

WATER LOSS AND
NITROGEN EXCRETION IN SHARP-NOSED REED FROGS
(*HYPEROLIUS NASUTUS*: ANURA, HYPEROLIIDAE)

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SUMMARY

Sharp-nosed African reed frogs, *Hyperolius nasutus* Gunther, are small (0.4 g) hyperoliids which have minimal rates of evaporative water loss ($4.5 \text{ mg g}^{-1} \text{ h}^{-1}$; $0.3 \text{ mg cm}^{-2} \text{ h}^{-1}$) that are only 1/10 to 1/20 that of a typical frog, *Hyla regilla*, of comparable size ($171 \text{ mg g}^{-1} \text{ h}^{-1}$, $4.8 \text{ mg cm}^{-2} \text{ h}^{-1}$). The surface-area-specific resistance to water flux of *H. nasutus* dorsal skin ($96\text{--}257 \text{ sec cm}^{-1}$) is similar to that of other 'waterproof' frogs (300-400), of cocooned frogs (40-500), and of desert reptiles (200-1400). However, *H. nasutus* can greatly increase the rate of evaporative water loss during radiative heat stress by mucous gland discharge, and by exposing the ventral skin.

Urea is the principal nitrogenous waste product of *H. nasutus* and uric acid comprises less than 1% of the total nitrogen excretion for both *H. nasutus* and *H. regilla*. Other 'waterproof' frogs, in contrast, are uricotelic.

Lethal dehydration requires less than two weeks in *H. nasutus*, despite its low surface-area-specific rate of water loss, because of its small size and concomitantly high surface-to-volume ratio. The rate of urea accumulation during dehydration was $23 \text{ mM g}^{-1} \text{ day}^{-1}$, which is sufficiently low that urea accumulation would not be lethal before the frog had succumbed to dehydrational death. Consequently, there appears to be little or 'no selective advantage for uricotelic in small 'waterproof' frogs, such as *H. nasutus*.

INTRODUCTION

Recent reports that two unrelated genera of tree frogs (*Chiromantis* and *Phyllomedusa*) are 'waterproof' and uricotelic (i.e. evaporative water from their skins at much lower rates than other amphibians, and excrete the bulk of their nitrogen loss as uric acid) have opened new horizons for amphibian environmental physiologists (Loveridge, 1970; Shoemaker, Balding & Ruibal, 1972; Shoemaker & McClanahan,

1975; Drewes *et al.* 1977). Loveridge (1976) subsequently reported seasonally reduced rates of evaporative water loss (EWL) in a third unrelated amphibian *Hyperolius nasutus* (Family Hyperoliidae) of about $0.6 \text{ mg g}^{-1} \text{ h}^{-1}$. *H. nasutus* differs from the previously described 'waterproof' frogs in that it is a very small species (about 0.4 g), and occurs in mesic environments throughout South, Central and East Africa (Loveridge, 1957; Broadley, 1971; Loveridge, 1976; Passmore & Carruthers, 1979). We have investigated the water relations of *H. nasutus* to determine its EWL under conditions similar to those used in studies of *Chiromantis* and *Phyllomedusa*, to determine whether its mechanism for 'waterproofing' is similar to that of *Chiromantis* or *Phyllomedusa*, and to document whether *H. nasutus* is uricoletic.

MATERIALS AND METHODS

Hyperolius nasutus were collected at Chemilil, Kenya and shipped to the California Academy of Sciences for study. Body mass of the adult frogs was from 0.4 to 0.5 g . Specimens of the Pacific tree frog (*Hyla regilla*) of similar body mass were collected locally in Oregon, and were studied in the same fashion as *Hyperolius*.

Evaporative water loss (EWL) was measured gravimetrically and hygrometrically. The gravimetric method consisted of placing frogs in cylindrical vials ($2.1 \text{ cm} \times 3.8 \text{ cm}$ high) enclosed at the end with plastic mesh ($1 \times 1 \text{ mm}$) in a desiccator over drierite. Vials and frogs were weighed daily, or more frequently, to $\pm 0.1 \text{ mg}$. Weight loss was assumed to equal water loss. Hygrometric measurement used a flow-through system (flow rate = $350 \text{ cm}^3 \text{ min}^{-1}$; velocity = 3 m min^{-1}) with the frog confined in a section of tygon tubing. Air entering the chamber was dried to a dew point of $-22 \text{ }^\circ\text{C}$ using drierite. Dew point of the excurrent air was continuously monitored with an EG & G (Model 911) dew-point hygrometer and a Honeywell strip-chart recorder. Absolute water content of the incurrent and excurrent air was calculated from the dew point using the equations of Parish & Putnam (1977) and water loss calculated from the difference between excurrent and incurrent absolute humidities.

The surface area of the frogs was calculated from the equation, $SA (\text{cm}^2) = 9.9 \text{ g}^{0.56}$ (McClanahan & Baldwin, 1969). We determined that one-third of the predicted surface area of *H. nasutus* was adpressed to the substratum, hence we assumed that two-thirds of the total surface area was exposed. This same assumption was made in calculating the cutaneous resistance of the skin to water flux for *H. regilla* and other amphibians and reptiles.

H. nasutus were also heat stressed with a 150 W infrared heat lamp during the measurement of EWL in order to determine whether the frogs could increase EWL for cooling.

The concentrations of nitrogenous wastes in the urine were measured for individuals either given access to, or deprived of, water. Urine samples were periodically collected directly from the bladder into $100 \mu\text{l}$ glass capillary tubes. Urea and ammonia concentrations were determined with a modified urease assay (Sigma Chemicals) and uric acid concentration was determined from the absorbance at 290 nm of urine samples with and without uricase digestion (Sigma Chemicals).

Table 1. Rates of total evaporative water loss (EWL) for *Hyperolius nasutus* and *Hyla regilla*, and calculated values for resistance of the skin to water flux, for frogs in still air with EWL measured gravimetrically, and for frogs in flowing air ($\sim 3 \text{ m min}^{-1}$) with EWL measured with a dew-point hygrometer

(Values are $\bar{X} \pm$ standard error, with the number of observations. Values for EWL of a free water surface are included for comparison.)

		<i>H. nasutus</i>	<i>H. regilla</i>	Free water surface
Mass-specific EWL ($\text{mg g}^{-1} \text{ h}^{-1}$)	Still air	1.7 ± 0.5 (7)	28.5 ± 1.8 (6)	—
	Flowing air	4.5 ± 1.5 (6)	171 ± 34 (4)	—
Surface-specific EWL ($\text{mg cm}^{-2} \text{ h}^{-1}$)	Still air	0.28	4.8	4.6
	Flowing air	0.75	28.5	45.7
Resistance (s cm^{-1})	Still air	257	15.2	15.6
	Flowing air	96	2.0	1.6

The integument was studied using frozen-section, paraffin and epon techniques. Frogs were frozen in liquid nitrogen and frozen-sectioned at $12\text{--}24 \mu$ in an AEC cryostat, and the sections were stained with Sudan III or IV (Chiffelle and Putt's technique; Humason, 1972). Paraffin sections were prepared and stained with Hematoxylin and Eosin (Humason, 1972). Epon sections were also prepared and examined with light and transmission electron microscopy. Scanning electron microscopy was also employed.

RESULTS

Evaporative water loss. The rate of EWL was clearly higher for *Hyla regilla* than *Hyperolius nasutus* by a factor of 15–40, regardless of the method of measurement (Table 1; Figure 1), providing the reed frog assumed its typical 'hunched' posture which conceals the ventral surface. With the flow-through hygrometric system, the EWL increased rapidly to over $1000 \text{ mg g}^{-1} \text{ h}^{-1}$ whenever the ventral skin was exposed to the dry airstream (Fig. 1). The relative humidity of the chamber air for *H. nasutus* was normally 0%, but would have been about 20% for *H. regilla* because of their higher EWL.

Hyperolius nasutus were able to increase EWL from the dorsal skin by $300 \times$ (to $1350 \pm 88 \text{ mg g}^{-1} \text{ h}^{-1}$) when thermally-stressed with a radiant heat load. The colour and texture of the dorsal skin changed simultaneously with the increase in EWL due to glandular discharge of fluid as observed under a dissecting microscope.

Mass-specific EWL was converted to surface-area-specific EWL assuming that two-thirds of the predicted surface area was exposed to the airstream. *Hyperolius* had about one-twentieth the surface-area-specific EWL of *Hyla*, and a free water surface (Table 1). The cutaneous resistance to water flux (R ; s cm^{-1}) was calculated after Nobel (1974) as

$$R = \Delta C_{\text{wv}} / \text{EWL},$$

where ΔC_{wv} is the water vapour concentration deficit (mg cm^{-3}) and EWL is surface-area-specific EWL ($\text{mg cm}^{-2} \text{ s}^{-1}$). The cutaneous resistance for *H. nasutus* skin is only $\frac{1}{10} - \frac{1}{20}$ that of *H. regilla* and a free water surface (Table 1).

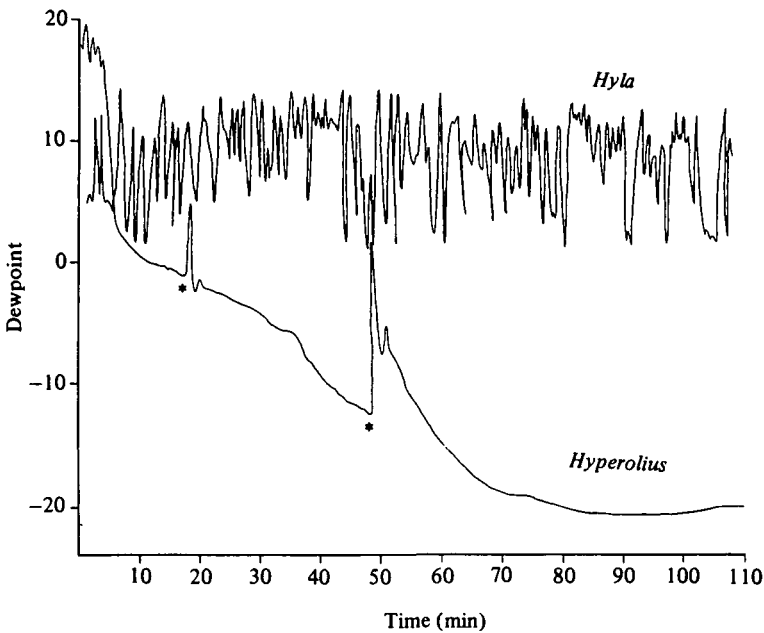


Fig. 1. Dewpoint ($^{\circ}\text{C}$) of excurrent air from animal chamber for a 'waterproof' *Hyperolius nasutus* and a typical anuran amphibian, *Hyla regilla*. * indicates activity by *Hyperolius nasutus* which exposed the ventral skin. Dewpoint of the incurrent air was about -20°C . Excurrent dewpoint of $+20^{\circ}\text{C}$ corresponds to approximately $1000\text{ mg g}^{-1}\text{ h}^{-1}$ rate of evaporative water loss.

Table 2. Millimolar concentrations and % partitioning of nitrogenous wastes in the bladder urine of *Hyperolius nasutus* and *Hyla regilla*

(Values are $\bar{X} \pm$ standard error with the number of observations.)

	<i>H. nasutus</i> (n = 11)	<i>H. regilla</i> (n = 8)
Ammonia (mM)	5.4 ± 1.5	2.3 ± 1.2
Urea (mM)	71.3 ± 16	90.2 ± 12
Uric acid (mM)	0.02 ± 0.01	0.09 ± 0.02
N excreted as ammonia (%)	3.6%	1.1%
N excreted as urea (%)	96.3%	98.6%
N excreted as uric acid (%)	0.1%	0.3%

Skin histology. Frozen sections of *H. nasutus* skin, stained for lipids with Sudan III or IV, revealed neither a lipid layer nor lipid glands, nor was body wiping behaviour observed. Surface lipids were also discounted as a waterproofing mechanism by observing that swabbing the dorsal surface with acetone did not increase the EWL. The skin of *H. nasutus* has few melanophores, apparently no xanthophores although the frogs are bright green in colour, and a tight layer of flat iridophores. It is tempting to speculate that the tight iridophore layer confers the waterproofing properties of *H. nasutus*, but this hypothesis awaits further investigation.

Nitrogenous waste products. Initial observations of captive frogs revealed no obvious signs of urate deposition, in contrast to the conspicuous white or yellow urate past

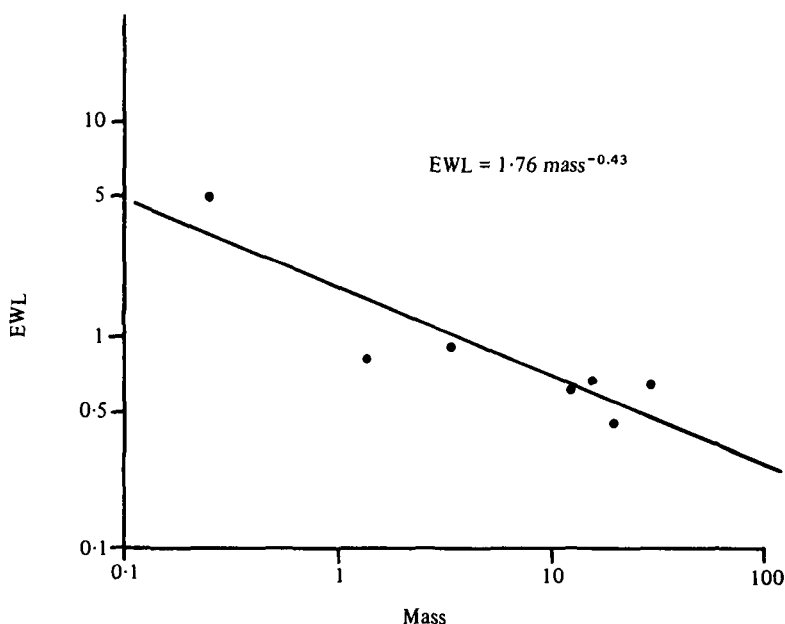


Fig. 2. Relationship between evaporative water loss (EWL; $\text{mg g}^{-1} \text{h}^{-1}$) and body mass (g) for 'waterproof' frogs, *H. nasutus* (~ 0.4 g; present study), *Hyperolius marmoratus* (~ 1.2 g; Withers *et al.* in preparation), *Chiromantis xerampelina* (~ 35 g; Loveridge, 1970) and *Phyllomedusa* spp (Shoemaker & McClanahan, 1975). The regression equation for the \log_{10} -transformed data is $\log EWL = 0.246 - 0.428 \log BM$ ($n = 7$; $r^2 = 0.75$).

excreted by *Chiromantis* and *Phyllomedusa*. We analysed urine (collected directly from the bladders of hydrated and dehydrated *H. nasutus*), faecal pellets, and a discolouring substance which accumulated on their perching sites. Both *H. nasutus* and *Hyla regilla* excreted over 95% of their nitrogenous waste as urea, only 1–4% as ammonia, and even less (< 1%) as uric acid (Table 2). Only trace amounts of uric acid were detected in faecal pellets and faeces accumulated on the perching sites. There is therefore no evidence for substantial uricotelism in this third taxon of 'waterproof' frogs.

DISCUSSION

The minimum EWL of *H. nasutus*, measured with the flow-through method ($4.5 \text{ mg g}^{-1} \text{h}^{-1}$, Table 1) was much greater than that for *Chiromantis* (0.44 ; Loveridge, 1970) and *Phyllomedusa* (0.6 ; Shoemaker *et al.* 1972), but *H. nasutus* is a smaller species with a higher surface-to-volume ratio. The calculated surface-area-specific EWL was more similar, at 0.3 – $0.8 \text{ mg cm}^{-2} \text{h}^{-1}$ for *H. nasutus* and 0.2 and 0.3 for *Chiromantis* and *Phyllomedusa* respectively. Other hyperoliids also have low EWL (Withers *et al.*, in preparation). A small (3.5 g) phyllomedusine, *P. hypochondrialis*, has a higher EWL than larger phyllomedusines ($0.9 \text{ mg g}^{-1} \text{h}^{-1}$) but has the same surface-area-specific rate ($0.2 \text{ mg cm}^{-2} \text{h}^{-1}$, calculated from Shoemaker & McClanahan, 1975). These values are not directly comparable to ours for *H. nasutus* since they were made at a lower flow rate and air velocity. Nevertheless, there is a clear relationship between EWL and body mass for 'waterproof' frogs (Fig. 2). Comparable

Table 3. *Values for resistance of skin (s cm⁻¹) to water flux calculated for waterproof frogs, normal frogs, cocooned frogs and reptiles*

(Calculated after Nobel (1974) assuming that all water loss occurs via the skin for amphibians, but only cutaneous EWL was used in the calculation for reptiles.)

Species	Resistance		Reference
	Still air	Flowing air	
Waterproof frogs			
<i>Hyperolius</i>	257	96	Present study
<i>Chiromantis</i>	990	400	Loveridge, 1970; Drewes <i>et al.</i> 1977
<i>Phyllomedusa</i>	—	300	Shoemaker <i>et al.</i> 1972
Normal frogs			
<i>Hyla</i>	15	2	Present study
<i>Xenopus</i>	7	—	Loveridge, 1970
<i>Rana</i>	12	—	Loveridge, 1970
<i>Bufo</i>	9	—	Loveridge, 1970
<i>Pyxicephalus</i>	7	—	Loveridge, 1970
Cocooned frogs			
<i>Pyxicephalus</i>	190	190	Loveridge & Withers, 1981
<i>Leptopelis</i>	—	40	Loveridge & Craye, 1979
<i>Pternohyla</i>	—	457	Ruibal & Hillman (1981)
<i>Lepidobatrachus</i>	—	163	McClanahan <i>et al.</i> 1976
Reptiles			
<i>Amphibolurus</i>	521	—	Dawson <i>et al.</i> 1966
<i>Gehyra</i>	198	—	Dawson <i>et al.</i> 1966
<i>Sphenomorphus</i>	248	—	Dawson <i>et al.</i> 1966
<i>Sauromalus</i>	1360	—	Bentley & Schmidt-Nielsen, 1966
<i>Iguana</i>	370	—	Bentley & Schmidt-Nielsen, 1966
<i>Anolis</i>	565	—	Claussen, 1967
<i>Uta</i>	1080	—	Claussen, 1967
Water	16	2	Present study

surface-area-specific rates of EWL for *Hyla* and a free water surface are much greater at about 4.7 mg cm⁻² h⁻¹, or 10–20 × that of *H. nasutus* (Table 1).

Surface-area-specific rates of minimum EWL were converted to resistance (calculated after Nobel, 1974) to compare skin permeability in a variety of amphibians and reptiles. Direct comparisons of cutaneous resistance are only approximations because of variations in technique among investigators and because of various assumptions in calculating resistance (e.g. exposed surface area). Nevertheless, the dorsal skins of *Hyperolius*, *Chiromantis* and *Phyllomedusa* clearly have high resistances similar to that of many desert reptiles, and 10–40 × greater than those for the skin of other amphibians and a free water surface (Table 3). Some aestivating amphibians form cocoons and thereby gain high values for cutaneous resistance to water flux (Table 3), but this is not possible during activity.

H. nasutus ventral skin, which is normally kept adpressed to the substratum, has a low resistance to water flux, as demonstrated by the rapid and marked increase in EWL when the ventral skin is exposed (Fig. 1). A similar disparity in EWL for dorsal and ventral skin has been reported for *Chiromantis* (Drewes *et al.* 1977) but not for *Phyllomedusa*, which covers its entire body surface area with lipid secretion, using complex behaviour patterns (Blaylock, Ruibal & Platt-Aloia, 1976).

All *H. nasutus* individuals did not have the same EWL, when it was determined gravimetrically. Some individuals dehydrated at rates comparable to *H. regilla* and succumbed to dehydration after about 40% loss of body mass. Others showed a marked decline in EWL during dehydration. Loveridge (1976) also reported wide variations in EWL between 'summer' and 'winter' *H. nasutus*. However, we cannot exclude alteration in locomotor activity, and exposure of the ventral surface as an explanation for this variable and high EWL, since the ventral skin is not 'waterproof'. With the hygrometric method, a 1–2 h period was required for the minimum EWL to be attained, even if the frog was not active. This may reflect the time required for evaporation of water from the thin hydrated mucous film on the skin, and from whatever high resistance barrier to water loss presumably lies within the skin (see also Zucker, 1980 for comparable reptile data). Consequently, it is not clear from our study whether *H. nasutus* has the capacity to alter the permeability of its dorsal skin to water, as Loveridge's (1970) data indicate on a seasonal basis.

The thermoregulatory response to radiative heat loading of *H. nasutus* is a marked increase in EWL, a similar response to that of other 'waterproof' frogs, *Chiromantis petersi* (Hillman & Putnam, unpublished), *Phyllomedusa* (Shoemaker, 1980) and normal amphibians (Lillywhite, 1971; Lillywhite & Licht, 1975). The mechanism for increased EWL in many of these instances is presumably through mucous gland discharge. *Phyllomedusa savagei* also increases EWL when heat-stressed, but the mechanism involves a temperature-dependent change in the properties of the cutaneous lipids (McClanahan, Stinner & Shoemaker, 1978).

The mechanism for reduction of EWL from *H. nasutus* is unclear. Microscopic examination of the skin revealed no lipid glands or lipid layers, no wiping behaviour was observed, and EWL was not increased if the frogs were washed with acetone. In contrast, *Phyllomedusa* is 'waterproof' by virtue of a waxy secretion which is wiped over the entire body surface, with stereotyped wiping behaviour (Blaylock *et al.* 1976). Washing with acetone does increase the EWL of *Phyllomedusa* (V. H. Shoemaker, personal communication). The skin of *H. nasutus* has a tight layer of stacked iridophores, and it is tempting to speculate that this confers the waterproofing properties. A stacked iridophore layer has been implicated as the waterproofing mechanism for *Chiromantis* (Drewes *et al.* 1977). Nevertheless, the hypothetical role of iridophore layers in reducing EWL is at present circumstantial and remains to be verified.

Xeric *Chiromantis* and *Phyllomedusa* are in many respects ecological equivalents in Africa and South America. Both inhabit dry woodland savannah and are waterproof, uricotelic, and similar in body mass. Low EWL and uricotely are clearly useful adaptations in their habitats. In contrast, *Hyperolius nasutus* and congeners are much smaller frogs, and are found near permanent water, often perching on reeds during the daytime (Passmore & Carruthers, 1979). Such an exposed perching site poses a marked dehydrational threat to small amphibians. Dehydration tolerance of *H. nasutus* is typical of most amphibians at about 40% loss of body mass at a critical activity point. *H. nasutus* have large bladders and can store up to about 26% of their body mass as urine ($\pm 3\%$; $n = 5$). *H. nasutus*, which could perch in still, dry air for about 7 days, or about 3 days in a 3 m min^{-1} breeze with no bladder

reserves, would have corresponding survival times of about 14 and 5 days respectively if the bladders were initially full. If the reed frogs were not 'waterproof', the respective times would be 11 and 2 h with no bladder stores, and 20 and 3 h with full bladders. The low EWL of *H. nasutus* therefore profoundly influences important aspects of its ecology such as potential time spent perching relative to time spent hidden and inactive when they are presumably incapable of feeding.

It is not surprising that *H. nasutus* is not uricoletic, despite its being 'waterproof', in view of the relatively short time it can survive dehydration. The body fluid urea concentration of hydrated *H. nasutus* was about 40 mM, and urea accumulated at a rate of about 23 mM g⁻¹ day⁻¹, which is similar to that in other amphibians (Shoemaker & McClanahan, 1980). Nitrogenous wastes could therefore accumulate in the body fluids of *H. nasutus* for over 14 days without rising to lethal levels, assuming similar urea tolerance to other amphibians of about 400 mM (Cleworth, 1967). Since *H. nasutus* apparently has to rehydrate more frequently than every 14 days, there would be little selective pressure for the evolution of uricotely, particularly as uricotely imposes a greater energetic load than ureotely. Similarly, the small mesic *Phyllomedusa hypochondrialis* has about the same surface-area-specific EWL as its larger congeners, but excretes less of its nitrogen wastes as uric acid (25%) than *P. pailona* and *P. iherengi* (45%) and *P. sauvagei* (80%; Shoemaker & McClanahan, 1975).

Cocoon-forming frogs have a markedly reduced EWL, similar to that of the 'waterproof' frogs, and are not uricotelic during their dormant periods (McClanahan *et al.* 1976; Loveridge & Withers, 1981). The low rates of urea synthesis during dormancy (McClanahan *et al.* 1976; Loveridge & Withers, 1981) also prevents the lethal accumulation of urea. Consequently, there seems to be no selective advantage for uricotely in these cases either.

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