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The urinary bladder as a physiological reservoir that moderates dehydration in a large desert lizard, the Gila monster *Heloderma suspectum*

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Summary

Animals inhabiting xeric environments use a variety of behavioral and physiological strategies to balance water budgets. We studied the potential contribution of the urinary bladder to osmoregulation in a large desert lizard, the Gila monster *Heloderma suspectum*. Here we present results of a series of *in vivo* laboratory experiments which tested the hypothesis that the Gila monster urinary bladder serves as a physiological reservoir, as in amphibians and chelonians, providing water that buffers increases in plasma osmolality when food and water are unavailable. Adult Gila monsters absorbed water from the urinary bladder into circulation and absorption of water from the urinary bladder and drinking water provided similar osmoregulatory benefits within 24 h, although drinking water provided a more immediate osmotic benefit. During food and water deprivation, plasma osmolality increased 2.5 times faster in lizards with an empty urinary bladder compared with those with a full bladder. During rehydration, stereotyped binge drinking behavior increased body mass nearly 22%, which resulted in a 24% reduction in plasma osmolality and a substantial increase in bladder water within 24 h. These results support our hypothesis and demonstrate for the first time in an adult lizard that the urinary bladder can function as a long-term physiological water reservoir. This trait can provide a critical benefit to osmoregulation during the 2- to 3-month summer dry season characteristic of the deserts that Gila monsters inhabit.

Key words: water economy, hydrostasis, dehydration, osmoregulation, reptile.

Introduction

Water is a scarce, but critical resource for vertebrates in xeric environments; thus desert animals use numerous behavioral and physiological means to find, store, and economically use water. Mammals can effectively concentrate urine; however, the morphology of reptilian and amphibian nephrons does not facilitate the production of hyperosmotic urine. However, post-renal modification of urine occurs in the urinary bladder or cloaca of many reptilian and amphibian species, improving water economy (Shoemaker and Nagy, 1977; Bentley, 1979; Jorgensen, 1998). The use of the urinary bladder as a physiological reservoir – defined here as an anatomical location where water can be sequestered and later returned to general circulation – has been described and empirically studied in amphibians and chelonians for centuries (Townson, 1799; Darwin, 1839) (see also Jorgensen, 1998; Minnich, 1976; Shoemaker and Nagy, 1977). These and recent studies have shown that dilute urine stored in the urinary bladder of amphibians helps to offset relatively high cutaneous evaporative water loss (EWL) during terrestrial activity, and bladder volume can vary significantly on a fine (minutes to hours) or extended temporal scale (weeks to months) (Ruibal, 1962; McClanahan, 1967; Delson and Whitford, 1973; Sinsch, 1991; Jorgensen, 1994). Other studies examining the long-term osmoregulatory benefits of bladder water storage provide physiological explanations for classic examples of drought tolerance in chelonians (Minnich, 1976; Nagy and Medica, 1986; Peterson, 1996; Jorgensen, 1998) and toads (McClanahan, 1972; Bradshaw, 1997).

The use of the urinary bladder as a physiological reservoir is atypical in other taxa. Birds, crocodilians, and snakes lack a urinary bladder entirely (Bentley, 1979). Moreover, mammals are not known to use the urinary bladder as a physiological reservoir (Bentley, 1979). Because mammalian kidneys can produce hyperosmotic urine rich in urea, which does not readily precipitate, the bladder is impermeable to water and solutes to maintain the concentration of urine and primarily serves to store urine until micturition. Many lizards, like amphibians and chelonians, have a urinary bladder and all produce hypoosmotic urine containing nitrogenous waste as uric acid, which readily precipitates. Beuchat (Beuchat, 1986) described the presence of urinary bladders among lizards and concluded that no phylogenetic or habitat generalizations could be made regarding the occurrence of urinary bladders. Although the organ is relatively widespread among lizards, its functional significance has been examined only twice. Cooper and

Robinson suggested that the small lacertid lizard *Aporosaura anchietae* uses its diminutive urinary bladder (3% of body mass) as a physiological reservoir (Cooper and Robinson, 1990). The neonate *Sceloporus jarrovii*, a small phrynosomatid lizard, possesses an ephemeral urinary bladder that contains dilute urine at birth (36 mmol kg^{-1}; 14% of body mass) (Beuchat et al., 1986). The urine is absorbed over several days helping neonates maintain stable plasma osmolality, but no urine is added to the bladder, which degenerates into a non-functional vestigial organ when the fluid is exhausted (Beuchat et al., 1986). This work confirmed that a lizard was capable of using the urinary bladder as a physiological reservoir, yet the ephemeral bladder of *S. jarrovii* prevents this organ from contributing to survival of the adult animal. Thus, long-term osmoregulatory benefits of the urinary bladder in an adult lizard have not been demonstrated.

The Gila monster *Heloderma suspectum* is a large (adult body mass 350–600 g, snout–vent length 300–360 mm), long-lived (~20 years), venomous lizard that predominantly inhabits the Sonoran Desert of Arizona and Mexico (Bogert and Martín del Campo, 1956; Beck, 2005). The Gila monster provides an ideal model for studies examining the potential contribution of the urinary bladder to osmoregulation because it occupies xeric environments and uses a novel suite of behavioral, morphological and physiological strategies to survive (Beck and Jennings, 2003; DeNardo et al., 2004; Beck, 2005). Notably, despite having to forage long distances to locate widely dispersed prey (vertebrate nestlings and eggs) (Bogert and Martín del Campo, 1956; Beck, 2005), these lizards reduce exposure to the most desiccating conditions by behavioral avoidance. Gila monsters become predominantly nocturnal during the warmest months of the year (May–August), but because of a low preferred body temperature (29°C) (Bogert and Martín del Campo, 1956; Beck, 2005), may be thermally challenged by air temperatures that typically exceed 40°C at sunset and can remain above 35°C for several hours. Perhaps to cope with these high temperatures, Gila monsters have a relatively high EWL rate compared with other lizards of their size. In fact, the Gila monster uses cloacal evaporative cooling to reduce body temperature when it becomes critically elevated (>37.5°C) (DeNardo et al., 2004). Gila monsters also conserve water by foraging infrequently (3–17% of the time during dry months) and selecting thermally and hydrically favorable refugia when inactive (Beck, 1990; Beck and Jennings, 2003) (J.R.D. and D.F.D., unpublished). Despite this, the combination of environmental conditions and the natural history of the Gila monster produce considerable seasonal osmotic perturbations, resulting in a plasma osmolality increase from 290 mOsmol kg^{-1} to 360 mOsmol kg^{-1} between April and July in free-ranging Gila monsters (J.R.D. and D.F.D., unpublished).

The Gila monster possesses a relatively large urinary bladder (Beuchat, 1986) that we, using portable ultrasonography, have determined can be distended with large quantities of dilute urine for several weeks [up to 75 ml, 20% of body mass, 28 mOsmol kg^{-1} (J.R.D. and D.F.D., unpublished)]. Thus, we hypothesized that dilute urine stored in the urinary bladder of the Gila monster serves as a physiological reservoir, which provides water necessary to buffer increases in plasma osmolality during drought. We first determined whether the Gila monster urinary bladder is permeable to water by serially monitoring the radioactivity of plasma in anesthetized lizards given tritiated water $[^3H]_2O$ transurethrally into the urinary bladder. Second, we compared the relative rate of water absorption from the urinary bladder of dehydrated lizards by serially measuring plasma osmolality of dehydrated Gila monsters following introduction of water into the urinary bladder or stomach. We then evaluated an osmoregulatory role of dilute urine storage by comparing the rate of dehydration between lizards with full and empty urinary bladders during food and water deprivation. Finally, we quantified acute rehydration dynamics by comparing relevant variables (i.e. body mass, plasma osmolality, tail volume and urinary bladder dimensions) of dehydrated lizards to the same variables after 24 h free access to water.

Materials and methods

Wild-caught *Heloderma suspectum* (Cope 1869) were obtained from the Arizona Game and Fish Department and held under permit # SP627813. All experiments were conducted at 25°C and in accordance with Arizona State University's Institutional Animal Care and Use Committee (IACUC) under protocol 783R.

Tritiated water absorption

Seven adult Gila monsters (mean mass=534 g; range=481–644 g) were anesthetized by delivering 2% isoflurane through an endotracheal tube until a surgical plane of anesthesia was established. Once anesthetized, the lizard was placed in dorsal recumbency and its urinary bladder catheterized. First, the vent was opened with a vaginal speculum and then a #14 fr. Foley catheter was inserted through the cloaca and directed dorsally into the colon. Once in the colon, a 60 ml syringe was attached to the catheter port, slight negative pressure was applied to the syringe, and proper catheter placement was verified by observing fecal matter in the catheter. The catheter cuff was then inflated with 2–3 ml deionized water, the catheter was pulled gently to partially evert the cloaca and allow visualization of the urethral opening, and the catheter was secured to the tail with surgical tape. A #8 fr. Foley catheter was inserted through the urethral opening and into the urinary bladder. To verify proper positioning, a 60 ml syringe was attached to the catheter, gentle negative pressure was applied, and urine and urates or both were observed in the catheter. The catheter cuff was inflated with 2–3 ml distilled water and gently pulled caudally until it no longer moved to seal the neck of the urinary bladder. The catheter was taped to the lizard's tail and the colonic catheter was then deflated and removed. Water can be absorbed from the cloaca of some species (Peaker et al., 1968; Braysher and Green, 1970) and this would confound the results of this

experiment; thus ultrasonography (Concept/MLV; Dynamic Imaging, Ltd, Livingston, Scotland, UK) and a visual inspection were used during infusion of $[^3H]_2O$ into the bladder and subsequent blood collections to verify that the catheter sealed the opening of the cloaca and prevented urine flow from the urinary bladder into the cloaca.

Experimental infusion medium was created by diluting 1.5 g ^3H (1 mCi g^{-1}; Moravek Biochemicals, Inc., Brea, CA, USA) in 103.5 ml nanopure water. A 60 ml syringe was used to infuse 20 ml of the $[^3H]_2O$ medium directly into the urinary bladder *via* the catheter and then left attached to the catheter port to prevent backflow into the catheter. Heparinized 1 ml syringes were used to collect 0.25 ml blood samples from the caudal vein of each lizard <10 min before treatment (time 0) and 30, 60 and 90 min following infusion of $[^3H]_2O$ medium. After collecting the 90 min sample, the remaining fluid was removed from the urinary bladder using the attached 60 ml syringe and then the catheter cuff was deflated and catheter removed. Anesthetic was discontinued and the lizard was placed on a warm heating pad until fully recovered. Within 15 min of blood collection, blood samples were centrifuged to separate plasma from whole blood and each plasma sample was divided into three 20 μl aliquots. Three 20 μl aliquots of $[^3H]_2O$ medium were also collected to determine radioactivity (counts per minute; c.p.m.) infused into the urinary bladder. Aliquots were frozen in 5 ml scintillation vials at –80°C until analyzed. Aliquots were thawed, 4 ml scintillation fluid (ScintSafe +50; Fisher Scientific International, Pittsburgh, PA, USA) added to each vial, and the radioactivity of each sample measured using a Beckman 1500 liquid scintillation counter (Beckman Coulter, Inc., Fullerton, CA, USA).

Data analysis

All data were subjected to tests of normality and homogeneity of variances prior to inference and statistical analyses were completed using JMP IN (Version 5.1, SAS Institute, Inc., Cary, NC, USA). Alpha-level was 0.05 unless noted. The mean counts min^{-1} (c.p.m.) of each triplicate sample was calculated and a repeated-measures analysis of variance (RM-ANOVA) model was used to analyze differences in plasma radioactivity among individuals and over time, with individual as the between-subjects factor, time as the repeated measure, and plasma radioactivity as the dependent variable. Mauchly's Criterion for Sphericity was violated; therefore a Greenhouse–Geisser correction was applied prior to interpretation (Zar, 1999). Tukey–Kramer tests (adjusted for experiment-wise Type I error rate; $\alpha=0.05/N$) were used *post hoc* to identify significant differences in mean plasma radioactivity at each time point.

Relative rehydration rate

Twelve adult Gila monsters (mean mass=537 g; range=463–676 g) were dehydrated in the laboratory by withholding food and water until each animal reached 80% of its initial body mass (iM_b). Once at 80% iM_b, each lizard was randomly assigned to one of two treatment groups (N=6) using a random numbers generator: (1) bladder (BLDR) – 30 ml deionized water were introduced into the urinary bladder *via* a transurethral catheter (placed as described above), and (2) Oral (ORAL) – 30 ml deionized water were introduced into the stomach *via* an intragastric tube. All treatments were carried out under anesthetic as described above. For both treatments, 0.25 ml blood was collected from the caudal vein using heparinized 1 ml syringes on day 1 of dehydration (pre-dehydration), immediately before introduction of water (time 0), and while anesthetized at 30, 60 and 90 min post-infusion. Animals were allowed to recover from anesthesia as described above and additional 0.25 ml blood samples were collected and processed at 24 and 48 h post-infusion. Within 1 h of collection, plasma was separated from whole blood using centrifugation and samples were frozen at –80°C until analyzed.

Plasma samples were thawed and osmolality (mOsmol kg^{-1}) of 20 μl samples was determined in triplicate using vapor pressure osmometry (model 5500xr; Wescor, Inc., Logan, UT, USA). Prior to use, the osmometer was calibrated using the three-step factory recommended calibration procedure and sealed osmolality standards (290 and 1000 mOsmol kg^{-1}). Additionally, a pooled plasma sample was collected from several captive Gila monsters not included in this study, its osmolality was measured immediately after calibration, and this pooled sample was used to check for osmometer variation every 40–50 samples. If pooled sample osmolality varied more than the limits of the osmometer (±6 mOsmol kg^{-1}), the osmometer head was cleaned, the osmometer recalibrated, and the pooled sample re-analyzed before continuing triplicate analysis beginning with the last sample prior to cleaning the head to verify correct measurement of the sample.

Data analysis

The mean plasma osmolality of each triplicate was calculated and differences in plasma osmolality between groups and over time were analyzed using a RM-ANOVA model with treatment as the between-subjects factor, time as the repeated measure, and plasma osmolality as the dependent variable. Mauchly's Criterion for Sphericity was met for this analysis. Tukey–Kramer tests (corrected for experiment-wise Type I error rate; $\alpha=0.05/N$) were used *post hoc* to identify significant differences at each time point between treatment groups. Finally, paired *t*-tests were used to compare final (48 h) osmolality to pre-dehydration osmolality for each treatment to verify whether animals' plasma osmolality returned to initial, unmanipulated levels.

Bladder water contribution to osmoregulation

To evaluate an osmoregulatory role of dilute urine stored in the urinary bladder, the osmoregulatory abilities of Gila monsters with and without a full urinary bladder were compared. Pilot studies showed that artificially filling the urinary bladder *via* a transurethral catheter as described above may cause lizards to void the bladder prematurely, possibly due to irritation caused by the catheter. Therefore, ultrasonography

was used to assess urinary bladder condition (full or empty) of Gila monsters housed in our laboratory and the first six animals with large distended urinary bladders (minimum 30 mm length and 10 mm depth) were assigned to one treatment group (FULL) and the first six lizards with empty urinary bladders (no fluid detectable in bladder) to another (EMT).

For the duration of the study, lizards were housed in pairs in glass-fronted plastic terraria (100 cm×50 cm×30 cm; Vision Products, Canoga Park, CA, USA) with room temperature (22°C), subsurface heat, and a 75 W overhead light bulb providing a heterogeneous thermal environment ranging from 22 to 45°C. Lighting was programmed to produce a 07:00 h–19:00 h photophase. To induce dehydration in both treatment groups, seasonal drought conditions typical of deserts inhabited by Gila monsters (Beck, 2005) (below), were simulated by depriving the lizards of food and water. During assignment to treatment groups and every 2 weeks thereafter, lizards were weighed (±0.1 g; Acculab GS-2001 electronic scale, Edgewood, NY, USA), tail volume was measured (±1 ml; water displacement) as an index of energy storage because this species primarily stores fat caudally (Bogert and Martín del Campo, 1956; Beck, 2005), 0.2 ml blood was collected from the caudal vein using a heparinized 1 ml syringe, and cranial–caudal length and dorsal–ventral depth (±0.1 mm) of the urinary bladder were measured using ultrasonography to determine when fluid was expended. Blood samples were processed using osmometry as described above and data were collected until individual lizards reached a plasma osmolality of 360 mOsmol kg^{-1}; plasma osmolality becomes elevated to this level in free-ranging Gila monsters during seasonal drought in the Sonoran Desert population that has been studied by our group for several years. When each animal reached this dehydrated state, final measurements were collected and water was provided *ad libitum* (see rehydration dynamics below).

Data analysis

Initial and final standard body mass (total mass – mass of stored urine), tail volume and plasma osmolality between FULL and EMT treatment groups were compared using Student's *t*-tests. Paired *t*-tests were used to compare initial and dehydrated plasma osmolality within treatment groups. The overall osmoregulatory benefit of dilute urine stored in the urinary bladder was assessed by using *t*-tests to compare the number of days required for each lizard to reach 360 mmol kg^{-1}. Because FULL lizards took varying times to expend urinary bladder water and all lizards took varying times to reach 360 mmol kg^{-1}, the daily rate of plasma osmolality change (change in osmolality per day; ΔOsmol day^{-1}) was compared over three subsets of the deprivation period. First, all FULL lizards took at least 28 days to expend bladder water, thus FULL and EMT lizards' ΔOsmol day^{-1} during the first 28 days of the study were compared using a *t*-test. Once FULL lizards absorbed all bladder water, ΔOsmol day^{-1} was predicted to increase substantially and be similar to that of EMT lizards. Thus, paired *t*-tests were used to compare the initial ΔOsmol day^{-1} of the FULL lizards to the ΔOsmol day^{-1} of the same lizards during the 28 d following confirmation that the bladder was empty. Finally, ΔOsmol day^{-1} of FULL lizards with empty urinary bladders and EMT lizards were compared using a *t*-test.

Rehydration dynamics

Following dehydration to evaluate an osmoregulatory role of bladder water (described above), rehydration of eleven of the Gila monsters was scrutinized to identify water storage locations and determine the volume of water consumed and its effects on plasma osmolality. One EMT lizard was excluded from this study because it dehydrated first, was provided *ad libitum* water, and immediately displayed a stereotyped binge-drinking behavior, which prompted this assessment of rehydration dynamics. Final dehydrated measurements of body mass, tail volume, plasma osmolality, and urinary bladder dimensions were made as described above. Lizards were provided with tapwater (28 mOsmol kg^{-1}) *ad libitum* and after 24 h, each measurement was repeated (rehydrated). Because only length and depth (not width) of urinary bladders were measured, fluid presence could be verified, but accurate estimates of volume could not be calculated.

Data analysis

There were no treatment or sex differences in the following analyses, so data were pooled and comparisons between dehydrated and rehydrated conditions were made. The total volume of water consumed in 24 h was calculated by subtracting individuals' rehydrated mass from dehydrated mass (1 ml water=1 g) and a paired *t*-test was used to compare body mass of dehydrated lizards to rehydrated lizards. The osmotic benefit of consumed water was assessed by comparing plasma osmolality of dehydrated to that of rehydrated lizards using a paired *t*-test. The relationship between volume of water consumed and change in osmolality was analyzed using linear regression. Because dehydrated body mass and volume of water consumed were correlated (r^2=0.48; P=0.011), the relative proportion that mass and osmolality changed was calculated (value of change/dehydrated value) for each individual and used for regression analysis. Potential water storage locations were evaluated by comparing lizards' tail volume (paired *t*-test) and urinary bladder condition when dehydrated and rehydrated. Finally, *post-hoc* comparisons of rehydrated and initial (pre-dehydration) mass, plasma osmolality, and tail volume were made using paired *t*-tests to assess the degree to which lizards rebounded from these dehydration-associated changes.

Results

Tritiated water absorption

Mean plasma radioactivity varied significantly over the sampling period (time effect: $F_{1,2}$=20.95, P=0.045) following infusion of 17 000 c.p.m. at time 0. However, both the individual ($F_{4,2}$=1.29, P=0.48) and time-individual interaction

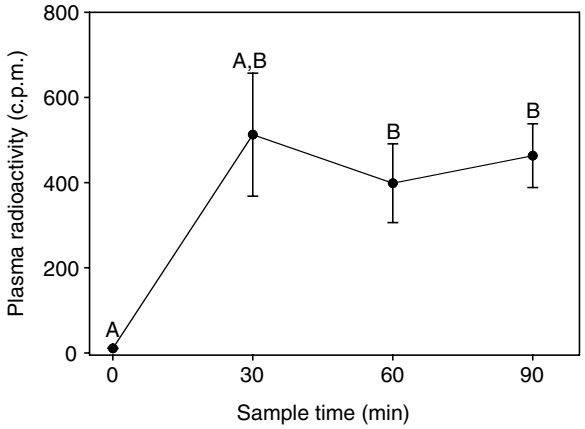

Fig. 1. Mean increase in plasma radioactivity following infusion of 17 000 c.p.m. $[^3H]_2O$ transurethrally into the urinary bladder of *H. suspectum* at time 0. Values shown are means ± 1 s.e.m. of seven lizards. Different letters denote statistically significant differences between time points.

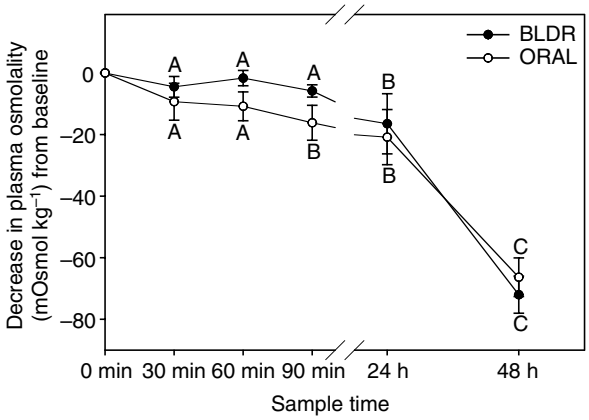

Fig. 2. Relative rehydration rate of dehydrated *H. suspectum* following introduction of 30 ml water orally (ORAL) or into the bladder (BLDR). Absolute changes in plasma osmolality is plotted to clarify relative ORAL to BLDR rehydration rates because initial osmolality differed slightly between groups. Values shown are means ± 1 s.e.m. of six lizards per treatment. Different letters denote significantly different values over time and between groups.

($F_{4,2}$=1.45, P=0.44) were not significant (Fig. 1). Tukey's *post-hoc* analyses indicated that plasma radioactivity was not elevated significantly above baseline until 60 min post-infusion (t=3.48, d.f.=6, P=0.013; t=4.20, d.f.=6, P=0.006; t=6.06, d.f.=6, P=0.0009, at 30, 60 and 90 min, respectively) and plasma radioactivity was stable at 30, 60 and 90 min (P>0.08; Fig. 1).

Relative absorption rate

The RMANOVA model detected a significant effect of time on plasma osmolality ($F_{5,6}$=31.72, P=0.0003). The treatment effect ($F_{1,10}$=3.89, P=0.077) and treatment–time interaction ($F_{5,6}$=0.52, P=0.75) were not significant, indicating that the plasma osmolality of the two groups did not change differently over time (Fig. 2). *Post-hoc* analyses indicated that plasma osmolalities of ORAL and BLDR treatment groups were similar at 30 and 60 min (P>0.05), but the ORAL group plasma osmolality had decreased significantly further below baseline than the BLDR group by 90 min (t=2.86, d.f.=10, P=0.017). Within 24 h, however, plasma osmolalities following both treatments had decreased similarly (t=0.83, d.f.=10, P=0.43) and significantly below baseline (ORAL: t=4.29, d.f.=5, P=0.008; BLDR: t=3.99, d.f.=5, P=0.01). At 48 h, plasma osmolalities were still similar (t=0.38, d.f.=10, P=0.71) and significantly lower than baseline (ORAL: t=22.32, d.f.=5, P<0.0001; BLDR: t=9.86, d.f.=5, P=0.0002), and neither differed from pre-dehydration osmolalities (ORAL: t=–1.49, d.f.=5, P=0.20; BLDR: t=–1.25, d.f.=5, P=0.27; Fig. 2).

Bladder water contribution to osmoregulation

Despite non-random assignment of treatments, treatment groups did not differ in standard body mass, plasma osmolality, or tail volume, initially or following dehydration, suggesting that groups were suitable for comparison, and dehydration protocol affected FULL and EMT treatment groups similarly. Notably, there was a significant difference in the number of days FULL and EMT lizards took to reach 360 mOsmol kg^{-1} plasma osmolality; FULL lizards took nearly 2.5 times longer than EMT lizards (78.5 *versus* 33.3 days; t=–3.19, d.f.=10, P=0.0096; Table 1). Daily rates of plasma osmolality increase varied depending on urinary bladder condition such that osmolality of FULL lizards initially increased significantly slower than EMT lizards (ΔOsmol day^{-1}=0.64 *vs* 1.91; t=4.02, d.f.=10, P=0.002; Fig. 3). Moreover, once FULL lizards absorbed all urinary bladder fluid, their ΔOsmol day^{-1} nearly doubled, but remained significantly below that of EMT lizards (ΔOsmol day^{-1}=1.13 *vs* 1.91; t=3.03, d.f.=10, P=0.013) and was not different from initial FULL values (ΔOsmol day^{-1}=1.13 *vs* 0.64; t=–1.63, d.f.=5, P=0.16). These data support the hypothesis that dilute urine stored in the urinary bladder contributes to osmoregulation by moderating dehydration during resource restriction.

Rehydration dynamics

After 24 h *ad libitum* access to water, dehydrated lizards' body masses had increased significantly (295 *vs* 378 g; t=–14.8, d.f.=10, P<0.0001; Table 2), indicating that the mean volume of water imbibed was 83 ml (range: 54–112 ml). Plasma osmolality decreased significantly (359.2 *vs* 289.4 mmol kg^{-1} day^{-1}; t=28.3, d.f.=10, P<0.0001; Table 2) and the change in plasma osmolality was inversely related to the volume of water consumed (r^2=0.46; P=0.022), indicating that lizards that drank more water decreased plasma osmolality more. Both mass and osmolality of rehydrated lizards were similar to initial, pre-dehydration values (mass: t=1.62, d.f.=10, P=0.13; osmolality: t=–0.67, d.f.=10, P=0.51), but tail volume remained significantly below initial values (tail volume: t=6.03, d.f.=10, P=0.0001; Table 2). Tail volume did not differ between dehydrated and rehydrated lizards (36 *vs* 37 ml;

Table 1. *Summary of initial and final body condition indicators of Gila monsters (*Heloderma suspectum*) and the number of days required for them to reach an ecologically relevant dehydration state having either a full or empty urinary bladder*

	Days to dehydrated plasma osmolality	Standard body mass (±1 g)	Tail volume (±1 ml)	Plasma osmolality (mOsmol kg^{-1})
Initial:				
FULL	–	366±89 (269–506)	51±8 (43–62)	290±5 (284–298)
EMT	–	330±52 (264–383)	43±11 (30–57)	293±2 (291–296)
d.f.	–	10	10	10
t-statistic	–	–1.22	–1.30	1.21
P value	–	0.25	0.22	0.25
Final:				
FULL	79±8 (41–120)	307±25 (221–395)	38±4 (27–50)	358±3 (351–372)
EMT	33±5 (29–41)	267±18 (204–327)	35±3 (24–44)	362±2 (354–370)
d.f.	10	10	10	10
t-statistic	–3.19	–1.30	–0.40	1.09
P value	*0.01	0.22	0.70	0.30

FULL, animals with a full bladder at the start of the experiment; EMT, animals with an empty bladder at the start of the experiment.
Values shown are mean ± 1 s.e.m. (minima and maxima in parentheses); *N*=6 per treatment.
*Significant difference (α=0.05) between treatment groups.

t=–1.69, d.f.=10, *P*=0.121; Table 2). By contrast, copious fluid was observed in the urinary bladder of each animal just 24 h after the urinary bladder had been empty. Although bladder volume could not be accurately estimated, the length (52.5 mm) and depth (16.1 mm) of the urinary bladder increased substantially (Table 2).

Discussion

Tritiated water absorption and relative absorption rate

The Gila monster urinary bladder can function as a physiological reservoir because it is permeable to water, and long-term urine storage suggests that bladder membrane permeability is likely a regulated process. Plasma radioactivity of all lizards increased significantly and similarly within 60 min and then remained stable. This result verified that individuals absorbed water from the urinary bladder into circulation over time at approximately the same rate and suggested that bladder fluid and plasma reached equilibrium within 30–60 min (Fig. 1). Though initially not as rapid, absorption of water from the urinary bladder relieved dehydration at 24 and 48 h as effectively as absorption from the gastrointestinal tract (Fig. 2). These data suggest that drinking water provides a more immediate hydric benefit to Gila monsters, yet absorption of dilute urine from the urinary bladder can provide osmoregulatory benefits over 24–48 h equaling those associated with drinking. Rehydration naturally occurs in Gila monsters exclusively *via* water uptake from the gastrointestinal tract; however our data demonstrate that unlike the impermeable mammalian bladder, the Gila monster urinary bladder is not a permanent barrier to water absorption. Whether permeability to water is characteristic of the lizard urinary bladder is unknown. In other taxa possessing a permeable urinary bladder (e.g. anurans and chelonians), permeability is often regulated in response to water availability by insertion of aquaporins; however regulatory mechanisms controlling urinary bladder permeability in lizards are unknown and deserve study.

Bladder water contribution to osmoregulation

Absorption of water stored in the urinary bladder provided a direct osmoregulatory benefit to Gila monsters by significantly delaying plasma osmolality elevation when food and water were unavailable. In fact, the plasma osmolality of the EMT group increased at nearly triple the initial rate of the FULL group, and, although in the FULL group the rate nearly doubled when bladder fluid was expended, plasma osmolality

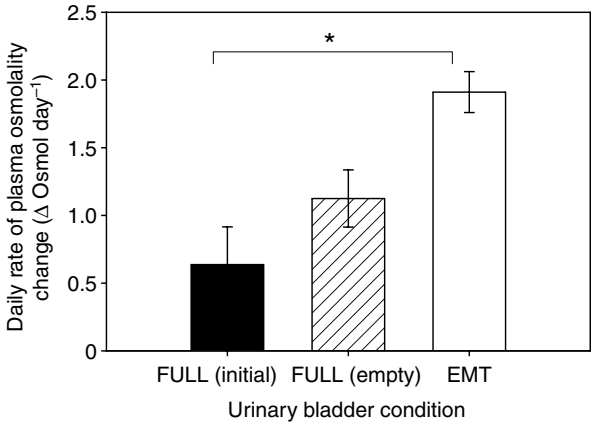

Fig. 3. Comparison of the daily rate of plasma osmolality increase during food and water deprivation of *H. suspectum* under three conditions: (1) full urinary bladder initially (FULL initial; filled bar), (2) following complete depletion of the same lizards' full urinary bladder (FULL empty; hatched bar), and empty urinary bladder initially (EMT; open bar). Values shown are means ± 1 s.e.m. of six lizards per group. *Significant differences in daily rate between conditions.

Table 2. *Summary of rehydration dynamics for 11 Gila monsters (Heloderma suspectum) when dehydrated to an ecologically relevant plasma osmolality and then provided water ad libitum for 24 h*

	Body mass (±0.1 g)	Plasma osmolality (mOsmol kg^{-1})	Tail volume (±1 ml)	Urinary bladder length (±0.1 mm)	Urinary bladder width (±0.1 mm)
Initial	401.0±29.1 (284–298)	292±1 (284–298)	48±3 (30–62)	–	–
Dehydrated	294.8±15.1*,† (221–395)	359±2*,† (351–372)	36±2* (23–50)	0	0
Rehydrated	378.1±19.6 (286–507)	289±3 (275–311)	37±2‡ (25–48)	52.5±3.1 (41.6–76.2)	16.1±1.6 (9.7–28.1)

Rehydrated values are also compared to initial, pre-dehydration values for the same animals.
Values shown are mean ± 1 s.e.m. (range in parentheses).
*Significant difference (P<0.05) between initial and dehydrated groups.
†Significant difference (P<0.05) between dehydrated and rehydrated groups.
‡Significant difference (P<0.05) between initial and rehydrated groups.

of lizards in the FULL group still increased at only 60% of the EMT rate. Over time, these differences enabled lizards in the FULL group to delay significant osmotic perturbation 135% longer than those in the EMT group (79 *vs* 33 days; Table 1). Delaying dehydration may have broad implications as dehydration can cause hypotension and increased plasma viscosity and hematocrit, which can negatively affect oxygen and nutrient transport, metabolism and muscle coordination (Etzion et al., 1984; Wilson and Havel, 1989; Rogowitz et al., 1999; Hochachka and Somero, 2002). Moreover, hydration state may influence the thermal biology (Baker, 1989; Ladyman and Bradshaw, 1989; Plummer et al., 2003), activity patterns (McClanahan, 1967; Nagy and Medica, 1986; Lorenzon et al., 1999) and reproductive output (Vleck and Priedkalns, 1985; Coe and Rottenberry, 2003) of animals, all of which can affect fitness. Whether hydration state influences critical physiological and behavioral processes in free-ranging Gila monsters remains to be clarified.

In addition to providing a direct osmoregulatory advantage, the physiological reservoir may benefit other critical processes by balancing hydric costs associated with physiological trade-offs. For example, energetic demands may stimulate Gila monsters to leave refugia where EWL and heat load are reduced (Beck and Jennings, 2003) to forage for widely dispersed prey, which exposes these lizards to xeric surface conditions for long activity periods, exacerbating EWL (Mautz, 1982; DeNardo et al., 2004). This critical surface activity can expose Gila monsters to temperatures substantially above their 29°C preferred body temperature (Bogert and Martín del Campo, 1956; Beck, 2005). If necessary, Gila monsters can reduce body temperature *via* EWL. EWL rates of Gila monsters are affected by hydration state (DeNardo et al., 2004), but whether reservoir volume can also influence EWL is unknown. Some toads are able to assess bladder volume and preemptively respond to decreased bladder volume by increasing water absorption behavior (Tran et al., 1992) and water flow across the seat patch (Parsons et al., 1993). Identifying a similar mechanism in lizards in which bladder condition is monitored and water-conserving responses (e.g. reduced EWL) preempt changes in plasma osmolality could help elucidate the dynamic condition-dependent links between competing fundamental physiological processes (e.g. energy acquisition, thermoregulation and osmoregulation).

In nature, the Gila monster experiences seasonal drought and high temperatures throughout much of its range. For example, between late-March and mid-July at our Sonoran Desert field site 50 km north-northwest of Tucson, AZ, USA, drought averaged 95 days with less than 2.0 mm rainfall (4 yr mean). From mid-May to mid-July, drought conditions were exacerbated by relatively high mean daily minimum (21.3°C) and maximum (39.2°C) air temperatures (1 m above ground). Based on our results, we predict that a Gila monster beginning this seasonal drought with a voluminous urinary bladder would likely experience little change in plasma osmolality initially and then a gradual plasma osmolality increase once the bladder is empty (Fig. 3). If laboratory conditions sufficiently simulated field conditions, this perturbation would take about 80 days (Table 1) and result in a plasma osmolality of free-ranging Gila monsters that will remain relatively stable March–April, peak just prior to the start of summer monsoon rains in July, and return to March–April levels in August–September during the rainy season. Peterson (Peterson, 1996) documented a similar seasonal plasma osmolality pattern in a Mojave Desert population of desert tortoises (*Gopherus agassizii*) and found that tortoises balanced water and electrolyte budgets annually by storing water to endure drought and capitalizing on seasonal rainfall and vegetation when available. Because Gila monsters inhabit seasonal habitats, are capable of ample water and energy storage, and can endure significant physiological perturbations, we predict that Gila monsters also balance water and energy budgets annually, rather than daily or weekly.

Lizards beginning seasonal drought with no bladder water probably face considerable osmotic challenges. We predict that plasma osmolality of lizards starting with no bladder water would increase rapidly (Fig. 3), resulting in elevated plasma osmolality that may reach 360 mOsmol kg^{-1} after just 1 month (Table 1). If drought persists, lizards may be unable to tolerate further elevation of plasma osmolality and may die. Under similar conditions in the Mojave Desert, a significant increase in *G. agassizii* mortality was attributed to sustained drought (Longshore et al., 2003). The duration of drought and reliability of seasonal rainfall clearly play an important role in these and many other organisms' lives. Elucidating consequences of forecast shifts in the timing of precipitation associated with global

climate change provides a timely and much needed avenue for further investigation, particularly in xeric environments.

Storing a large volume of water in the coelomic cavity may also have negative consequences. For example, similar coelomic cavity space is needed to accommodate the increased mass and volume associated with reproduction or consumption of a large meal, both of which can have deleterious effects on locomotor performance (Seigel et al., 1987; Shine, 2003) or predator avoidance (Shine, 1980; Lee et al., 1996; Veasey et al., 2001). It is unknown whether a fluid-filled bladder has similar deleterious effects on locomotion or anti-predator behavior, which could be critical in widely foraging predators like the Gila monster.

Rehydration dynamics

Following dehydration, binge-drinking enabled Gila monsters to significantly increase body mass, reduce plasma osmolality, and begin replenishing the physiological reservoir. Lizards imbibed enough water to increase body mass nearly 22%, causing a concomitant 24% decrease in plasma osmolality, which returned osmolality to baseline values (Table 2). Moreover, binge-drinking may contribute to future osmoregulation as each lizard's urinary bladder was distended with fluid after just 24 h. This result demonstrates the Gila monster's exceptional ability to capitalize on unpredictable and infrequent water availability in order to rapidly balance its long-term water deficit and prepare for future drought. In nature, Gila monsters may rehydrate and replenish the physiological reservoir by responding to rainfall and drinking from ephemeral pools as documented in *G. agassizii* (Nagy and Medica, 1986). In fact, we have noted a widespread and rapid increase in surface activity in response to the first summer monsoon rainfall (13 of 16 and 17 of 20 lizards were surface active during the first monsoon rains of 2004 and 2006, respectively). Together, these observations support the notion that Gila monsters may use temporary pools to rehydrate and suggest that lizards can rapidly reduce plasma osmolality and begin replenishing the physiological reservoir.

Water is clearly stored in the urinary bladder, however, caudal water storage seems unlikely in Gila monsters because tail volume did not differ significantly between dehydrated and rehydrated lizards (Table 2). Instead, our data support the idea that the tail is a primary lipid storage location because tail volume decreased significantly following food and water deprivation (Table 1) and although rehydrated body mass approached initial values, consumption of water did not cause tail volume to rebound from dehydrated values (Table 2). Thus, we postulate that monsoon rains provide Gila monsters two temporally distinct critical benefits. Immediately, monsoons may enable Gila monsters to balance long-term water budgets by decreasing osmolality and increasing stored water volume. Subsequently, monsoons stimulate reproduction of prey species, providing eggs and nestlings that Gila monsters consume and assimilate to increase tail volume and contribute to annual energy budgets.

Conclusions

Our data support the hypothesis that dilute urine stored in the urinary bladder of the Gila monster serves as a physiological reservoir, which allows Gila monsters to manage water budgets on a long-term (months) rather than short-term (daily) basis. Gila monsters store large volumes of dilute urine in the bladder and absorb it into circulation to delay dehydration during food and water deprivation. Furthermore, dehydrated Gila monsters can capitalize on infrequent water availability by binge drinking, which enables these lizards to rapidly rehydrate and replenish urinary bladder volume. Some reptiles and amphibians use the urinary bladder as a physiological reservoir to cope with xeric conditions (e.g. Ruibal, 1962; McClanahan, 1967; Minnich, 1976; Beuchat et al., 1986; Peterson, 1996; Jorgensen, 1998). Our study, however, is the first to verify that an adult lizard uses its urinary bladder as a physiological reservoir to moderate dehydration.

List of abbreviations

BLDR	treatment group in which 30 ml deionized water was introduced into the urinary bladder
EMT	treatment group beginning the experiment with an empty urinary bladder
EWL	evaporative water loss
FULL	treatment group beginning the experiment with a full urinary bladder
iM_b	initial body mass
ORAL	treatment group in which 30 ml deionized water was introduced into the stomach
RM-ANOVA	repeated-measures analysis of variance

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References

Baker, M. A. (1989). Thermoregulation in dehydrated vertebrates. In *Milestones in Environmental Physiology* (ed. M. K. Yousef), pp. 101-107. The Hague, Netherlands: SPB Academic.

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

Beck, D. D. (2005). *The Biology of Gila Monsters and Beaded Lizards*. Berkley: University of California Press.

Beck, D. D. and Jennings, R. D. (2003). Habitat use by Gila monsters: the importance of shelters. *Herpetol. Monogr.* **17**, 111-129.

Bentley, P. J. (1979). The vertebrate urinary bladder: osmoregulatory and other uses. *Yale J. Biol. Med.* **52**, 563-568.

Beuchat, C. A. (1986). Phylogenetic distribution of the urinary bladder in lizards. *Copeia* **1986**, 512-517.

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 Martín del Campo, R. (1956). The Gila monster and it allies. *Bull. Am. Mus. Nat. Hist.* **109**, 1-238.

Bradshaw, S. D. (1997). *Homeostasis in Desert Reptiles* (ed. J. L. Cloudsley-Thompson). Berlin: Springer-Verlag.

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

Coe, S. J. and Rottenberry, J. T. (2003). Water availability affects clutch size in a desert sparrow. *Ecology* **12**, 3240-3249.

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.

Darwin, C. (1839). *The Voyage of a Nauralist Around the World in H.M.S. Beagle*. London: Routledge.

Delson, J. and Whitford, W. G. (1973). Adaptation of tiger salamander, *Ambystoma tigrinum*, to arid habitats. *Comp. Biochem. Physiol.* **46**, 631-638.

DeNardo, D. F., Zubal, T. E. and Hoffman, T. C. M. (2004). Cloacal evaporative cooling: a previously undescribed means of increasing evaporative water loss at higher temperatures in a desert ectotherm, the Gila monster, *Heloderma suspectum*. *J. Exp. Biol.* **207**, 945-953.

Etzion, Z., Meyerstein, N. and Yagil, R. (1984). Tritiated-water metabolism during dehydration and rehydration in the camel. *J. Appl. Physiol.* **56**, 217-220.

Hochachka, P. W. and Somero, G. N. (2002). Water-solute adaptations: the evolution and regulation of the internal milieu. In *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*, pp. 217-289. Oxford: Oxford University Press.

Jorgensen, C. B. (1994). Robert Townson: observations on amphibian water economy revived. *Comp. Biochem. Physiol.* **109A**, 325-334.

Jorgensen, C. B. (1998). Role of urinary and cloacal bladders in chelonian water economy: historical and comparative perspectives. *Biol. Rev.* **73**, 347-366.

Ladyman, M. and Bradshaw, D. (2003). The influence of dehydration on the thermal preferences of the western tiger snake, *Notechis scutatus*. *J. Comp. Physiol.* **173B**, 239-246.

Lee, S. J., Witter, M. S., Cuthill, I. C. and Goldsmith, A. R. (1996). Reduction in escape performance as a cost of reproduction in gravid starlings, *Sturnus vulgaris*. *Proc. R. Soc. Lond. B Biol. Sci.* **263**, 619-623.

Longshore, K. M., Jaeger, J. R. and Sappington, J. M. (2003). Desert tortoise (*Gopherus agassizii*) survival at two eastern Mojave Desert sites: death by short-term drought? *J. Herpetol.* **37**, 169-177.

Lorenzon, P., Clobert, J., Oppliger, A. and John-Alder, H. (1999). Effect of water constraint on growth rate, activity and body temperature of yearling common lizard (*Lacerta vivipara*). *Oecologia* **118**, 423-430.

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

McClanahan, L. L. (1967). Adaptations of the spadefoot toad, *Scaphiopus couchi*, to desert environments. *Comp. Biochem. Physiol.* **20**, 73-99.

McClanahan, L. L. (1972). Changes in body fluids of burrowed spadefoot toads as a function of soil water potential. *Copeia* **1972**, 209-216.

Minnich, J. E. (1976). Water procurement and conservation by desert reptiles in their natural environment. *Isr. J. Med. Sci.* **12**, 740-758.

Nagy, K. A. and Medica, P. A. (1986). Physiological ecology of desert tortoises in southern Nevada. *Herpetologica* **42**, 73-92.

Parsons, R. H., McDevitt, V., Aggerwal, V., LeBlang, T., Manley, K., Kim, N., Lopez, J. and Kenedy, A. (1993). Regulation of pelvic patch water flow in *Bufo marinus*: role of bladder volume and Ang II. *Am. J. Physiol.* **264**, R1260-R1265.

Peaker, M., Wright, A., Peaker, S. J. and Phillips, J. G. (1968). Absorption of tritiated water by cloaca of domestic duck (*Anas Platyrhynchos*). *Physiol. Zool.* **41**, 461-465.

Peterson, C. C. (1996). Anhomeostasis: seasonal water and solute relations in two populations of the desert tortoise (*Gopherus agassizii*) during chronic drought. *Physiol. Zool.* **69**, 1324-1358.

Plummer, M. V., Williams, B. K., Skiver, M. M. and Carlyle, J. C. (2003). Effects of dehydration on the critical thermal maximum of the desert box turtle (*Terrapene ornata luteola*). *J. Herpetol.* **37**, 747-750.

Ruibal, R. (1962). The adaptive value of bladder water in the toad, *Bufo cognatus*. *Physiol. Zool.* **35**, 218-223.

Rogowitz, G. L., Cortes-Rivera, M. and Nieves-Puigdoller, K. (1999). Water loss, cutaneous resistance, and effects of dehydration on locomotion of *Eleutherodactylus* frogs. *J. Comp. Physiol. B* **169**, 179-186.

Seigel, R. A., Huggins, M. M. and Ford, N. B. (1987). Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia* **73**, 481-485.

Shine, R. (1980). 'Costs' of reproduction in reptiles. *Oecologia* **46**, 92-100.

Shine, R. (2003). Locomotor speeds of gravid lizards: placing 'costs of reproduction' within an ecological context. *Funct. Ecol.* **17**, 526-533.

Shoemaker, V. H. and Nagy, K. A. (1977). Osmoregulation in amphibians and reptiles. In *Annual Review of Physiology*. Vol. 39 (ed. E. Knobil), pp. 449-471. Palo Alto, CA: Annual Reviews.

Sinsch, U. (1991). Reabsorption of water and electrolytes in the urinary-bladder of intact frogs (genus *Rana*). *Comp. Biochem. Physiol.* **99A**, 559-565.

Townson, R. (1799). *Tracts and Observations in Natural History and Physiology*. London: printed for the author by J. White.

Tran, D.-Y., Hoff, K. V. and Hillyard, S. D. (1992). Effects of angiotensin II and bladder condition on hydration behavior and water uptake in the toad, *Bufo woodhousei*. *Comp. Biochem. Physiol.* **103A**, 127-130.

Veasey, J., Houston, D. and Metcalfe, N. (2001). A hidden cost of reproduction: the trade-off between clutch size and escape take-off speed in female zebra finches. *J. Anim. Ecol.* **70**, 20-24.

Vleck, C. M. and Priedkalns, J. (1985). Reproduction in zebra finches: hormone levels and effect of dehydration. *Condor* **81**, 37-46.

Wilson, B. S. and Havel, P. J. (1989). Dehydration reduces the endurance running capacity of the lizard *Uta stansburiana*. *Copeia* **1989**, 1052-1056.

Zar, J. H. (1999). Two-factor analysis of variance. In *Biostatistical Analysis* (ed. T. Ryu), pp. 231-272. Upper Saddle River, NJ: Prentice-Hall.