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Water relations of the burrowing sandhill frog, *Arenophryne rotunda* (Myobatrachidae)

Abstract *Arenophryne rotunda* is a small (2–8 g) terrestrial frog that inhabits the coastal sand dunes of central Western Australia. While sand burrowing is a strategy employed by many frog species inhabiting Australia's semi-arid and arid zones, *A. rotunda* is unique among burrowing species because it lives independently of free water and can be found nocturnally active on the dune surface for relatively extended periods. Consequently, we examined the physiological factors that enable this unique frog to maintain water balance. *A. rotunda* was not found to have any special adaptation to reduce EWL (being equivalent to a free water surface) or rehydrate from water (having the lowest rehydration rate measured for 15 Western Australian frog species), but it was able to maintain water balance in sand of very low moisture (1–2%). Frogs excavated in the field were in dune sand of 4.4% moisture content, as a consequence of recent rain, which was more than adequate for these frogs to maintain water balance as reflected by their low plasma and urine osmotic concentrations. We suggest that in dry periods of the year, *A. rotunda* can achieve positive water balance by cutaneous water uptake by burrowing deeper into the substrate to where the percent water content is greater than 1.5%.

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Introduction

Despite the low and irregular rainfall, frogs are found in most Australian desert regions and are often the most abundant vertebrate species in a given area (Main 1968; Read 1999). Most frogs inhabiting Australia's semi-arid and arid regions burrow into the soil to reduce desiccation. Some of these burrowing frogs (*Neobatrachus* and *Cyclorana* spp.) form a cocoon by accumulating layers of shed skin (Lee and Mercer 1967; Withers 1995) to reduce evaporative water loss (Withers 1998). Other species (including *Arenophryne rotunda*, *Myobatrachus gouldii*, *Notaden* spp., *Uperoleia* spp. and *Heleioporus* spp.) do not form a cocoon and are therefore reliant upon the hygric properties of the surrounding soil to maintain water balance while burrowed. Few studies have examined the osmotic relationship of non-cocooning frogs with their surrounding soil or quantified the ability of non-cocooning species to absorb water from soil.

Arenophryne rotunda Tyler 1976 is a small burrowing frog (males 26–30 mm, females 28–33 mm snout-vent length; Tyler et al. 2000) found on coastal sand dunes of the mid-west of Western Australia, from Kalbarri to Dirk Hartog Island (Tyler et al. 1980). These dunes border the arid zone and experience severe summer drought (Burbidge and George 1978; Roberts 1984). *A. rotunda* lives completely independent of free water, with eggs deposited underground in mid-autumn followed by direct development of young froglets, which emerge in time for winter rain (Roberts 1984).

Arenophryne rotunda have been found nocturnally surface active without rain during the months of August–November (Tyler et al. 1980; Roberts 1990). Water lost during nightly activity must be replenished from soil

moisture while burrowed during the day. During the dry summer months, frogs do not emerge but rather burrow deeper to access water as the water table of the dune recedes (Roberts 1990). Burrowed *A. rotunda* have been reported to have a plasma osmolality intermediate between that of fully hydrated frogs (surface active during rainfall) and partially dehydrated frogs (surface active without rainfall) (Roberts 1990), suggesting that burrowing allows partial but not necessarily full rehydration.

The aim of this study was to describe the capacity of *A. rotunda* to maintain water balance. In this study we: (a) measured rates of evaporative water loss (and cutaneous resistance); (b) measured rates of rehydration in water and in soil of varying moisture contents to determine the minimum soil moisture level necessary for osmotic balance; (c) excavated *A. rotunda* in the field and measured soil moisture content and the matric water potential of the surrounding soil; and (d) related the osmotic concentration of the plasma of excavated frogs to the soil moisture content and water potential of the surrounding soil to determine if *A. rotunda* were in osmotically favourable conditions. *A. rotunda* burrow in coarse dune sand, which is likely to have high water potential even at low moisture levels, so we predicted that frogs would be able to maintain hydration at relatively low moisture contents. Even in the absence of free-standing water, *A. rotunda* should be able to rehydrate from soil while burrowed and this should relieve the pressure on the evolution of any special adaptation for the reduction of evaporative water loss or rapid uptake of water from soil.

Materials and methods

Field studies

Arenophryne rotunda were studied in the field at False Entrance (26°24′S, 113°18′E), Western Australia, during 6–8 August 2004. Frogs were observed moving about on steep dune faces during the night, and their tracks were easily followed in the mornings to their burrowing site. Burrows were located both on the top and sides of steep sand dunes; while tracks were also noted in the swale between dunes, no burrows were located there. Frogs were excavated from sand dunes and soil samples collected from the surface (0.5 cm), adjacent to the burrowed frog, and at depths of 5, 10 and 20 cm for analysis of soil moisture content. Temperature (°C) was also measured at each depth. All frog excavations, soil collection and temperature measurements took place between 1030 and 1330 hours. A total of 29 frogs was excavated (soil samples and temperature measurements were taken for a subset of these excavations, see Fig. 1 for sample sizes). Eight excavated frogs were dissected in the field for collection of plasma, while 16 were returned to the laboratory for other water balance experiments. Sex could only be determined for dissected individuals, with seven of eight individuals being females. Mean
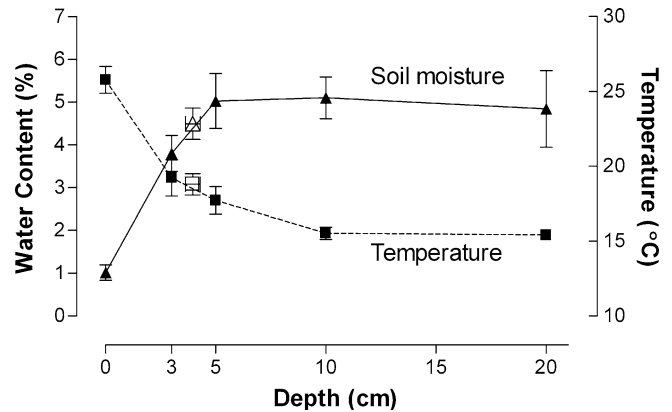
Fig. 1 Gravimetric soil moisture content (%) and temperature (°C) profiles to 20 cm depth for sand dunes from which *Arenophryne rotunda* were excavated. *Open triangle/open square* is mean depth and moisture/temperature at which frogs were excavated. Values are mean ± SE (soil moistures: $n = 6, 5, 5, 6, 5$; temperature: $n = 6, 4, 6, 16, 15, 12$)

urine-free mass of excavated individuals was 5.1 g ($n = 21$, range 2.1–7.8) and the snout-vent length was 32.1 mm ($n = 21$, range 24–36).

Preceding the field study (21–23 July), 50.3 mm of rain was recorded at the Denham weather station 45 km northeast of False Entrance. Average overnight temperature during the field study was 11.1°C and the daily average maximum was 20.2°C.

Plasma and urine osmotic concentrations

Plasma and urine samples were collected from eight frogs 0.5–3 h following excavation in the field; frogs were held until sampling in a sealed plastic zip-lock bag to prevent desiccation. Blood and urine samples were frozen for return to the laboratory for assay. Total osmotic concentration was determined for 15 μl samples of the plasma and urine by freezing point depression (Gonotec Osmomat 030). Concentrations of sodium and potassium ions were measured in 5 μl samples by flame photometry using a Varian model 475 atomic absorption spectrophotometer, and chloride was determined for 5 μl (plasma) or greater (urine) samples with a Buchler-Cotlove 4-2000 automatic titrating chloridometer. Urea was measured for 5 μl samples by the photometric urease method of Fawcett and Scott (1960) using a Varian DMC80 spectrophotometer.

Soil properties

All sand moisture data are expressed as percent gravimetric water content (i.e. the difference between the wet and dry sand masses divided by the dry sand mass, expressed as a percentage). Dry sand masses were obtained by placing weighed soil samples in a 105°C oven overnight and reweighing. A water retention curve (relationship between soil moisture content and soil water

potential) was determined for sand from the dunes where frogs were excavated. Saturated sand samples were placed on a porous ceramic plate and exposed to a given pressure inside a sealed pressure chamber. Pressures of 10, 100 and 1,500 kPa were used, and the moisture content of saturated sand at atmospheric pressure was taken as 0 kPa. Sand samples remained in chambers until no additional water could be forced from them (i.e. an equilibrium between forces retaining water in the soil sample and that applied by pressure was reached). Sand samples were then removed and the gravimetric water content of the soil samples was determined by drying and reweighing.

Frog-soil water balance

We could determine whether excavated frogs were in osmotic balance from their plasma osmotic concentration and the moisture content of the soil where they were burrowed ($n=6$). For each excavated frog to be in osmotic balance (i.e. neither gaining nor losing water) the water potential of the soil should equal that of the plasma. The osmotic concentration [C (mOsm)] of body fluids was converted to an equivalent osmotic pressure as $P = RTC$ (Nobel 1983), where P is the osmotic pressure (MPa), R the gas constant (8.314×10^{-6} m^{-3} MPa mol^{-1} K^{-1}) and T the temperature (298 K). P then represents the hydraulic pulling force of the frog's body fluids on the water in the surrounding soil. This equivalent osmotic pressure (MPa), when substituted into the water retention curve, gives the gravimetric water content of the soil necessary for the frog to be in water balance.

Laboratory experiments

Rehydration in water and soil

Normally hydrated *A. rotunda* were washed free of sand, dried with a paper towel and induced to urinate by inserting a polished glass cannula into the cloaca, then weighed to ± 0.001 g with a Sartorius Handy balance. Frogs were then dehydrated over a period of about 4 h in dry containers by exposure to laboratory conditions with a fan forcing air across the top of the container. Body mass was reduced to approximately 80% of the initial mass by evaporative water loss. Experiments were conducted at approximately 23°C and 52% relative humidity.

For rehydration from water, frogs were placed individually in separate containers (100 mm diameter) with 25 ml of distilled water. They were removed at intervals for a period of 16.5 h, gently blotted dry with tissues and reweighed. Their bladders were not drained at each reweighing so that the water balance response could be suppressed naturally by the accumulation of bladder urine. Most frogs did not urinate during reweighing as they were handled gently. However, data from those that did urinate during the experiment, as indicated by a loss of mass between successive weighings, were ignored except to calculate their rehydration rate.

For rehydration from soil, frogs were randomly assigned to a sand moisture content treatment of 1, 2, 5 or 10% and housed individually in sand in plastic containers (11 cm high×9 cm round base diameter). The sand was collected from dunes where the frogs were found burrowed. All frogs burrowed almost immediately and were left in their soil for 5 h. Frogs were then removed, rinsed of encrusted soil and blotted dry with paper towel before weighing. Rates of water uptake and loss are expressed as a percentage of the fully hydrated, bladder empty, body mass lost or gained per hour (% h^{-1}), and as mg cm^{-2} h^{-1} for interspecies comparison (Fig. 6).

Cutaneous evaporative water loss and resistance

Frogs were washed in tap water, weighed (bladder empty) and placed individually in a small glass cylinder (2.7 cm diameter). The EWL was measured at room temperature (23°C) by flow-through hygrometry. Compressed air, dried using a silica gel column, was passed through the glass cylinder containing the frog (which sat on a plastic-mesh floor to expose the ventral skin to the air stream) at a flow rate of 50 ml min^{-1}, controlled by a Bronkhorst CE Hitec mass-flow controller/meter (calibrated against an Alltech 4700 Digital Flow Checker). The water content and temperature of the excurrent air stream were measured for baseline conditions before and after each frog was placed in the glass cylinder and for steady-state conditions with the frog at rest in the glass cylinder, using a Vaisala HMP358 thin-film capacitance humidity/Pt resistance thermometer probe and HMI36 meter. The RS232 output from the meter was logged by a PC using a custom Visual Basic data acquisition program and data saved to file. Data files were later analysed to determine the EWL using a custom Visual Basic program. Total resistance to water loss (s cm^{-1}) was calculated as $(\chi_{sat} - \chi_{ex})$/SAEWL, where χ_{ex} and χ_{sat} are the excurrent and saturation water vapour densities (mg H$_2$O cm^{-3}) and SAEWL is the surface-area-specific EWL (mg H$_2$O cm^{-2} s^{-1}) with surface area (cm^2) calculated as $9.9\,g^{0.567}$ (McClanahan and Baldwin 1969). Rates of water loss are expressed as a percentage of the fully hydrated, bladder empty, body mass lost per hour (% h^{-1}) and mg g^{-1} h^{-1} for interspecies comparison (Fig. 7).

Statistical analysis

All data are presented as mean $\pm$ SE with the sample size. Sand moisture, temperature, osmotic and rehydration data were analysed by ANOVA with post hoc comparisons between groups made using the Student–Newman–Keuls test. All analyses were conducted using
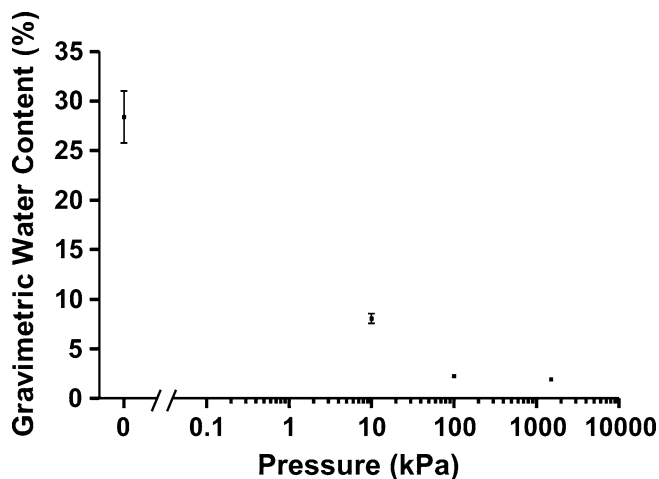
Fig. 2 Water retention curve for sand from *Arenophryne rotunda* burrowing sites. Values are mean ± SE ($n = 4$)

StatistiXL v1.4 (http://www.statistixl.com). An alpha level of 0.05 was taken to indicate significance.

Results

Field studies

Arenophryne rotunda ($n = 12$) were excavated in the field from an average depth of 4.0 ± 0.34 cm and were found in sand of moisture $4.4 \pm 0.37\%$. Soil moisture (Fig. 1) increased significantly with depth ($F_{4,22} = 10.6$, $P < 0.001$). Post hoc tests indicated that the moisture content at 3 cm was significantly greater than at the surface. However, the moisture content at depths of 5, 10 and 20 cm were not significantly different from 3 cm. Therefore, at 4.0 cm frogs had access to the maximum moisture available in the top 20 cm of sand. Sand temperature decreased significantly with depth ($F_{5,63} = 41.5$, $P < 0.001$), with all depths having a temperature lower than surface. The average temperature where frogs were found was $18.8 \pm 0.7°$C, which was significantly lower than the surface temperature of $25.8 \pm 2.9°$C (post hoc $P < 0.001$).

The water retention curve for sand taken from *A. rotunda* burrows (Fig. 2) shows that sand held 28.4% moisture at saturation point (0 kPa) but quickly lost water with pressure to 8.1% at 10 kPa, 2.3% at 100 kPa and 1.9% at 1,500 kPa.

Field plasma and urine osmotic concentrations

Excavated *A. rotunda* ($n = 8$) had a mean plasma concentration of 280 ± 16.0 mOsm and a significantly lower mean urine concentration of 147 ± 21.7 mOsm ($F_{1,14} = 24.3$, $P < 0.001$; Table 1). Concentrations of sodium ($F_{1,14} = 43.6$, $P < 0.010$) and chloride ($F_{1,14} = 18.7$, $P < 0.01$) were significantly higher in plasma than urine, while urea was significantly lower in urine than in plasma ($F_{1,14} = 10.5$, $P < 0.01$). Potassium concentration did not differ significantly between the urine and plasma.

Frog-soil water balance

For all individuals the field soil moisture content was higher than required for osmotic balance (Table 2). An average sand moisture content of 2.01% was required for a frog to be in osmotic balance with the sand; however, the average moisture in which frogs were found in the field was significantly higher at 4.98% (paired *t* test, $t_5 = 5.448$, $P < 0.01$) indicating that the burrowed frogs were not water-limited (Table 2).
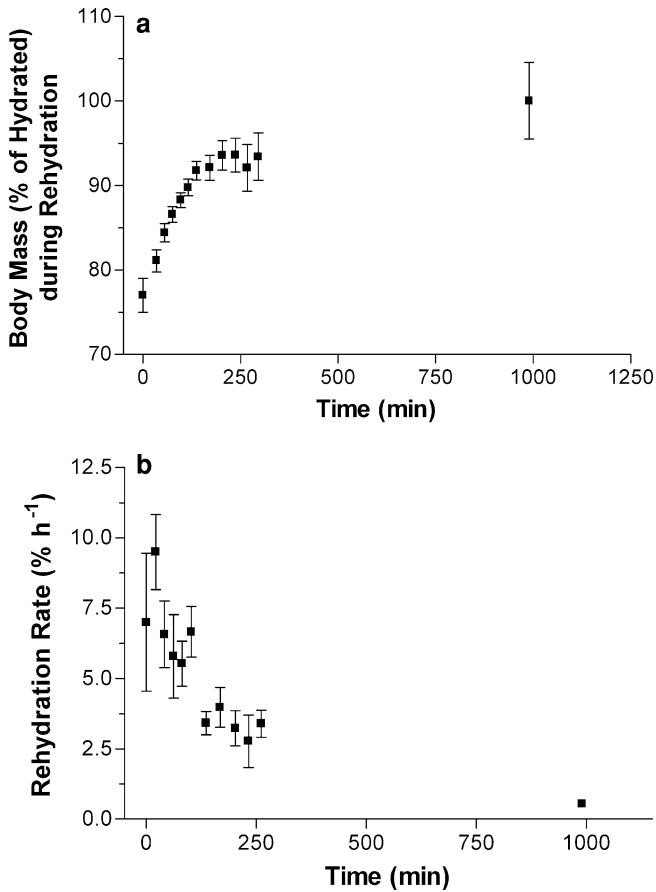
Laboratory experiments

Rehydration in water and soil

Initial hydrated body mass of frogs was 2.2 ± 0.22 g ($n = 8$) and frogs were dehydrated to 1.7 ± 0.20 g or 77% of their fully hydrated mass before rehydrating from water. When placed in distilled water, dehydrated frogs rehydrated to 93.4% of their initial body mass after about 290 min (Fig. 3a). After 16.5 h, their mass was

Table 1 Total osmotic concentration and concentrations of contributing osmolytes in the plasma and urine of excavated *Arenophryne rotunda* ($n = 8$)

	Plasma	Urine
Na$^+$ concentration (mM)	95.9 ± 4.17	24.8 ± 9.92[**]
Cl$^-$ concentration (mM)	86.3 ± 6.35	31.2 ± 11.03[*]
K$^+$ concentration (mM)	5.0 ± 0.21	5.9 ± 1.41[ns]
Urea concentration (mM)	16.8 ± 2.02	40.6 ± 7.07[*]
Other[a]	76	45
Total osmotic concentration (mOsm)	280.4 ± 15.97	147.5 ± 21.74[**]

Values are mean ± SE. Significant differences between plasma and urine are indicated: *ns* not significant; [*]$P < 0.05$; [**]$P < 0.001$ [a]Other includes Ca^{2+}, Mg^+, glucose, HCO_3 and plasma proteins, etc. These osmolytes make up the difference between the sum of Na$^+$, Cl$^-$, K$^+$, urea and the total osmotic concentration
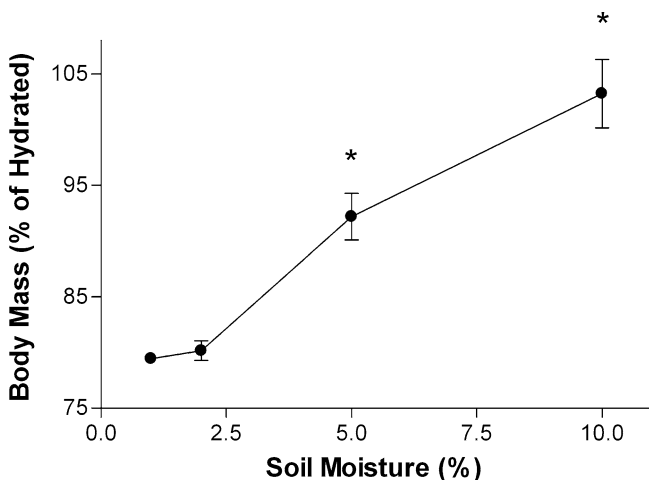
Table 2 Plasma osmolality and calculated equivalent pressure for excavated *Arenophryne rotunda*, soil moisture content corresponding to this pressure (balancing soil moisture) and the actual soil moisture content from the site of excavation (field soil moisture)

	Plasma osmolality (mOsm)	Equivalent pressure (kPa)	Balancing soil moisture (%)	Field soil moisture (%)
1	300	743	2.00	6.04
2	295	731	2.01	4.86
3	316	783	2.00	7.03
4	327	811	1.99	3.66
5	280	693	2.02	3.71
6	242	601	2.04	4.58
Mean	293	727	2.01	4.98

9.49% h^{-1} from 30 to 60 min, then declined to 2.77% h^{-1} by 260 min (Fig. 3b).

Body mass of frogs dehydrated to 80% of their hydrated mass increased significantly after 5 h in sand with moisture contents of 5% ($F_{1,12} = 29.7$, $P < 0.001$) and 10% ($F_{1,12} = 49.5$, $P < 0.001$). However, the body mass of frogs in 1 and 2% treatments did not significantly increase following dehydration (Fig. 4). Frogs only fully rehydrate within the 5 h period in the 10% soil moisture treatment (Fig. 4).
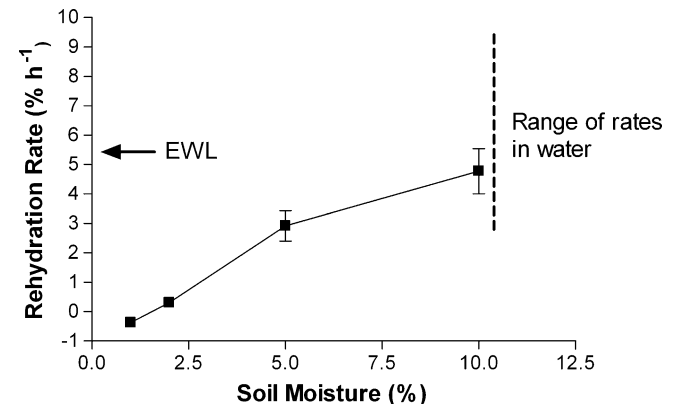
The rate of water uptake for frogs in 5 and 10% soil moisture falls within the range of rates calculated instantaneously for frogs in water (Fig. 5). As rehydration rates in sand are averages calculated over the 5 h period they are likely to be lower than the maximal rates (just as was observed for uptake from water), and this is particularly the case for frogs in the 10% soil treatment since they might have reached 100% of their hydrated body mass prior to the end of the 5 h period.

Cutaneous evaporative water loss and resistance

For EWL experiments, the mean mass of 17 *A. rotunda* was 5.4 ± 1.31 g. EWL was $5.33 \pm 0.997\%$ h^{-1}, corresponding to a cutaneous resistance of 1.5 ± 0.20 s cm^{-1}. However, because of the relatively slow air-flow rate (to enable concomitant measurement of VO_2) the excurrent air was saturated, so these measures of EWL must be considered absolute minimum rates, and for resistance they are absolute maximum values.

Fig. 3 a Increase in percent body mass of *Arenophryne rotunda* during rehydration from water following dehydration. Values are mean $\pm$ SE ($n = 8$). **b** Rehydration rate for *Arenophryne rotunda* in water. Values are mean $\pm$ SE ($n = 5$–8)

$101 \pm 4.5\%$ of their initial mass. The instantaneous rate of rehydration, determined from the weight gain between consecutive measurements, was highest at

Discussion

Studies determining rates of rehydration by arid-adapted frog species have generally measured the rate of rehydration from free water (e.g. Bentley et al. 1958; Main and Bentley 1964), as fast rehydration following emergence may be considered an important adaptive

Fig. 4 Body mass (percentage of hydrated mass) of *Arenophryne rotunda* rehydrating in soil of differing moisture content. Frogs were dehydrated to 80% of hydrated mass before being placed in soil for a period of 5 h. Values are mean $\pm$ S.E ($n = 8$). *Indicates significant difference from dehydrated mass ($P < 0.05$)


Fig. 5 Rate of water uptake for *Arenophyrne rotunda* in soil of varying moisture contents for a period of 5 h. The range of rehydration rates calculated during the first 5 h of rehydration in water (Fig. 3b) and minimum EWL are also presented. Values are mean $\pm$ SE ($n = 8$)
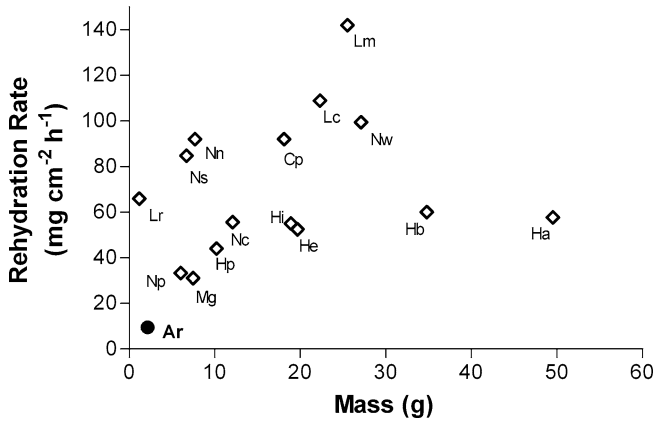
Fig. 6 Comparison of rehydration rates (initial 60 min) from water for *Arenophryne rotunda* (*circle* this study), *Myobatrachus gouldii* (Mg) and other frogs *Neobatrachus pelobatoides* (Np), *N. centralis* (Nc), *N. wilsmorei* (Nw), *N. sutor* (Ns), *Heleioporus psammophilus* (Hp), *H. inornatus* (Hi), *H. eyrei* (He), *H. barycragus* (Hb), *H. albopunctatus* (Ha), *Notaden nichollsi* (Nn), *Cyclorana platycephala* (Cp), *Litoria rubella* (Lr), *L. caerulea* (Lc) and *L. moorei* (Lm) (from Bentley et al. 1958; Main and Bentley 1964; Withers and Roberts 1993)

feature of desert species. However, many frog species inhabiting arid or semi-arid areas utilise soil moisture while burrowed, which may reduce the need to rehydrate quickly from temporary water bodies. *A. rotunda* had the lowest rehydration rate in water (7.20% h^{-1} over the first 60 min, from 80% hydrated mass; Fig. 6) of 15 Western Australian frog species spanning a variety of habitat types from mesic to arid (*Litoria, Cyclorana, Notaden, Neobatrachus, Heleioporus, M. gouldii*; Bentley et al. 1958; Main and Bentley 1964; Withers and Roberts 1993). The turtle frog, *M. gouldii*, is similar to *A. rotunda* in that it lives independently of free water (Roberts 1981; How and Dell 1989; Withers and Roberts 1993) and is the closest relative of *A. rotunda* within the Myobatrachidae (Read et al. 2001). It also has a much lower rehydration rate than the majority of other Western Australian species. Withers and Roberts (1993) suggest
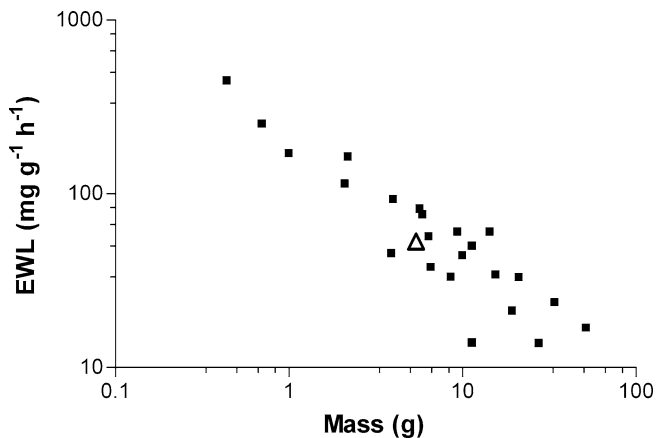
Fig. 7 Comparison of evaporative water loss of *Arenophryne rotunda* (*open triangle* this study) and 23 other Australian frog species (*squares* Withers 1998)

that the low rehydration rate of the turtle frog reflects the absence or rarity of free water in its natural habitat, resulting in a lack of selective pressure for a quick water balance response from free water. This hypothesis may be equally applicable to explain the slow rate of rehydration from water found for *A. rotunda* in the current study.

Few studies have compared the rates of rehydration in water to that in soil for burrowing species but there is some evidence that a reliance on soil moisture by burrowing species has resulted in increased capacity for water uptake from soil. For example, *Heleioporus eyrei* placed in wet soil (13.4%) rehydrates at a rate not significantly different from that in water (Packer 1963). Our current study found that *A. rotunda* in 10% soil moisture had a rate of water uptake not significantly different from that in water when averaged over the 5 h period (water: 0.047% min^{-1} vs. 10% soil moisture: 0.081% min^{-1}, $F_{1,14} = 4.19$, $P = 0.06$). The mechanism of increased water uptake from soil in burrowing species is unknown and not congruent with data comparing uptake rates from water. For example, *Scaphiopus hammondii* rehydrate more slowly in water than both the aquatic *Rana clamitans* and semi-terrestrial *R. pipiens* (Thorson 1955). Similarly, for *A. rotunda* the rate of rehydration in water was slow when compared to other Western Australian species (Fig. 7), being most similar to *M. gouldii*, the only other myobatrachid species living independently of free water. The reduced rate of water uptake in sand burrowing species may reflect a reduced vascularisation in the cutaneous pelvic patch region. When describing the morphology of the vasculature of the pelvic region of aquatic and terrestrial species, Roth (1973) noted that although most terrestrial species had increased pelvic circulation, *S. couchi* was an exception and has poor circulation perhaps reflecting the more mesic microhabitat of burrowed *Scaphiopus*. A similar lack of pelvic vascularisation may explain the slow water uptake rate of *A. rotunda* as only the ventral surface of the frog was submerged in water during the rehydration from water experiment of this study. For burrowing species, increased dorsal cutaneous vascularisation would be of benefit as it is in contact with the soil while burrowed. This and the reduced need for rapid water uptake could explain the reduced vascularisation of the pelvic patch and the low water uptake rates when only the pelvic patch region is submerged in water. Whatever the mechanism, effective soil water uptake by burrowing species is likely to contribute to their successful exploitation of the burrowing niche.

Our soil rehydration experiment indicated that *A. rotunda* could rehydrate in sand moisture contents as low as 1–2%. This threshold is in agreement with the theoretical calculation of the moisture content necessary to be in osmotic balance (2.01%, Table 2). In our field study, tracks visible on the dunes in the mornings indicated that *A. rotunda* were active during the nights and it is likely that they would become dehydrated by evaporation during this exposed activity

period. Frogs generally lose water at the same rate as a free surface of water (Shoemaker et al. 1992) and there is little evidence that terrestrial species have higher resistance EWL than aquatic species (Thorson 1955; Heatwole 1984) with the notable exception of species of the arboreal genera *Phyllomedusa*, *Chiromantis*, *Hyperolius*, *Hyla* and *Litoria* which have much reduced rates of EWL (Christian and Parry 1997; Loveridge 1970; Shoemaker et al. 1972; Shoemaker and McClanahan 1975; Withers et al. 1982, 1984; Wygoda 1984). For 23 Australian species, variation in EWL was mostly explained by size (larger species have a smaller size-specific surface area; Withers 1998). The EWL of *A. rotunda* falls well within the range of these 23 species (Fig. 7) and the resistance of 1.5 s cm^{-1} is indicative of a free water surface.

The high level of moisture in the dune sand at the time of our field study (4.4% at a burrow depth of 4 cm) and the rate of rehydration from similar moisture in the laboratory (approximately 3% h^{-1}) indicated that *A. rotunda* would have been able to fully rehydrate while burrowed for 12 h during the day, from even 40% mass lost during nightly activity. The level of moisture in the dune sand at the time of year of this study (August 2004) is likely to be much higher than at most other times of year, as significant rain had fallen only a few days earlier. Rainfall at Shark Bay is almost entirely restricted to the period May–August (Roberts 1984, 1990). There are only two other reports of the moisture levels where *A. rotunda* have been found burrowed. Tyler et al. (1980) excavated *A. rotunda* from 1.5% moisture content sand at a depth of 10 cm in August, and Roberts (1984) found two males sitting on eggs in soil of 1.44% moisture content at a depth of 80 cm in August. The similar moisture found in both these studies may indicate that about 1.5% is the minimum moisture at which *A. rotunda* can maintain water balance. From the moisture retention curve of the sand (Fig. 2), it is clear that little additional moisture can be forced from the soil between 2.3% moisture (100 kPa) and 1.9% moisture (1,500 kPa), indicating that the curve was approaching the asymptote of the minimum matric moisture at around 1.5% moisture content. That the moisture content found by both Tyler et al. (1980) and Roberts (1984) is similar to the minimum matric potential suggests that frogs are burrowing to the point at which the sand first becomes moist, at around 1.5% moisture content, and this point deepens during the dry summer months. From the laboratory rehydration experiment, the rehydration rate at 1.5% moisture by interpolation (Fig. 4) is approximately 0.18% h^{-1}, which would maintain water balance. However, the rate would probably not be sufficient to maintain full hydration if the frogs were nocturnally active every night and experiencing EWL at the rate calculated in this study. The surface moisture recorded in this study (1.0%) is likely to be an overestimate of the moisture experienced by the frogs on the surface as it was difficult to obtain a surface soil sample without collecting moister sand from immediately beneath the surface. However, even if frogs were to shuffle into the moister subsurface soil for periods while nocturnally active, this would not counter the rate of EWL from the dorsal surface, which was shown in this study to be conservatively 5.33% h^{-1}. It is therefore not surprising that *A. rotunda* do not undergo nocturnal activity during the summer months as the considerable depth to which they would need to burrow during the day (e.g. 80 cm to reach 1.5% moisture content) would incur substantial energetic cost and the moisture available in the dune at that depth would not allow them to rehydrate sufficiently rapidly.

The plasma osmolality values of excavated *A. rotunda* confirm that they were able to fully rehydrate while burrowed. The lowest osmolality reported for *A. rotunda* is 257 mOsm for individuals active on the surface during heavy rain (Roberts 1990). Higher osmolalities were reported for frogs excavated during early summer (307 mOsm in November) and late summer (291 mOsm in February); the highest osmolality is for individuals found active on the surface in the absence of rainfall (326 mOsm in August; Roberts 1990). *A. rotunda* excavated during the current study had an average plasma osmolality of 280 mOsm which is closer to the values obtained for frogs during rain providing further evidence that *A. rotunda* were able to rehydrate substantially while burrowed at the relatively high sand water content of 4.4% at 4 cm burrow depth.

The plasma and urine solute composition of excavated *A. rotunda* (Table 1) is typical of hydrated frogs (e.g. McClanahan 1972; Withers and Roberts 1993; Withers and Guppy 1996). The higher urine urea concentration (40 mM) compared to plasma (20 mM) of excavated *A. rotunda* is unusual but might reflect the day–night hydration–rehydration cycle of the frogs; loss of water by evaporation at night could promote water but not urea reabsorption from the urinary bladder, resulting in the slightly elevated urine urea levels. Higher urine urea levels than plasma have also been reported for other frogs (e.g. McClanahan 1972; Withers and Roberts 1993).

Arenophryne rotunda is one of only a very small number of frog species that survives independently of standing (free) water in an arid region. We have shown that the dunes they burrow in provide water in excess of the requirements necessary to maintain osmotic balance. *A. rotunda* have a slow rate of rehydration from water, much lower than has been demonstrated for other sand burrowing species such as *Heleioporus* (Bentley et al. 1958) and *Notaden nichollsi* (Main and Bentley 1964), but unlike these species they have access to soil moisture and rarely (if ever) have access to free water and therefore there has been no selection pressure to absorb free water quickly. In the field, *Arenophryne* seem to be able to reabsorb water from as little as 1.5% sand moisture content, which is inadequate for rehydration after nocturnal activity but is adequate during long-term burrowing.

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