RESEARCH ARTICLE

Cutaneous water collection by a moisture-harvesting lizard, the thorny devil (*Moloch horridus*)

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ABSTRACT

Moisture-harvesting lizards, such as the Australian thorny devil, *Moloch horridus*, have the remarkable ability to inhabit arid regions. Special skin structures, comprising a micro-structured surface with capillary channels in between imbricate overlapping scales, enable the lizard to collect water by capillarity and transport it to the mouth for ingestion. The ecological role of this mechanism is the acquisition of water from various possible sources such as rainfall, puddles, dew, condensation on the skin, or absorption from moist sand, and we evaluate here the potential of these various sources for water uptake by *M. horridus*. The water volume required to fill the skin capillary system is 3.19% of body mass. Thorny devils standing in water can fill their capillary system and then drink from this water, at approximately 0.7 µl per jaw movement. Thorny devils standing on nearly saturated moist sand could only fill the capillary channels to 59% of their capacity, and did not drink. However, placing moist sand on skin replicas showed that the capillary channels could be filled from moist sand when assisted by gravity, suggesting that their field behaviour of shovelling moist sand onto the dorsal skin might fill the capillary channels and enable drinking. Condensation facilitated by thermal disequilibrium between a cool thorny devil and warm moist air provided skin capillary filling to approximately 0.22% of body weight, which was insufficient for drinking. Our results suggest that rain and moist sand seem to be ecologically likely water sources for *M. horridus* on a regular basis.

KEY WORDS: Lizard, Water uptake, Skin, Moist sand, Capillary, Micro-ornamentation

INTRODUCTION

The availability of water is typically a limiting factor for species living in arid regions, and special abilities to cope with such conditions are often required. Desert lizards, such as the Australian thorny devil (Agamidae: *Moloch horridus* Gray 1841) and the Texas horned lizard (Iguanidae: *Phrynosoma cornutum*), are spectacularly adapted to harvest environmental moisture using their body surface as a water collector (Bentley and Blumer, 1962; Pianka and Pianka, 1970; Pianka and Parker, 1975; Sherbrooke, 1990; Pianka et al., 1998). Special surface micro-structures of the Oberhäutchen (i.e. outer layer of epidermis) and small capillary channels in between the scales allow these so-called 'moisture-harvesting' lizards to collect water into their skin capillaries and transport it to their mouth for drinking (Gans et al., 1982; Withers, 1993; Sherbrooke, 2004; Sherbrooke et al., 2007; Comanns et al., 2011).

The physical ability to harvest moisture is associated with a network of capillary channels, in between the overlapping scales, that enables passive and sometimes directional transport of the collected water to the mouth for drinking (Withers, 1993; Sherbrooke et al., 2007; Comanns et al., 2011, 2015). Transportation to the mouth for ingestion is necessary, because the integument is substantially waterproof to minimize evaporative water loss (Bentley and Blumer, 1962; Withers, 1993) and this precludes water absorption across the skin. Fundamental for capillary water transportation is a water source that provides a sufficient volume of water for uptake by the skin to fill the capillary channels to a sufficient extent that capillary water finally reaches the mouth for ingestion.

So far, five possible sources for water uptake have been considered for moisture-harvesting lizards, namely falling rain, puddles, water droplets from dew or fog condensation, moist soil and thermally facilitated condensation (Withers, 1993). To encompass these various potential water sources, the term moisture-harvesting has recently been introduced (Comanns et al., 2011). Some of the potential water sources are probably very effective for water-harvesting lizards, e.g. falling rain and surface water puddles, but likely occur infrequently and irregularly. Based on their stereotypic skin drinking posture, *Phrynosoma*, *Trapelus* and *Phrynocephalus* likely specialize on drinking rain water (Schwenk and Greene, 1987; Sherbrooke, 1993, 2004; Veselý and Modrý, 2002). Water puddles are probably an effective water source for *M. horridus*, although they are not commonly found in their sandy habitat (Pianka and Pianka, 1970; Gans et al., 1982; Withers, 1993). Thorny devils and horned lizards placed in a water puddle readily uptake water via capillary channels, which they subsequently drink (Bentley and Blumer, 1962; Withers, 1993; Sherbrooke, 2004). These strategies for water uptake require that the lizard remains relatively immobile for an extended period of time, during which they drink using periodic mouth movements. Whether environmental water droplets, moist soil or facilitated condensation are likely water sources is less clear.

Water droplets on vegetation or rocks, e.g. from fog condensation or dew (Beysens, 1995), were suggested to possibly be licked up by moisture-harvesting lizards (Withers, 1993), but their specialised jaws and tongues for myrmecophagy might make it difficult or impossible to lick water droplets. In contrast, water droplets from advective fog condensation, rain, sleet or snow are licked up routinely by various other lizards and snakes (Louw, 1972; Repp and Schuett, 2008). The skin itself has also been considered to facilitate condensation. Especially in the early morning, when the skin surface temperature is below air temperature, water could condense on the skin, facilitated by thermal imbalance between the

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cold skin and warmer, humid air (Lasiewski and Bartholomew, 1969; Beysens, 1995; Lee et al., 2012). The specialized epidermal structure of moisture-harvesting lizards might facilitate such condensation, e.g. the spiny scales of *M. horridus* have been suggested to act as condensation foci (Gans et al., 1982). Condensation of humid air has also been suggested to occur for the less spiny lizard *Phrynocephalus helioscopus* (Schwenk and Greene, 1987), and a tropical tree frog (Tracy et al., 2011). However, Comanns et al. (2011) have found little confirmation for a facilitated condensation hypothesis for water-harvesting lizards. Nevertheless, we also investigate further whether facilitated cutaneous condensation might provide a water source for thorny devils on a regular basis.

Moist sand has also been considered to be a possible water source for drinking. Water is absorbed via physical contact of the feet or ventral body surface with moist sand, but less readily than from a water puddle (Withers, 1993). Similar passive water collection and skin capillary transport had been found for some toads (Lillywhite and Licht, 1974). However, in its natural habitat, *M. horridus* was observed to use an unusual behaviour, different from the moisture-harvesting stance, wherein the lizard rubbed its ventral body surface on the moist substrate and shovelled moist sand onto its back, presumably to enhance capillary water uptake and hence drinking (Sherbrooke, 1993). Consequently, we measured the water-harvesting capability of thorny devils from moist sand of varying water content, and used skin replicas to mimic sand-shovelling and water uptake from dorsal skin surfaces.

So far, only overall weight gain during water uptake has been used to demonstrate capillary filling and water ingestion by thorny devils (Bentley and Blumer, 1962; Sherbrooke, 1993, 2004; Withers, 1993).

The overall questions of this study are related to how effective different water sources are in filling the capillary channels to provide water for drinking. In particular, we investigate: (1) the skin capillary volume for thorny devils; (2) how much water is ingested in total, and per jaw movement; (3) how much water can be absorbed from moist sand, and is it adequate for drinking to occur; (4) the role of the Oberhäutchen and pre-wetting of the skin; and (5) how significant facilitated condensation is for filling the skin capillaries and drinking.

MATERIALS AND METHODS

Moloch horridus (snout–vent length: 94.5±4.7 mm, body mass: 39.86±6.39 g; means±s.e.m., *n*=6) were collected at Mt Gibson (Western Australia) and transported to the laboratory at the University of Western Australia. They were maintained in terraria with sand and vegetation for environmental enrichment, and fed locally collected ants every 1–2 days; they were returned to their capture site after 18 days. Each lizard was involved in experiments less often than every second day, and only one experiment was conducted per lizard per day to minimize any handling stress. Thorny devils were weighed to the nearest ±0.0001 g (A&D Electronic Balance ER-182A).

Water uptake from puddles

Thorny devils were dehydrated by approximately 0.3% of initial mass prior to measurement of water uptake from puddles, by exposing them to a dry air stream (using laboratory compressed air) at an ambient temperature of 30°C. They were then placed in a terrarium with 3 mm of water for 60 min, at laboratory temperature (∼22°C), and observed constantly for jaw movements, indicating drinking. The lizards were then placed in a plastic crate in the

laboratory for 60 min to evaporate any surface water, and were then re-weighed. The water content of the skin capillary channels was calculated as the difference between the 60-min wet weight and 60-min air-dried weight, and ingested water as the difference between air-dried and initial weights. For comparison of individuals, the percent body weight changed is used. All six lizards were examined twice, within 7 days, to count jaw movements and measure drinking uptake during the 60 min of observations.

Sand water uptake

Red sand from thorny devil habitat was wet-sieved to 250–500 µm grain size and oven-dried before use in experiments. Thorny devils were placed on sand with differing water contents. A near-saturated water content of 22% (saturation=23.5% water content) was chosen as the experimental maximum for moist sand, with 0% water content as a dry reference; intermediate water contents of sand were 3, 5, 10, 16, 18 and 20%. The lizards were placed on the sand substrate and observed constantly during experiments to watch for jaw movements, indicating drinking. After 60 min on sand, the lizard was removed and any remaining sand was gently dislodged with a brush before the lizard was weighed. The lizards were then placed in a plastic crate in the laboratory for 60 min to air-dry, and were then re-weighed. As for thorny devils in a water puddle, mass differences were used to determine the filling volume of the skin capillary channels and amount of water ingested.

Pre-wetting by moist sand

Skin casts were made for various body locations of two preserved *M. horridus* specimens (58063, 51985; Zoological Research Museum Koenig, Bonn, Germany) using polyvinylsiloxane (President light body dental casting material, Coltene, Switzerland). A standardized mould diameter of 1.5 cm was achieved using small polypropylene tubes. We then made skin replicas from these casts using epoxy resin (10:4 mix, resin to hardener; Toolcraft, Conrad Electronic, Hirschau, Germany) filled into the casts, vacuum-degassed in a desiccator, and hardened at room temperature. After 48 h, skin replicas were removed from casts. To adjust the wetting properties of epoxy resin closer to that of skin, replicas were coated with one drop of 3% poly(2-vinylpyridine) (1820 g M^{-1}, Polymer Standards Service GmbH, Mainz, Germany) in ethanol, then dried at room temperature and hardened under UV light for 20 min.

The skin replicas were used to measure wettability after contact with moist quartz sand. The replicas were first cooled with a Peltier element (1.5×1.5 cm) to 15 or 20°C, as measured using an infrared thermometer (Voltcraft IR 800-20D, Conrad Electronic). Moist quartz sand was then placed on top of the skin replica with a funnel, and then gently removed with a fine metal probe after 5 min. Wettability was then tested for these pre-wetted replicas by measuring the spreading behaviour of 5 µl droplets of distilled water applied on replicas after contact with sand of varying water content; the spreading response is correlated with wettability (Butt et al., 2003; Bonn et al., 2009). The observed spreading behaviour was categorized for further analyses: 0=no wettability (no spreading), 1=medium wettability (half spreading) and 2=good wettability (full spreading).

Quartz sand (grain size: 1–2 mm, Quarzsand GmbH, Nudersdorf, Germany) was oven-dried, and then water was added to obtain the required 2, 4, 6, 8 or 10% water content before experiments. The quartz sand was saturated at a water content of approximately 11.0%.

Water condensation

Thorny devils were maintained in the laboratory at approximately 22°C for more than 90 min, and then weighed. They were then placed in a chamber at 30°C and 95% relative humidity to facilitate condensation, and weighed after 30 and 60 min. They were then returned to the laboratory, kept in room-temperature air for drying, and then re-weighed after 60 min. Condensation was calculated as the highest weight gain in warm humid air (generally after 30 min).

Statistical analyses

All data are presented as means±standard error unless indicated otherwise. Data were analyzed by a Student's *t*-test and ANOVA followed by a least significant difference (LSD) test for multiple comparisons between pairs of means, or a Kendall rank-order correlation for water spreading on replicas. All tests were carried out using SPSS 21.0. A *P*-value of <0.05 was regarded as significant.

Ethics and licensing approvals

All experiments on thorny devils were approved by the Animal Ethics Committee of the University of Western Australia (RA/3/100/1260). Thorny devils were collected under license from the Western Australian Department of Parks and Wildlife (SF009451).

RESULTS
Water uptake from puddles

Live thorny devils placed in a 3 mm water puddle, and observed continuously for 60 min, were classified into two groups: drinking (five cases) and non-drinking (seven cases; Fig. 1). Lizards that were not observed to have any jaw movements, indicating absence of drinking, had a total body weight gain of 3.66±0.29%. Body weight gain after drying in air was reduced to 0.47±0.08%; this was significantly different from zero (one-sample *t*-test: *P*=0.001), i.e. these lizards had a small but significant weight gain despite not apparently drinking. Accordingly, we calculated the water content of the skin capillary channels as 3.19±0.25% body weight for non-drinking lizards (i.e. 3.66–0.47). This is equivalent to 9.19 mg cm^{-2} body surface area (surface area calculated as cm^2=12.6 g$^{0.642}$; after Mautz, 1982).
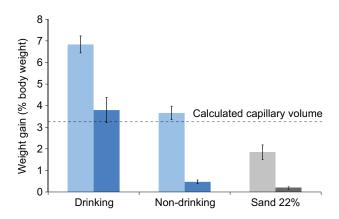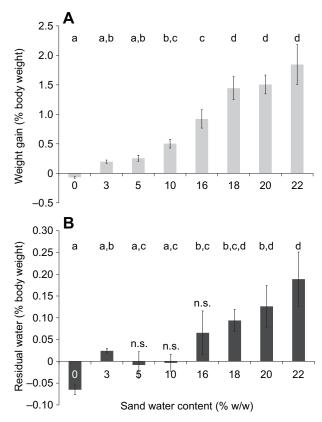

Fig. 1. Water uptake by *Moloch horridus* standing in a 3 mm water puddle. Weight gain after 60 min (light bars) and remaining water uptake (dark bars) measured after an additional 60 min air-drying. 'Drinking' indicates five cases in which the lizards were observed drinking (jaw movements); 'non-drinking' indicates seven cases in which the lizards were not observed to drink. Values for lizards on 22% moist sand are shown for comparison. The horizontal dashed line indicates the calculated capillary volume (see Results, 'Water uptake from puddles'). Error bars are ±s.e.m. All values are significantly different from zero (one-sample *t*-tests, *P*<0.05).

Lizards that had rhythmic jaw movements (up to 2411 h^{-1}, at a frequency of ~1 s^{-1} while drinking) had a total body weight gain of 6.84±0.35%, which is significantly more than the 3.66% for non-drinking lizards (*t*-test: *P*<0.001; Fig. 1). For these drinking lizards, we calculated their drinking water gain as 3.32±0.52% body mass, assuming the dry weight gain (3.79±0.58%) is the sum of ingested water and residual water (0.47% of body weight for non-drinking lizards). Jaw movements were counted during the second trial; for three lizards that did drink, the average volume per jaw movement was 0.7±0.1 µl, which is 17.9±1.0 nl g^{-1} body mass.

Water collection from moist sand

On dry sand, thorny devils actually lost weight (−0.065±0.011%), presumably reflecting evaporation, but with increasing sand water content there was a fairly linear increase in water uptake (Fig. 2A). Even on sand with only 3% water content, there was an initial weight gain of 0.20±0.03% body weight, and water collection increased to 1.84±0.34% body weight for sand of 22% water content. This weight gain on 22% moisture sand is only a little more than half of (and significantly different from; *P*=0.005) the capillary volume determined for non-drinking lizards in a water puddle (3.19% body weight; Fig. 1). Their weight gain after drying in room air was reduced to 0.19±0.06% body weight, which is significantly different from zero (one sample *t*-test: *P*=0.021), i.e. these lizards had a small but significant weight gain. Water collection from sand

Fig. 2. Water collection and uptake of *M. horridus* on moist sand. (A) Maximum weight gain after 60 min on moist sand. Water uptake was significantly different from zero for all sand water contents. Significant differences between sand water contents are indicated by different letters (ANOVA: $F_{2.32}$=16.85, *P*<0.001; LSD test). (B) Residual water uptake from moist sand after 60 min drying in air. Significance indicated as in A (ANOVA: $F_{2.32}$=4.63, *P*=0.001; LSD test). n.s., not significant. *N*=6; error bars are ±s.e.m.

with ≥10% water content differed significantly from dry sand, and water collection from sand with ≥18% water content was significantly different from that of sand with lower water contents (ANOVA: $F_{2,32}$=16.85, P<0.001; LSD test; see Fig. 2A).

The water uptake remaining after air-drying, measured by the difference between final and initial weights, indicates a maximum gain of 0.19±0.06% body weight on sand with 22% water content, which is less than for non-drinking lizards in a water puddle (0.47±0.08% body weight; t-test: P=0.017). Further, the lizards were observed continually during experiments and no swallowing movements were seen. So, presumably this water gain does not reflect any ingestion, but is residual water in the skin capillaries or absorbed into the stratum corneum. In contrast, the weight change was negative, and significantly different from zero (P=0.02), for lizards on dry sand, presumably reflecting a small water loss by evaporation or metabolism.

Wettability of skin

The micro-ornamentation on the Oberhäutchen of the skin can hold a thin water film and thus enhance the wettability of the skin by pre-wetting (Comanns et al., 2011, 2014). We therefore tested moist sand as a possible source for such pre-wetting of the skin using skin replicas. The spreading behaviour of water droplets applied to replicas that had been in contact with moist sand differed considerably between sand of different water contents (Fig. 3A). For 2% sand water content there was almost no spreading, whereas there was full spreading regardless of temperature for 10% water content. Wettability increased significantly with increasing sand water content (Kendall rank-order correlation: 15°C P=0.052; 20°C P=0.023); there was a positive effect of pre-wetting for sand water contents ≤6% (Fig. 3B).

A

No wettability (0)   Medium wettability (1)   Good wettability (2)
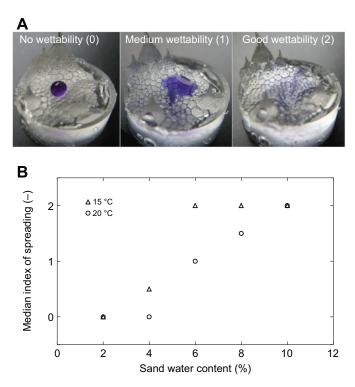
B

Fig. 3. Wetting behaviour of water droplets on skin replicas from _M. horridus._ (A) Spreading of water droplet as an indication of wettability, and assigned values for categorization: 0=no wettability, 1=medium wettability and 2=good wettability. In experiments, droplets were not coloured as for this figure. (B) Quantification of spreading behaviour on replicas cooled to 15°C (triangles) and 20°C (circles), as described in A. N=6.

Water collection by facilitated condensation

Water condensation was highest for _M. horridus_ that were moved from a 20°C environment to a chamber at 30°C and 95% relative humidity after 30 min for all except one lizard. Overall, condensation was 82.7±12.7 mg or 0.215±0.008% body weight (n=6). No mouth movements were observed and their residual weight change after drying for 60 min in room-temperature air of −0.061±0.012% body weight was significantly less than zero (P=0.002), suggesting that they lost mass by evaporation (mass loss by respiratory exchange of CO_2 for O_2 was estimated to be much lower, <0.000024% h^{-1}, based on a resting metabolic rate of 0.061 ml O_2 g^{-1} h^{-1}; Withers and Bradshaw, 1995).

DISCUSSION

We have found that the micro-structured skin capillary channels of thorny devils results in significant absorption of water onto the skin from liquid water, less so from moist sand, and even less so by facilitated condensation. When conditions were optimal (water puddle), drinking was 3.32±0.52% of body mass, at approximately 0.7 µl of water per jaw movement (or 17.9 nl g^{-1} jaw movement^{-1}). In comparison, the Texas horned lizard (_P. cornutum_) has a smaller skin capillary capacity of 2.04% (♀) to 2.47% (♂) but a higher ingestion per jaw movement (1.84 µl, or 65.3 nl g^{-1} jaw movement^{-1}; Sherbrooke, 2004) than thorny devils. However, water was applied in large amounts to the head of _P. cornutum_, probably resulting in a washing of water into the mouth that does not reflect capillary action. Water uptake, based on likely ingestion of water, was much less effective for thorny devils absorbing water from moist sand and particularly from facilitated condensation, reflecting the prerequisite necessity to fill the capillary channels with water before drinking occurs.

Drinking from water puddles

Water is transported passively in the half-open capillary channels between the scales by capillary force (Bentley and Blumer, 1962; Gans et al., 1982; Withers, 1993; Sherbrooke et al., 2007; Comanns et al., 2011). As soon as water reaches the scales surrounding the mouth, the lizard can but does not necessarily drink. Drinking movements were only observed and net water uptake was only measured five times (for three individual lizards) in puddles. Thus drinking appears to be an active choice and does not automatically take place if _M. horridus_ is in contact with a sufficient amount of water.

For lizards that had drinking (jaw) movements while sitting in a puddle, the maximum weight gain was much greater (6.84%) than for non-drinking individuals (3.66%; Fig. 1). However, if we assume that their capillary volume and residual mass gain were the same as for non-drinking lizards (3.19% and 0.47% body weight, respectively), then we calculate the volume of water drunk as 3.17±0.39% body weight. This actual water uptake by drinking was similar to the capillary volume (3.19% body weight; t-test: P=0.599) and the lizards stopped drinking after approximately 50 min. In total, 1.27 ml water was ingested, at approximately 0.7 µl of water uptake per jaw movement. These results suggest that _M. horridus_ can drink about the amount of water held by its integument during a single 60 min drinking period, meaning that it requires approximately double the amount of water to fill the capillary channels and drink this amount of water.

The integumental water capacity can be considered to be the maximum weight gain of a non-drinking lizard while in a water puddle, i.e. 3.66% body weight. This is more than the weight gain measured by Bentley and Blumer (1962) (2.67% within 5 min) and

about the same as the 3.7% body weight (Withers, 1993). However, all of this 3.66% weight gain was not lost when the lizards were dried in air for 60 min; there was a residual weight gain of 0.47%. It appears that capillary volume and weight gain are not quite the same. As 0.2% body weight was found to remain after moist sand exposure, and with condensation, this can be considered as a potential contributor to residual water gain (0.47% of the mass gain remained after puddle exposure). Subtracting this 0.2% from the residual weight gain of non-drinking lizards yields 0.27% body weight, which presumably reflects further residual water in deep recesses of the capillary system and micro-structures that are resistant to evaporative loss of water under our experimental conditions, or water absorbed into the stratum corneum (Lillywhite, 2006).

The cutaneous water-holding capacity of thorny devils ($\sim$9.19 mg cm^{-2}) is slightly higher than that of the Texas horned lizard ($\sim$5.9 mg cm^{-2}; calculated from Sherbrooke, 2004; 2.04–2.47% body mass assuming surface area calculated after Mautz, 1982). Values for granular skin of other vertebrates are also considerably lower, reflecting roles other than water transport to the mouth for ingestion. For an amphibian (Woodhouse toad, *Bufo woodhousei*), the cutaneous water-holding capacity is less ($\sim$0.43 mg cm^{-2}), presumably because its skin granularity holds water for direct uptake across the permeable skin (Lillywhite and Licht, 1974; Lillywhite and Stein, 1987). The file snake (*Acrocordis granulatus*) has granular, hygroscopic skin with a water-holding capacity of 5.4 mg cm^{-2}, to retard desiccation when out of water (Lillywhite and Sanmartino, 1993). Latex casts of African elephant (*Loxodonta africana*) skin hold 1.27 mg cm^{-2}, and of Asian elephants (*Elephas maximus*) approximately 0.81 mg cm^{-2}; this serves to maintain hydration of the skin (Lillywhite and Stein, 1987).

Presumably, similar biophysical constraints apply to water gain by thorny devils from other sources, such as fog condensation and dew. If the capillary volume needs to be 100% filled before there is any capacity for drinking, then these environmental water sources need to provide 3.66% of body mass before the thorny devil can drink. It remains to be shown, however, whether these water sources can provide sufficient water to fill the capillary channels, or how full the capillary channels need to be to suffice for drinking. Directly drinking water droplets enables various lizards and snakes to ingest environmental water (Louw, 1972; Repp and Schuett, 2008), but the jaw and tongue specializations of thorny devils and horned lizards presumably prevents them from doing this.

Water uptake from moist sand
Water exchange with moist sand of different water contents from dry to 22% (just less than saturated, 23.5% water content) resulted in a small weight loss of 0.065% for 0% sand moisture to a maximum weight gain of 1.84% after 60 min on the moistest (22%) sand (Fig. 2A). The 0.065±0.011% weight loss after 2 h on dry sand and then in dry air probably results from evaporative weight loss. The 0.30±0.06 g d^{-1} weight loss measured for a 28.6 g thorny devil (Withers and Dickman, 1995) in dry air is equivalent to −0.087% body weight, which is similar to our measured weight loss on dry sand for our experimental duration of 2 h and mean body mass of 39.86 g.

The 'ingested' water uptake, i.e. the difference between air-dry and initial weights for thorny devils on moist sand, was greatest for the highest water content (22%; Fig. 2B), but even this was only 0.19±0.06% body weight and thus much less than for non-drinking lizards after sitting in water (3.66%) and their residual mass gain of 0.47%. In addition, no jaw movements were seen for thorny devils during any of the moist sand experiments. The residual water uptake

of approximately 0.19% body weight for 22% moisture sand likely results from water still held by skin structures such as capillary channels, Oberhäutchen or absorption of water into the skin stratum corneum (Lillywhite, 2006), rather than from drinking.

A water collection of approximately 0.2–0.3% body weight on sand with 3–5% water content definitely seems insufficient for drinking, because it does not differ from water still held by skin structures for thorny devils from a puddle, after drying in air (0.47%). Even the maximum water collection from moist sand (22% water content) was only 1.84% of body weight, which would only fill approximately 59% of the skin capillary channels (Fig. 1). However, as drinking was found to be an active choice depending on motivation, it is not possible at present to determine the minimum required percentage filling of capillary channels (if it is not 100%), and hence the lowest sand water content sufficient for drinking. So, the question of whether water collection from moist soil can, in principle, be sufficient for drinking remains unresolved.

Sand shovelling and soil water uptake
Even though it seems that even a high sand water content is insufficient for drinking, sand shovelling has been observed for thorny devils in the field after rain (Sherbrooke, 1993), so it seems likely that this can increase water collection above the 1.84% body weight gain for near-saturated sand, and be sufficient for drinking. Sand shovelling onto the dorsal surface increases the area of contact and would presumably increase the rate of water collection from the sand (Withers, 1993). This also provides two further physical benefits that facilitate water uptake. If moist sand or soil is to be an effective source for water uptake, then soil water potential has to be overcome to transfer the water from one system (sand) to the other (skin). The soil water potential Ψ_{Soil} (MPa) is defined by its components, mainly matrix potential Ψ_M and gravitational potential Ψ_G (Taiz and Zeiger, 2007): $\Psi_{Soil} = \Psi_M + \Psi_G$. Hence, the first physical effect of sand shovelling is that the loosened substrate has a reduced sand capillarity (higher Ψ_M). That way, water could be extracted even from sand that normally has a higher capillary force than the skin, i.e. with less water content or smaller grain size spectrum. The second effect is that moist sand on the dorsal body surface provides a gravitational force (Ψ_G is positive) to fill skin capillary channels, instead of requiring a suction force (Ψ_G is negative) for uptake of water from moist sand on the ventral side.

Water collection by facilitated condensation
In comparison to the skin water-holding capacity of 3.66% body weight, there was a weight gain of only 0.2% by thermally facilitated condensation, driven by the initial thermal disequilibrium between the cool lizard and the warm air. This is only about half as much as the previously described residual mass gain of 0.47%, and is similar to the pre-wetting volume, and therefore very insufficient for drinking. Such a small amount of water can still be useful for pre-wetting, by which the wettability of the skin and thus the uptake of water is enhanced (Comanns et al., 2011). The ecological role of a pre-wetted skin is likely a preparation for faster uptake of water from more useful water sources, e.g. dew-wetted sand. Especially in desert environments, where temperatures increase extremely rapidly in the morning, this could enable the lizard to rapidly collect a sufficient amount of water before evaporation precludes further water uptake.

Wettability of skin replicas to mimic influence of pre-wetting
The super-wettable properties of pre-wetted skin were further quantified using skin replicas to test for the spreading behaviour of

applied water droplets. In summary, the moister the sand, the better the water droplets spread on the sand-wetted replicas (Fig. 3B), which suggests pre-wetting by water collection from moist sand. As we found an increase in spreading behaviour of water droplets between sand of 4 and 8% water content, a water content less than saturation (here, approximately 8% water content for quartz sand saturated at 11%) appears sufficient for full wettability and thus maximum facilitation of water uptake. Thermal facilitation of possible condensation using replicas cooled to 15°C suggested that maximum facilitation of water uptake occurs at a lower (~6%) sand water content (Fig. 3B). These findings suggest that consequent water collection is positively enhanced by pre-wetting. However, above a specific sand water content, this positive enhancement is not further increased. A similar pre-wetting mechanism can be assumed for maximum water collection on wet sand of the natural habitat, where water collection did not significantly increase further for ≥18% sand water content (Fig. 2A). As pre-wetting takes place much earlier than collection of a water amount sufficient for drinking, it is likely a preparation for faster uptake of water from more efficient sources.

The outermost skin layer (Oberhäutchen) exhibits a hexagonal surface structure, which has a diameter of approximately 10 to 20 μm and a depth of a few micrometres. Comanns et al. (2011) suggested that this micro-ornamentation of the Oberhäutchen is responsible for pre-wetting the scale surface. Maximum pre-wetting is reached when all surface microstructures are filled with a water film. In the case of skin replicas tested with moist quartz sand, such maximum pre-wetting appears above 8% sand water content. For maximum filling of the surface micro-ornamentation, a skin water coverage of ≥73% had been calculated to render the skin super-hydrophilic, i.e. extremely wettable (Comanns et al., 2011). Such an increase in wettability would enable a thorny devil to collect a greater amount of water with its skin, if the opportunity arises. Considering a hexagonal model of surface micro-ornamentation suitable for such water coverage, a cavity area fraction of 73% (wall area fraction of 27%) is required. If completely filled with water, this would be sufficient to render the skin super-hydrophilic. A hexagon diameter of 15.86 μm and a wall width of 2 μm satisfy this model. These model values are within the natural size range observed for Oberhäutchen micro-ornamentation (10 to 20 μm; Comanns et al., 2011). Assuming a structure depth of 5 μm, the measured average condensation of 0.2% of mass (82.7 mg) is sufficient to fill more than 226 cm² of micro-ornamentation. We calculate the body surface of a 39.86 g thorny devil (after Mautz, 1982) to be 134.3 cm², so water coverage is probably more than 1.7 times the body surface. This provides evidence that thermally facilitated condensation, although insufficient for drinking, enables pre-wetting that is sufficient to render the skin super-hydrophilic. This increased wettability can be provided not only by facilitated condensation, but also by a very small water collection of 0.2% body weight from other sources, e.g. sub-saturated moist sand (Fig. 2A).

Drinking mechanism
Regardless of how water is transported to the mouth, the mechanism for water ingestion is unclear. Bentley and Blumer (1962) suggested that drinking might involve hygroscopic mucus secretion from the lips, or that microstructures associated with the capillary channels that continue from the thorny devil's skin into the buccal cavity might assist ingestion (Sherbrooke et al., 2007). Regardless, jaw movements are crucial for drinking (Bentley and Blumer, 1962; present study). Assuming the capillary channels that enter the inter-jaw spaces are roughly elliptical, with a width of 200 μm and a

height of 100 μm (Sherbrooke et al., 2007), we calculate a cross-sectional area of 15.7×10^{-3} mm² and thus a required length of 44.6 mm for a volume of 0.7 μl of water to be drunk per jaw movement. Considering that each jaw has approximately 40 to 50 capillary channels, of approximately 0.5 mm in length, then the measured water uptake of 0.7 μl per jaw movement appears physically reasonable. Despite this morphological plausibility for the volume of jaw-adjacent capillaries contributing to the ingested volume per jaw movement, a mechanism for how water is transferred into the buccal cavity remains uncertain. When the capillaries are entirely filled, there is no free liquid–air interface to generate further capillary forces for water ingestion, so we suggest a physical squeezing mechanism. Further studies are required to resolve the mechanism for water transfer from the jaw capillary channels into the buccal cavity for swallowing.

Conclusions
Drinking occurred only for thorny devils in a water puddle, i.e. with filled capillaries (~3.19% body mass). As only some lizards drank while in a puddle, drinking appeared to be an active choice. It is unlikely that other sources of water, such as fog condensation, dew or thermally induced condensation, can provide sufficient water to fill the skin capillary channels to provide water for drinking. Even water uptake from moist sand can only fill the capillary network to approximately 59%, and this appears insufficient for drinking. However, additional contact of dorsal skin with moist sand could provide water collection from moist sand. Using skin replicas to mimic sand-shovelling behaviour suggests that enhanced water collection is positively influenced by sand water content. Considering that a reduction of soil water potential is required for water collection from soil, *M. horridus* benefits from sand shovelling in three ways: larger contact area, reduced matric potential and utilization of gravitational force. Consequently, water collection from moist substrate also seems likely to be a routine water source for thorny devils to meet their water demand.

A small integumental water collection of 0.2% body weight (from thermally facilitated condensation or contact with moist soil) was calculated to be sufficient to pre-wet the skin surface of *M. horridus*, and render the skin super-hydrophilic (contact angle ≤10 deg). This change in surface wettability would enhance the ability of thorny devils to use their skin to collect water from various other sources.

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