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Capacity for thermal adaptation in Nile tilapia (*Oreochromis niloticus*): Effects on oxygen uptake and ventilation

Jonas Ndau Leonard [a,b], Peter Vilhelm Skov [b,*]

[a] *Sokoine University of Agriculture, College of Agriculture, Department of Animal, Aquaculture and Range Sciences, Section of Aquaculture, Morogoro, Tanzania*
[b] *Technical University of Denmark, DTU Aqua, National Institute of Aquatic Resources, Section for Aquaculture, North Sea Science Park, 9850, Hirshals, Denmark*

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ABSTRACT

Standard metabolic rate (SMR) and maximum metabolic rate (MMR) were determined for Nile tilapia acclimated to six different experimental temperatures from 18 °C to 38 °C. SMR increased exponentially with temperature, from 79.8 mg O_2 kg^{-1} h^{-1} at 18 °C, to 255.1 mg O_2 kg^{-1} h^{-1} at 38 °C (Q_{10} = 1.79). The main increase in Q_{10} occurred within the highest temperature range, whereas in the lower temperature from 18 °C to 22 °C, temperature did not significantly affect SMR. MMR showed a hyperbolic correlation with increasing temperature, rising from 240.5 mg O_2 kg^{-1} h^{-1} at 18 °C to a peak of 373.8 mg O_2 kg^{-1} h^{-1} at 30 °C, before decreasing again at higher temperatures. Absolute aerobic scope (AAS) peaked at 26.0 °C, which we conclude to be the optimal temperature for Nile tilapia. The optimal temperature range, defined as the thermal range where 80% or more of the metabolic scope (MS) can be maintained, occurred between 19.5 and 32.1 °C. The lower (TC_{MIN}) and upper (TC_{MAX}) critical temperatures occurred at 13.1 °C and 38.8 °C. Nile tilapia showed a 4-fold scope for increasing ventilation frequency from 24 opercular beats min^{-1} (OB min^{-1}) during SMR at 18 °C, to a maximum of 100 OB min^{-1} which occurred during MMR at 34 °C. f_V during MMR increased with temperature, but above 30 °C became uncoupled with MO_2, as fish were unable to sustain their rates of oxygen consumption despite a high f_V. There was a strong correlation between f_V and SMR (r^2 = 0.83) across all temperatures indicating that f_V is a good predictor of SMR. However, the correlation between MMR and f_V was weak (r^2 = 0.06), due to a strong interacting effect of temperature. When selecting data from the thermal optimum range, a good correlation between f_V and MO_2 was obtained (r^2 = 0.74).

1. Introduction

Aquaculture production of Nile tilapia, *Oreochromis niloticus*, has increased 4-fold over the last decade. The species is cultured in simple production systems in more than 140 countries, representing one of the most important farmed freshwater fish. Annual global production exceeds 4.5 million tons, and represents 8.3% of global production volume of all finfish (FAO, 2020). Nile tilapia is a tropical species native to northern, western, and eastern Africa, but has been introduced to Asia and the Americas, where they are mainly farmed in shallow earthen ponds.

The distribution of Nile tilapia is limited by a lower lethal temperature of 11–12 °C, and an upper lethal temperature of 42 °C (FAO, 2021), while an optimal rearing temperature is not well defined. For fingerlings, a thermal optimum of 25–30 °C has been reported (El-Sherif and El-Feky, 2009), and for juveniles 27–32 °C (Pandit and Nakamura,

1970). FAO (2021) reports a preferred temperature range of 31–36 °C, but temperature ranges from 20 to 30 °C have also been reported (El-Sayed and Kawanna, 2008; Mirea et al., 2013). Water temperature is one of the most significant environmental factors to influence the physiology and energetics of fish, and defines the metabolic scope of an individual (Fry, 1947). The ability of fish to behaviourally regulate their occupied temperature has been shown for several species; in Atlantic cod (*Gadus morhua*) using a shuttle-box system (Petersen and Steffensen, 2003), for minnows (*Phoxinus phoxinus*) in a vertical temperature gradient (Ward et al., 2010), and for largemouth bass (*Micropterus salmoides*) in a horizontal gradient (Díaz et al., 2007). Fish are thought to behaviourally select temperatures that maximise their physiological performance (Dalla Valle et al., 2003; Ward et al., 2010), although numerous other factors (e.g. risk of predation, preferred water depth, food availability, hypoxia) are known to be influencing factors. If thermal preference of a species coincides with the temperature where

physiological performance and fitness are at an optimum (Martin and Huey 2008), it may reflect the temperature at which the greatest proportion of energy can be channeled towards growth, because maintenance costs are minimized (Brauner and Richards, 2020; Bryan et al., 1990).

Experimentally, the thermal optimum can be determined from the temperature at which a species has its maximum aerobic scope. This is expressed as the difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR) (Farrell, 2016), from which a Fry aerobic scope curve can be produced to illustrate aerobic scope at different acclimation temperatures, expressed either in absolute terms (e.g. net oxygen uptake capacity) or in relative terms (e.g. factorial capacity). The upper and lower boundaries of the thermal range can be defined based on the degree of scope conservation. Commonly, such thresholds are set relatively high, i.e. 80-90% of maximum (Eliason et al., 2013; Frisk et al., 2012).

Whether the purpose is to assess impacts of climate change, species distribution, or optimal rearing conditions for different fish species in aquaculture, it is essential to determine how different environmental variables affect the performance or fitness of a species, which ranges are safe for the fish, and when water quality parameters begin to limit or mask the metabolic scope of a species (Claireaux and Lefrançois, 2007). Numerous water quality variables play a role in this regard, although dissolved oxygen and temperature probably represent the most widely studied. Temperature is perhaps the main determinant of habitat suitability for a fish species, because it affects oxygen transport capacity (Pörtner and Farrell, 2008) through changes in cardiac and ventilation performance, and thereby the ability to extract oxygen from the water and delivery to mitochondrial tissues. Thermal tolerance varies considerably between species. Some, such as equatorial species of damselfish (*Acanthochromis polyacanthus* and *Dascyllus melanurus*) can only occupy extremely narrow temperature ranges of 2–3 °C (Rummer et al., 2014) while eurythermal species such as pike perch (*Sander lucioperca*) (Frisk et al., 2012) and European eel (*Anguilla anguilla*) (Methling, 2013) have much broader thermal optima.

For ectotherms, metabolic rates and ventilation frequencies change in response to changes in demand driven by environmental conditions, and such data are important in the construction of bioenergetic models, and conveys important information on the effects of activity levels and stress (Cech Jr and Brauner, 2011). Furthermore, such data can be used to determine feeding levels for maintenance and growth in aquaculture (Jobling, 1981), and potential requirements for supplemental oxygen of a species (Fitzgibbon et al., 2007). Information on the influence of temperature on oxygen consumption rates of Nile tilapia are limited. Turker (2011) investigated the influence of temperature on SMR and routine metabolic rate (RMR) of Nile tilapia acclimated to 5 temperatures ranging from 19 to 31 °C and found that both SMR and RMR increased exponentially with increasing temperature, while any temperature dependence of MMR was not determined.

Determining the metabolic rates of fish is routinely performed in the laboratory, using intermittent respirometry, whereas obtaining such data under field conditions provides estimates of metabolic rates that are prone to errors (Millidine et al., 2008; Soofiani and Hawkins, 1985). The ability to estimate metabolic rates of fish directly in the rearing tanks of an aquaculture system is a desirable tool for general husbandry and welfare practices and can be used as a management tool in production systems. One suggested approach for estimation of metabolic rates in the field is based on the measurements of respiratory variables, such as ventilation frequency or amplitude. While amplitude is near impossible to assess without instrumentation, ventilation frequency (f_V) can often be directly observed. A relationship between ventilation frequency (f_V) and oxygen consumption rate (MO_2) has been explored for a number of fish species, including Atlantic salmon, *Salma salar* (Millidine et al., 2008), stoplight parrotfish, *Sparisoma viride* (Van Rooij and Videler, 1996), and pike perch (Frisk et al., 2012). While dependencies between f_V and MO_2 exist, it appears to be species specific to which extent

temperature is an interacting factor.

The objective of the present study was to determine the influence of temperature on the metabolism and aerobic scope of Nile tilapia. This was achieved through measurements of SMR and MMR at six different acclimation temperatures, ranging from 18 to 38 °C. We also wanted to determine to what extent ventilation was a limiting factor as temperatures approached critical levels, and finally, whether ventilation frequency (f_V) could be correlated to MO_2 in Nile tilapia. For this, we measured operculum beats during the SMR and MMR values determined at the different acclimation temperatures.

2. Materials and methods

2.1. Experimental animals and acclimation

Nile tilapia were obtained from a laboratory stock originating from a commercial breeder (Til-Aqua International, Velden, Netherlands). The fish were acclimated to 25 °C, and initially stocked in triplicate groups of 8 individuals in 60L glass aquariua at 25 °C. Temperature was adjusted at a rate of 1 °C per day until the desired experimental temperatures of 18, 22, 26, 30, 34, or 38 °C was reached. The temperature in each aquarium was regulated using temperature controllers (T 2001 HC, Aqua medic). Fish were fed twice daily to satiation using a commercial extruded Tilapia feed (EFICO Alpha 845F, BioMar A/S, Denmark), and oxygen levels were maintained above 75% saturation by continuous aeration. Approximately 50% of the water volume in each aquarium was replaced daily, using aerated water thermostatted to the experimental temperature. Fish were allowed to acclimate to their final experimental temperature for a minimum of 3 weeks prior to any measurements.

2.2. Respirometry measurements

Oxygen consumption measurements were performed using four respirometers immersed into a 260L holding tank, supplied by a 600L reservoir where water was continuously pumped over a trickling filter. Inlet water passed through a UV unit (9W UV-C Aqua Cristal GmbH, Neuhofen, Germany), and the experimental temperature was maintained at ± 0.1 °C by a 1600W heater controlled by programmable relay (PR-5714, PR Electronics, Denmark).

Oxygen consumption rates were measured using computerized intermittent flow respirometry (Steffensen, 1989) in loops of 7–10 min depending on the experimental temperature. One loop consisting of 3–4 min flush period followed by a 1-min waiting period prior to the 3–5 min measuring period. The fish used ranged in mass from 76.9 to 99.3 g (mean 85.4 g).

The oxygen level in each respirometer was measured using fibreoptic sensors connected to an oxygen meter (OXY-4 mini, Precision Sensing GmbH, Regensburg, Germany). Oxygen data was recorded and processed on a PC using AutoResp 2.2.0 software, (Loligo Systems, Denmark), according to the method described by Steffensen et al. (1984).

Prior to introducing fish to the respirometers, feed was withheld for 24 h. In the morning, 4 fish were randomly selected from each treatment and introduced into a respirometer for overnight MO_2 measurements. The following day, the maximum metabolic rate (MMR) of individual fish was determined following the protocol provided by (Killen et al., 2007). Briefly, a continuous flush mode of the loop was activated, and one fish at a time was acquired from a respirometer and transferred to a container with aerated water at the experimental temperature. The fish was chased by hand until unresponsive to handling, and then quickly transferred to the respirometer. The flush cycle was ended, and MO_2 measurements resumed.

The following morning, fish were removed and respirometers were thoroughly cleaned prior to the introduction of new fish for the next experiments. When changing from one experimental temperature to another the entire system was allowed to stabilize overnight without

fish. SMR was quantified from MO_2 measured by fitting the MO_2 values into frequency distribution (Skov et al., 2011) while MMR was extracted by using quantile approach (q = 0.99) (Dupont-Prinet et al., 2013).

For all fish, oxygen uptake was adjusted to a common body size of 85g using the allometric equation described by Steffensen et al. (1994).

$$MO_{2(85)} = MO_{2(BM)} (BM/85)^{(1-A)}$$

Where $MO_{2(BM)}$ is the oxygen consumption rate of fish with a body mass (BM), $MO_{2(85)}$ is the oxygen consumption of fish adjusted to a mass of 85g, A is the allometric exponent quantifying the relationship between the metabolic rate and body mass. In the present study, the value for A was set at 0.79 (Clarke and Johnston, 1999). The relationship between SMR obtained for each individual fish and the experimental temperature was fitted using the exponential function

$$SMR = a \times e^{(bT)},$$

while individual values for MMR and absolute aerobic scope (AAS) were fitted to a second order polynomial function

$$MMR = aT^2 + bT - c$$

Where, a, b and c are constants estimated from regression analysis, and T is the experimental temperature (°C). AAS was calculated as the difference between MMR and SMR for each individual, and the relationship between experimental temperature and AAS was determined by fitting all values to a quadratic function.

The sensitivity of SMR to temperature was then calculated as Q_{10} (Eccles, 1985; Mayfield and Cech, 2004)

$$Q_{10} = (R_2/R_1)^{(10/(T_2 - T_1))}$$

Where, Q_{10} is the factor by which the SMR changes when temperature is changed from T_1 to T_2, R_1 and R_2 are SMR at temperature T_1 and T_2 respectively.

2.3. Ventilation frequency measurements

To obtain the ventilation frequency (f_V), opercula beat frequencies were monitored by camera at each experimental temperature, during collection of data for SMR and MMR. Ventilation frequency was determined according to the procedure provided by Cerezo and Garcia (2004), in which the opening and closing of the operculum of the fish was counted over a 30s period and expressed as opercular beats per minute (OB min^{-1}).

2.4. Data analysis and statistics

Statistical analyses were performed in SigmaPlot (v. 14.5, Systat Software, Germany). One-way ANOVA was used to test for statistical difference between treatments. Data was tested for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe). In some instances, it was necessary to log-transform data to obtain equal variance. All-pairwise multiple comparison procedures were performed using the Holm-Sidak method, and statistical significance was taken at P < 0.05. The confidence interval for optimal temperature was obtained using the function lm () in the Stats package of the open-source software R, by resampling with replacement for 1000 iterations, fitting the polynomial model to each resampled dataset, predicting the AAS for temperatures between the observed values in steps of 0.1 °C, extracting the corresponding temperature for the maximum AAS, and calculating the 5 and 95 percentile of the optimum temperature from all 1000 iterations.

3. Results

3.1. Oxygen consumption and aerobic scope

SMR and MMR of Nile tilapia at each acclimation temperature are shown in Table 1, along with the corresponding absolute aerobic scope (AAS) and ventilation frequency (f_V). SMR showed an exponential increase with temperature described as

$$SMR = 24.423e^{0.0582T} \quad (r^2 = 0.83) \tag{1}$$

where T is the acclimation temperature (°C).

The temperature coefficient (Q_{10}) across the entire temperature range was 1.79. Q_{10} between temperature increments showed modest effects of temperature in the lower range (e.g. Q_{10} = 1.02 between 18 and 22 °C, whereas the main contribution came from the higher temperature increments, where Q_{10} was 3.30 between 34 and 38 °C.

Mean MMR peaked at an acclimation temperature of 30 °C, although not significantly different from 34 °C (Table 1). At 38 °C, MMR decreased significantly and approached SMR. The relationship between temperature and MMR was expressed as

$$MMR = -0.959T^2 + 56.033T - 469.84 \quad (r^2 = 0.52) \tag{2}$$

The intercepts of the extrapolation of SMR and MMR showed a loss of metabolic scope at 13.1 °C (CT_{MIN}) and 38.8 °C (CT_{MAX}) (Fig. 1A). The relationship between acclimation temperature and AAS, calculated as the difference between Eqs. (1) and (2) is shown in Fig. 1B. AAS followed a parabolic curve with a peak at 26.0 °C, with a 95% confidence interval from 25.6 to 26.3 °C, at which AAS was 238.8 mg O_2 kg^{-1} h^{-1}. The lower and upper temperatures at which 80% or more of the AAS could be maintained were found to be 20.2 and 31.7 °C, respectively.

3.2. Ventilation frequency

Ventilation frequencies (f_V) during SMR and MMR at each experimental temperature are presented in Table 1, along with the absolute scope for ventilation (ASf_V). There was a modest correlation between f_V and SMR across all experimental temperatures (SMR = 1.792 × f_V + 45.57, r^2 = 0.61), and the amount of oxygen extracted during SMR did not change significantly between temperatures (0.05–0.06 mg O_2 kg^{-1} OB^{-1}). However, the correlation between f_V and MO_2 at MMR was poor (r^2 = 0.06), and the amount of oxygen extracted per ventilation decreased by 25–40% (from 0.07 to 0.08 mg O_2 kg^{-1} OB^{-1}) as temperatures approached the upper range. Absolute scope for ventilation (ASf_V), the difference between f_V at MMR and SMR, increased significantly with temperature. ASf_V peaked at 34 °C at 47.6 OB min^{-1}, but

Table 1
Metabolic rates and associated ventilation frequencies for Nile tilapia at different acclimation temperatures.

	18 °C	22 °C	26 °C	30 °C	34 °C	38 °C
SMR (mg O_2 kg^{-1} h^{-1})	79.7 ± 3.9[a]	80.2 ± 3.1[a]	110.8 ± 7.6[b]	136.8 ± 5.8[c]	158.3 ± 6.3[c]	255.1 ± 11.4[d]
f_V *SMR (OB min^{-1})*	24.3 ± 0.8[a]	29.5 ± 1.1[b]	32.8 ± 0.8[c]	37.9 ± 0.3[d]	52.4 ± 1.7[e]	83.9 ± 2.9[f]
MMR (mg O_2 kg^{-1} h^{-1})	240.5 ± 6.3[a]	281.8 ± 12.5[ab]	319.7 ± 8.7[bc]	373.8 ± 9.1[d]	333.3 ± 21.8[cd]	265.5 ± 9.4[a]
f_V *MMR (OB min^{-1})*	51.7 ± 1.8[a]	62.2 ± 4.2[b]	74.6 ± 2.3[c]	84.3 ± 5.0[cd]	100.0 ± 2.8[d]	94.0 ± 3.4[d]
AAS (mg O_2 kg^{-1} h^{-1})	160.8 ± 5.6[a]	221.5 ± 12.1[ab]	208.9 ± 11.1[bc]	237.0 ± 9.1[b]	155.4 ± 20.0[ac]	10.4 ± 5.9[d]
ASf_V *(OB min^{-1})*	27.3 ± 2.1[a]	32.7 ± 4.6[ab]	41.8 ± 2.3[bc]	46.3 ± 5.2[bc]	47.6 ± 2.6[c]	10.1 ± 3.9[d]

Standard metabolic rate (SMR), ventilation frequency at SMR (f_V SMR), maximum metabolic rate (MMR), and ventilation frequency at MMR (f_V MMR). Absolute aerobic scope (AAS), and absolute scope for ventilation frequency (ASf_V). All values are presented as mean ± S.E.M., under normoxic conditions. Values that do not share a common superscript are significantly different between temperature treatments ($p < 0.05$).
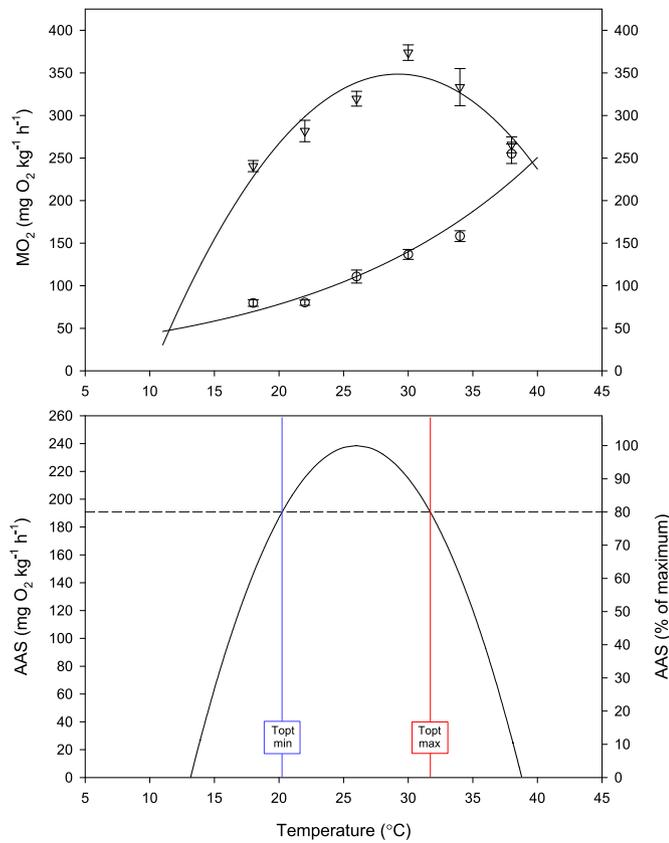
Fig. 1. Relationship between acclimation temperature and standard metabolic rate (SMR), maximum metabolic rate (MMR), and absolute aerobic scope (AAS). (A) Effects of acclimation temperature on standard metabolic rate (SMR, circles) and maximum metabolic rate (MMR, triangles). Solid lines represent the exponential regression between temperature and SMR, and the polynomial regression with MMR, respectively. n = 12 for each data point. Values represent mean ± S.E.M. (B) Absolute aerobic scope (AAS) as mg O_2 kg^{-1} h^{-1} (left hand axis) or as percentage of maximum (right hand axis), based on the regression between acclimation temperature and the difference between MMR and SMR for all individual fish (n = 72). The correlation is extrapolated beyond the tested temperatures to indicate TC_{MIN} and TC_{MAX}, while the thermal range where 80% of AAS is conserved is bordered by lines indicating T_{OPT} min and T_{OPT} max.

then decreased nearly 5-fold at 38 °C due to a considerable increase in ventilation effort during SMR. Fig. 2A shows the overall relationship between mean f_V and SMR and MMR for each experimental temperature, and illustrates the uncoupling between ventilation effort and MMR that sets in at 34 °C.

Due to the lack of correlation between ventilation effort and MO_2 at the temperature extremes, we limited the data analysis to include the temperatures within the thermal optimum (i.e. 22, 26, and 30 °C) (Fig. 2B). This resulted in an improved correlation between f_V and SMR and MMR, described as

$$MO_2 = 4.434 \times f_V - 18.002 \ (r^2 = 0.74) \tag{3}$$

where f_V is ventilation frequency as opercular beats per minute (OB min^{-1}).

4. Discussion

The relationship between acclimation temperature and SMR observed in the presented study follows the exponential relationship that has been reported for numerous other species (Beamish, 1964; Clarke and Johnston, 1999; Frisk et al., 2012; Ikeda et al., 2001; Johnston et al., 1991). The increase in SMR (Q_{10} = 1.79) across the thermal
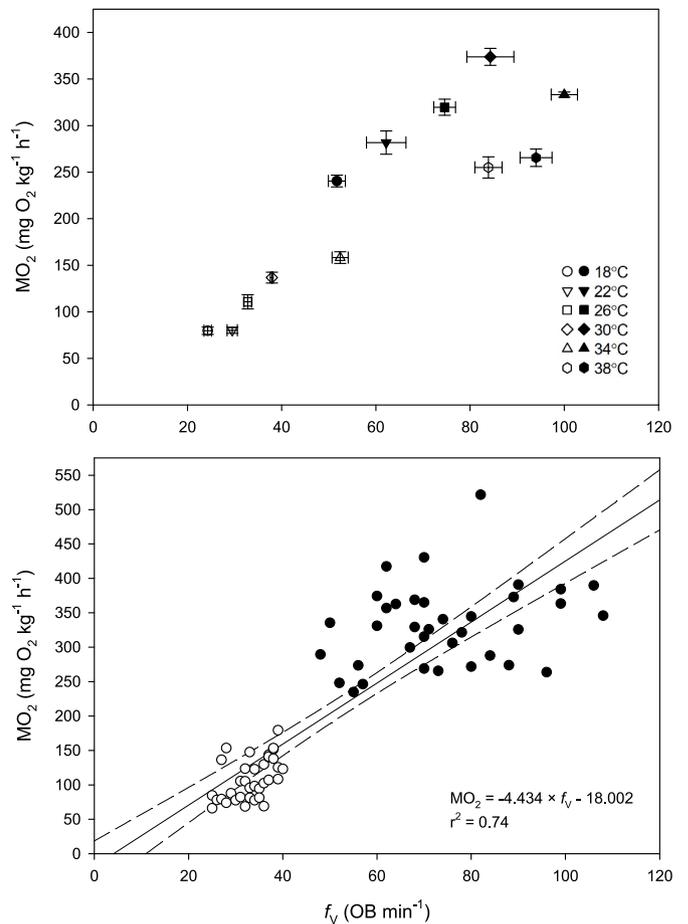

Fig. 2. Relationship between ventilation frequency (f_V) and standard metabolic rate (SMR) and maximum metabolic rate (MMR) at different acclimation temperatures. (A) Ventilation frequency (f_V) at standard metabolic rate (SMR, open symbols) and maximum metabolic rate (closed symbols) for each acclimation temperature from 18 to 38 °C. A moderate correlation was evident for f_V and SMR, while ventilation during MMR became compromised at high temperatures. n = 12 for each data point. Values are mean ± S.E.M. (B) f_V and MO_2 for data selected within the optimal thermal range. Open symbols represent values for SMR, closed symbols represent MMR values. The solid line represents the collective correlation between f_V and MO_2, while dashed lines show 95% confidence intervals.

range shows a smaller thermal sensitivity than reported for other eurythermal species, such as the tropical barramundi (*Lates calcarifer*) with a Q_{10} of ~2.5 (Norin et al., 2014), as well as temperate round goby (*Neogobius melanostomus*, Q_{10} = 2.6) (Christensen et al., 2021), or pike perch (*Sander lucioperca*, Q_{10} = 2.0) (Frisk et al., 2012). However, in some of these studies, SMR was not measured at temperatures near CT_{MAX}, thereby not including any large increases in Q_{10} that would be expected from the upper thermal increments.

The increase in MMR of Nile tilapia with increasing temperature followed the dominant response type in fishes, in that it peaked at lower acclimation temperature than SMR. Beyond 30 °C, MMR failed to increase further, instead declining rapidly until acclimation temperature reached 38 °C, at which point SMR and MMR were not statistically distinguishable. The observed increase in MMR as temperature approached T_{OPT} can be accommodated by a high oxygen diffusion rate across the fish gills (Dejours, 1990), but above T_{OPT}, decreases in MMR are caused by insufficient oxygen transport capacity related to either cardiac output (Brett, 1964; Pörtner and Knust, 2007; Pörtner and Farrell, 2008), limitations in blood oxygen carrying capacity (Gallaugher et al., 1995), gill oxygen flux (Gallaugher et al., 2001), or combinations thereof.

The resultant AAS for Nile tilapia shows that the species is eury-thermal with a quite broad thermal optimum from 20.2 to 31.7 °C, able to survive temperatures ranging from 13 to 39 °C. Compared to active species such as e.g. rainbow trout (*Oncorhynchus mykiss*) (Skov et al., 2011) or sockeye salmon (*O. nerka*) (Eliason et al., 2013) that have MMRs in the order of 600 mg O_2 kg^{-1} h^{-1}. As such, the MMR of Nile tilapia falls in the lower range of reported values for teleosts (Killen et al., 2017). The present findings for Nile tilapia show a wide performance breadth (temperature) and a relative low performance maximum (AAS), suggesting that this species is a temperature generalist (Seebacher et al., 2015). From earlier studies on the metabolic costs associated with feeding and digestion, we know that Nile tilapia of a similar size to those used in the present experiment, are able to utilize their full aerobic scope to fuel specific dynamic action (SDA) when fed to satiation (Obirikorang et al., 2017; Skov et al., 2017). This has also been observed for catfish (*Siluris meridionalis*) (Fu et al., 2008), so that in these species there is little or no scope left to fuel other activities. As we did not measure SDA or feed intake in the present study, we cannot conclude about the effects of temperature hereon, but the present results provide some indication that feed intake may become compromised at higher and lower acclimation temperatures as AAS is reduced, and that the thermal breadth with respect to feeding, and combined feeding and activity, may be narrower than assumed here.

The estimated T_{OPT} and optimal temperature range in the present study overlap with the works of El-Sherif and El-Feky (2009), Pandit and Nakamura (1970), and El-Sayed and Kawanna (2008), who reported that temperatures within the range of 25–32 °C were optimal for growth performance in Nile tilapia juveniles and fingerlings. Overall, it is not unlikely that the thermal preference of Nile tilapia is size-dependent (Morita et al., 2010). It has been suggested that maximal growth rates in bigger fish require lower temperatures, supported by growing evidence that thermal optima are associated with ontogenetic shifts. Considerable changes in T_{OPT} occur in response to even modest changes in fish size, as has been shown among others for Atlantic cod (*Gadus morhua*) (Lafrance et al., 2005), brown trout (*Salmo trutta*) (Elliott and Allonby, 2013), and European perch (*Perca fluviatilis*) (Christensen et al., 2020). Therefore, future studies may find lower T_{OPT} values for larger size classes of Nile tilapia.

An additional objective of the present study was to investigate any correlation between ventilation frequency and oxygen consumption, that could be used to estimate of oxygen consumption rates of Nile tilapia in the rearing facilities. To have any validity, it is important that such a correlation is valid for minimum and maximum rates of MO_2. While the correlation between f_V and SMR across the experimental temperatures was modest, the correlation with MMR was poor due to a large interaction of temperature. As such, f_V is not a useful indicator of MO_2 in Nile tilapia across all temperatures. However, when only considering the experimental temperatures that fell within the thermal optimum (i.e., 22, 26 and 30 °C), it was found that f_V was strongly correlated with MO_2 ($r^2 = 0.74$). From earlier studies on the relationship between f_V and MO_2 it is apparent that there are species specific differences in the mechanisms used to increase gill ventilation volume. Frisk et al. (2012) showed that the relationship between f_V and MO_2 (SMR and MMR) was independent of temperature in pike perch between 13 and 28 °C, and that 95% of the change in MO_2 could be accounted for by changes in f_V. For Atlantic salmon, the relationship between f_V and MO_2 is highly temperature dependant, but more importantly, f_V only contributes marginally (~30%) to changes in MO_2 regardless of temperature (Millidine et al., 2008). Instead, buccal volume (V_B) appears to play a larger role in regulating gill ventilation volume (V_G) in Atlantic salmon. It has been shown that Nile tilapia uses combinational changes in f_V and V_B to regulate V_G. For example, during hypoxia (40% O_2 sat.), V_G increases from 300 to 1300 ml kg^{-1} h^{-1}, which is achieved by a 25–100% increase in f_V (Zeraik et al., 2013), in combination with a 3–4 fold increase in V_B (Obirikorang et al., 2020). A combined mechanism for increasing V_G has also been shown when Nile tilapia acclimated to

15 °C are exposed to 35 °C over a 5-h period. Here, V_G increased 10-fold, through a doubling in f_V and a 5-fold increase in V_B (Maricondi-Massari et al., 1998). As with MO_2, V_B and f_V and their combined effects on V_G may also be affected by ontogenetic changes. Ken-Ichi (1992) showed that for Nile tilapia, V_G scaled positively with size with the traditional exponent of 0.7 (Clarke and Johnston, 1999), but that body size correlated negatively with f_V (−0.3), but positively with V_B (1.0).

Maricondi-Massari et al. (1998) showed that, as Nile tilapia experience temperatures exceeding T_{OPT}, changes in V_G are primarily driven by increasing V_B, and less so by f_V. This supports the observation in the present study that f_V becomes progressively uncoupled from MO_2 at higher temperatures. Despite this, the amount of oxygen extracted per breath decreased during MMR at high temperatures. We did not measure heart rate or cardiac output, and therefore we can only hypothesize about the cause of reduced oxygen extraction at the highest experimental temperatures. It is a possibility that gill surface area is a limiting factor for maximum oxygen extraction in Nile tilapia at critically high temperatures. Future investigations examining cardiac output and oxygen extraction efficiencies could shed further light on this, and contribute to the ongoing discussions on the gill-oxygen limitation theory (Pauly, 2021; Scheuffele et al., 2021). At present, we presume that all gill lamellae are recruited, while f_V and blood oxygen carrying capacity are at maximum, and that therefore it is the internal oxygen transport system that is failing (Farrell, 2016).

5. Conclusion

The present study showed that T_{OPT} for Nile tilapia is 26.0 °C, with an optimal thermal range from 20.2 to 31.7 °C. Within the optimal thermal range, f_V is a good predictor of MO_2 during normoxic conditions. The lower and upper critical temperature was found to be 13.1 and 38.8 °C. At acclimation temperatures above the optimum range, oxygen extraction efficiency began to decline, despite no change in f_V, suggesting the onset of oxygen transport failure. It is possible that SMR, MMR and f_V are associated with ontogenetic shifts. As such, the values reported in the present study apply to fish with a body mass near 85 g, and future studies should be directed at investigating how these may be influenced by size.

Author contribution statement

PVS - Conceptualization.
JNL & PVS Formal analysis.
PVS Funding acquisition.
JNL & PVS Investigation.
PVS & JNL Methodology.
PVS Project administration.
PVS Resources.
PVS Supervision.
JNL & PVS Validation.
JNL & PVS Visualization.
JNL Roles/Writing - original draft.
PVS & JNL Writing - review & editing.

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