Novel approaches to the calculation and comparison of thermoregulatory parameters: Non-linear regression of metabolic rate and evaporative water loss in Australian rodents

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ABSTRACT

The calculation and comparison of physiological characteristics of thermoregulation has provided insight into patterns of ecology and evolution for over half a century. Thermoregulation has typically been explored using linear techniques; I explore the application of non-linear scaling to more accurately calculate and compare characteristics and thresholds of thermoregulation, including the basal metabolic rate (BMR), peak metabolic rate (PMR) and the lower (T_{lc}) and upper (T_{uc}) critical limits to the thermoneutral zone (TNZ) for Australian rodents. An exponentially-modified logistic function accurately characterised the response of metabolic rate to ambient temperature, while evaporative water loss was accurately characterised by a Michaelis-Menten function. When these functions were used to resolve unique parameters for the nine species studied here, the estimates of BMR and TNZ were consistent with the previously published estimates. The approach resolved differences in rates of metabolism and water loss between subfamilies of Australian rodents that haven't been quantified before. I suggest that non-linear scaling is not only more effective than the established segmented linear techniques, but also is more objective. This approach may allow broader and more flexible comparison of characteristics of thermoregulation, but it needs testing with a broader array of taxa than those used here.

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1. Introduction

Many of the processes that structure populations and drive patterns of ecology are rooted, at least partially, in energetics (Bradshaw, 2003; McNab, 2002; Seebacher and Franklin, 2012; Tomlinson et al., 2014). Similarly, where populations diverge these energetic drivers provide some basis for evolutionary trajectories, either as ecological constraints on the capacity to manage an energy budget, or as inherited basal requirements (phylogenetic patterns; Cruz-Neto et al., 2001; Elgar and Harvey, 1987; Hayssen and Lacy, 1985; White and Seymour, 2003). Measurement of endothermic basal metabolic rate (BMR) provides insight into the minimum maintenance requirement of an endotherm (IUPS, 2003; Withers, 1992a), and a large literature has grown around the allometric scaling of this metric, and the ecological and evolutionary correlates of divergence from expected values (regression residuals). The estimation of RMR, where the rate of heat production is equal to the rate of metabolism, with no associated costs of digestion, movement, growth or reproduction (IUPS, 2003) is essentially a study of thermal performance. This is because, as ambient temperature (T_a) decreases, the rate of heat loss from the animal, reflecting wet thermal conductance (C_{wet}), increases due to increased thermal differentials between the warm body and the environment. Therefore, since C_{wet} is defined by metabolism divided by the temperature differential, a high temperature differential will imply a high metabolic rate, assuming a constant C_{wet}:

$$C_{wet} = \frac{VO_2}{T_b - T_a} \qquad (1)$$

Increased heat loss in colder conditions is offset by increased metabolic activity to maintain homeostasis, and the BMR occurs over a T_a range where no metabolic effort is required to do this. This raises expectations that specific thresholds (the upper and lower critical temperatures, T_{lc} and T_{uc}) may be significant, as recognised by the conceptualisation of the thermoneutral zone (TNZ; IUPS, 2003; Withers, 1992a), within which metabolic effort is not significantly increased to facilitate thermoregulation. While these thresholds are often resolved, their direct comparison is

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difficult because they are defined as limits to thermoregulatory patterns, rather than elements of them.

Thermal performance has long been understood to be a non-linear process for ectotherms, yielding asymmetrical, unimodal performance functions (Angilletta, 2006; Kovac et al., 2007; Tomlinson et al., 2015; Tomlinson and Menz, 2015; Tomlinson and Phillips, 2015). While these might traditionally have been estimated by linear regression models of transformed data, recent discussions have moved away from that (Gurka and Edwards, 2011; Hayes and Shonkwiler, 2006), towards non-linear approaches to function fitting (Kovac et al., 2014, 2007; Tomlinson et al., 2015; Tomlinson and Phillips, 2015), which has facilitated specific estimation of critical thermal thresholds. In endotherms, however, the thermal performance of metabolic rate is dominated by endogenous thermoregulation, and the critical elements of the most well-established conceptualisation of thermoregulation (the Scholander-Irving model) are a lower limit to energetic expenditure (BMR) and a scaling effect that facilitates increased metabolic heat production by increasing metabolic rate as ambient temperature declines. The slow, apparently constant increase in RMR as temperature falls below the TNZ has, for a long time, beguiled researchers into fitting linear regressions to RMR (e.g. Antinuchi and Busch, 2000; Bennett and Spinks, 1995; Cooper and Withers, 2010; Cooper et al., 2009; Dawson, 1973; Hosken and Withers, 1999; Scholander et al., 1950; Tomlinson et al., 2007, 2012b; Withers, 1992b; Withers et al., 1990, 2000), and either using a break-point regression to estimate the lower end of the TNZ, or not using any statistical procedure to do so at all, where the data did not extend to high enough experimental temperatures. The linear analysis of increases in RMR below the TNZ results largely from a separation between chemical (metabolic) and physical (posture, insulation and peripheral vasoconstriction) processes of thermoregulation imposed by Scholander et al. (1950). From this assumption, if no metabolic efforts are deployed until the physical processes are exhausted, then BMR should be unchanging throughout the TNZ. Once physical processes are fully deployed, then endogenous heat generation should be engaged, and it should increase in linear fashion proportional to the thermal challenge represented by falling T_a.

The Scholander-Irving model of thermoregulation has substantial appeal, both for its parsimonious underlying logic, and for its simple mathematical estimation of BMR and TNZ, such that is has formed the basis of most of the ensuing studies of the comparative energetics of endothermy (McNab, 1980). Despite its general appeal, the linear concept of the Scholander-Irving model has been acknowledged as an over-simplification of the mechanics of heat transfer (Porter et al., 2000; Rezende and Bacigalupe, 2015), and has proven to be imperfect in some applications. The most obvious exceptions to the model occur in species that do not thermoregulate perfectly (Angilletta et al., 2010; Lovegrove et al., 1991; McNab, 1980), where high levels of variation around T_b and C_{wet} do not imply complete dependence on increasing metabolic heat production below the TNZ. Despite increasing evidence that this may be the norm, rather than the exception (Angilletta et al., 2010), the implicit complexity of this scenario probably engenders more extensive discussion than I intend to devote here. In more "standard" endothermic models, Lovegrove et al. (1991) explored the role of an upper limit to thermogenic capacity (the peak metabolic rate; PMR), and the influence that its incorporation has upon thermal conductance and the thermal performance curve of RMR predicted by the linear concept Scholander-Irving model. One of the implications of the linear concept of the Scholander-Irving model is that the slope of the increase in RMR below the TNZ should extrapolate to the optimal body temperature when RMR is zero, but such fits usually overestimate T_b (McNab, 1980). By extension, estimating C_{wet} from the slope of this fit is usually

unreliable (McNab, 1980). The basis of many of these inaccuracies is the simplification of a single, constant conductance coefficient (Lovegrove et al., 1991; McNab, 1980). As McNab (1980) notes, this is due to the assumed sequential separation of physical and metabolic processes of thermoregulation. It is unlikely that these processes are entirely separated (McNab, 1980), and RMR theoretically cannot follow a linear increase below the TNZ (Lovegrove et al., 1991), because, amongst other things, deployment of metabolic and physical elements of thermoregulation in parallel engenders a non-linear relationship, where small increases in metabolic rate should be made towards the limits of the effectiveness of physical changes, and as the effectiveness of physical changes are exceeded, the contribution of metabolic rate should increase. Furthermore, the slight increase in RMR above the TNZ implies a second non-linear element to the overall thermal performance function of RMR, usually explained by hyperthermia and a Q_{10} effect (Schmidt-Nielsen, 1983; Withers, 1992a).

The fitting of regression functions is largely dependent upon the theory upon which the function is constructed (Bates and Watts, 1988; Ritz and Streibig, 2008). In the context of traditional linear regression there is only one form to the function, described by slope and intercept, and so the assumption is only that this form legitimately models the data, although typically within a limited range of treatment values. In the context of endothermic RMR and thermoregulation, the Scholander-Irving model of thermoregulation (Scholander et al., 1950) explicitly states a minimum level of energetic expenditure (BMR) within a zone where temperature regulation is maintained by physical means (TNZ). It also states an increase in energy expenditure below this temperature region, which can be variable depending upon body size, insulation, phylogeny and climatic adaptation (Scholander et al., 1950). While not explicitly discussed, PMR is evident in some of the empirical results reported (Scholander et al., 1950). The most parsimonious base function to incorporate both a lower asymptote (BMR) and an upper asymptote (PMR) is a sigmoidal, logistic curve. Such a formulation of a logistic curve is entirely consistent with the Scholander-Irving model of thermoregulation, incorporating both the critical elements of a lower, asymptotic limit to metabolic rate, and a parameter facilitating the increase of metabolic heat production with decreasing temperature below a critical threshold. Compared to the traditional linear approach, however, a logistic function incorporates a graded interchange between BMR and metabolic heat production, implying parallel deployment of physical and chemical elements of thermoregulation. Finally, the increase above a critical threshold can be served by the addition of an exponential modifier to the function (Fig. 1), similar to the exponential penalties applied to ectothermic thermal performance to induce a reduction beyond thermal tolerance thresholds (Kovac et al., 2014, 2007; Tomlinson et al., 2015; Tomlinson and Menz, 2015; Tomlinson and Phillips, 2015).

Metabolic rate is, however, only a single element of thermoregulatory physiology, complimented by evaporative water loss (EWL) to facilitate evaporative cooling at high T_a. This implies another avenue of physiological constraint to thermoregulation by the water budget and the water use efficiency (WUE) of an organism (Cooper and Withers, 2008, 2009, 2014; Withers and Cooper, 2014; Withers et al., 2006, 2012). The thermal performance of both EWL and WUE are also theoretically non-linear in nature, and have also usually been estimated by linear analyses. There is a great deal less theoretical information on which to construct performance functions for these physiological traits, because there has been a great deal less research into them. Evaporative water loss may be characterised by a Michaelis-Menten function (López et al., 2000; Ritz and Streibig, 2012), where EWL remains relatively constant until the upper limit of the TNZ, where it rapidly increases.