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Influence of temperature on evaporative water loss and cutaneous resistance to water vapour diffusion in the orange-thighed frog (*Litoria xanthomera*)

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Abstract

We evaluated the effect of ambient temperatures between 25 and 43°C on the rate of evaporative water loss (EWL) in eight adult *Litoria xanthomera* (average body mass = 7.3 ± 0.6 g). Frogs were placed in a cylindrical chamber that permitted them to fully conceal their ventral surfaces using a water-conserving posture. Their EWL was 7.1 ± 0.7 mg g^{-1} h^{-1} at 25°C and reached 28.0 ± 2.5 mg g^{-1} h^{-1} at 43°C. Agar replicas of the frogs were used to evaluate boundary-layer resistances associated with the EWL measurements and, thus, to permit evaluation of cutaneous resistance to vapour diffusion (r_c) in live frogs. The r_c of *L. xanthomera* was stable over the temperature range of 25–35°C, averaging about 28 s cm^{-1}, and then declined directly with ambient temperatures above 37°C. The highest r_c recorded for each individual over the range of temperatures studied averaged 32.0 ± 1.2 s cm^{-1}. The thermolabile nature of r_c demonstrates a well developed thermoregulatory control of EWL in this species, a trait very similar in pattern and extent to that previously measured in the closely related *Litoria chloris*.

Introduction

The hylid anurans of Australia are highly diverse with respect to the range of habitats they occupy, ranging from species associated with permanent water to arid-zone frogs that remain underground for several years between rains. Another aspect of hylid diversity is found in the physiological traits they display. Notable in this regard is the substantial variation in skin permeability to water vapour displayed by frogs in the genus *Litoria*. Whereas most frogs that have been evaluated have skin that is so permeable to water that it behaves as a free-water surface (Wygoda 1984), several Australian hylids have skin that is highly resistant to vapour flux (summarised in Buttemer *et al.* 1996). Perhaps surprisingly, the lowest cutaneous vapour permeability among Australian frogs is not found in arid-zone species, but instead has been measured in frogs associated with rain forests. Included in this group are the dainty green tree frog (*Litoria gracilenta*) and the red-eyed tree frog (*L. chloris*), with cutaneous resistances (r_c) to evaporation that are over 100-fold and 40-fold greater, respectively, than those found in 'typical' ranid and bufonid frogs (Withers *et al.* 1984; Buttemer 1990). The functional significance of the higher r_c found in *L. gracilenta* is not clear, but the frogs studied were residents of much warmer regions of New South Wales than the site where the *L. chloris* were collected and *L gracilenta* is a much smaller species (Barker *et al.* 1995). The orange-thighed frog (*L. xanthomera*) is closely related to *L. chloris* (Davies *et al.* 1986), but it is smaller and occurs at lower latitudes (Barker *et al.*1995). This presents an opportunity to see whether interspecific differences in climate regime and/or body size influence cutaneous vapour resistance.

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Material and Methods

Experimental animals

The eight orange-thighed tree frogs used in this study were captured in rainforest north of Townsville, Queensland, in February and shipped by air to Wollongong, New South Wales. They were placed in glass terraria maintained at room temperature (18–23°C). Silver-backed, 60-W incandescent lights were positioned within the terraria above potted plants to provide opportunity for basking. All frogs maintained or gained mass on a diet of blowflies (*Lucilia cuprina*) and had free access to water. Body masses of these frogs averaged 7.3 ± 0.6 g (range = 6.2–9.2 g). All experiments were conducted in accord with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, as approved by the University of Wollongong Animal Ethics Committee (AE94/19).

Evaporative water loss measurement

Rates of evaporative water loss at 25, 30, 35, 37, 39, 41 and 43°C were measured gravimetrically using materials and procedures described in detail elsewhere (Buttemer 1990). Briefly, frogs, having had free access to water beforehand, were placed in a Plexiglas cylinder (10.8 cm diameter, 12 cm length) that, in turn, was placed within an isothermal, water-jacketed cylinder of larger diameter. The innermost cylinder housing the frog was attached to a threaded rod that extended through a water-tight collar to a pedestal positioned on an electronic balance (Mettler P400; 0.001 g sensitivity). The entire apparatus was housed within a temperature-regulated cabinet that permitted thermal matching of air and water-jacket temperatures.

Chamber humidity was held at low levels by passing compressed air through a drying tube containing desiccant (Drierite) and then into the chamber at a rate of 7 L min^{-1}. A section of porous foam rubber was placed between the air inlet and the cylinder containing the frog to assure laminar airflow. Air temperature just anterior to the frog was monitored using a calibrated thermocouple in conjunction with a DataTaker DT100F datalogger (Data Electronics P/L). Air humidity was recorded downstream using a Vaisala humidity probe.

Frogs were placed inside the chamber and then given about 1 h to thermally equilibrate before measurements were recorded. We noted each frog’s posture during these evaluations and recorded its body temperature by inserting a calibrated thermocouple into its cloaca within 1 min of removing the frog from the chamber. Rates of evaporative water loss (EWL) were calculated from the slope of least-squares regression of the change in body mass over time. The rates of EWL we report were recorded during the last hour of a frog’s 2–2.5-h exposure to a given temperature and are based on a 10-min measurement period that had both a statistically significant correlation coefficient and a similar mass-loss rate to at least one other 10-min period of recording.

Cutaneous resistance evaluation

The rate of cutaneous water loss (E_c) is driven by the difference in vapour pressure between the inside and outside of the skin. This vapour pressure potential, in turn, is restricted by two vapour resistance elements in series: the resistance of the skin to vapour flux (r_c) and the boundary layer resistance (r_b) associated with air adhering to the outside surface of the animal (Spotila and Berman 1976). These relations are expressed as:

$$E_c = ({}_sd_s - RH\ {}_sd_{a)}\ /\ (r_b + r_{c)} \qquad (1)$$

where E_c is the rate of cutaneous water loss (g cm^{-2} s^{-1}), ${}_sd_s$ is the saturation vapour density (g cm^{-3}) of water in air at skin temperature (T_s), ${}_sd_a$ is the saturation vapour density (g cm^{-3}) at the air temperature (T_a) of the EWL measurement, RH is the relative humidity of the measured air (expressed fractionally), and r_b and r_c are boundary and cutaneous resistances to water vapour flux, respectively (both in s cm^{-1}).

Apart from air speed, the boundary layer resistance depends mostly on the physical geometry of the animal and it is possible to evaluate this term using an agar replica of a frog (Spotila and Berman 1976). We anaesthetised the smallest and largest frogs using tricaine methanesulfonate (Sigma) and made moulds of them in their water-conserving posture (Pough *et al.* 1983) using dental alginate impression material (Buttemer 1990). We poured 3% agar into these moulds and determined rates of water loss and body temperatures at 30°C for these agar models under the same conditions as experienced by live frogs during their EWL measurements. Because the agar replicas behave as free-water surfaces and thus lack cutaneous resistance (Spotila and Berman 1976), r_b may be evaluated by rearrangement of Equation 1. Boundary-layer resistance values for the other frogs were interpolated from these two measurements according to their body mass relative to these two frogs.

We estimated body surface area exposed to air of each frog using an equation derived by McClanahan and Baldwin (1969) and modified for frogs in water-conserving postures by Withers *et al.* (1982*a*) ($SA = 6.6\ mass^{0.56}$, where SA = surface area (in cm^2) and mass is in grams). This equation has been shown to predict accurately the exposed surface areas of *Litoria caerulea* and *L. chloris* (Buttemer 1990).

Data analysis

We compared physiological responses of *Litoria xanthomera* to those of *L. chloris* (data from Buttemer 1990) using repeated-measures ANOVA (JMP ver. 5; SAS). Interspecific comparison of maximum individual cutaneous resistance values was performed using an unpaired *t* test (JMP ver. 5). Unless stated otherwise, values presented in this paper are means ± s.e. and number of animals is represented as *N*.

Results

All frogs remained in the water-conserving posture (Pough *et al.* 1983) throughout the EWL measurements. The average rate of water loss at 25°C was 7.1 ± 0.7 mg g^{-1} h^{-1} ($N = 8$). The rate of EWL rose steadily between 25 and 35°C and then increased substantially at temperatures higher than 35°C (Fig. 1). This pattern is somewhat similar to that shown by *L. chloris*, but the rate of EWL on a mass-specific basis was consistently and significantly higher in *L. xanthomera* than in *L. chloris* ($F_{1,96} = 112.8$, $P < 0.001$) at all temperatures examined (Fig. 1). The thermal sensitivity of EWL was somewhat higher in *L. xanthomera* than in *L. chloris*, as indicated by the 4-fold versus 3.5-fold increase in EWL for these

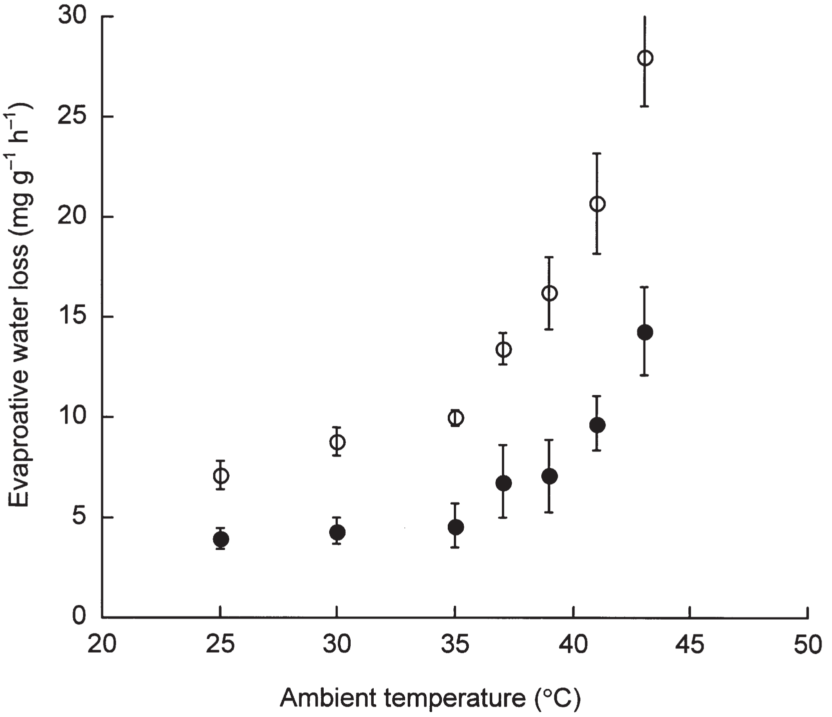

Fig. 1. The relationship between rate of evaporative water loss and ambient air temperature in *Litoria xanthomera* (open circles) and *L. chloris* (filled circles). The symbols designate the mean value recorded and the vertical line through each symbol describes the standard error of the mean. Values presented for *L. chloris* are from Buttemer (1990).

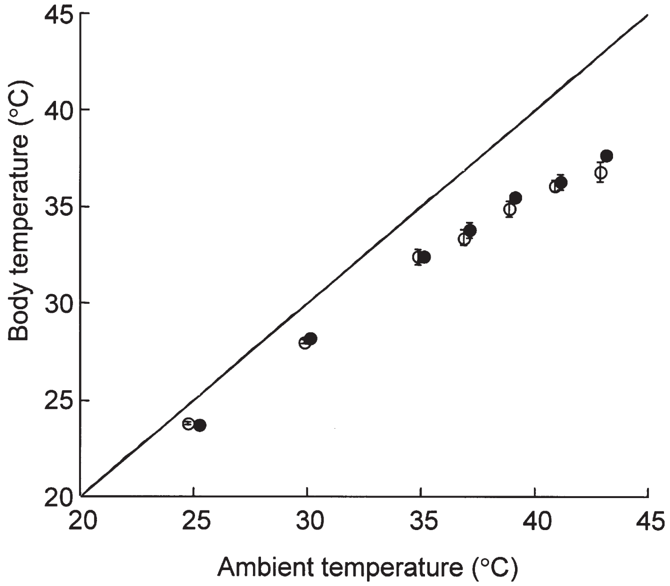

Fig. 2. The relation between ambient temperature and body temperature in *Litoria xanthomera* (open circles) and *L. chloris* (filled circles). Symbols represent mean values and vertical lines depict one standard error. Data for L. *chloris* are from Buttemer (1990).

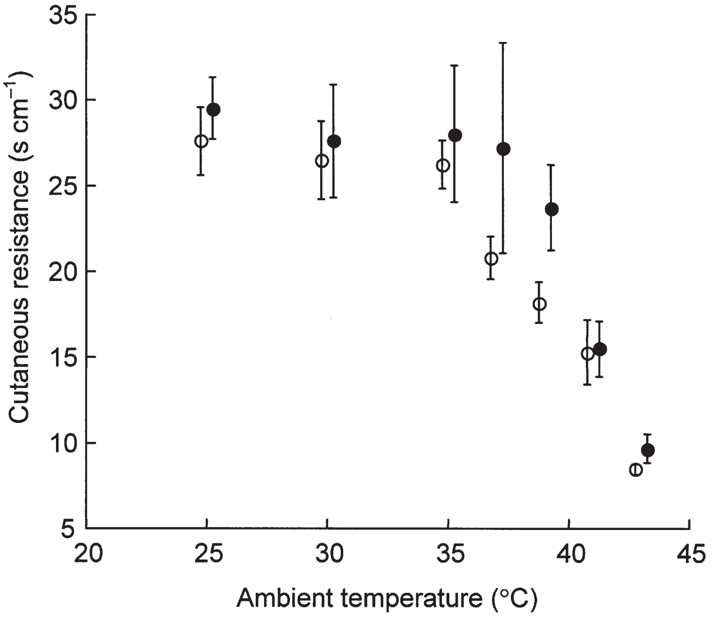

Fig. 3. Cutaneous resistance to water vapour flux as a function of air temperature in *Litoria xanthomera* (open circles), and *L. chloris* (filled circles). Symbols represent mean values and vertical lines depict one standard error. Data for L. *chloris* are from Buttemer (1990).

species, respectively, over the 18°C interval. Body temperature (T_b) was within 3°C of ambient temperature (T_a) between T_as of 25–35°C in both species and then showed progressively more divergence from T_a at temperatures above 35°C (Fig. 2). *L. xanthomera* tended to have lower T_bs than *L. chloris* at T_as above 35°C, but this difference was not statistically significant ($F_{1,96} = 2.47$; $P = 0.12$).

The boundary layer resistance (r_b) of the two agar replicas of *L. xanthomera* was 1.8 and 2.3 s cm^{-1} for the 6.2-g and 9.2-g frog models, respectively. The cutaneous water vapour resistance (r_c) values of *L. xanthomera* were statistically indistinguishable for T_as of 25–35°C and averaged 28.8 ± 0.9 s cm^{-1} over this T_a range. There was an abrupt and significant decline in r_c at 37°C (Tukey–Kramer HSD, $P < 0.05$) and a pronounced reduction of r_c at each 2°C rise in T_a above 37°C (Fig. 3). This pattern differed somewhat in *L. chloris* in that its r_c did not drop significantly until T_as of 41°C and higher (Tukey–Kramer HSD, $P < 0.05$) (Fig. 3). Despite this difference, there was much greater inter-individual variation in *L. chloris* than in *L. xanthomera*, resulting in there being no statistical difference between these species' r_c over this range of T_as ($F_{1,96} = 0.41$, $P = 0.52$). The highest r_c attained by each frog studied was significantly lower ($t = -2.25$, d.f. = 12, $P = 0.04$) in *L. xanthomera* than in *L. chloris*, averaging 32.0 ± 1.2 ($N = 8$) and 39.1 ± 3.3 s cm^{-1} ($N = 6$), respectively.

Discussion

Although *L. chloris* and *L. xanthomera* have sufficiently different morphological properties to distinguish them as separate species (Davies *et al.* 1986), they have very similar life histories. Both species spend most of the year in rainforest or wet sclerophyll forest canopies and are usually encountered only after heavy rains when they descend to breed (Barker *et al.* 1995; Morrison *et al.* 2001). These frogs are also very similar in the extent of their cutaneous resistance to water vapour diffusion and in how this resistance varies with temperature. The higher r_c shown by *L. chloris* was not expected as the animals used in that study (Buttemer 1990) were from a more temperate habitat than the *L. xanthomera* and, thus, are expected to experience lower ambient temperatures. It is important to note, however, that summer periods are accompanied by much more rain in northern Queensland, where *L. xanthomera* resides, than at Gosford, New South Wales, which is near our *L. chloris* collection site at Ourimbah Creek. For example, the long-term average rainfall during December through March is nearly three times greater at Cairns, which is within *L. xanthomera's* distribution, than at Gosford (364 *v.* 134 mm, respectively) and it has 1.5 times the average number of rainy days per month during this period (17.5 *v.* 10.8 days, respectively) (data from Australian Bureau of Meteorology 2002). This higher rainfall and associated higher humidity will result in a lower potential for EWL, which will likely offset the slightly lower r_c found in *L. xanthomera* than in *L. chloris*.

The magnitude of r_c in *L. xanthomera* is quite high for a frog and would place this species among a group of frogs considered to be moderately waterproof (Shoemaker *et al.* 1992). To put this in perspective, most frogs that have been examined have very high rates of EWL because their skin offers little or no barrier to vapour loss. Adolph (1931) demonstrated this unambiguously in ranid frogs by showing that they had the same rate of EWL before and after skin removal. Later studies have confirmed minimal r_c for many ranid and bufonid species (Spotila and Berman 1976; Wygoda 1984), but there have been some remarkable discoveries of frogs with skins as resistant to water loss as terrestrial reptiles, having r_c values of 200–300 s cm^{-1}. Examples of such frogs include *Phyllomedusa* spp. (Shoemaker *et al.*

1972; Shoemaker and McClanahan 1975), *Chiromantis* spp. (Loveridge 1970; Withers *et al.* 1984), and *Hyperolius marmoratus* (Withers *et al.* 1982*b*), all of which are arboreal.

In addition to sharing vapour-resistant skins, *L. chloris* and *L. xanthomera* have very similar thermolability in r_c. Both species show very stable r_c values at temperatures below 35°C and then show progressive decreases in r_c as ambient temperature rises (Fig. 3). While it is not clear whether this thermal variation in r_c is simply a consequence of physicochemical changes in compounds used to reduce water loss within the skin, the progressive decline in r_c with increasing heat loads will stabilise T_b when T_a is high. A similar pattern of body-temperature regulation has also been identified in *Chiromantis* and *Phyllomedusa* (Shoemaker *et al.* 1987). It is possible that the thermolability in r_c shown by *L. chloris and L. xanthomera* is achieved by varying their rate of discharge of cutaneous mucus. This response is shown by *L. aurea*, which varies its rate of glandular mucus discharge from 0 to 9.4 discharges per min as its body temperature rises from 22 to 34°C (Lillywhite and Licht 1975). Such careful control of r_c conserves body water over a very broad range of ambient temperatures, while permitting protection from hyperthermia when ambient temperatures are extreme.

There are now sufficient studies of EWL in Australian frogs to examine patterns of r_c among *Litoria* spp. in relation to their habitat and their size. Because of the substantial differences in r_c between ventral and dorsal skin (Withers *et al.* 1984), these comparisons are restricted to studies that have allowed frogs to conceal their highly permeable ventral skin by assuming a water-conserving posture (Pough *et al.* 1983) on a solid substrate. From available studies, it is clear that r_c in *Litoria* shows no consistent relation with body size, either intraspecifically, as found in this study, or interspecifically (Fig. 4). It is also noteworthy that the *Litoria* spp. with the highest r_c values are coastal arboreal species

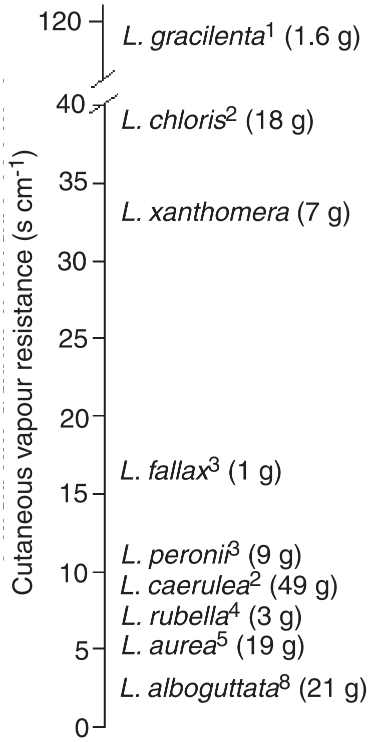

Fig. 4. Cutaneous resistance of Australian *Litoria* spp. The values alongside each species represent the average body mass of frogs used in these measurements. Superscripts indicate the sources of data: 1, Withers *et al.* (1982*b*); 2, Buttemer (1990); 3, Amey and Grigg (1995); 4, Withers (1995); 5, Buttemer *et al.* (1996); and 6, Withers and Richards (1996). The value shown for *L. fallax* was estimated from Amey and Grigg (1995, fig. 1) and assumes a body temperature of 28°C and a 5% relative humidity.

(*L. gracilenta*, *L. chloris*, and *L. xanthomera*), whereas the lowest values are found in more terrestrial representatives, including the only burrowing *Litoria* species (*L. alboguttata*) (but see Meyer *et al.* 1997) and a species commonly found in arid habitats (*L. rubella*) (Fig. 4). This may seem counterintuitive, but the resistance to water loss comprises both boundary layer (r_b) and cutaneous (r_c) elements. Frogs that spend the day at canopy level (*L. chloris* and *L. xanthomera*) or on small reeds (*L. gracilenta*) are exposed to wind which profoundly reduces r_b (Shoemaker *et al.* 1992). By contrast, frogs that reside at ground level experience much lower wind velocities, especially if in a heterogeneous landscape, and will consequently have much higher r_b than their arboreal counterparts. This means that total resistance to water loss may not be very different for frogs at these different locations, despite their large difference in r_c. Given the uncertainty as to whether *Litoria* spp. are monophyletic (Tyler 1979) or polyphyletic (Cogger 2000), it is unclear to what extent phylogeny influences the apparent association between habitat and cutaneous resistance in this group.

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