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The relationship between temperature and standard rate of metabolism in African lungfish, *Protopterus aethiopicus*, from Uganda

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The relationship between temperature and metabolism among ancient (non-teleost) fishes, while largely unknown, is essential to an understanding of the effects of temperature on fish energetics. This study quantifies the effect of temperature on the standard rate of metabolism in the African lungfish, *Protopterus aethiopicus*. We present a Q_{10} value of 3.3 for individuals ranging in body mass from 42–222g across an ecologically-relevant 10°C temperature range. *Protopterus aethiopicus* showed a positive bilogarithmic (log-log) relationship between the rate of oxygen consumption and body mass (range = 44–222g) at three water temperatures (20, 25, 30°C). However, adjusted mean rates of oxygen consumption differed among all three temperatures. A Q_{10} value derived as the means of Q_{10} values calculated for each individual lungfish averaged 3.3 ± 0.4 (SE) for 20–30°C. A comparison of literature-derived Q_{10} values for both tropical and temperate fishes suggests a higher Q_{10} in tropical species (n = 3) than in temperate species (n = 10) across an ecologically-relevant thermal range.

Keywords: air-breathing fish, ecophysiology, metabolic rate, Q_{10}, respirometry

Introduction

Among the myriad of environmental factors known to affect the metabolism of ectotherms, few are as significant as temperature. The study of this relationship, from both a molecular and whole-organism perspective, yields insight into the intimate connection between animal energetics and the environment. An understanding of how temperature affects metabolic rate is also critical for comparative studies that seek to examine effects of other factors (e.g. phylogeny, ecology) on animal energetics. For fishes, Winberg (1956) derived a series of coefficients that could be used to adjust metabolic rates of species by applying the Van't Hoff equation:

$$[Q_{10} = (K_2/K_1)^{(10/\,t2-t1)}]$$

where K_1 and K_2 are the metabolic rates measured at two different temperatures, t_1 and t_2, for changes in temperature across intervals of 10°C. This permitted adjustment of metabolic rates, measured at a specific temperature to a second specific temperature of biological interest, and also provided a description of how an individual's metabolic rate responded to an increase or decrease in temperature. As long as an ectotherm is acclimated for an adequate length of time to each new temperature, this rate reflects the long-term compensatory effects associated with metabolism that maintain a homeostatic balance within the individual despite a change in environmental conditions.

Metabolic studies that have focused on temperature effects in fishes have been largely restricted to non-air-breathing species from temperate and polar waters (Clarke and Johnston 1999), and the Q_{10} coefficients for fishes derived by Winberg were based largely on data collected for temperate teleosts. The degree to which these coefficients apply to tropical fishes that experience a smaller range of natural temperature fluctuation is largely unknown. Researchers investigating species from these systems are faced with the decision to calculate their own Q_{10} values or to use published values measured on other species. Based on the experiments of Johnston *et al*. (1991) and Clarke and Johnston (1999), utilising Q_{10} relationships for organisms with different lifestyles, and/or from unrelated taxa, can clearly lead to erroneous adjustments to calculated metabolic rates. In addition to a temperate bias, our understanding of temperature effects on fish metabolism is based on literature focused primarily on non-air-breathing teleosts. Air breathers, with less dependence on aquatic oxygen, may differ in their metabolic response to temperature variation. The objective of this study was to determine the Q_{10} value for the African lungfish, *Protopterus aethiopicus*, an ancient air-breathing fish from a tropical freshwater habitat. We present a Q_{10} value based on six individuals, ranging in body mass from 42–222g, evaluated across a ecologically-relevant 10°C temperature range.

Materials and methods

Six juvenile lungfish, *Protopterus aethiopicus* (Heckel 1851), ranging in size from 44–222g, were used in this study. Individuals were collected in June 2001 from Lake Nabugabo, Uganda, and live-transferred to the University of Florida. Lake Nabugabo lies just south of the equator and is a small satellite lake (surface area = 24km^2, mean depth = 4.5m) that was isolated from Lake Victoria approximately 4 000 years ago (Greenwood 1965). The lake lies within the extensive Lwamunda Swamp that was formerly a bay on the western shore of Lake Victoria (Greenwood 1965, Ogutu-Ohwayo 1993). In the Lake Nabugabo system, Goudswaard *et al*. (2001) found that 90% of the lungfish captured in minnow traps in the swamp were between 20 and 45cm TL. Larger lungfish in the system are found in more open waters (LJC, pers. obs.). Water temperatures, measured throughout the year at a lagoon deep within the swamp where lungfish occur, averaged between 21.3°C and 25.2°C in the early morning and between 21.3°C and 27.2°C in the early afternoon (Chapman *et al*. 2002).

Prior to metabolic trials, individuals were held for ~1.5 years at 23°C ± 0.5°C under normoxic conditions in four partitioned 76-litre tanks with no two lungfish occupying the same space within a tank. Tank temperatures were controlled with aquarium heaters, and temperatures were measured daily. Tanks were maintained on a 12h/12h photoperiod and individuals were fed fresh chicken liver once a week. Six individuals were acclimated across a temperature range of 20°C to 30°C under normoxia over a five-month period beginning in January 2003. The water temperature of individuals, initially kept at 23°C, was gradually increased to 30°C over a one-week period. Once at 30°C, individuals were acclimated for at least two weeks with a daily fluctuation in temperature of less than ±0.5°C. Each lungfish was assigned a number and a random number table was used to select the order in which individuals were measured for their standard metabolic rate (SMR). For these lungfish, the SMR is 'the respiration rate of an unfed fish resting quietly in the experimental chamber' (Clarke 1991, 1993). Spontaneous activity, as noted by qualitative observations, did not change as a function of temperature and all fish were observed resting quietly during trials, with movements usually being associated solely with respiration. We believe this accurately reflects the standard rate of metabolism for *Protopterus aethiopicus* and the reported SMR. Individuals were unfed for one week prior to the start of metabolic tests. The SMR was measured via the measurement of oxygen consumption in a two-phase respirometer following the protocol described in Seifert and Chapman (2006), which permitted simultaneous measurement of oxygen consumed from both water and air. The respirometer was constructed using PVC, oxygen-impermeable vinyl tubing, and Swagelock brass compression fittings. Several chambers of different sizes were built and were interchangeable within the system. The chamber where fish were placed consisted of a length of PVC connected to a T-junction that served as an air chamber. The T-junction was constructed from clear PVC to allow periodic observation of fish during respiratory trials. The length of PVC that constituted the bulk of the water chamber was opaque. A 1.5amp Mag-drive water pump, Omega flow meter, oxygen-sensing probe and thermistor, were all placed in series for the water portion of the system. A stopper with an oxygen probe, thermistor and a small fan inserted through it was used to seal the air portion of the system. Thus, each phase of the respirometer contained an associated oxygen-sensing probe and thermistor, which provided for the simultaneous measurement of oxygen consumption and temperature from both phases of the respirometer. The respirometer utilised Ocean Optics Oxygen Sensing probes to measure oxygen concentration in both water and air.

The probes were calibrated to 0% dissolved oxygen in water containing sodium sulfite and to 100% dissolved oxygen for the water chamber in air-saturated water and for the air chamber in air. The probes were calibrated on a per cent scale of oxygen saturation in the medium. Per cent oxygen saturation was then converted to units of mg O_2 l^{-1} hr^{-1} with corrections for ambient air pressure and water vapour pressure. The respirometer was designed as a closed system with the capacity to flush out and replace the contents of either phase. This allowed the removal of any metabolic waste products that accumulated during the acclimation period.

Individual fish were acclimated overnight at the appropriate temperature and to the system with free access to air. The dissolved oxygen concentration in the water chamber was allowed to drop to near 0% over this time period. Upon beginning each run in the morning, the system was flushed with air-saturated water until the dissolved oxygen concentration in the system was approximately 50%. This served to remove any metabolic wastes that may have accumulated overnight and to raise the oxygen saturation in the water chamber. The air chamber was then closed and the run was started. Oxygen measurements were made every 10secs over the course of the run and were recorded automatically by the computer software running the system. The SMR was measured when DO levels were near 0%. In the case of the smallest fish, where DO levels did not drop to 0%, the SMR was measured when acquisition of oxygen from the water 'plateaued' at a constant rate. The metabolic run was monitored and the percent saturation of oxygen in the air chamber was never allowed to drop below 80%.

Respirometer temperature was maintained to within ±0.5°C during each experimental run. After all six individuals had been measured for their SMR, the temperature was reduced so that each fish was gradually subjected (over a three-day period) to a lower temperature regime, after which they were again acclimated for at least a two-week period. In this way, the above metabolic experiments were performed at 30°C, 25°C and 20°C.

Linear regression analysis was used to examine the relationship between SMR and body mass within each temperature category. Analysis of covariance was used to detect differences in the slopes and intercepts of the bilogarithmic (log-log) relationships between metabolic rate and body mass, and we report the adjusted means for each group. The Q_{10} value relating SMR to water temperature over the 10°C range (20–30°C) was calculated using the following equation:

$$Q_{10} = (K_2/K_1)^{(10/\,t2-t1)}$$

where K_2 and K_1 are metabolic rates at temperatures t_2 and t_1 and adjusted accordingly to determine Q_{10} values for 20–25°C and 25–30°C. This was done for each individual fish, and we report the mean and standard error for each Q_{10} value.

Results

Protopterus aethiopicus showed a positive bilogarithmic relationship between the rate of oxygen consumption (mg O_2 g^{-1} hr^{-1}) and body mass (range = 44–222g) at all three water temperatures (20°C: r^2 = 0.798, p = 0.016, slope = 0.71, intercept = –1.51; 25°C: r^2 = 0.847, p = 0.009, slope = 0.94, intercept = –1.69; 30°C: r^2 = 0.862, p = 0.007, slope = 1.20, intercept = –2.02, Figure 1a). There were no significant differences in the slopes of the bilogarithmic relationships between the rate of oxygen consumption and body mass (F = 1.375, p = 0.290). However, there was a difference in the intercepts of the relationships (F = 25.500, p = <0.001). Adjusted mean rates of oxygen consumption (adjusted to the common body mass of 111g and a common regression line using ANCOVA) differed among all three temperatures (Sidak tests, p < 0.05, antilog adjusted mean metabolic rate: 20°C = 0.88mg O_2 g^{-1} hr^{-1}; 25°C = 1.69mg O_2 g^{-1} hr^{-1}; 30°C = 2.75mg O_2 g^{-1} hr^{-1}, Figure 1b). Q_{10} values were derived as the means of Q_{10} values calculated for each individual lungfish and averaged 4.1 ± 0.8 (SE) for 20–35°C; 3.2 ± 0.8 (SE) for 25–30°C and 3.3 ± 0.4 (SE) for 20–30°C. Respiratory allocation to water and air remained relatively stable between the three temperature regimes with allocation of oxygen from air meeting close to the total oxygen requirement.

Discussion

Protopterus aethiopicus exhibited a strong positive increase in the SMR with an increase in temperature across an ecologically-relevant temperature range. To our knowledge, this study is the first to quantify the relationship between temperature and the resting rate of metabolism in an air-breathing fish.

Clarke and Johnston (1999) sought to refine the relationship between metabolic rate and water temperature for teleost fishes utilising literature-derived data that met a specific set of criteria (i.e. accurate determination of resting metabolism, adequate acclimation time, etc: see Clarke and Johnston 1999 for full description of criteria). Using an Arrhenius model to compare resting metabolic rates adjusted for a 47g fish across a temperature range, they found that tropical fish require 6.2 times more oxygen at 30°C than do polar fish at 0°C (Clarke and Johnston 1999). In their model, water temperature explained 59% of the variance in resting metabolic rate among 69 species of teleosts, with the remaining variance attributed to the combined effects of phylogeny, ecology and/or lifestyle. Clarke and Johnston (1999) also derived a Q_{10} value of 1.83 from their curve based on 69 species of teleosts (a value derived from an interspecific curve) and reported a median Q_{10} value of 2.40 from 14 published values on individual species that were exposed to different temperature regimes. Our Q_{10} value for the African lungfish falls above both these values, suggesting a relatively high Q_{10} in this species.

We reviewed studies of tropical and temperate fishes that measured the resting metabolism within the same species across a gradient of temperatures that met the criteria outlined by Clarke and Johnston (1999), but also where the fishes had been acclimated to various temperatures for at least one week prior to measurement of metabolic rate. This acclimation is important and increases comparability of the data. Eight of these studies involved 11 species of temperate fishes, while this study and the remaining two studies examined three tropical species (Table 1). Data provided by Saint-Paul (1983) indicated a Q_{10} value of 2.97 for the tropical frugivorous characid *Colossoma macropomum*, and data provided by Caulton (1978) indicated a Q_{10} value of 2.74 for the tropical tilapiine cichlid *Oreochromis mossambicas*, both relatively high values compared to the

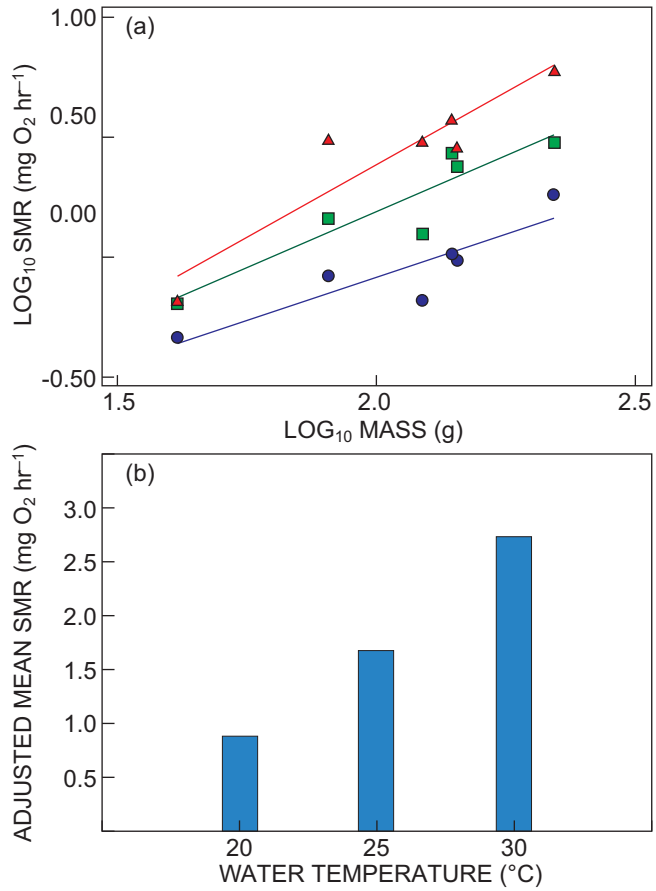

Figure 1: (a) Bilogarithmic relationship between SMR and body mass for six individuals of *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda, measured at three different temperatures: 30°C, 25°C and 20°C. Lungfish were acclimated for at least two weeks prior to measurement. Triangles represent 30°C, open squares represent 25°C, and closed circles represent 20°C. (b) Antilogged adjusted mean SMR for juvenile *P. aethiopicus* adjusted to 110.5g fish (mean for six individuals) for three temperatures: 30°C, 25°C and 20°C

Table 1: Q_{10} values calculated for various species of teleost fishes. Resting rates of metabolism were provided by Andrew Clarke (British Antarctic Survey), adjusted to a 47g fish. Q_{10} values were calculated across temperature ranges listed. Citations indicate initial source of raw metabolic data. All studies included measured resting rate of metabolism (mg $O_2 l^{-1} hr^{-1}$) and acclimated individuals for sufficient periods of time prior to measurement at different water temperatures

Species	Temperature range (°C)	Q_{10}	Study
Tropical			
Colossoma macropomum	20–30	2.97	Saint-Paul (1983)
Oreochromis mossambicas	19–28	2.74	Caulton (1978)
Protopterus aethiopicus	20–30	3.30	This study
Temperate			
Scophthalmus maximus	6.0–22.0	3.06	Mallekh and Lagardere (2002)
Gambusia affinis	10.0–30	2.24	Cech *et al*. (1985)
Anguilla anguilla	20.0–27.0	2.48	Degani *et al*. (1989)
Anguilla rostrata	15.0–25.0	3.67	Degani and Gallagher (1985)
Cyprinus carpio	10.0–30.0	2.60	Beamish (1964)
Carassius auratus	10.0–30.0	2.29	Beamish and Mookherjii (1964)
Catostomus commersonii	10.0–30.0	2.44	Beamish (1964)
Oncorhynchus nerka	5.0–20.0	2.02	Brett and Glass (1973)
Salvelinus fontinalis	10.0–20.0	2.84	Beamish (1964)
Pleuronectes platessa	2.0–22.0	2.06	Fonds *et al*. (1992)
Platichthys flesus	2.0–22.0	2.24	Fonds *et al*. (1992)

median value (2.40) reported by Clarke and Johnston (1999), and higher than eight of the 11 temperate species (median = 2.44, range = 2.02–3.67, Table 1).

Studies of Q_{10} values, and comparisons of these values across species, are useful in illustrating (a) if the rate of change follows the standard derived relationships calculated from interspecific data, and (b) whether the rate of change varies among species, or groups of ecologically- or phylogenetically-related taxa. Although there is clearly a need for a broader comparison, it is possible that the trend towards a higher Q_{10} value for lungfish and two other tropical species, compared to temperate fishes for which comparable data are available, reflects the lower degree of annual thermal variability that is generally characteristic of tropical waters. Comparing our derived Q_{10} value for African lungfish, and those reported for other tropical species, to the Q_{10} value of 1.83 derived by Clarke and Johnston (1999) for all teleosts (whether polar, temperate or tropical) highlights the problems associated with using published Q_{10} values to adjust metabolic rates in the absence of species-specific values. It is a common practice to adjust metabolic rates using Winberg's Q_{10} coefficients derived primarily from temperate fishes and it has been argued that a Q_{10} value of 2 is an appropriate adjustment for this relationship among diverse species (Fry and Hochachka 1970, Miller and Mann 1973). However, there is clearly a high degree of interspecific variation and while this variation is interesting, a lack of appropriate data for a diverse array of fishes limits our understanding of its source.

Although the number of fish species for which Q_{10} data are available, and their phylogenetic breadth is small, this comparison highlights the need for further examination of phylogenetic and geographic trends in Q_{10} data. Preliminary examination of resting metabolic rates in primitive non-teleost fishes suggests that a low resting rate of metabolism is a primitive characteristic (AWS, unpubl. data). It will be of interest to investigate if a lower thermal sensitivity in terms of Q_{10} is correlated with taxonomic position, or if it is strictly a function of habitat. In addition, future work that examines effects of temperature outside of the range experienced in nature will address whether metabolic compensation approaches the higher and lower bounds of thermal tolerance. A few studies suggest that there is metabolic depression above some critical temperature, but mechanisms for these observations are not well understood (Saint-Paul 1983). Further investigations will shed light on the contributions of protein turnover, membrane homeostasis, and cellular ion balance to metabolism. While these processes presumably play an important role, it is unclear what the individual contributions of these processes are to maintenance metabolism or their relative relationship to temperature and lifestyle (Clarke and Johnston 1999). A more complete picture of the relationship between temperature and its effect on resting metabolic rate across all groups of fishes will yield a deeper understanding of environmental factors upon physiological processes in ectotherms.

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