

RESEARCH ARTICLE

Energetic costs of bill heat exchange demonstrate contributions to thermoregulation at high temperatures in toco toucans (*Ramphastos toco*)

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ABSTRACT

Body temperature regulation under changes in ambient temperature involves adjustments in heat production and heat exchange rates between the animal and the environment. One mechanism involves the modulation of the surface temperature of specific areas of the body through vasomotor adjustment. In homeotherms, this thermoregulatory adjustment is essential for the maintenance of body temperature over a moderate temperature range, known as the thermal neutral zone (TNZ). The bill of the toco toucan (*Ramphastos toco*) has been described as a highly efficient thermal window and hypothesized to assist in the thermal homeostasis of this bird. Herein, we directly evaluated the contribution of heat exchange through the bill of the toco toucan and role of the bill in the delimitation of the TNZ. To do this, we measured metabolic rate (MR), via oxygen consumption, over a range of ambient temperatures from 0 to 35°C. MR measurements were made in birds with the bill intact and with the bill insulated. The limits of the TNZ did not differ between treatments, ranging from 10.8 to 25.0°C. The MR differed among treatments only at elevated temperatures (30 and 35°C), reaching 0.92 ± 0.11 ml O₂ g⁻¹ h⁻¹ (mean \pm s.d.) for the intact group and 1.13 ± 0.13 ml O₂ g⁻¹ h⁻¹ for the insulated group. These results indicate that although heat dissipation through the bill does not contribute significantly to widening of the TNZ, it may well be critically important in assisting body temperature regulation at higher temperatures extending above the upper limit of the TNZ.

KEY WORDS: Thermal window, Heat exchange, Metabolic rate, Thermal neutral zone, Thermal homeostasis

INTRODUCTION

Body temperature regulation in endothermic animals involves the balance between the rate of metabolic heat production (HP) and the rate of heat exchange between the animal and the environment (Dawson and Whittow, 2000; McNab, 1974). The latter is dictated by the animal's thermal conductance, which, in turn, is influenced by body size and shape, both of which depend on surface/volume relationships, body composition and effectiveness of insulation (Tattersall et al., 2012). Within specific limits of ambient temperatures (T_a), body temperature (T_b) can be maintained

reasonably constant by the modulation of thermal conductance, effected by changes in body posture or insulation thickness, or resulting from vasomotor adjustments (Gordon, 2012; IUPS, 2001; Stager et al., 2020). This thermoneutral zone (TNZ) is limited by a lower and upper critical temperature (LCT and UCT, respectively), beyond which the regulation of T_b will require increases in energy expenditure (McNab, 2012). Although little controversy exists about the LCT, the definition and identification of the UCT is complicated by the fact that it is often defined as the point at which evaporative cooling starts to increase (IUPS, 2001), which does not always coincide with the increment in metabolism, and usually does not appear as an obvious or marked threshold (Gordon, 2012; Long et al., 2014). In any case, the more efficiently an animal can modulate its thermal conductance, the broader its TNZ should be. This is obviously of adaptive value by allowing animals to regulate T_b at minimum cost (in terms of energy or body water) as T_a varies (see Bozinovic et al., 2014).

In birds, a widespread thermoregulatory response contributing to the modulation of sensible heat exchange is the adjustment in the insulative value of the plumage by varying plumage thickness or depth in response to variation in T_a , which alters the thickness of the feather/air insulative layer (Saarela et al., 1984): a mechanism akin to piloerection in mammals (e.g. Hohtola et al., 1980). Heat dissipation by feather fluffing and postural changes, such as wing drooping (Cooper and Siegfried, 1976), can be potentiated by concurrent vasomotor adjustments. Indeed, birds can modulate peripheral heat exchange by altering blood perfusion to peripheral organs, such as the feet, legs, bill and head ornaments (Eastick et al., 2019; Hagan and Heath, 1980; Steen and Steen, 1965). These appendages are uninsulated and, as their superficial temperature is altered – via vasomotor adjustments, conductive and radiative heat exchange can be modulated (e.g. Jessen, 2001). In this context, bill size has been demonstrated to vary with latitudinal gradient in environmental temperature (i.e. Allen's rule; Symonds and Tattersall, 2010), possibly under the influence of the most critical temperatures experienced at different seasons by different species or populations at different geographic locations (see Danner and Greenberg, 2015). Altogether, an adaptive association between appendage (e.g. bill, limbs) size and environmental temperatures, and, consequently, heat exchange capability, seems to be supported (Larson et al., 2018; Playa-Montmany et al., 2021; Symonds and Tattersall, 2010; Tattersall et al., 2018). This may involve energetic considerations and we posit that bill size will correlate with the amplitude of the TNZ in birds, particularly in the setting of the UCT threshold. However, the contribution and ability to modulate heat exchange through the bill to keep thermal balance remains empirically untested in birds.

The thermoregulatory role played by avian bills is epitomized by the toco toucan, whose ostentatious appendage can account for up to

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50% of total body surface area (external) and through which this bird has been estimated to dissipate as much heat as 4 times its basal rate of HP (Tattersall et al., 2009). As such, the toucan bill is recognized as one of the most potent thermal windows through which sensible heat exchange can be adjusted that has been identified in the animal kingdom. In the present study, we aimed to quantify the contribution of the toucan bill in establishing the limits to which T_b can be maintained at minimum costs. Is the impressive capacity for sensible heat exchange through the bill reflected in the breadth of its TNZ? If that is the case, does having such a large heat exchange organ extend the LCT, UCT or both thermal limits of the TNZ? We tested these questions by measuring the rates of oxygen uptake (\dot{V}_{O_2}) of adults of toucans at a range of ambient temperatures under two conditions. First, measurements were made in intact birds, able to use their bill for modulating sensible heat exchange to its full extent. Second, we artificially insulated the entire bill of the birds, effectively disabling the contribution of this heat exchange avenue to the global maintenance of the bird's thermal balance. By comparing the two conditions, we were able to isolate the bill's contribution to the amplitude and the limits of the TNZ.

MATERIALS AND METHODS

Animals

Experiments were carried out on six adult toucans, *Ramphastos toco* Müller 1776, of undetermined sex. Birds were obtained by donation from accredited animal facilities in São Paulo state (Instituto Chico Mendes de Conservação da Biodiversidade permit #27171-1). In captivity, toucans were maintained in outdoor wired mesh cages (6×10 m in area, 3 m high) at the Comparative Animal Physiology Laboratory, Universidade Estadual Paulista, Rio Claro municipality, São Paulo state, southeastern Brazil. Cages were provided with shelters, perches, water and a few small trees; the ground surface was mostly covered with grass and other low greens. Toucans were fed with fresh fruits and commercial pelleted food (Labcon Club Toucan Tucanes) offered twice a day.

Experimental protocol

We measured \dot{V}_{O_2} in birds subjected to temperatures varying from 0 to 35°C, at 5°C increments. All birds were measured at all experimental temperatures under two conditions: 'intact', in which birds were measured without any manipulation of the bill's thermal conductivity; and 'insulated', in which the entire external surface of the bill was covered with a layer of heat exchange-insulating material. To create this insulation, we used a 2 mm felt cloth tailored to match the exact shape and size of each individual bird's bill. Felt molds were lined internally with an aluminium foil tape and fixed to the bill with double-sided tape (Fig. 1). Thermal conductivity of this heat-insulating material was experimentally determined and averaged $0.0729 \pm 0.006 \text{ W m}^{-1} \text{ K}^{-1}$ (mean \pm s.d.) at temperatures varying from 4.3 to 31.5°C (see Supplementary Materials and Methods for details on how this was determined). Upper and lower bill parts were independently covered, so birds could freely engage in gaping and panting during experimentation.

All measurements were taken during the night, which corresponds to the circadian phase when *R. toco* is inactive (Sick and Barruel, 1988). Birds were fasted for 6–8 h prior to the experiments to ensure that they were post-absorptive at the time of measurements (Silva et al., 2008). In a typical trial, a fasted bird was transferred from the maintenance cage in the evening, weighed, and placed in a custom-made respirometric chamber (46.5 l, 44×44×24 cm) housed inside a temperature-controlled chamber (FANEM model 347 CD, Guarulhos, São Paulo, Brazil) for

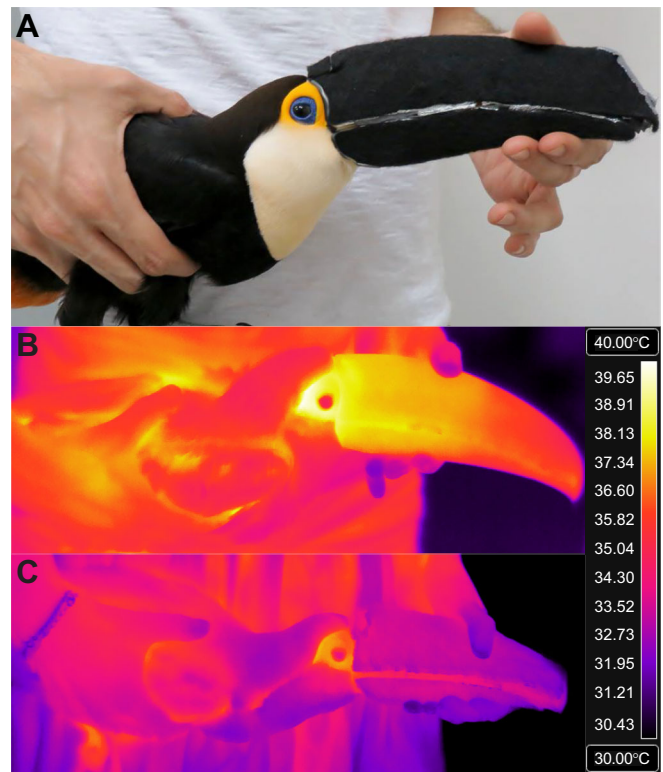


Fig. 1. Sample images of insulating material used to reduce heat exchange from the bill of toucans (*Ramphastos toco*). (A) A live image of an insulated bill. (B,C) Thermal images of a toucan (exposed to 35°C) with an uninsulated (intact) bill with a higher surface temperature (B) compared with that of a toucan (exposed to 30°C) with an insulated bill (C).

temperature control. Inside this chamber, we placed a small wood perch, 10 cm from the ground, on which the birds invariably sat. The respirometry system was immediately activated but, in all cases, a habituation period of at least 2 h was observed before the beginning of data collection. Once started, data collection persisted for approximately 2.5 h, which proved adequate in yielding consistent steady-state readings. At the end of the experiments, birds were removed from the respirometric chamber and their core T_b was measured by the insertion of an external temperature probe into their cloaca (TechLine, model TS-101).

Individual birds were measured once in each treatment/temperature combination, in random order, and were allowed to recover for at least 3 days between trials. All procedures were approved by the Animal Use and Ethics Committee (CEUA) of the Bioscience Institute, São Paulo State University (UNESP), campus of Rio Claro, Brazil (protocol #5373).

Respirometry

We determined the rates of oxygen uptake using an open-flow respirometric system (Lighton, 2008; Voigt and Cruz-Neto, 2009). In our setup, a supply line of pressurized air was directed through a column filled with drying agent (Drierite, W.A. Hammond Drierite Co. Ltd) and then ventilated to the animal chamber at a rate of 2.4 l min^{-1} , the air flow being measured and controlled using a mass flow controller/meter (MFC-2, Sable Systems International). From the outflow port, we used a custom-made 60 ml syringe manifold to sub-sample the excurrent airflow leaving the respirometric chamber at a rate of 150 ml min^{-1} (SS-4 Sub-Sampler Pump, Sable Systems International). This sub-sampling

line was directed through a drying column (same as above) and then into an oxygen analyzer (FoxBox Respirometry System, Sable Systems International). Immediately before and after each experimental trial, we also determined the baseline levels of fractional oxygen concentration by running our respirometry system under the identical experimental conditions, but with no animal inside the respirometric chamber. The oxygen analyzer was calibrated periodically against pure nitrogen and dry air, according to the manufacturer's recommendations.

\dot{V}_{O_2} was calculated by the change in fractional oxygen concentration between the baseline and that averaged for a steady-state period of at least 15 min measured in the last hour of the measurement period for each bird. Calculations followed Koteja (1996), assuming a respiratory exchange ratio of 0.8.

Energetic costs, heat storage and conductance calculations

HP (W) was estimated from \dot{V}_{O_2} assuming an oxycaloric equivalent of 20 J ml⁻¹ O₂ (Blaxter, 1989), and dividing the resulting values by 3600 (to convert h⁻¹ to s⁻¹; yielding J s⁻¹ or W). At each measurement temperature, because experiments were conducted pairwise on the same individuals, we subtracted the HP of the control condition HP from the HP of the insulated bird to assess the change, if any, in the energetic costs resulting from minimizing bill heat transfer. These values allowed us to estimate the energetic costs of compensatory mechanisms that might have come into play when the bill heat exchange capacity was disabled. Heat storage (q ; kJ) for the highest temperature condition was calculated based on the difference in T_b (ΔT) between the thermoneutral condition and 35°C, assuming a tissue heat capacity (c_p) of 3.45 kJ kg⁻¹ K⁻¹ (Giering et al., 1996) and incorporating animal body mass (m) using the heat capacity equation:

$$q = c_p m \Delta T. \quad (1)$$

We calculated wet thermal conductance at temperatures below and at the LCT using the formula described by McNab (1980):

$$C = \dot{V}_{O_2} / (T_b - T_a), \quad (2)$$

where C is the conductance (ml O₂ g⁻¹ h⁻¹ °C⁻¹), \dot{V}_{O_2} is the metabolic rate (MR; ml O₂ g⁻¹ h⁻¹) and T_b and T_a are the body and ambient temperatures, respectively.

Data analysis

Statistical analyses were performed in R version 4.2.0 (<https://www.R-project.org/>). Residuals from model fits were visually inspected to ensure that assumptions of normality and equal variance were met. To examine simultaneously the effects of T_a and insulation treatment, linear mixed-effects models were fitted using the package *lme4* (Bates et al., 2015), including the interaction term and animal identity as a random effect, and the significance of the models was tested using the *lmerTest* package, correcting for degrees of freedom using the Satterthwaite method (Kuznetsova et al., 2017). Segmented regressions were used to estimate LCT and UCT from \dot{V}_{O_2} data, with respect to T_a , using the *segmented* package (Muggeo, 2017). Plots were generated using the package *ggplot2* (Wickham, 2016).

RESULTS

The thermal conductivity (k) of the bill insulating material was positively related to the T_a ($r=0.987$; $P=0.013$) and yielded a mean value of 0.0729±0.006 W m⁻¹ K⁻¹ among the four tested temperatures (Table S1). The variation in the relative humidity

Table 1. Lower and upper critical temperatures for the control group (intact bill) and experimental group (insulated bill) determined from the mass-specific \dot{V}_{O_2} data using a manual intersection method contrasted with segmented regression approaches

	Control group		Insulated group	
	Manual	Segmented	Manual	Segmented
Lower critical temperature (°C)	11.5±2.8	10.9±3.5	10.5±2.6	10.8±2.5
Upper critical temperature (°C)	24.6±2.2	25.0±4.6	23.6±1.8	24.1±2.0

Data are means±s.d.

inside the climatic chamber did not influence the k values ($r=-0.649$; $P=0.351$).

The body mass of the toucans was slightly lower (by 16.7 g) in the bill-insulated group (619.5±60.6 g) compared with the intact condition (636.1±65.0 g; $F_{1,6}=7.93$; $P=0.031$), although there was no interaction between treatment and T_a on body mass ($F_{7,90}=1.58$; $P=0.151$). The lower and upper limits of the toucans' TNZ, calculated by manual regression line intersection or according to the three-phase regression (Table 1), did not differ between treatments. The TNZ limits were 10.9–25.0°C for the intact group and 10.8–24.1°C for the bill-insulated group (Table 1). Owing to a significant interaction between T_a and treatment ($F_{7,90}=1.58$;

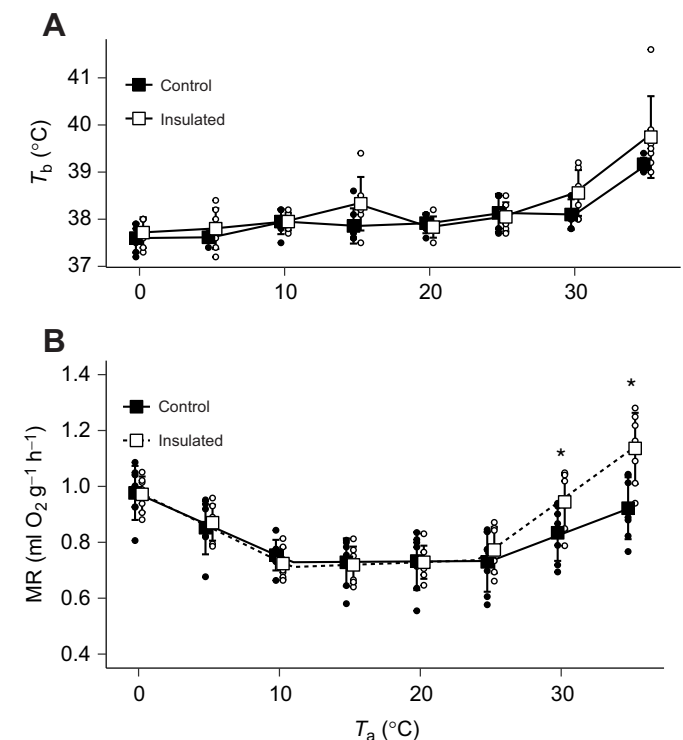


Fig. 2. Body temperature (T_b) and metabolic rate (MR) of the toucan (*R. toco*) as a function of ambient temperature (T_a) measured with the bill intact and the bill insulated. (A) T_b . (B) MR. The asterisks represent the temperatures at which the treatments differed statistically via *post hoc* test. Error bars reflect the means±s.d. and symbols represent the individual data points. Lines in the bottom plot reflect the segmented regression fits. $n=6$ per group. Two-way generalized linear mixed-model (GLMM) results for T_b : $T_a \times$ treatment ($P=0.17$), T_a ($P<2 \times 10^{-16}$), treatment ($P=0.0190$). Two-way GLMM results for MR: $T_a \times$ treatment ($P=1.11 \times 10^{-8}$), T_a ($P<2 \times 10^{-16}$), treatment ($P=2.6 \times 10^{-5}$).

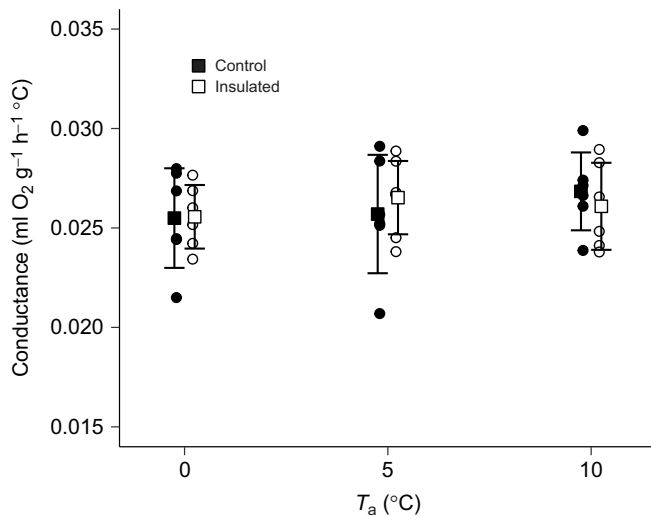


Fig. 3. Thermal conductance of toco toucan (*R. toco*) as a function of T_a below and at the lower critical temperature. Measurements were made with the bill intact (control) and with the bill artificially insulated. Error bars reflect the means \pm s.d. and symbols represent the individual data points. $n=6$ per group. Two-way GLMM results for conductance: $T_a \times$ treatment ($P=0.385$), T_a ($P=0.259$), treatment ($P=0.927$).

$P=1.1 \times 10^{-8}$), the mass-specific MR as a function of air temperature was different between treatments as a result of a greater increase in MR in the group with the insulated bill at temperatures of 30°C ($P=0.0076$) and 35°C ($P<0.0001$). At 35°C, the mean MR of the individuals with the insulated bills was 1.13 ± 0.13 ml O_2 g^{-1} h^{-1} $^{\circ}C^{-1}$, whereas the intact group presented a MR equal to 0.92 ± 0.11 ml O_2 g^{-1} h^{-1} $^{\circ}C^{-1}$ (Fig. 2). At T_a below 30°C, MR did not differ between treatments. The basal MR (BMR), measured within the TNZ, was equal to 0.779 ± 0.04 ml O_2 g^{-1} h^{-1} $^{\circ}C^{-1}$ in the intact group and 0.733 ± 0.05 ml O_2 g^{-1} h^{-1} $^{\circ}C^{-1}$ in the bill-insulated group ($F_{1,6}=0.004$; $P=0.99$).

T_b as a function of T_a did not differ between treatments (treatment \times temperature interaction: $F_{7,77.9}=1.53$; $P=0.17$), although it was significantly related to T_a ($F_{7,78}=36.28$; $P<2 \times 10^{-16}$) in both treatment groups, showing a rise in T_b as T_a rose, and being higher overall in the insulated condition (main effect of treatment: $F_{1,78.2}=5.69$; $P=0.019$; Fig. 2). Within the TNZ, the mean T_b of the birds was equal to $38.0 \pm 0.3^{\circ}C$ in the intact group and $38.2 \pm 0.5^{\circ}C$ in the bill-insulated group.

At T_a between 0 and 15°C, thermal conductance was similar between the two treatments, with mean values of 0.0276 ± 0.003 ml O_2 g^{-1} h^{-1} $^{\circ}C^{-1}$ in the intact group and 0.0271 ± 0.002 ml O_2 g^{-1} h^{-1} $^{\circ}C^{-1}$ in the bill-insulated group (Fig. 3). Overall, conductance was not affected by temperature ($F_{2,25.9}=1.42$; $P=0.259$), by treatment ($F_{1,25.9}=0.00862$; $P=0.927$) or by a temperature \times treatment interaction ($F_{2,25.9}=0.991$; $P=0.385$).

The mean HP within the TNZ (i.e. BMR) was 2.66 ± 0.41 W and 2.51 ± 0.21 W in the control and bill-insulated conditions, respectively. Energetic costs, assessed from the difference in HP between insulated and control conditions, were significantly influenced by temperature (Fig. 4; $F_{7,34.6}=9.01$; $P=2.7 \times 10^{-6}$) and this effect was mostly caused by the large HP differential at the highest temperatures tested (i.e. 30 and 35°C; see Fig. 4), as, in general, it hovered near 0 W for all other temperatures. At 35°C, for example, the cost of managing heat exchange in the bill-insulated group was 0.797 W above that of the control group, which represented $\sim 31.7\%$ of the BMR.

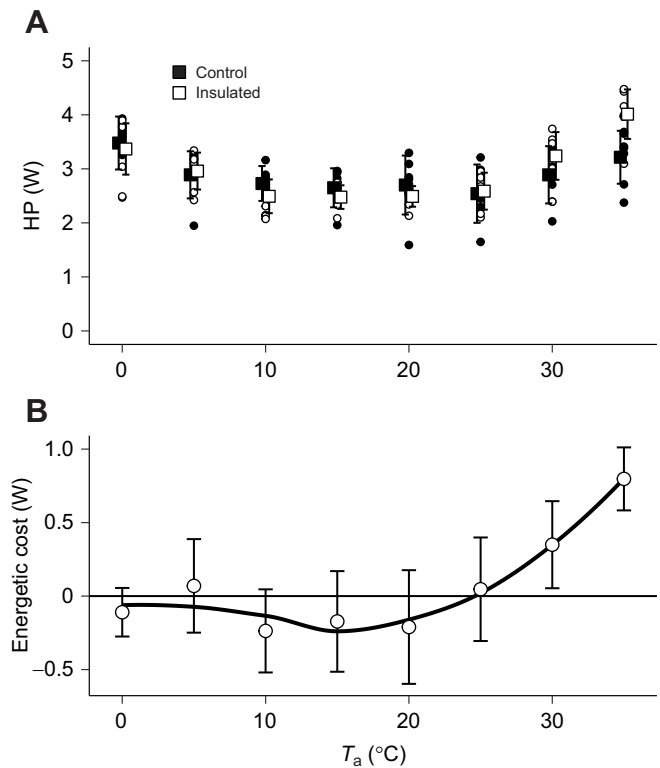


Fig. 4. Heat production (HP) and apparent energetic cost associated with insulating the bill of toco toucan (*R. toco*) as a function of T_a . (A) Data depict the means \pm s.d., with raw data points included ($n=6$ per group), for HP from both intact birds (control) and birds that had their bills covered (insulated). (B) The energetic cost (means \pm s.d.) represents the difference between the respective (pairwise) measurements conducted during the insulated trial minus those of the control trial. The solid line depicts a simple smooth curve to aid in visualizing the rise in energetic costs at higher temperatures.

Body heat storage (i.e. heat storage) at 35°C compared with thermoneutral conditions was 3.21 ± 0.33 kJ for the control condition and 3.99 ± 0.31 kJ for the bill-insulated birds; however, these were not significantly different between conditions ($F_{1,11}=2.85$; $P=0.12$).

DISCUSSION

Aside from the obvious role it plays in food acquisition, the role of the avian bill as a thermoregulatory organ has been well established for a diverse assemblage of birds subjected to different thermal habitat constraints (reviewed in Tattersall et al., 2017). Indeed, broad patterns correlating bill size and T_a (McQueen et al., 2022; Ryding et al., 2021) provide further evidence that heat exchange considerations are of adaptive relevance and have played a role in the evolution of the anatomical attributes of this multi-task appendage (Tattersall et al., 2017). The bill assists in the maintenance of thermal homeostasis by efficiently modulating dry heat exchange via vasomotor adjustments as T_a varies, diminishing in importance as temperatures approach core T_b (Hagan and Heath, 1980; Tattersall et al., 2009). An intuitive and underappreciated consequence of this response, which causes an undetectable increment in MR, is that it alleviates the energetic cost of T_b regulation (Gordon, 2012; McNab, 1974). Thus, one could expect that the greater the capacity of a bird to exchange heat through the bill, a trait that would co-vary with bill size (e.g. surface area), the larger the range of T_a the animal is capable of withstanding without

recruiting more energetically expensive thermoregulatory responses (i.e. the wider would be its TNZ). The results we obtained in the toco toucan clearly revealed that this was not the case, as we found no difference in the TNZ width between intact birds able to explore their extraordinary capacity for heat exchange through the bill at the fullest extent and those in which such a capacity was limited. Although contrary to our primary hypothesis, the closer examination of the patterns of metabolic variation at the full range of temperatures tested may yield some insights that we will cover in detail below.

The extreme capacity of toco toucans to dissipate heat through their bills provides an important advantage in assisting the birds in maintaining thermal homeostasis under moderately warm ambient temperatures (i.e. above the UCT but below their T_b) or under situations in which HP is increased (Tattersall et al., 2009). However, at the other extreme, at low temperatures, the possession of a large uninsulated appendage may represent a potential liability to heat conservation as the ramphotheca, underlying the most external bill layer, is composed of living tissue (Midtgard, 1984a,b; Van Hemert et al., 2012). This living tissue would require some moderate level of circulatory irrigation even if this circulation was dramatically reduced by a thermoregulatory-triggered vasoconstrictive response (e.g. Johansen and Bech, 1983). Therefore, we were expecting that intact toucans would lose more heat to the environment than the bill-insulated ones under low-temperature conditions and that this would cause MR to increase at a higher LCT in the intact condition. We found, however, that there was virtually no difference in the LCT and birds in both treatments exhibited an increase in MR as the temperature dropped below ~ 10.5 – 11.5°C . This observation indicates that toco toucans are indeed able to finely tune heat dissipation through the bill and overall HP to keep T_b constant at low temperatures, a response that was only previously implied (Tattersall et al., 2009). It also reveals how effective the vasoconstrictor response of the toco toucan bill could be in promoting heat conservation at cold temperatures. Infrared imaging, however, shows that toucans keep a temperature differential of approximately 1 and 4°C between their distal and proximal bill surface, respectively, and T_a within the 10 – 20°C range (Tattersall et al., 2009). Therefore, some heat must be lost through the bill at low temperatures, and the reason why this does not lead to differences in MR when the bill is insulated may be that toco toucans combine their autonomic vasomotor thermoregulatory response with behavior. When sleeping, toucans generally place the bill under one of the wings or between the interscapular feathers and raise the tail in order to cover the bill (Alvarenga, 2004). Indeed, in virtually all trials run below 10°C and whenever the birds were asleep at temperatures below 25°C , we found the birds at the end of the experiment with their bill tucked between their wings, a widespread response that has been shown to mediate thermoregulation in many bird species (Pavlovic et al., 2019), particularly in those with larger bills (Ryeland et al., 2017). The experimental bill insulation we devised had a thermal conductivity comparable with that provided by avian feathers (Rogalla et al., 2022; Walsberg, 1988; Wolf and Walsberg, 2000); therefore, we can conclude that the vasomotor and behavioral responses were as effective at heat conservation as having the bill surface entirely insulated, as tested in the present study.

Toco toucans start to exhibit a bill vasodilatory response at a temperature around 20°C , which becomes prominent throughout the entire bill surface as the temperature rises above 25°C (Tattersall et al., 2009). The temperature of 25°C agrees well with the UCT values we found for toucans, both in intact and in bill-insulated

birds. Also, we found little difference ($<0.2^\circ\text{C}$) in T_b between treatments, and no interaction between treatment and air temperature, therefore discounting heat accumulation in the bill-insulated group. Accordingly, birds with insulated bills must have recruited other heat-dissipating mechanisms to compensate for the disablement of their main thermal window, the bill, and maintained thermal balance within the TNZ. These compensatory responses may have included a vasodilator response to the feet and legs (J.N.C., G.J.T. and D.V.A., unpublished data) and the modulation of feather fluffing (e.g. Downs and Ward, 1997; Saarela et al., 1984; Weathers et al., 2001). Unfortunately, we were unable to collect data on the rates of evaporative water loss during the metabolic measurements and, therefore, we cannot ascertain whether evaporative cooling played a significant role in the regulation of T_b within the TNZ limits. If that was the case, we suspect that it may have been restricted to a moderate increase in respiratory frequency (Tattersall et al., 2009) and, perhaps, to gaping (Neumann, 2016) rather than active panting as it did not translate into a noticeable increment in MR. Previous research has shown that panting does not normally commence until above 33°C in toco toucans (Tattersall et al., 2009). Indeed, it has been demonstrated that the increment in evaporative cooling is often dissociated from the metabolically determined UCT (Gordon, 2012; Mitchell et al., 2018).

At the two temperatures tested above the UCT, 30 and 35°C , we found that bill-insulated birds had a significantly higher MR than that of birds with intact bills. Again, there was no difference in T_b between treatments or in body heat storage and, therefore, it seems likely that insulated birds were heavily panting at these temperatures (Tattersall et al., 2009; van de Ven et al., 2016), which would explain their higher MR. Thus, insulating the bill impacted the energetics of thermoregulatory responses to heat stress, presumably because bill-insulated birds were forced to recruit compensatory thermoregulatory mechanisms to ensure heat balance at a greater intensity compared with that of birds in the control group, in which the thermoregulatory role of the bill was kept functional. We have not quantified these mechanisms, but they are likely to include changes in posture, behavior and alternative thermal window recruitment, and increased levels of evaporative water loss from panting or gular fluttering. Thus, having access to the bill as a thermoregulatory organ at higher temperatures has important implications for energetics and water balance. In terms of energetics, our estimate is that the beneficial consequence of being able to access the normal heat exchange through the bill at 35°C corresponds to $\sim 32\%$ of the BMR; in other words, the recruitment of alternative thermolytic responses in the absence of bill heat exchange represents a large energetic cost. The most likely cause of this increment is the mechanical costs of increased panting, known to occur at these temperatures (see Tattersall et al., 2009) in the insulated condition. Thus, we demonstrated formally that access to a potent peripheral heat exchanger minimized the energetic cost of thermoregulation at higher temperatures.

The toco toucan has a neotropical distribution, from open fields to high tropical forests (Short and Horne, 2002). In Brazil, large populations are found in Cerrado vegetation (Sick and Barriel, 1988), where high temperatures (40 – 41°C) are recorded during spring and summer (Alves and Rosa, 2008; Silva et al., 2008). In these environments, it is possible that the bill of the toco toucan contributes in an important way to the maintenance of thermal homeostasis. Although our measurements in the lab and at rest did not show any profound influence of bill heat exchange on the limits of the TNZ, under more natural conditions (e.g. activity, higher convective heat exchange, solar heat exposure), the importance of

this heat exchange organ is likely to differ. For example, under certain conditions, the performance of the toucan's bill as an efficient thermal window could alter the duration of time they spend active during the hot hours of the day or exposed to solar radiation, impacting ecologically relevant activities, such as defense of territory, foraging and reproduction (Luther and Danner, 2016; van de Ven et al., 2019). Indeed, toucans appear to partition habitat usage seasonally, preferring to forage in open habitats during the cooler, drier winters of Brazil and to spend more time in gallery forests where fruit are more plentiful in the summer (Ragusa-Netto, 2008). Although foraging concerns appear to drive this habitat usage, the large bill might limit the capacity for toucans to select habitats with high solar radiation in the summer, when the bill would cease to facilitate heat loss and, potentially, would contribute to radiative heat gain (Tattersall et al., 2009).

In conclusion, the incredible vasomotor control over the bill blood supply exhibited by the toucan suggests that they can simultaneously have an effective radiator of body heat at high temperatures without paying an obvious cost at low temperatures. In other words, the fact that we found no difference in thermal conductance, MR or T_b from insulated and intact birds clearly shows that the cold-induced vasoconstriction of the bill was as good at preventing heat loss as having the bill covered with a highly insulating material. Although we did not find differences in the limits of TNZ associated with insulation of the bill, we did find a very clear effect at the highest temperatures tested. At temperatures above UCT, access to the bill as a heat exchange avenue promoted a lower energetic cost while birds were defending T_b . As the toucan has sufficient capacity to thermoregulate in the absence of convective/radiative bill heat exchange, these results speak to an argument that the enlarged bill is an exaptation for heat exchange rather than a thermoregulatory adaption *per se* (Tattersall et al., 2017). We also observed a large difference in the MR of insulated birds at temperatures above the UCT, with minimal difference in T_b , suggesting that toucans can almost fully compensate for the absence of bill heat exchange by adopting more costly thermolytic mechanisms, such as evaporative water loss through gaping and panting.

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Competing interests

The authors declare no competing or financial interests.

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Author contributions

Conceptualization: G.J.T., D.V.A.; Methodology: J.N.C., D.V.A.; Software: G.J.T.; Validation: J.N.C., G.J.T.; Formal analysis: J.N.C., G.J.T.; Investigation: J.N.C., D.V.A.; Resources: D.V.A.; Data curation: G.J.T.; Writing - original draft: J.N.C., G.J.T., D.V.A.; Writing - review & editing: J.N.C., G.J.T., D.V.A.; Visualization: G.J.T.; Supervision: D.V.A.; Project administration: D.V.A.; Funding acquisition: D.V.A.

Data availability

Datasets from this study are available from the Brock University repository: <http://hdl.handle.net/10464/16884>.

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