

RH: Energetics of Pangolins

Energetics Suggest Cause for Even Further Conservation Concern for Temminck's Ground Pangolin

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Abstract

Conservation of rare and declining species requires reliable information about life history traits and population growth characteristics. Unfortunately, long-term studies necessary to obtain such data are often difficult or impossible for species of concern. In such cases, data that can be collected during limited capture events can serve as valuable proxies. We measured metabolic characteristics of Temminck's ground pangolin (*Smutsia temminckii*) because metabolic traits provide clues about relative gestation length, reproductive output, and population growth, all of which were largely unknown for this species. As expected, both basal and field metabolic rates are considerably lower than would be predicted from body mass alone, suggesting pangolins are likely to have slow life histories and low intrinsic population growth. Such characteristics suggest Temminck's ground pangolins (and likely all pangolins) are less able to persist under heavy poaching and trafficking than other similarly sized mammals and will likely recover slowly even if poaching and trafficking are stopped. While physiological data cannot be used to directly calculate population growth rates, we believe they may provide a tangible data source to inform management decisions for critically endangered, difficult-to-study species.

Keywords: conservation physiology, doubly labelled water, Manidae, Pholidota, respirometry, resting metabolic rate

Introduction

There is an increasing appreciation for the relevance of functional energetics in conservation of rare and difficult-to-study species because energetics mediate survival, reproduction, population growth, and ultimately, species-level responses to changing environments (Wikelski & Cooke 2006, Tomlinson *et al.* 2014, Pagano *et al.* 2018). Considerable effort has been expended to understand the factors that drive metabolic expenditure in mammals (Speakman 2000), but as with other physiological traits, there have been research biases towards northern hemisphere species, species from mesic environments, and taxa that are easily captured and maintained in captivity (e.g., rodents; Lovegrove 2012). This is unfortunate because measurements of physiological characteristics of highly endangered and quickly declining species may allow for important inferences about life history characteristics that would be difficult to directly measure (Fei *et al.* 2016).

Pangolins (Pholidota: Manidae) are among the most trafficked mammals in the world (Challender *et al.* 2014, Ingram *et al.* 2019). Unfortunately, pangolins are also difficult to study in the wild because they are shy and predominately nocturnal (Willcox *et al.* 2019), so surprisingly little is known about their basic biology. Much of the limited information we do have about longevity, gestation, reproductive output, etc., comes from captive individuals (Hua *et al.* 2015). Therefore, we have limited ability to predict how resilient pangolins are to current conservation pressures and how quickly they might recover if those pressures are lessened. Pangolins are unique phylogenetically, ecologically, morphologically, and likely physiologically. Notably, pangolins maintain body temperatures (28–35 °C) several degrees below normal eutherian temperatures (Jones 1972, Heath & Hammel 1986, Pietersen 2013) and resting

metabolic rates considerably below those predicted from body mass alone (Heath & Hammel 1986), suggesting they exhibit a suite of traits indicative of a slow life history.

Estimates of population-level parameters important to species persistence (e.g., intrinsic growth rates) are lacking for pangolins, and will be difficult and time consuming to collect. To this end, we evaluated the metabolic function of Temminck's ground pangolins (*Smutsia temminckii*) under natural conditions and in relation to ambient temperature in captivity. Based on previous work in other pangolin species (Heath & Hammel 1986), we expected basal (BMR) and resting metabolic rates (RMR) lower than other mammals of comparable size. Additionally, we provide the first estimates of field metabolic rate (FMR), or the energetic cost of living under natural conditions, for a pangolin. If the expected low BMR and RMR translate into low FMR, it verifies that pangolins exhibit a suite of metabolic traits characteristic of a slow life history.

Methods

We opportunistically hand-captured Temminck's ground pangolins in the Sabi Sand Game Reserve in Mpumalanga Province, South Africa (24°06'55"S 30°51'11"E) during July–September 1998 (three males and one female for measurements of RMR) and December 2000–January 2002 (one male and three females for measurements of FMR; three of these animals were captured and measured on multiple occasions several months apart so we have seven total FMR values). The habitat is typical bushveld terrain comprising sub-tropical woodland. Upon capture, we fitted VHF radio-transmitters to large scales on the tails to aid in recapture (Swart *et al.* 1999). Body mass was similar between sexes (M: 12.8 ± 1.44 kg; F: 11.6 ± 0.64 kg).

Experimental Procedures

Temperature-Metabolic Rate Relationship

We used standard open-flow respirometry techniques to measure the relationship between ambient temperature and metabolic rate. The respirometer comprised of a metal galvanised box with a Perspex front (46 x 48 x 48 cm) placed inside a temperature-controlled cabinet (0.11 m³; Labotec). A pump pulled outside air from a storage reservoir (30 x 50 cm) upstream of the respirometer, through the respirometer, scrubbers (CO₂ trap of soda lime), water trap (of colour-indicator silica gel), and oxygen analyser (Applied Electrochemistry S-3A 2-channel oxygen analyser) at a flow rate of 6–10 L min⁻¹. We determined air flow with a bubble flowmeter constructed using a modified burette containing soapy water.

We collected oxygen consumption data between 08h00 and 18h00 (i.e. the inactive period) to minimize effects of endogenous rhythms of metabolism. Animals were deprived of food 3h prior to measurement of metabolic rate to achieve a post-absorptive state. We recorded data every minute for up to 90 min (but usually ~60 min) at each experimental temperature from 5–35°C in approximately 2.5°C increments. This temperature range represents a realistic range of temperatures likely to be experienced by ground pangolins. Maintaining pangolins in captivity is difficult, and we did not want to risk holding an animal long enough to record data at all temperatures at one time, so we released and recaptured animals at intervals of no less than two weeks. We measured metabolic rates at 2–3 ambient temperatures during each run, so unknown seasonal effects are possible. Before beginning each measurement, we allowed animals to acclimatise to the experimental temperature for at least an hour, during which time oxygen consumption readings stabilized and animals became inactive (activity was verified via behavioural observations every five minutes). After this initial hour, variation in oxygen

consumption within a run was low (standard deviations were < 7% of the mean in every run), so we included data for the entire measurement period in calculations. We calculated oxygen consumption ($\dot{V}O_2$) as a mass-specific rate ($\text{mL O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) following Lighton (2008) and averaged it across the entire sampling period. We converted $\dot{V}O_2$ to energy expenditure assuming 1 mL of oxygen releases 20.1 J of energy (Nagy 1983). The low variation in fractional O_2 data meant the calculated $\dot{V}O_2$ was insensitive to the method of averaging data. We measured rectal temperature using copper-constantan thermocouples (2 mm in diameter) inserted 2.5 cm into the animal's rectum at the end of measurements; temperature was taken after the readings had stabilized.

We used piecewise regression (program Joinpoint v 4.6.0.0; Statistical Research and Applications Branch, National Cancer Institute) to determine bounds of the thermoneutral zone (TNZ) for each individual and for all four individuals combined. We calculated best fit models assuming 0, 1, and 2 joinpoints and selected the best model based on permutation tests.

Field Metabolic Rate

We used the doubly labelled water (DLW) technique to measure FMR of pangolins captured from 2000–2002. Because animals were fitted with radio-transmitters, we were able to recapture them multiple times for sampling. The curled defensive posture of pangolins makes sampling blood difficult, so at each sampling event, we anaesthetised pangolins with isoflurane. To do so, we initially placed a cotton wool swab doused with 5 mL isoflurane with the animal in a sealed 20 L container. This initial dose was enough to permeate air in the container to speed the anaesthetisation process. Throughout the process, a flow of clinical O_2 was passed through the container at a rate of 5 L min^{-1} and an additional 0.2 mL of isoflurane was injected into the

container at 30 s intervals until the animal relaxed enough to be uncurled, usually in 3–5 min. We sampled blood from the caudal vein at the base of the tail, and at first capture, injected animals intraperitoneally with a known mass of DLW (c. 7×10^5 ppm ^{18}O and 3×10^5 ppm ^2H ; Rotem Industries Ltd, Beer Sheva, Israel and Isotec Inc. Miamisburg OH, USA). Animals were fully anaesthetised for 3–10 min and fully recovered after 20–30 min, when they were released at the capture site. The entire process from capture to release was ca. 1 h. To quantify initial enrichment, we drew a blood sample (c. 2.0 mL) 7 h after initial isotope enrichment. Afterwards, we tracked animals nightly and recaptured them approximately every four days for 28 d to draw additional blood samples. For each sample, we heat-sealed 200 μL of blood into glass capillaries and stored them at room temperature until processing. We vacuum distilled capillaries (Nagy 1983) and water from the resulting distillate was used to produce CO_2 and H_2 [methods in Speakman (1997) for CO_2 and Speakman and Król (2005) for H_2]. The isotope ratios ^{18}O : ^{16}O and ^2H : ^1H were then analysed using gas source isotope ratio mass spectrometers (Optima, Micromass IRMS and Isochrom μG , Manchester, UK), prior to calculation of daily energy expenditure (Speakman & Lemen 1999). ^{18}O and ^2H injectate enrichments (ppm), elimination constants (k_o and k_d), dilution spaces (N_o and N_d), and initial and final pool sizes were calculated as recommended in (Speakman 1997). The "plateau" method was used to estimate initial isotope enrichments with the "percentage mass" method of calculating final pool sizes. We applied the 2-pool calculation method to calculate CO_2 production using multi-point samples (Speakman *et al.* 1993). We assumed an respiratory quotient of 0.85, which is appropriate for an obligate insectivore (Cortés *et al.* 2009). The research was undertaken under the contemporary research code prescribed by the University of Pretoria and the Sabi Sand Ecological Committee.

Results

We measured oxygen consumption of four individuals (three males: one female). The lower bound of TNZ was clearly definable in the combined dataset and for each of the four pangolins individually, and in each case, was at 18°C (Figure 1). This temperature also corresponded to minimum $\dot{V}O_2$ ($123.6 \pm 12.6 \text{ mL h}^{-1} \text{ kg}^{-1}$). At this rate of oxygen consumption, energy expenditure would be 709.5 kJ day⁻¹ for an 11.9 kg individual. Interestingly, there was no distinguishable upper bound to TNZ for any individual. However, the relationship between T_a and metabolic rate was positive above 18°C ($\dot{V}O_2 \text{ mL h}^{-1} \text{ kg}^{-1} = 102.2 + 1.553 T_a$; $p = 0.022$). Rectal temperatures at the end of metabolic measurements ($33.9 \pm 0.85 \text{ }^\circ\text{C}$ across all experimental temperatures) were lower than normal eutherian body temperatures and were linearly related to experimental temperatures ($T_b = 32.9 + 0.05 T_a$; $R^2 = 0.71$; $p < 0.001$).

We were able to get successful estimates of FMR for four pangolins (one male; three females; body mass = $12.6 \pm 2.0 \text{ kg}$; in three cases, the same animal was measured twice). Small sample sizes preclude meaningful statistical analyses, but five of the resulting seven estimates of daily energy expenditures (1175, 1602, 1208, 1092, and 1173 kJ/day) were similar and do not appear related to season or body mass, although the highest value of this group was recorded in the largest individual (a 15.5 kg male in winter). One of the other measurements was much lower (324 kJ/day) and one was much higher (5475 kJ/day). There was nothing noteworthy about these individuals, and although we are sceptical of these latter two measurements, we nevertheless report them for thoroughness.

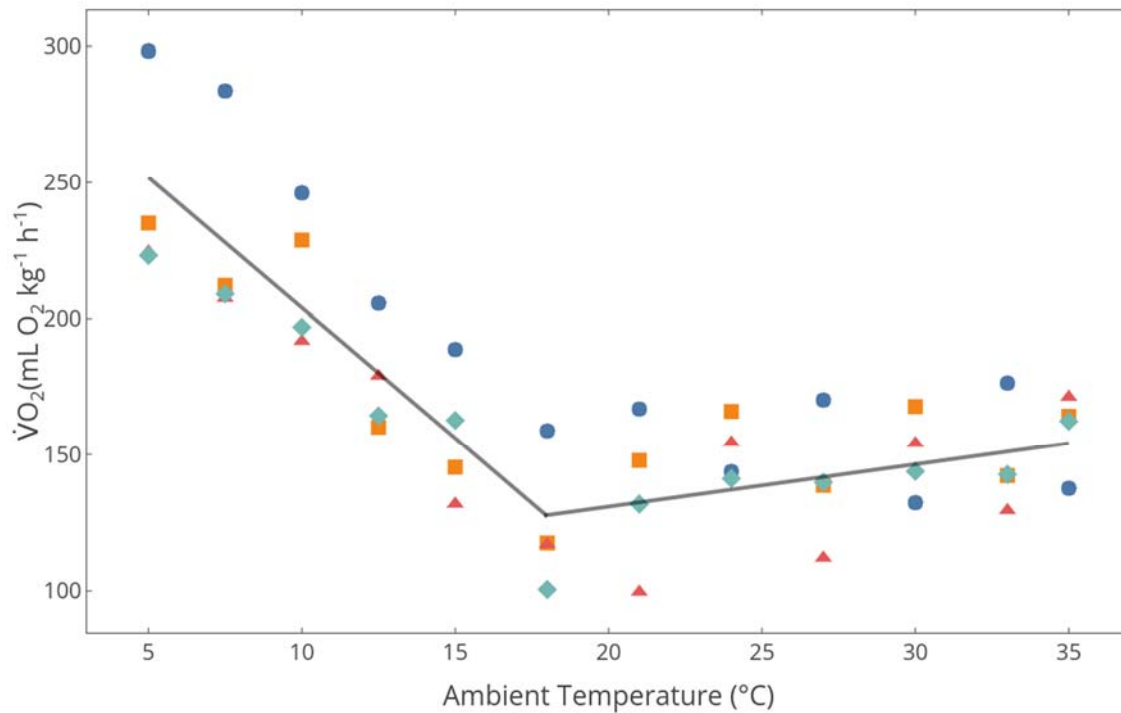


Figure 1. Relationship between ambient (chamber) temperature and metabolic rate for four Temminck's ground pangolins (represented by different shapes and colours). The line represents the best fit piecewise regression model. No upper critical temperature was identified in the piecewise regression.

Discussion

Our metabolic and body temperature data provide important clues about the pace of life of pangolins. Specifically, they suggest Temminck's ground pangolins exhibit low metabolic expenditures expected for a species with a slow life history. Mass-specific BMR is similar to, or slightly lower than that measured for other pangolins species, likely because Temminck's ground pangolins are several times larger than previously studied species (Heath & Hammel 1986).

Assuming the minimum $\dot{V}O_2$ value is indicative of BMR, BMR of Temminck's ground pangolins is less than 75% of that expected based on body mass (Koteja 1991, Speakman 2000).

Pangolins feed exclusively on ants and termites (Richer *et al.* 1997, Pietersen *et al.* 2016), and another similar-sized myrmecophagous species in southern Africa, the aardwolf (*Proteles cristatus*), has BMR similarly below expected values, so diet may be driving the low metabolic rates (Anderson *et al.* 1997). The lack of a distinguishable upper bound on TNZ, even at ambient temperatures as high as 35°C, suggests pangolins are likely well adapted to hot climates. This is of note because while Temminck's ground pangolins inhabit some of the hottest regions of Africa, they generally avoid the hottest parts of the daily cycle owing to their nocturnal habits and frequent use of burrows. Future measurements at higher temperatures will be valuable, but the positive relationship between T_a and metabolic rate suggests the highest test temperatures were near the upper critical temperature for this species. While care must be taken in interpreting point measurements of body temperature at the end of metabolic measurements, the generally low body temperatures we measured are further indicative of a low BMR and a slow life history.

The measured field metabolic rates for Temminck's ground pangolins also suggest a slow life history. Ignoring the unusually high and low values, the daily energy expenditure we measured is 25–50% of that expected based on body mass alone (Koteja 1991, Nagy *et al.* 1999, Speakman 2000). The ratio of mean FMR to mean BMR, which represents energy expended beyond maintenance energy, was 1.76. This value is lower than in most placental mammals and is comparable to some marsupials (e.g., tammar wallaby, *Macropus eugenii*), other placental mammals with slow life histories (e.g., brown-throated sloth, *Bradypus variegatus*), and other myrmecophagous species (e.g., aardwolf)(Koteja 1991, Capellini *et al.* 2010).

The metabolic characteristics reported herein combined with other physiological and observational data on reproductive traits (e.g., Heath & Hammel 1986, Weber *et al.* 1986, Challender *et al.* 2014, Hua *et al.* 2015) clearly suggest pangolins fall on the slow end of the

slow-fast life history continuum. Metabolic rates correlate to length of gestation, reproductive output, and population growth rates in mammals (McNab 1980). It is questionable to assume the relationships are causal (Glazier 2015), but the strong correlations still allow for powerful inferences about population-level characteristics of a species. Species with low metabolic rates often have low intrinsic population growth rates, which is of serious concern given the intense hunting and trafficking pressure pangolins are currently experiencing (Challender *et al.* 2014, Ingram *et al.* 2018). Species with slow life histories and low intrinsic growth rates are especially susceptible to hunting and poaching, and even if pressure is lessened, recovery periods for populations can be quite long (e.g., Turkalo *et al.* 2017). Thus, conservation assessments of pangolins (the four African species are IUCN vulnerable and the four Asian species are endangered or critically endangered) might be overly optimistic.

Capturing rare, declining, and otherwise elusive species for research purposes can be difficult. Following individuals or populations of such species across time to determine factors important for population growth might be nearly impossible. In this study, it was feasible to track a small number of pangolins over extended periods and recapture them for multiple measurements, but we exerted considerable effort to do so. In some studies on rare or endangered species, use of one-time measurements of morphological or physiological parameters as proxies for ecologically important parameters might be the most viable option to inform management decisions (e.g., Hilbers *et al.* 2017). Even a subset of data we collected here, like a single BMR or FMR measurement, can provide valuable information, and collecting such data is much more feasible than long-term monitoring projects. Those long-term projects are desperately needed for every pangolin species, but may not bear fruit on timescales necessary for making important decisions about the status of these species. We hope other researchers will begin to use

data that can be collected in limited capture events to draw inferences about important population- and species-level parameters of rare and endangered species, thus aiding in their conservation.

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