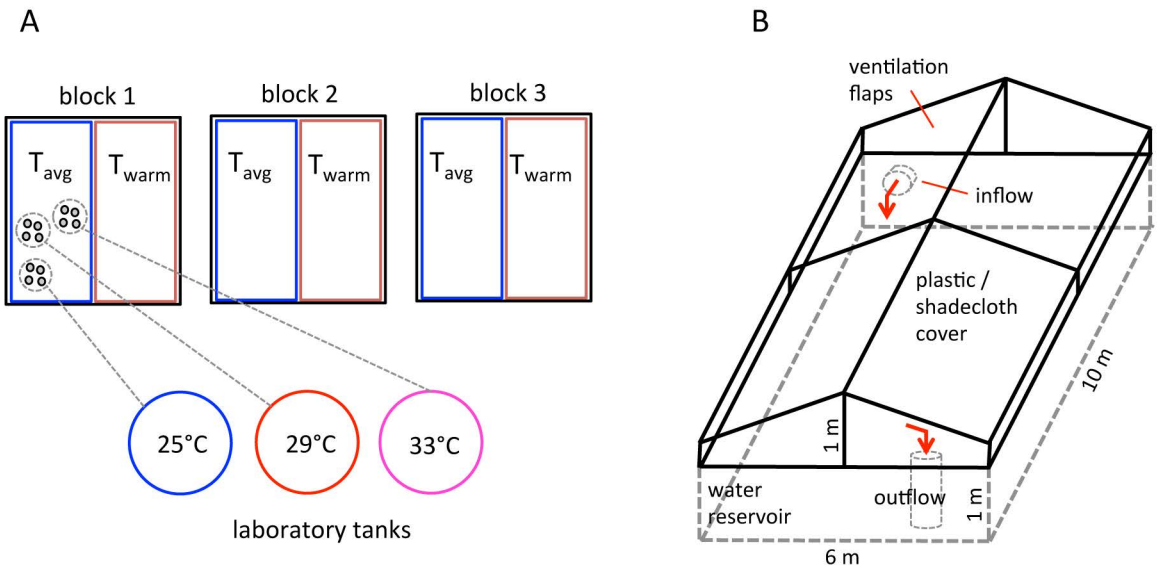

Figure S1. A schematic diagram of the experimental layout. Panel A show 3 blocks containing one replicate of the T_{warm} and one replicate of the T_{avg} rearing ponds. 2 - 4 fish (represented by grey dots) from each rearing pond were exposed to each of the 3 laboratory temperatures, as represented by the dashed grey lines from the T_{avg} pond in block 1. Panel B is a diagram of one plastic- or shade cloth-covered rearing pond highlighting key components of the heating and cooling design.

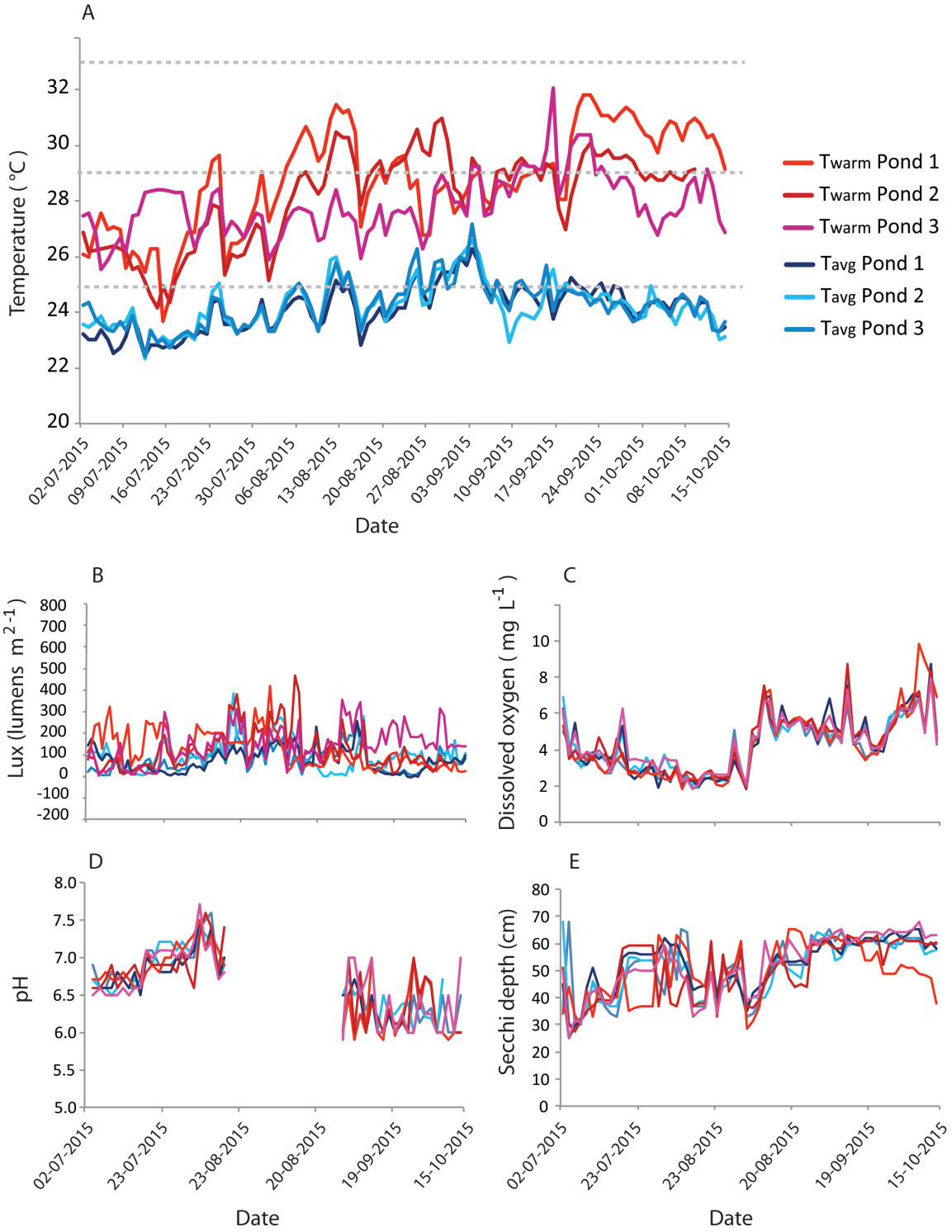

Figure S2. Plots of raw pond water quality data over the 3-month rearing period (19-Jul. –25-Oct., 2015). In all panels each line represents a pond replicate, with T_{warm} tanks in

red tones and T_{avg} tanks in blue tones. Panel A shows daily average maximum temperatures. Grey dotted lines represent the 3 experimental temperatures (25, 29 and 33°C) used in the laboratory for respirometry tests. Panels B - E show daily averages for Lux (B), dissolved oxygen (DO) concentrations (C), pH (D), and turbidity as measured by Secchi depth (E).

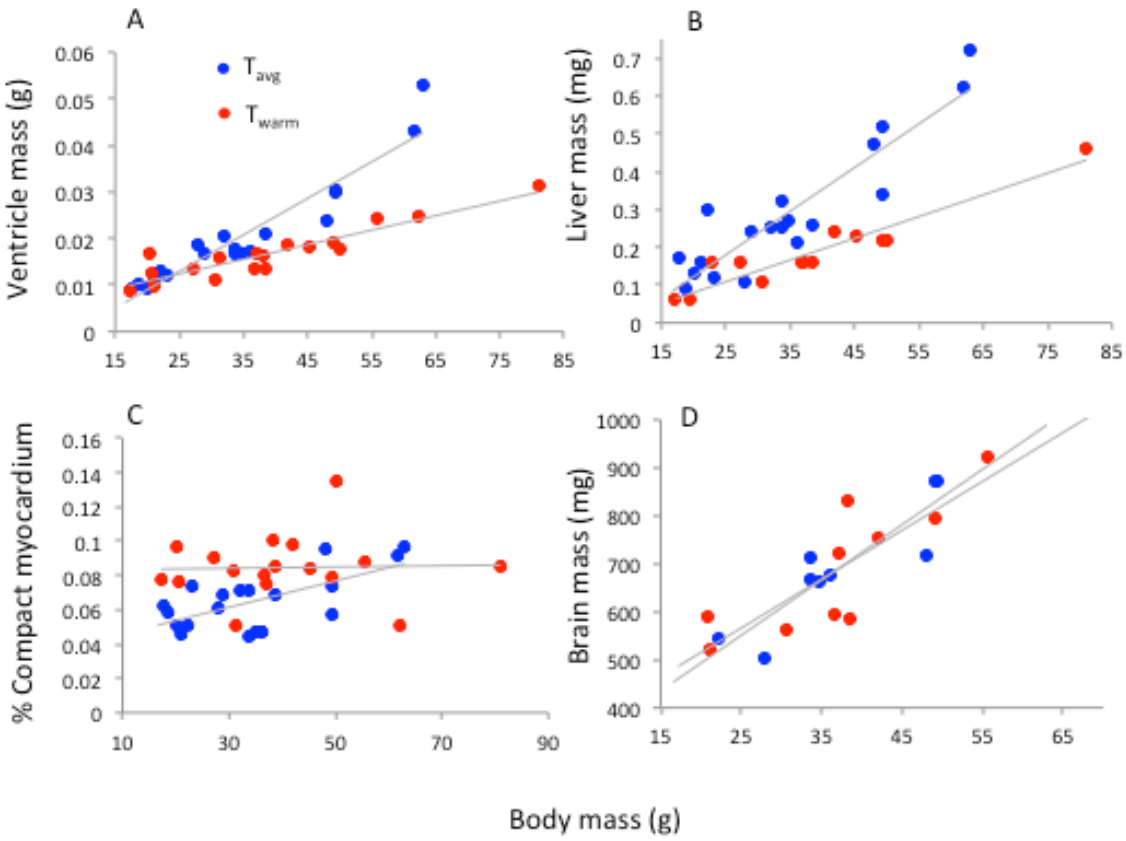

Figure S3. Linear relationships between body mass and ventricle mass (A), liver mass (B), % compact myocardium (C), and brain mass (D) raised under T_{warm} and T_{avg} conditions for 3 months.