The Journal of Experimental Biology 207, 945-953
Published by The Company of Biologists 2004
doi:10.1242/jeb.00861

Cloacal evaporative cooling: a previously undescribed means of increasing evaporative water loss at higher temperatures in a desert ectotherm, the Gila monster *Heloderma suspectum*

Dale F. DeNardo*, Tricia E. Zubal and Ty C.M. Hoffman
Department of Biology, Arizona State University, Tempe, AZ 85287-4501, USA
Author for correspondence (e-mail: denardo@asu.edu)

Accepted 31 December 2003

Summary

The Gila monster *Heloderma suspectum* is an active forager in an environment that, at times, can be extremely hot and arid. Thus, Gila monsters face extreme thermostatic and hydrostatic demands. For a desert ectotherm routinely risking dehydration, evaporative water loss (EWL) is typically viewed as detrimental. Yet evaporation simultaneously dehydrates and cools an animal. We explored EWL in Gila monsters by measuring cutaneous, ventilatory and cloacal EWL at five ambient temperatures between 20.5°C and 40°C. Our results show that Gila monsters have high EWL rates relative to body mass. Cutaneous EWL underwent a consistent, temperature-dependent increase over the entire range of test temperatures (Q_{10}=1.61, with EWL ranging from 0.378 to 0.954 mg g^{-1} h^{-1}). Ventilatory EWL did not show a significant temperature-dependent response, but ranged from 0.304 to 0.663 mg g^{-1} h^{-1}. Cloacal EWL was extremely low and relatively constant between 20.5°C and 35°C, but rose dramatically above 35°C (Q_{10} >8.3×10^7, from 0.0008 at 35°C to 7.30 mg g^{-1} h^{-1} at 40°C). This steep rise in cloacal EWL coincided with an increasing suppression of body temperature relative to ambient temperature. Dehydration to 80% of initial body mass led to a delay in the onset and an attenuation of the dramatic increase in cloacal EWL. These results emphasize the potential value of EWL for thermoregulation in ectotherms and demonstrate for the first time the role of the cloaca in this process.

Key words: evaporative water loss, temperature, reptile, cloaca, cutaneous evaporation, ventilatory evaporation, lizard, Gila monster, *Heloderma suspectum.*

Introduction

One of the fundamental physiological dichotomies among vertebrates is that of endothermy and ectothermy (Hensel et al., 1973; Hayes and Garland, 1995). While ectothermy is metabolically inexpensive, it allows for little independence from the thermal vagaries of the environment (Crompton et al., 1978; McNab, 1978). Ectotherms have insufficient heat production to elevate body temperature (T_b) above ambient temperature (T_a), and therefore must obtain heat from the environment *via* radiation, conduction and convection.

In addition to having limited capabilities for internal heat production, ectotherms are thought to have limited physiological mechanisms for significantly reducing T_b when environmental temperatures are high (Schmidt-Nielsen, 1964). Evaporative water loss (EWL) represents the predominant means by which any organism can cool its body when T_a exceeds T_b. Not surprisingly, then, EWL has been shown to be critical to thermoregulation in endotherms (for reviews, see Dawson and Bartholomew, 1968; Calder and King, 1974). However, EWL of ectotherms is rarely investigated in terms of its potential for suppressing body temperature. Instead, it is widely accepted that the only means by which reptiles can lower T_b is to move to a cooler environment such as a burrow.

While EWL could provide a means for reptiles to reduce T_b when T_a exceeds T_b, EWL is typically viewed merely as a detriment to water balance (for a review, see Mautz, 1982a). Reptiles living in arid environments tend to have reduced EWL compared to more mesic species, and this low EWL rate is considered to be an adaptive response to xeric conditions (Cohen, 1975; Mautz, 1982a,b; Dmi'el, 1998, 2001). While seldom studied, the use of EWL for thermoregulation by ectotherms has been reported in several species. Cicadas can effectively reduce T_b below T_a by actively increasing cutaneous EWL (Toolson, 1987). Additionally, some arid-environment lizards increase EWL by panting at thermally challenging temperatures (Templeton, 1960; Dawson and Templeton, 1963; Warburg, 1965; Crawford and Kampe, 1971).

Gila monsters *Heloderma suspectum* are relatively large, active foraging lizards of the Sonoran Desert of Arizona and northern Mexico. Single foraging bouts can cover considerable distances (in excess of 1 km) over an extended period of time (12 h or more) in search of vertebrate nests, the contents of which comprise their diet (Bogert and Del Campo, 1956; Beck, 1990). The Sonoran Desert summer consists of two distinct

climatic seasons. From mid-April through mid-July, the Sonoran Desert is hot (daytime high temperatures of 35–45°C) and dry (no rainfall). However, a summer rainy season commences in approximately mid-July and extends into mid-September. During this summer rainy season, temperatures remain high but there is a relatively reliable, albeit limited, rainfall (approximately 10 cm). While Gila monsters are predominantly crepuscular or nocturnal during the summer to avoid peak temperatures, air temperatures frequently exceed 40°C at sunset and remain warm throughout much of the night. Requiring lengthy surface activity in an environment that is hot and dry for several consecutive months suggests that Gila monsters should have low cutaneous evaporation to benefit water balance. Nevertheless, Gila monsters are said to have 'leaky skin' (Lowe et al., 1986; Brown and Carmony, 1991), though published data in support of this contention are lacking. From the perspective of water balance, leaky skin in a xeric environment is maladaptive because it increases the rate of desiccation (Brown and Carmony, 1991). Consequently, the purported existence of leaky skin is used as evidence to support the hypothesis of a tropical origin for Gila monsters (Lowe et al., 1986).

Since EWL rates of Gila monsters have physiological, ecological, and even evolutionary implications, we examined evaporative water flux in this species. In addition to measuring total EWL, we investigated the relative contribution by the skin and other potential routes of water loss. We designed a means by which we could partition cutaneous, ventilatory and cloacal EWL. We use the term 'ventilatory' to refer to evaporation occurring from the buccopharyngeal epithelia, whether or not such evaporation is being enhanced by breathing. While cloacal EWL has not previously been described, we considered it a viable means by which water could be evaporated from the body. While usually confined within the body cavity, the mucous membranes of the cloaca can be exposed to the environment through the vent (with or without eversion; D. F. DeNardo, personal observation). Furthermore, water permeability of the lizard cloaca has been demonstrated in the context of post-renal concentration of urine (Braysher and Green, 1970). While previous studies of EWL in reptiles have neglected or intentionally prevented cloacal EWL (for a review, see Mautz, 1982a), we chose to examine this mucosal surface as a possible route for EWL.

We hypothesized that EWL could provide thermal advantages to an actively foraging ectotherm that inhabits a hot, arid environment. Evaporation would be especially advantageous if there were a fairly predictable water resource. In fact, despite living in an arid environment, cicadas are able to invest large volumes of water into EWL because of their high tolerance of desiccation (Toolson, 1987) and their ability to regularly obtain water from the xylem of bushes (Cheung and Marshall, 1973). The predictable late summer rains of the Sonoran Desert provide a water resource to replenish water expended during the dry spring and early summer months. Additionally, the Gila monster possesses a large urinary bladder that might serve as a water reservoir during extended dry periods, as it does in the desert tortoise *Gopherus agassizii* (Dantzler and Schmidt-Nielsen, 1966; Minnich, 1976). Therefore, we predicted that Gila monsters would have a relatively high EWL rate and that elevated water flux would be especially apparent at thermally challenging temperatures, when hyperthermia would be more of an immediate physiological threat than dehydration. We further predicted that, by providing a mechanism for shedding body heat, high EWL rates would allow the animal to maintain sub-ambient T_b, at least in the short term. Lastly, since water is especially critical during dehydration, we predicted that dehydration would lead to a reduction in EWL.

Materials and methods

Animals

Eighteen adult Gila monsters *Heloderma suspectum* Cope (432–691 g) from a colony acquired from the Arizona Game and Fish Department (AZ G&F holding permit #SP689454), were housed individually in 91 cm × 71 cm × 46 cm cages with a basking light at one end of the cage to provide a thermal gradient. The room was maintained at 25±1°C with a 12 h:12 h light:dark cycle. This thermal regime (25°C room temperature with a basking lamp provided for 12 h of the day) allowed the Gila monsters to maintain the species' selected body temperature (29.4°C, Bogert and Del Campo, 1956) during the day, but drop to a typical active season night-time temperature (D. F. DeNardo, unpublished data). Except during the dehydration experiment, animals were provided water *ad libitum* and fed one dead adult mouse approximately biweekly (however, animals were deprived of food for at least one week prior to any experimental trial).

Body surface area was estimated by representing the animal as a collection of simple geometric volumes. We made actual body measurements that allowed us to calculate the surface areas of the geometric constituents: a square pyramidal frustum (the head), five right regular cylinders (the body and four limbs), and a right circular cone (the tail). Estimated whole body surface areas were between 622 and 958 cm^2.

Experimental apparatus

Ambient temperature was maintained throughout trials by housing the test chamber in an environmental chamber fitted with an electronic temperature controller (Omega Engineering CN2011, Stamford CT, USA). The test chamber was thus a chamber within a chamber, and it experienced very little thermal oscillation throughout trials (T_a was maintained at ±0.2°C during a given trial and ±0.5°C among trials).

We partitioned EWL into two components (head and body) by placing Gila monsters individually into a two-compartment test chamber fitted for separate flows of air into and out of the compartments. The test chamber was custom-made to fit the test species, thereby minimizing the time for turnover of air and maximizing the temporal resolution of our hygrometric measurements. The chamber was constructed almost entirely of borosilicate glass (Pyrex), because glass is minimally

hygroscopic, and it allowed for continuous visual monitoring of the test animal using an infrared camera connected to a remote monitor. The overall geometry of the test chamber was a horizontally placed, right circular cylinder (overall length 52 cm, inside diameter 9.5 cm) with closed, flat ends. To allow for partitioning, the main cylinder consisted of two open-ended cylinders of unequal length (body compartment: 39.5 cm long, 2800 ml volume; head compartment: 12 cm long, 850 ml volume). A two-part neck stock composed of aluminum plate was attached perpendicularly to a horizontal base and served to safely hold the venomous lizard in place while the investigator installed the compartments and thereafter during trials. Attached to the open end of the head compartment was a latex sheet (#07315 Heavy Dental Dam, Hygenic, Akron, OH, USA) perforated with an elliptical hole (17 mm × 21 mm) through which the head was passed. With the animal in place, the cylinders were clamped against the stock using a bar clamp. A closed-cell foam gasket formed a seal between the body compartment and the stock. The compliant latex sheet sealed the head compartment and prevented mixing of air between compartments even if the animal moved. The lack of mixing of air between the head and body chamber was verified during test trials that delivered 100% saturated air into one chamber without causing any change in dewpoint in the other chamber.

Each compartment was fitted with three threaded, borosilicate glass hose connectors (#7 Chem-Thread, Chemglass, Vineland, NJ, USA). Two connectors accepted non-hygroscopic tubing (Bev-A-Line, Thermoplastic Processes Inc., Stirling, NJ, USA) for both influent and effluent air. The third hose connector served as a port for passage of type T (copper–constantan) thermocouple cables, permitting continuous recording of animal temperatures and ambient temperatures. A small, outward leak at the thermocouple port, required to allow for play in the cables, allowed us to equalize pressures between compartments (a higher flow rate was used in the body compartment). The leak did not affect the sub-sampled effluent in our positive-pressure setup, and equalization of pressures further reduced the chance of mixture of air between compartments.

Influent air was first passed through an industrial purifier (#PCDA11129022, Puregas, Denver, CO, USA) that removed carbon dioxide and water vapor. Dried air was sent through a manifold to supply separate air lines for each of the compartments. Mass-flow controllers (#FMA-A2406 and #FMA-A2409, Omega Engineering, Stamford, CT, USA) were placed in the air lines upstream of the compartments to maintain separate and constant influxes (head: 1000 ml min^{-1}; body 4000 ml min^{-1}). We calibrated the mass-flow controllers for the experimental air mixture (dry and CO_2-free) using soap film flow meters, and we generated calibration curves describing STP (standard temperature and pressure) volumetric flow (ml min^{-1}) as a function of electrical potential difference (mV). At the flow rates selected, the air in the head and body compartments underwent 99% turnover (Lasiewski et al., 1966) every 3.4 and 3.2 min, respectively.

Each compartment's effluent was sent to its own hygrometer (#RH100, Sable Systems, Las Vegas, NV, USA) and then vented to the room. The hygrometers were calibrated with bottled nitrogen (zero gas) and experimental air that was bubbled through three serially placed columns of distilled water, each approximately 150 cm deep, before being sent individually to the hygrometers (span gas). A copper–constantan thermocouple measured the water temperature in the columns, and each hygrometer was individually heated to be warmer than the water, thus preventing condensation. The hygrometers were set to output dewpoint and were adjusted so that the dewpoint reading equaled the water temperature. We verified the linearity of the hygrometers and the veracity of the calibrations by later sending air through the columns when the water was comparatively cooler, and the hygrometers indicated the correct (and lower) dewpoints. The hygrometers remained powered throughout the entire experiment to minimize calibration drift, and calibrations were checked occasionally and readjusted when necessary. While the hygrometers showed little or no drift, we minimized the effects of any drift by calculating evaporative fluxes based on elevations in dewpoint above separate baseline values obtained by flowing air through the sealed, empty compartments before each trial. Measurements were sampled every second and averaged every minute by a computer-interfaced datalogger (CR23×, Campbell Scientific, Logan, UT, USA) that received inputs from five thermocouples, two mass-flow controllers, and two hygrometers.

Experiment 1: Effects of T_a on EWL

In order to monitor T_b throughout the experimental trials, each of six lizards (mean body mass 606±26.8 g) was implanted with a thermocouple array. Each array consisted of three 30-gauge, type T thermocouple cables (#TT-T-30-SLE, Omega Engineering) extending from three (two male, one female) subminiature connectors (#SMP-W, Omega Engineering). To prevent injury to the animal, the thermocouples terminating the long cable and one of the short cables were thinly covered with pourable rubber coating (Plasti-Dip, PDI Inc., Circle Pines, MN, USA).

With the animal under isoflurane anesthesia, an approximately 1 cm incision was made ventro-laterally in the abdominal region. From the incision site, a metal trocar was routed subcutaneously until it was exteriorized on the dorsum at mid-body. The two thermocouples coated with Plasti-Dip were inserted from the dorsum retrograde into the trocar. The trocar was removed, leaving the short thermocouple situated subcutaneously at the back. The body wall was punctured at the superficial ventro-lateral incision site, and the long thermocouple was placed 1 cm deep into the body cavity and sutured to the body wall. The array was triply sutured to the skin where it emerged on the dorsum to keep it in place and reduce tension at the dorsal incision site. Both the dorsal and ventro-lateral incisions were closed with everting mattress sutures (3-0 Vicryl, Ethicon, Somerville, NJ, USA). The third thermocouple was attached to the skin surface directly superficial to the

subcutaneous thermocouple using cyanoacrylate glue and then covered with a thin coating of Plasti-Dip. When connected to the datalogger, these three thermocouples could provide continuous measurements of core body, subcutaneous, and skin temperatures. Because of the failure of several subcutaneous and skin thermocouples, only core T_b results are reported here. Each animal was given at least 3 days to recover from surgery prior to participating in the experiment trials.

Each Gila monster was tested once at each of five ambient temperatures (approximately 20.5, 30.0, 35.0, 37.5 and 40.0°C). T_a = 30°C approximates the body temperature selected by Gila monsters in a laboratory thermogradient (29.4°C; Bogert and del Campo, 1956) as well as the mean T_b obtained from free-ranging Gila monsters (29°C; Lowe et al., 1986; 28.5°C, Beck, 1990), while the other temperatures lie near or beyond the extremes of the species' active T_b range (24–37°C; Beck, 1990). Animals were used only when they were not undergoing or about to undergo ecdysis, as ecdysis can affect EWL. Trials for an individual were separated by at least 24 h, and the five treatment temperatures were randomized. Animals were moved from the housing room to the environmental chamber and allowed at least 2 h to adjust to the trial temperature. Based on pilot tests, this time was sufficient for T_b to stabilize while the animal was kept in the new thermal environment. During this stabilization time, air was flowed through the sealed but empty compartments to obtain baseline compartment air temperatures and dewpoints. Compartment vapor densities calculated from the baseline dewpoints were subtracted from vapor densities calculated from dewpoints during the experimental trial, and the resulting differences (along with flow rates and body mass) were used to determine EWL (see 'Calculations' below).

Animals were then placed in the partitioned chamber for at least 40 min to allow them to adjust to the new environment and for stabilization of dewpoints and body temperatures. The three body temperatures, ambient temperatures of the two compartments, and separate dewpoints of air flowing over the head and air flowing over the rest of the body were recorded for 20 min while the animal was at rest. Upon collection of these data, a cotton wad was placed in the cloaca, and an H-shaped piece of latex was tied around the hind limbs to cover the vent. This 'diaper' prevented moisture from leaving the cloaca, while minimally impeding integumentary evaporation (the diaper covered approximately 1% of the animal's total surface area). After being fitted with the diaper, the animal was returned to the test chamber and a second set of data was collected in the same fashion as the original set. For any trial in which moisture (e.g. urine) was visible on the animal or the walls of the chamber during or at the end of the trial, the data were discarded and the trial was repeated at a later time. The presence of such liquid was also easily detectable as a rapid rise on the plot of body chamber dewpoint.

Experiment 2: Effects of dehydration on EWL

We recorded the mass of six adult Gila monsters not used in experiment 1 (mean body mass=520±27.9 g) and then deprived them of food and water for 6–10 weeks, until they reached approximately 80% of initial mass. Six additional adult Gila monsters (mean body mass=523±29.5 g) were provided water *ad libitum* but no food for 10 weeks. We were thereby able to assess the fraction of mass-loss attributable to energetic demands (catabolism), rather than to dehydration. To assess the effect of dehydration on serum osmolality, a blood sample was collected from the caudal vein of each animal after the 6–10 week period. We centrifuged the samples and stored the serum in sealed tubes at –80°C for later analysis. We measured serum osmolality twice for each sample with a vapor pressure osmometer (#5500, Wescor, Logan, UT, USA) that we calibrated immediately prior to measurements using standard solutions (290 mmol kg^{-1} and 1000 mmol kg^{-1}; Wescor).

The six dehydrated Gila monsters underwent experimental trials similar to that of experiment 1, except that animals were not implanted with thermocouple arrays, and trials were limited to 37.5°C and 40°C. Imposing these limitations allowed for much faster completion of the trials (to minimize the duration of the dehydrated state) while still providing valuable data for assessing the effect of dehydration on EWL at the most thermally challenging temperatures.

Calculations

For each trial, we determined values for dewpoint and temperature by calculating the mean values over a 5 min period near the end of the trial when values were nearly constant. We used dewpoints to calculate ambient-temperature vapor pressures using an eighth order polynomial describing saturation vapor pressure as a function of air temperature (Flatau et al., 1992). Vapor pressures were used to calculate ambient-temperature vapor densities using the Ideal Gas Law (Campbell and Norman, 1998). Finally, evaporative fluxes (mg min^{-1}) were calculated by multiplying vapor density (mg ml^{-1}) by ATP (ambient temperature and pressure) rate of flow of air (ml min^{-1}) for each of the two compartments. We calculated absolute evaporative flux (mg H_2O h^{-1}) as well as fluxes relative to both mass (mg H_2O g^{-1} h^{-1}) and surface area (mg H_2O cm^{-2} h^{-1}) to account for variation in size between individuals. We assumed that a portion of the water vapor appearing in the head compartment was attributable to evaporation from the cranial integument (skin and conjunctivae) and that evaporative flux from the skin of the head equaled that from the skin of the body. We further assumed that, despite the probably greater evaporative flux from the moist eyes than from the dry skin, the small size of the eyes compared to the head made the absolute increase negligible. We therefore estimated the non-ventilatory component of the flux occurring in the head chamber based on the surface area of the head and on the area-specific value for evaporative flux from the skin in the body compartment during the diapered trial. The resulting non-ventilatory, head-chamber component was then subtracted from the total head-chamber flux to yield ventilatory flux, and it was added to the body-chamber flux to yield non-ventilatory flux. Finally, non-

ventilatory flux during the diapered trial was subtracted from non-ventilatory flux during the non-diapered trial to yield cloacal flux, and non-ventilatory flux during the diapered trial was taken to be cutaneous flux. Lastly, for experiment 1 we assessed the ability of Gila monsters to physiologically thermoregulate by subtracting the mean air temperature of the body compartment from the mean core T_b during each trial.

Statistical analysis

We used StatView (version 5, SAS Institute, Cary, NC, USA) for all statistical analyses. For experiments 1 and 2, we used repeated-measures analyses of variance (R-M ANOVA), with T_a and cloacal patency as within-subjects factors, and either T_b or water flux as the dependent variable. To compare EWL rates of hydrated animals in experiment 1 with dehydrated animals in experiment 2, we used R-M ANOVA with hydration as the between-subjects factor, T_a as the within-subjects factor, and water flux as the dependent variable. *Post-hoc* comparisons were made using paired Student's *t*-tests adjusted for an experimentwise Type 1 error rate of 0.05. The adjusted alpha for controlling Type 1 experimentwise error was 0.05/*N*, where *N* = the number of sampling periods (i.e. α=0.01 and α=0.025 for experiments 1 and 2, respectively). Osmolality results were analyzed using a Student's *t*-test. All values are presented as means ± S.E.M.

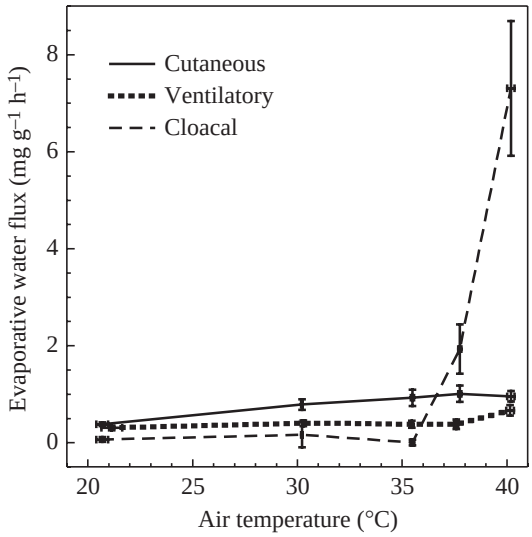

Fig. 1. Mean cutaneous, ventilatory and cloacal water loss rates in six Gila monsters at various experimental temperatures. Note that ventilatory EWL shows little temperature sensitivity, while cutaneous EWL increases gradually as air temperature (T_a) increases, and cloacal EWL shows a dramatic increase at T_a greater than 35°C. Vertical and horizontal error bars represent ± 1 standard error for water loss rates and chamber temperature, respectively.

Results

Experiment 1

EWL from both the head and body compartments increased with increasing T_a (head: $F_{4,5}$=10.07, P<0.0001; body: $F_{4,5}$=25.83, P<0.0001). The head compartment showed a linear increase across all temperatures, while the increase in the body compartment was linear between 20.5 and 35°C, and then showed a dramatic increase above 35°C. Applying the diaper significantly reduced EWL in the body compartment (cloacal patency main effect: $F_{1,5}$=30.27, P=0.0003), and this effect was temperature dependent (cloacal patency × T_a effect: $F_{1,5}$=22.42, P<0.0001). *Post-hoc* analyses indicated that the diaper significantly reduced body chamber EWL only at 40°C [mean reduction=7.30 mg g^{-1} h^{-1} (89%), P=0.0033], although the mean reduction in EWL at 37.5°C was also substantial [mean reduction=2.14 mg g^{-1} h^{-1} (75%), P=0.013]. Contrary to the suppressive effect on body EWL, applying the diaper had a positive effect on EWL in the head compartment (cloacal patency main effect: $F_{1,5}$=10.54, P=0.0088), but this effect was not temperature dependent [cloacal patency × T_a: $F_{1,5}$=0.80, P=0.53]. *Post-hoc* analyses showed that the increase was only significant at 37.5°C (mean increase=0.229 (48%), P=0.0065), although a considerable increase also occurred at 40°C [mean increase=0.209 (28%), P=0.054].

Increasing T_a led to a significant increase in both cutaneous and cloacal, but not ventilatory, evaporative fluxes (cutaneous: $F_{4,5}$=10.27, P=0.0001; cloacal: $F_{4,5}$=21.34, P<0.0001; ventilatory: $F_{4,5}$=2.38, P=0.086, Fig. 1, Table 1A). Cutaneous flux showed a relatively constant increase throughout all trial temperatures (Q_{10}=1.61), while cloacal flux was low and relatively constant between 20.5°C and 35°C, but rose dramatically above 35°C (Q_{10}=8.3×10^7).

Trial temperature affected the difference between chamber temperature and T_b, with increasing chamber temperatures leading to a greater suppression of T_b below chamber temperature ($F_{4,5}$=27.90, P<0.0001; Fig. 2). While applying the diaper consistently reduced the degree of temperature suppression at all higher temperatures, the lack of an effect at lower temperatures led to no overall effect of diaper application on temperature suppression ($F_{1,5}$=2.57, P=0.14). However, the interaction between chamber temperature and diaper application approached, but failed to reach, statistical significance ($F_{1,5}$=2.39, P=0.067).

Experiment 2

Restricting food and water to six experimental animals for 6–10 weeks led to a significantly greater loss in body mass compared to animals provided with no food but free access to water (water-deprived: 78±1% of initial body mass, range 75–81%; *ad libitum* water: 95±2%, range 87–101%; P<0.0001). Furthermore, serum osmolality of the water-deprived Gila monsters was significantly higher than that of the animals provided water *ad libitum* (water-deprived: 603±7 mOsm kg^{-1} H_2O; *ad libitum* water: 487±37 mOsm kg^{-1} H_2O; P=0.013). Combined, these results demonstrate that the majority of mass lost in the experimental group was water, and, although the degree of dehydration is not quantifiable, the water-deprived animals were considerably dehydrated.

Table 1. *Mean evaporative water flux of (A) six normally hydrated Gila monsters, each tested at five ambient temperatures, and (B) six dehydrated Gila monsters, each tested at two ambient temperatures*

	Evaporative water flux (mg g^{-1} h^{-1})			
T_a (°C)	Total	Cutaneous	Cloacal	Ventilatory
(A) Normally hydrated animals				
20.0	0.748±0.117	0.378±0.041 (51%)	0.066±0.053 (9%)	0.304±0.053 (41%)
30.0	1.345±0.315	0.785±0.107 (58%)	0.164±0.262 (12%)	0.397±0.061 (29%)
35.0	1.302±0.159	0.923±0.170 (71%)	0.001±0.062 (0%)	0.378±0.072 (29%)
37.5	3.316±0.588	1.008±0.172 (30%)	1.930±0.514 (58%)	0.378±0.104 (11%)
40.0	8.921±1.31	0.954±0.107 (11%)	7.303±1.39 (82%)	0.663±0.114 (7%)
(B) Dehydrated animals				
37.5	1.374±0.095	0.719±0.102 (52%)	0.008±0.059 (0%)	0.663±0.096 (48%)
40.0	4.176±0.764	0.964±0.104 (23%)	2.221±0.679 (52%)	0.991±0.108 (25%)

T_a, ambient temperature.
Values are means ± S.E.M.
Numbers in parentheses represent the percentage of total water flux at that temperature.
Note that temperature differentially affects cutaneous, cloacal and ventilatory water flux, leading to changes in the relative contribution of each, and that dehydration significantly decreased cloacal water flux.

As in experiment 1, T_a had a significant effect on EWL in both the head and body compartments for the dehydrated Gila monsters (head: $F_{1,5}$=51.50, P<0.0001; body: $F_{1,5}$=14.30, P=0.0036). Also, similar to the results of experiment 1, applying the diaper had a significant effect on EWL in the body compartment (cloacal patency main effect: $F_{1,5}$=8.44, P=0.016; cloacal patency × T_a: $F_{1,5}$=10.03, P=0.010), but not the head compartment (cloacal patency main effect: $F_{1,5}$=0.13, P=0.72; cloacal patency × T_a: $F_{1,5}$=0.37, P=0.56). *Post-hoc* tests indicate that significant results from the body compartment were due to a diaper-induced reduction in EWL at 40°C (P=0.021).

Increasing T_a had a positive effect on all fluxes (cutaneous: $F_{1,5}$=7.56, P=0.040; cloacal: $F_{1,5}$=10.67, P=0.022; ventilatory: $F_{1,5}$=15.54, P=0.011; Table 1B). Comparing results from experiments 1 and 2 reveals that dehydration had a significant effect on cloacal and ventilatory fluxes, but not on cutaneous flux (cutaneous: $F_{1,5}$=0.75, P=0.41; cloacal: $F_{1,5}$=19.74, P=0.0012; ventilatory: $F_{1,5}$=8.08; P=0.018; Fig. 3). Dehydration suppressed cloacal flux relative to that of hydrated animals at both temperatures tested (P=0.0038 and P=0.0082 at 37.5 and 40°C, respectively). While the effect of dehydration was negative for cloacal flux, it was positive for ventilatory flux (i.e. ventilatory flux in dehydrated animals was higher than that of hydrated animals).

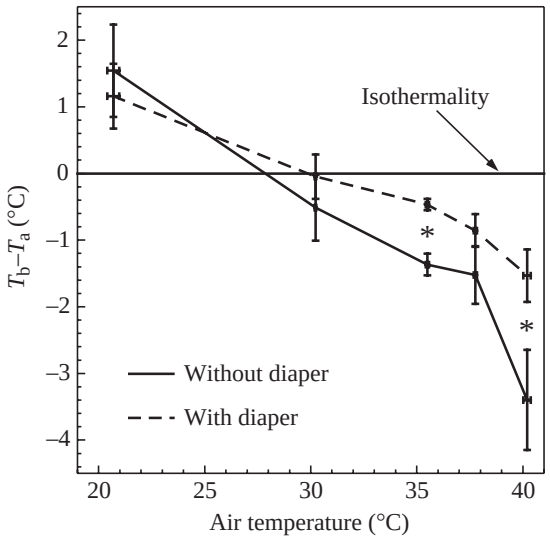

Fig. 2. Mean differences between air temperature (T_a) and body temperature (T_b) of six Gila monsters at five experimental temperatures. Increasing T_a led to increasing suppression of T_b (P<0.0001), and T_b was consistently lower than T_a at higher temperatures. Asterisks represent significant differences (P<0.01) between the control and diaper trial values. Vertical and horizontal error bars represent ± 1 S.E.M. for temperature suppression and chamber temperature, respectively.

Discussion

Like many reptiles, EWL of Gila monsters is highly sensitive to temperature, with Q_{10} values for cutaneous EWL comparable to other lizard species (Crawford and Kampe, 1971). Even at the cooler temperatures tested, Gila monsters have a high total EWL relative to other lizards from arid environments (for a comparative summary of EWL in reptiles, see Mautz, 1982a). The finding that EWL in Gila monsters compares most closely with that of lizards from mesic rather than arid environments might be viewed as support for the contention that Gila monsters evolved in a more tropical environment than they now inhabit (Lowe et al., 1986). However, when considering body size, which is inversely related to EWL rate (Mautz, 1982a), EWL of Gila monsters is

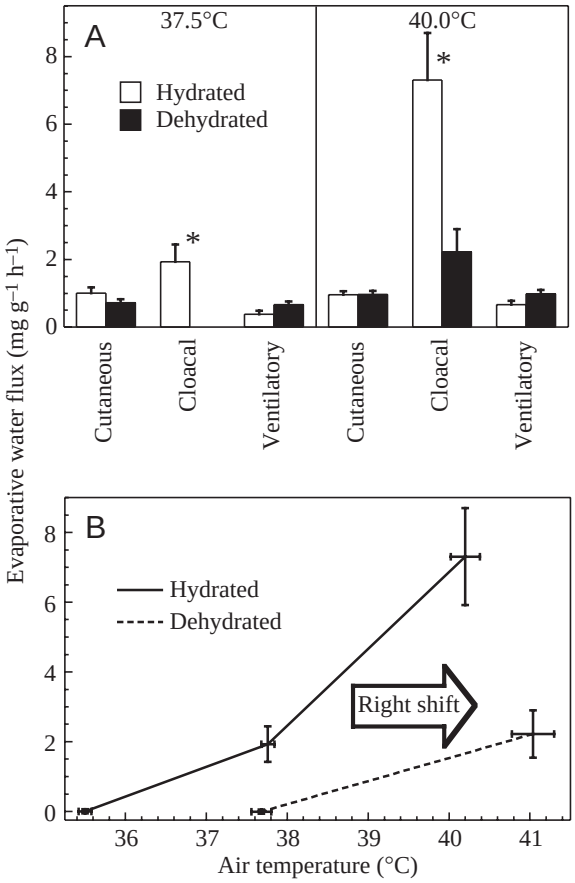

Fig. 3. The effect of dehydration on EWL by Gila monsters at thermally challenging temperatures. Each symbol indicates the mean value for six Gila monsters; error bars indicate ± 1 S.E.M. (A) Effect on cutaneous, ventilatory and cloacal EWL at 37.5°C and 40°C. An asterisk indicates a statistically significant (P<0.025) difference between values from hydrated and dehydrated animals. (B) Cloacal EWL of hydrated animals and dehydrated animals. Note the lack of elevated cloacal EWL at 37.5°C and attenuation of cloacal EWL at 40°C for dehydrated animals (i.e. a right shift in the EWL–temperature response curve).

considerably higher than that of other lizards regardless of habitat type. Therefore, the high evaporation rate of Gila monsters more likely exists for physiological reasons (i.e. thermal homestasis) than simply as a relic of this lizard's more tropical ancestry.

The current trend is to evaluate EWL simply for its negative impact on water balance (e.g. Eynan and Dmi'el, 1993; Dmi'el, 1998, 2001; Winne et al., 2001), but high EWL has the potential to be a major contributor to thermostasis, assuming sufficient water availability. This is especially true at higher temperatures, where EWL can help maintain T_b below the thermal maximum temperature (i.e. below those temperatures where locomotory activity is substantially impaired). Several lizard species have been shown to dramatically increase EWL at higher temperatures (Table 2). In each of the previously studied species, the increase in EWL is a result of increases in ventilatory flux (predominantly a reflection of panting). Gila monsters are apparently unique among studied species in that EWL rates at high temperatures are considerably higher than even those of panting lizards, and in that the source of this dramatic increase in EWL is the cloaca. Previous studies either ignored (e.g. Dawson and Templeton, 1963) or prevented (e.g. Templeton, 1960; Warburg, 1965; Crawford and Kampe, 1971) any EWL occurring from the cloaca. Cloacal EWL might be a unique physiological adaptation of Gila monsters, or it might be more widely spread among lizard taxa. It is worth noting that none of the Gila monsters in the current study panted during the trials, even at the highest T_a. This observation is reinforced by the small change in ventilatory EWL as temperature increased. While panting is common among lizards, other species also fail to pant at thermally challenging temperatures (Dawson, 1960). Cloacal evaporation might simply be an alternative mechanism by which lizards can decrease T_b. Lastly, the temperature at which Gila monsters exhibit extreme elevations in EWL is somewhat lower (37.5°C) than that of other species studied (typically ≥40°C). For some species this difference could be a result of a lack of data for temperatures between 37 and 40°C, but this difference might also be attributable to the relatively low selected body temperature of Gila monsters. While it might have been advantageous to examine the response of Gila monsters at temperatures higher than 40°C, such temperatures are extremely risky to the health of this species, especially when cloacal EWL is prevented.

Data regarding the ability of EWL to reduce T_b of lizards to below T_a are mixed. Evaporation seems to be of marginal importance in decreasing T_b in some species (Templeton, 1960; Crawford and Kampe, 1971), but it can significantly reduce T_b in others (Dawson and Templeton, 1963; Warburg, 1965; DeWitt, 1967; this study). Regardless of its effectiveness, EWL is probably not used to extend activity bouts for long durations at high T_a, because of the relative scarcity of water in habitats with such high temperatures. Nevertheless, it might allow the lizard to slightly extend the duration of activity (Dawson and Templeton, 1963), and even a slight extension could be important in an extreme environment.

The Sonoran Desert is characterized by an extended period of high temperature. Maximum daily temperature often exceeds 40°C from mid spring until early fall (i.e. April–October). Despite living in a hot environment, Gila monsters have a relatively low selected body temperature of approximately 29°C (Bogert and del Campo, 1956; D. F. DeNardo, unpublished data). Furthermore, Gila monsters are active foragers, preying on the contents of bird, mammal and reptile nests. Relying on such widely dispersed resources requires Gila monsters to forage over long distances (Beck, 1990). To regulate T_b during the summer, Gila monsters restrict activity to the cooler periods of the day. However, EWL might allow extension of the activity period to complete critical activities (e.g. locating shelter, consuming prey or engaging in combat) without reaching temperatures that approach their critical thermal maximum.

Table 2. *Total evaporative water fluxes (EWL) of various arid and semi-arid lizards at moderate and thermally challenging air temperature (T_a)*

Genus and Species	Body mass (g)	T_a (°C)	EWL (mg g^{-1} h^{-1})	Reference
Crotaphytus collaris	30	32	0.46	Dawson and Templeton, 1963
		40	0.73	
		44	4.70	
Dipsosaurus dorsalis	48	32	0.86	Templeton, 1960
		40	2.08	
		44	3.64	
Sauromalus obesus	140	26	0.22	Crawford and Kampe, 1971
		40	0.67	
		43.5	2.36	
Pogona barbatus (*Amphibolurus barbatus*)	241	30	0.47	Warburg, 1965
		40	1.04	
Trachydosaurus rugosus (Tiliqua rugosa)	315	30	0.67	Warburg, 1965
		40	1.12	
Heloderma suspectum	606	30	1.35	This study
		40	8.92	

All species, except Gila monsters *Heloderma suspectum*, are known to pant at higher temperatures, thus explaining the substantial increase in EWL at those temperatures. The dramatic elevation in EWL of Gila monsters at 40°C is attributable to cloacal water flux.

While water is a limited resource in all deserts, much of the Sonoran Desert has a reliable summer monsoon season (mid-July to mid-September) that provides relatively frequent access to water for at least the latter half of the hot summer. Therefore, the length of time during which Sonoran Desert residents cope with arid conditions (typically mid-April through mid-July) is reduced compared to many desert environments. The periodic availability of water and the concomitant increase in food availability associated with the summer monsoon season might underlie the predominant restriction of Gila monsters to these areas of the Sonoran Desert. Additionally, Gila monsters possess extremely large urinary bladders that potentially act as reservoirs for water during the dry periods. Previous studies support such a role for the bladder in other desert lizards (Beauchat et al., 1986; Cooper and Robinson, 1990), but water storage in the bladder remains unexplored in Gila monsters.

Despite experiencing a summer rainy season and perhaps possessing a water reservoir, Gila monsters are vulnerable to dehydration during the dry summer months (Bogert and Del Campo, 1956; Beck and Jennings, 2003), and high EWL rates at this time would be costly and possibly fatal. Therefore, it is not surprising that Gila monsters reduce cloacal EWL rates when dehydrated by increasing the minimum temperature at which significant cloacal EWL occurs and by reducing evaporative flux at higher temperatures. We recognize that the decrease in evaporative flux during dehydration is almost certainly due in part to the physical effect that the increased osmotic pressure of the blood has on the vapor-pressure gradient driving the evaporation. However, the direct effect of increased osmolality is unlikely to account for the full magnitude of the reduction in EWL. Instead, it is likely that much of the reduction in EWL is due to physiological adjustments made to minimize loss of body water. For example, alteration in cloacal perfusion and or vent gape could substantially affect the rate of cloacal EWL. While vent gape has been anecdotally observed in Gila monsters at high environmental temperatures, possible regulatory mechanisms remain to be tested. Similarly unknown yet interesting and deserving of future study are the regulatory parameters for cloacal EWL. In dehydrated desert iguanas *Dipsosaurus dorsalis*, an increase in plasma osmolality delays the onset and extent of panting, which induces a ‘right shift’ and blunting of the EWL–temperature response curve (Dupré and Crawford, 1985). While serum osmolality increased significantly in the dehydrated Gila monsters, it is unknown whether cloacal EWL in Gila monsters is similarly regulated by osmolality. However, since the presence of water in the urinary bladder might allow for water expenditure without changing plasma osmolality, regulation of cloacal EWL in Gila monsters might also be influenced by urinary bladder volume. While the results presented here do not provide insight into the underlying mechanisms or regulatory parameters involved in cloacal EWL, this study presents a previously undescribed means for controllable evaporative water loss and points out the possible importance of EWL for thermoregulation in ectotherms. The degree to which EWL can serve as a thermoregulatory mechanism depends on the availability of water (within both

the organism and the environment) and on the ability of the organism to regulate water loss. Further studies of this and other species are warranted to better understand how desert organisms trade off between thermostasis and hydrostasis.

We thank G. E. Walsberg for generously allowing us to use his equipment and for his valued input to the development of this study. We are grateful to C. A. Roeger and M. D. Wheeler for skillfully constructing the glass portions of the test chamber. We also thank D. D. Beck, G. E. Walsberg, and E. N. Taylor for providing insightful comments about earlier drafts of this manuscript. Lastly, we thank the Arizona Game and Fish Department for contributing the animals used in this study, and A. K. Mattlin for assisting in data collection and maintaining the animals during the study period. Support for this research was provided in part by grants from the Howard Hughes Institute and the ASU Office of the Provost through the Undergraduate Biology Enrichment Program, and from the National Science Foundation (IBN-0210804 to G. E. Walsberg). All work was approved by the ASU Institutional Animal Care and Use Committee (protocol # 01-617R).

References

Beck, D. D. (1990). Ecology and behavior of the Gila monster in southwestern Utah. *J. Herpetol.* **24**, 54-68.

Beck, D. D. and Jennings, R. D. (2003). Shelter-site selection and habitat use by Gila monsters. *Herpetol. Monogr.* **17**, 111-129.

Beuchat, C. A., Vleck, D. and Braun, E. J. (1986). Role of the urinary bladder in osmotic regulation of neonatal lizards. *Physiol. Zool.* **59**, 539-551.

Bogert, C. M. and Del Campo, R. M. (1956). The Gila monster and its allies. *Bull. Amer. Mus. Natur. Hist.* **109**, 1-238.

Braysher, M. and Green, B. (1970). Absorption of water and electrolytes from the cloaca of an Australian lizard, *Varanus gouldii* (Gray). *Comp. Biochem. Physiol.* **35**, 607-614.

Brown, D. E. and Carmony, N. B. (1991). *Gila monster: Facts and Folklore of America's Aztec Lizard*, 2nd Edition. Silver City, NM: High-Lonesome Books.

Calder, W. A. and King, J. R. (1974). Thermal and caloric relations of birds. In *Avian Biology*, vol. IV (ed. D. S Farner and J. R. King), pp. 260-413, New York: Academic Press.

Campbell, G. S. and Norman, J. M. (1998). *An Introduction to Environmental Biophysics*, 2nd Edition. New York: Springer.

Cheung, W. W. K. and Marshall, A. T. (1973). Water and ion regulation in cicadas in relation to xylem feeding. *J. Insect Physiol.* **19**, 1801-1816.

Cohen, A. C. (1975). Some factors affecting water economy in snakes. *Comp. Biochem. Physiol.* **51A**, 361-368.

Cooper, P. D. and Robinson, M. D. (1990). Water balance and bladder function in the Namib Desert sand dune lizard, *Aporosaura anchietae* (Lacertidae). *Copeia* **1990**, 34-40.

Crawford, E. C., Jr and Kampe, G. (1971). Physiological responses of the lizard *Sauromalus obesus* to changes in ambient temperature. *Am. J. Physiol.* **220**, 1256-1260.

Crompton, A. W., Taylor, C. R. and Jagger, J. A. (1978). Evolution of homeothermy in mammals. *Nature* **272**, 333-336.

Dantzler, W. H. and Schmidt-Nielsen, B. (1966). Excretion in fresh-water turtle (*Pseudemys scripta*) and desert tortoise (*Gopherus agassizii*). *Am. J. Physiol.* **210**, 198-210

Dawson, W. R. (1960). Physiological responses to temperature in the lizard *Eumeces obsuletus. Physiol. Zool.* **33**, 87-103.

Dawson, W. R. and Bartholomew, G. A. (1968). Temperature regulation and water economy of desert birds. In *Desert Biology*, vol. 1 (ed. G. W. Brown, Jr), pp. 357-394. New York: Academic Press.

Dawson, W. R. and Templeton, J. R. (1963). Physiological response to temperature in the lizard *Crotaphytus collaris*. *Physiol. Zool*. **36**, 219-236.

DeWitt, C. B. (1967). Precision of thermoregulation and its relation to environmental factors on the desert iguana, *Dipsosaurus dorsalis. Physiol. Zool.* **40**, 49-66.

Dmi'el, R. (1998). Skin resistance to evaporative water loss in viperid snakes: habitat aridity versus taxonomic status. *Comp. Biochem. Physiol.* **121A**, 1-5.

Dmi'el, R. (2001). Skin resistance to evaporative water loss in reptiles: a physiological adaptive mechanism to environmental stress or a phyletically dictated trait? *Israel J. Zool*. **47**, 55-67.

Dupré, R. K. and Crawford, E. C., Jr (1985). Control of panting in the desert iguana: roles of peripheral temperatures and the effect of dehydration. *J. Exp. Zool.* **235**, 341-347.

Eynan, M. and Dmi'el, R. (1993). Skin resistance to water loss in agamid lizards. *Oecologia* **95**, 290-294.

Flatau, P. J., Walko, R. L. and Cotton, W. R. (1992). Polynomial fits to saturation vapor pressure. *J. Appl. Meteorol.* **31**, 1507–1513.

Hayes, J. P. and Garland, T., Jr (1995). The evolution of endothermy: testing the aerobic capacity model. *Evolution* **49**, 836-847.

Hensel, H., Brück, K. and Raths, P. (1973). Homeothermic organisms. In *Temperature and Life* (ed. H. Prect, J. Christophersen, H. Hensel and W. Larcher), pp. 503-761. New York: Springer-Verlag.

Lasiewski, R. C., Acosta, A. L. and Bernstein, M. L. (1966). Evaporative water loss in birds. I. Characteristics of the open flow method of determination and their relation to estimates of thermoregulatory ability. *Comp. Biochem. Physiol.* **19**, 445–457.

Lowe, C. H., Schwalbe, C. R. and Johnson, T. B. (1986). *The Venomous Reptiles of Arizona.* Phoenix: Arizona Game and Fish Dept.

Mautz, W. J. (1982a). Patterns of evaporative water loss. In *Biology of the Reptilia*, Vol. 12 (ed. C. Gans), pp. 443-481. New York: Academic Press.

Mautz, W. J. (1982b). Correlation of both respiratory and cutaneous water losses of lizards with habitat aridity. *J. Comp. Physiol.* **149**, 25-30.

McNab, B. K. (1978). The evolution of homeothermy in the phylogeny of mammals. *Am. Nat.* **112**, 1-21.

Minnich, J. E. (1976). Water procurement and conservation by desert reptiles in their natural environment. *Isr. J. Med. Sci.* **12**, 854-861.

Schmidt-Nielsen, K. (1964). *Desert Animals: Physiological Problems of Heat and Water*. London: Oxford University Press.

Templeton, J. R. (1960). Respiration and water loss at the higher temperatures in the desert iguana, *Dipsosaurus dorsalis*. *Physiol. Zool*. **33**, 136-145.

Toolson, E. C. (1987). Water profligacy as an adaptation to hot deserts: water loss rates and evaporative cooling in the Sonoran Desert cicada, *Diceroprocta apache* (Homoptera: Cicadidae). *Physiol. Zool.* **60**, 379-385.

Warburg, M. R. (1965). The influence of ambient temperature and humidity on the body temperature and water loss from two Australian lizards, *Tiliqua rugosa* (Gray) (Scincidae) and *Amphibolurus barbatus* Cuvier (Agamidae). *Aust. J. Zool.* **13**, 331-350.

Winne, C. T., Ryan, T. J., Leiden, Y. and Dorcas, M. E. (2001). Evaporative water loss in two natricine snakes, *Nerodia fasciata* and *Seminatrix pygaea. J. Herpetol*. **35**, 129-133.