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RESEARCH ARTICLE

Meal consumption is ineffective at maintaining or correcting water balance in a desert lizard, *Heloderma suspectum*

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SUMMARY

Many xeric organisms maintain water balance by relying on dietary and metabolic water rather than free water, even when free water may be available. For such organisms, hydric state may influence foraging decisions, since meal consumption is meeting both energy and water demands. To understand foraging decisions it is vital to understand the role of dietary water in maintaining water balance. We investigated whether meal consumption was sufficient to maintain water balance in captive Gila monsters (*Heloderma suspectum*) at varying levels of dehydration. Gila monsters could not maintain water balance over long time scales through meal consumption alone. Animals fed a single meal took no longer to dehydrate than controls when both groups were deprived of free water. Additionally, meal consumption imparts an acute short-term hydric cost regardless of hydration state. Meal consumption typically resulted in a significant elevation in osmolality at 6 h post-feeding, and plasma osmolality never fell below pre-feeding levels despite high water content (~70%) of meals. These results failed to support our hypothesis that dietary water is valuable to Gila monsters during seasonal drought. When considered in conjunction with previous research, these results demonstrate that Gila monsters, unlike many xeric species, are heavily reliant on seasonal rainfall and the resulting free-standing water to maintain water balance.

Key words: dehydration, dietary water, water balance, reptile.

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INTRODUCTION

Energy and water balances are both crucial to organism survival, yet the degree to which energy and water represent discrete currencies varies among species. In the most dichotomous situation, food consumption provides the organism with energy, while drinking fulfils water requirements. However, because free-standing water can be both temporally and spatially limited in the environment, dietary water (i.e. the water present in the consumed meal) can make up a considerable portion of an organism's water intake. In fact, some species can heavily or fully rely on dietary water for maintaining water balance (Karasov, 1983; Golightly and Ohmart, 1984; Cooper, 1985; Nagy and Medica, 1986; Green et al., 1991; Nagy et al., 1991; Zhi-long et al., 1992; Nagy and Gruchacz, 1994; Znari and Nagy, 1997; Ostrowski et al., 2002).

Reliance on dietary water may have a considerable influence on foraging decisions (Kotler et al., 1998). Many xeric species will shift their diet to items with greater water content as forage dries up or as free water becomes increasingly limited (e.g. Karasov, 1983; Golightly and Ohmart, 1984; Nagy and Gruchacz, 1994). In addition to its influence on diet preference, dietary water can also influence activity. If dietary and metabolically produced water completely satisfies water requirements, as is the case for a variety of desert rodent species (Nagy and Gruchacz, 1994; Degen et al., 1997), then those organisms can continue to remain active even when free water is unavailable. However, if meal consumption does not enable an organism to maintain water balance, the organism may dehydrate, which can lead to a reduction in activity to minimize water loss. The desert tortoise (*Gopherus agassizii*), a xeric species that inhabits the American Southwest, relies on dietary water as an important contributor to water balance (Nagy and Medica, 1986; Peterson, 1996a; Peterson, 1996b; Henen et al., 1998). However, despite having food available during extended periods of drought, a desert tortoise experiences a dramatic increase in its plasma osmolality, reduces activity, and relies on fluid stored in its urinary bladder to survive. Despite being well adapted to their xeric lifestyle, desert tortoises are heavily reliant upon seasonal rainfall for survival (Nagy and Medica, 1986; Peterson, 1996a; Peterson, 1996b; Henen et al., 1998). Given the existing variation among species, understanding the extent to which a meal influences water balance can be important in determining the vulnerability of organisms to hydric limitations of their environment.

The role of dietary water in water balance has predominantly been investigated in herbivorous species, where different forage can have dramatically different water and protein content (the latter being critical due to protein catabolism, leading to the need to eliminate large amounts of nitrogenous wastes). In contrast, carnivorous diets tend to be more consistent in both water and protein content. While this consistency reduces the value of diet shifts, it does not address whether dietary water is a significant contributor to water balance in carnivorous species. Most prey consists of ~70% water, so meal consumption could contribute substantially to water balance.

To date, the vast majority of work on dietary water contributions to water balance has relied on indirect field assessments that use injections of isotopically labeled water (e.g. Karasov, 1983; Cooper, 1985; Green et al., 1991; Nagy et al., 1991; Zhi-long et al., 1992; Nagy and Gruchacz, 1994; Znari and Nagy, 1997; Ostrowski et al., 2002) or measurements of stable isotopes in body water (e.g. Wolf and Martinez del Rio, 2000). While these studies provide

considerable information on a larger scale, the specifics related to the intake of individual meals cannot be evaluated. Thus such work would benefit from complementary studies that make more direct assessments under tightly controlled conditions. Therefore, we conducted a set of laboratory experiments on an infrequently feeding carnivore to evaluate the short-term impact of meal consumption on plasma osmolality and to determine whether consumption of a meal can maintain the hydration state over extended periods of time.

The Gila monster (*Heloderma suspectum* Cope 1869) is an ideal species to examine the impact of meal consumption on hydration state. It is the largest lizard in North America and inhabits the xeric American Southwest where summer temperatures frequently exceed 40°C and free water can be unavailable for 2–3 months (Beck, 2005). To endure lengthy dry periods, Gila monsters use their urinary bladder as a water reservoir (Davis and DeNardo, 2007) and, upon depletion of the reservoir, tolerate considerable increases in plasma osmolality [>360 mosmol kg^{-1} (Davis and DeNardo, 2009; Davis and DeNardo, 2010)]. Furthermore, increased plasma osmolality leads to a reduction in surface activity, presumably to reduce evaporative water loss (Davis and DeNardo, 2009). At the onset of the first summer rains, Gila monsters will binge drink free water, rapidly returning their osmolality to a normosmotic state (Davis and DeNardo, 2007).

Although extensive work has examined the role of free water in the physiological ecology of Gila monsters, no work has examined how meal consumption may affect hydration state, especially during periods of extreme water limitation. Given the importance that dietary water plays in the water budgets of other animals, and that Gila monsters have a specialized diet of vertebrate nestlings and eggs (Beck, 2005) that contain ~70% water, we hypothesized that dietary water is a valuable supplemental water resource to Gila monsters during seasonal drought. Accordingly, we determined the impact of dietary water on rates of dehydration and rehydration in Gila monsters. In our first experiment, we examined to what extent dehydration rate is altered by a single meal and the effectiveness of different meal types in rehydrating Gila monsters. We predicted that the rate of dehydration in water-deprived Gila monsters would be significantly slower in animals provided with a meal compared with those given no food. Additionally, when animals are in an extremely dehydrated state, we predicted that meal consumption would considerably improve hydration state, but, unlike a single drinking event, would not fully rehydrate Gila monsters. In our second experiment, we examined the acute (first 48 h) hydric implications of consuming different meals at various stages of hydration (normosmotic, moderate dehydration and extreme dehydration) and determined how multiple meals influence the time it take Gila monsters to reach an extreme dehydration state. We predicted that ingestion of a meal results in an acute water cost at all hydration states, and that such negative effects would be more substantial in rodent meals because of the more complex requirements for digestion. Additionally, we predicted that egg meals would significantly extend the time to reach extreme dehydration relative to rodent meals.

MATERIALS AND METHODS

Study animals and experimental housing

For both experiments, we used 12 long-term captive, adult (experiment 1: mean initial mass 530 g, range 415–639 g; experiment 2: mean initial mass 469 g, range 405–690 g) Gila monsters obtained from the Arizona Game and Fish Department and held under holding licence SP577864. Additionally, all experiments were conducted in accordance with Arizona State University's Institutional Animal Care and Use Committee under protocol 09-1044R. Animals were housed in individual opaque containers (length, 34 cm; width, 21.5 cm; depth, 13.4 cm) with screen lids to allow for exposure to the environmental chamber conditions and to permit visual observation. As the environmental chamber had multiple levels, animal cages were rotated within the experimental chamber once or twice per week.

During the experiments, Gila monsters were housed in an environmental chamber at 30.0±0.2°C that received affluent air with a dew point of 3.5±1.3°C. These values approximate the preferred body temperature of the species (Beck, 2005) and the ambient humidity during the hot, dry season in the Sonoran Desert (authors' personal observations). Air temperature was maintained using a feedback design where a datalogger (21X micrologger, Campbell Scientific, Logan, UT, USA) received input from a thermocouple placed within the chamber and, based on this input, provided variable power to a heating element (iQ FlexHeat, CaloriQue LLC, West Wareham, MA, USA) within the chamber. A small fan placed adjacent to the heating element ran continuously to circulate the air within the chamber.

To achieve the desired dew point, room air was bubbled serially through two 1 liter humidifying bottles, the first being at room temperature (~25°C) and the second heated to ensure that exiting air was completely saturated when it cooled back to room temperature. Air then flowed serially through two 1 liter condensation bottles (both at room temperature, ~25°C) to remove excess moisture. This humidified air then flowed through a mass flow controller (UNIT Instruments, Yorba Linda, CA, USA) and into a small refrigerator set to attain the desired dew point. The air then exited the refrigerator and warmed to room temperature before flowing into the environmental chamber (mean flow rate, 1789±1 ml min^{-1}). The entire air flow system was plumbed with minimally hygroscopic Bev-a-line tubing.

Output of supply air flow rate from the mass flow controller and dew point of the environmental chamber from the hygrometer were recorded by the datalogger every minute. A small pump drew air from the environmental chamber to a flow-through hygrometer (RH-100, Sables Systems, Logan, UT, USA). Additionally, the dew point of the supply air was monitored daily using a bypass system connected to the flow-through hygrometer, enabling us to monitor the supply air dew point while minimizing disturbances to both the environmental chamber conditions and the animals. The supply air flow rate and the dew point of the supply air were adjusted as needed throughout the duration of both experiments (although both were quite stable throughout the duration of both experiments and rarely needed adjustment).

Experiment 1

Dehydration component – effect of a single meal on dehydration rate

The goal of the dehydration component of experiment 1 was to examine whether ingesting a single meal affected the rate of dehydration in captive, free water-deprived adult Gila monsters. We used a single meal since Gila monsters are infrequent binge feeders and thus might only ingest a single meal over the course of the hot, dry season.

For a minimum of 14 days prior to the start of the experiment, Gila monsters were maintained with *ad libitum* water but without food to ensure that animals were normosmotic (~290–300 mosmol kg^{-1}) and post-absorptive. As Gila monsters use water stored in their urinary bladders to buffer changes in plasma

osmolality (Davis and DeNardo, 2007), each Gila monster had its urinary bladder drained *via* trans-urethral bladder catheterization (for details, see Davis and DeNardo, 2007) just prior to beginning the experiment. Ultrasonography (Concept/MLV, Dynamic Imaging, Livingston, UK) was used to confirm that the urinary bladder was empty after catheterization. After catheterization, the animal was returned to its normal housing container, but without water. Twenty-four hours after trans-urethral bladder catheterization, an initial mass (g), tail volume (ml, serves as an estimate of energy stores), and a 0.1 ml blood sample (for plasma osmolality) were collected from each animal. Animals were then placed in their experimental containers in the environmental chamber without water. Body mass, tail volume and a blood sample were collected weekly throughout the experiment. Blood samples in both experiments were collected from the caudal vein using a heparinized 1 ml syringe.

As animals reached a moderately dehydrated state (~320–330 mosmol kg^{-1}), they were alternately assigned into one of two treatment groups: fed ('Fed') and non-fed ('Con') animals. Animals receiving a meal were fed two previously frozen but thawed juvenile rats (total mass 60.0±0.1 g). Sixty grams of thawed rat represents approximately two-thirds of the average monthly caloric demand of free-ranging Gila monsters (D.F.D., unpublished data). Following assignment into treatment groups, we continued to monitor plasma osmolality, mass and tail volume of animals weekly. However, to ensure the safety of the animals and to obtain a more precise estimate of days to dehydration, blood samples were collected more frequently as osmolality approached the upper limits of dehydration (>340 mosmol kg^{-1}). Once a lizard reached a plasma osmolality greater than 350 mosmol kg^{-1} [which approximates the near-maximum osmolality reached by free-ranging Gila monsters in the Sonoran Desert (Davis and DeNardo, 2009)], a final mass, tail volume, blood sample and ultrasound of bladder dimensions were collected, and the number of days to maximum dehydration was recorded. Animals then entered the rehydration experiment described below.

Rehydration component – effect of different meal types on rehydration

To address the degree to which different meal types rehydrate Gila monsters, the dehydrated animals from the first component of experiment 1 were alternately assigned to one of two rehydration treatments: animals were fed 60 g of either juvenile rat ('Rat') or blended chicken egg ('Egg'), excluding shell, immediately following the final blood sample of the dehydration component. Gila monsters prey nearly exclusively on the contents of vertebrate nests (Beck, 2005), so our treatments represent two ecologically relevant meals. Following feeding, the animals were returned to the environmental chamber and remained without free water. Given the high starting osmolality during this rehydration component, blood samples were collected prior to and 48 h after feeding. Once an animal reached either a normosmotic (<310 mosmol kg^{-1}) or extremely dehydrated (>350 mosmol kg^{-1}) state, the animal was removed from the study and provided *ad libitum* access to free water (Fig. 1A).

Experiment 2 – acute and long-term impact of multiple meals on hydration state

The goal of experiment 2 was to assess the acute hydration cost of digesting meals for adult Gila monsters at varying states of hydration, and examine the effect of consuming multiple meals on the rate of dehydration in these same animals. Similar to experiment 1, all animals were normosmotic, in a post-absorptive state, and had their urinary bladders drained via trans-urethral bladder catheterization prior to beginning experiment 2. As before, all animals were checked by ultrasound following catheterization to ensure that the urinary bladders were empty. Initial processing was completed 24 h after catheterization as described above.

The experimental design followed that of experiment 1 except for a change in the frequency and timing of meal treatments and post-feeding blood sampling (Fig. 1B). After baseline measurements were collected, animals were assigned to one of two treatments: Rats or Eggs meals. Each animal was scheduled to receive four supplementations while in the environmental chamber – a meal while in an normosmotic state (beginning of experiment), a meal when moderately dehydrated (320–330 mosmol kg^{-1}), oral free water supplementation (at a volume similar to the amount of dietary water in the meal, mean=42±2 ml) when extremely dehydrated (>350 mosmol kg^{-1}), and a meal when the animal returned to an extremely dehydrated state after free water supplementation. In addition to weekly processing of the animals, we collected blood samples 6, 24 and 48 h as well as 6 days (144 h) after each treatment (the 6-day sample was not collected after the third feeding). Mass and tail volume were collected at time 0, 48 h and 6 days post-treatment). Forty-eight hours after the third feeding, animals were provided with a bowl of water for 3 h, allowing them sufficient time to binge drink free water to satiation. Twenty-four hours post-binge drinking, a final blood sample and body mass were collected.

Determination of plasma osmolality

Plasma was separated from whole blood by centrifugation, and then plasma was stored in sealed containers at −80°C until the samples were analyzed. Weekly plasma samples were processed within 24 h of blood collection so that we could closely monitor hydration state. Frozen plasma samples were thawed and osmolality of samples was determined in triplicate using a vapor pressure osmometer (model 5500, Wescor, Logan, UT, USA). Before analyzing samples, the osmometer was calibrated using the three-step factory protocol using osmolality standards of 290 and 1000 mosmol kg^{-1}. To verify that the osmometer was consistent throughout the experiments, a sample of pooled plasma collected from well-hydrated captive adult Gila monsters (~290–300 mosmol kg^{-1}) was analyzed in triplicate after completing calibration procedures, as well as after every 20 triplicate samples. If the pooled plasma sample readings varied beyond the error range of the osmometer (±6 mosmol kg^{-1}), then the 290 standard was analyzed in triplicate as well. If the osmolality of both samples fell outside the error range of the osmometer, the osmometer head was cleaned, the osmometer was recalibrated, and pooled Gila monster plasma was re-run in triplicate prior to continuing analysis of samples, beginning with the 20 triplicate samples that fell between an accurate pooled sample reading and the pooled sample reading that varied beyond the osmometer error range.

Water content of meals

We determined the water content of both meal types (juvenile rat and shell-free chicken egg) using a sample size of five for each meal type. We also determined the water content of the two most commonly consumed prey of Gila monsters – desert cottontail rabbit (*Sylvilagus audubonii*) pups (*N*=1) and Gambel's quail (*Callipepla gambelii*) eggs (*N*=3). We could not use these natural preys for our experiments due to their limited availability, but we wanted to compare the water content of our experimental meals to that of the natural prey. Water content was determined by placing each item individually in a small, pre-weighed aluminum tray. Prey wet mass was calculated as initial total mass minus empty tray mass. The trays were then placed in a vacuum-sealed oven, and the prey dried

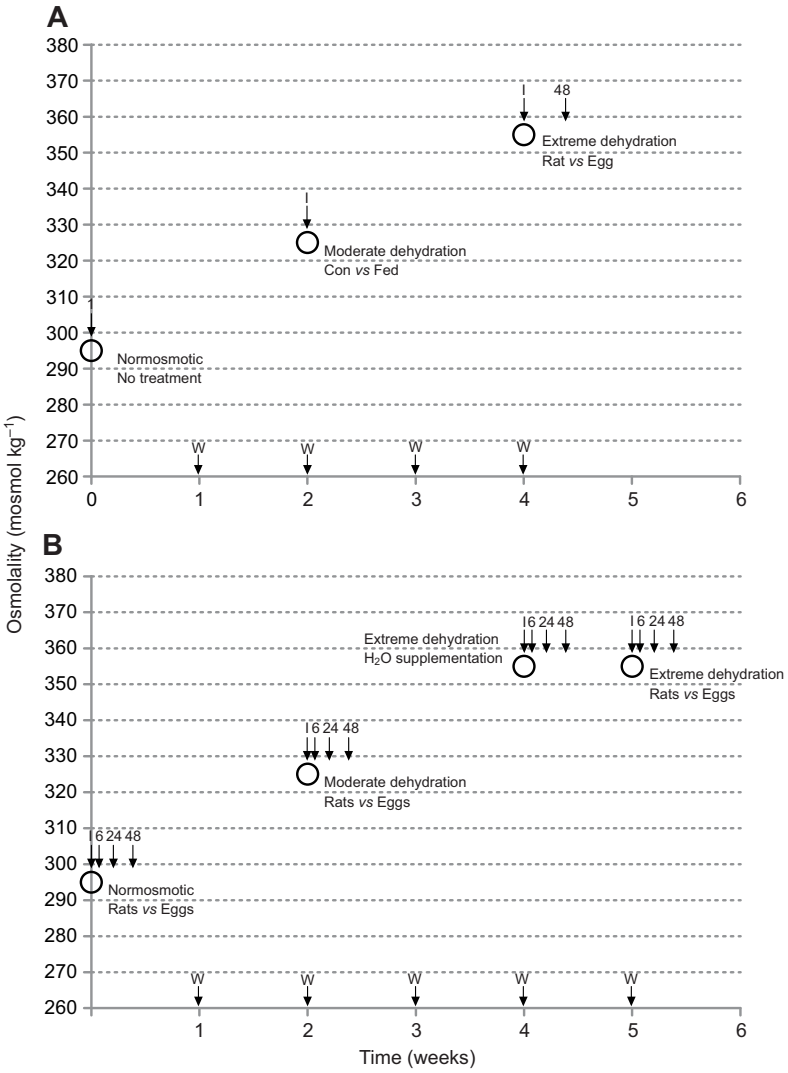

Fig. 1. Diagrammatic representation of the experimental designs for (A) experiment 1 and (B) experiment 2. Circles indicate the target hydration state for each manipulation; arrows and 'W' depict weekly processing of animals (see Materials and methods); arrows and 'I', '6', '24' and '48' represent sampling points prior to and at 6, 24 and 48 h after manipulation, respectively.

at 65°C until mass remained unchanged for at least 3 days. Prey dry mass was calculated as end total mass minus empty tray mass, and water content (%) was then calculated as (prey wet mass – prey dry mass)/(prey wet mass) × 100.

Data analysis

Experiment 1 – dehydration component

The effect of a single meal on dehydration time was assessed by comparing the number of days animals in the Con *versus* Fed treatment groups took to reach the extremely dehydrated state by using a non-parametric *t*-test (Mann–Whitney), since the assumptions of an unpaired Student's *t*-test and Welch's *t*-test were violated. Because the amount of time animals took to dehydrate varied, as did the final plasma osmolality, we also compared the rate of change in plasma osmolality of animals prior to and following treatment at the moderately dehydrated state using a repeated measures analysis of variance (rmANOVA).

Experiment 1 – rehydration component

To determine the effect of meal consumption on rehydration of extremely dehydrated Gila monsters, the mean plasma osmolality at times 0 and 48 h post-treatment were compared between Rat and Egg treatment groups using an rmANOVA.

Experiment 2

To determine the acute effect of meal consumption on hydration state, mean plasma osmolality at 0, 6 h, 24 h, 48 h and 6 days (144 h) were compared between treatments using an rmANOVA. This analysis was applied separately to the four manipulation events (feeding when normosmotic, second feeding when moderately dehydrated, free water supplementation when extremely dehydrated, third feeding when extremely dehydrated) plus the binge drinking event at the end of the experiment (comparing osmolality at time 0 and 24 h post-binge drinking). Snout–vent length (SVL) was initially used as a covariate in each analysis; however, because there was no significant effect of SVL in any of the aforementioned tests, the rmANOVAs were completed excluding SVL as a covariate.

The effect of multiple meals on dehydration time was assessed by comparing the number of days animals in the Rats *versus* Eggs treatment groups took to reach an extremely dehydrated state by using a non-parametric *t*-test (Mann–Whitney), since the assumptions of an unpaired Student's *t*-test and Welch's *t*-test were violated.

Water content of meals

The difference in the water content of our rat *versus* blended chicken egg meals was assessed by comparing the percent water content of

each meal type using an unpaired Student's *t*-test, as the variances were equal and the data were normally distributed. The variances between the blended chicken egg *versus* fresh quail eggs were not equal; however, the data were normally distributed, so we compared the difference in water content between blended chicken egg *versus* fresh quail eggs by using a Welch's *t*-test. As our sample size for the juvenile desert cottontail rabbits was N=1, we did not use a statistical test to compare its water content with the water content of our rat meals.

RESULTS

Water content of meals

There was no difference in mean water content of juvenile rats and chicken eggs (rats 70.4±0.3%, eggs 74.5±0.2%, unpaired *t*-test: P=0.374). Similarly, we found no difference in water content of fresh quail eggs relative to chicken eggs (quail eggs 72.3±0.7%, Welch's *t*-test: P=0.073). Finally, although we lacked a sufficient sample size for statistical comparison (due to difficulties in obtaining samples), a single nestling desert cottontail rabbit had a water content of 76.5%.

Experiment 1 – dehydration component

There was no difference in the number of days it took the Con *versus* Fed treatment groups to reach an extreme dehydration state (Con 32.5±4.86 days, Fed 32.5±2.66 days, Mann–Whitney test: P>0.20, Fig. 2). Similarly, after we calculated the rate of change in osmolality prior to and following treatment at the moderately dehydrated state, we found that there was no effect of time or treatment on rate of change in osmolality, nor was there an interaction between time and treatment (rmANOVA time: $F_{1,10}$=0.766, P=0.40; rmANOVA treatment: $F_{1,10}$=2.753, P=0.13; rmANOVA time and treatment: $F_{1,10}$=0.766, P=0.40).

Experiment 1 – rehydration component

There was a significant effect of treatment on the osmolality of extremely dehydrated Gila monsters fed Egg or Rat (rmANOVA treatment: $F_{1,10}$=9.37, P=0.012, Fig. 3), and there was an interaction between time and treatment (rmANOVA time and treatment: $F_{1,10}$=15.16, P=0.003, Fig. 3). There was no effect of time on osmolality of extremely dehydrated Gila monsters after feeding (rmANOVA time: $F_{1,10}$=4.558, P=0.059, Fig. 3). Tukey–Kramer *post hoc* analysis revealed that: (1) there was no difference in starting osmolality (pre-feed) between either treatment group, (2) the Egg group did not experience a significant change in osmolality 48 h post-feeding, (3) animals in the Rat treatment had a significantly higher osmolality 48 h post-feeding relative to pre-feeding, and (4) animals in the Rat treatment had a significantly higher osmolality 48 h post-feeding relative to those in the Egg treatment (Fig. 3).

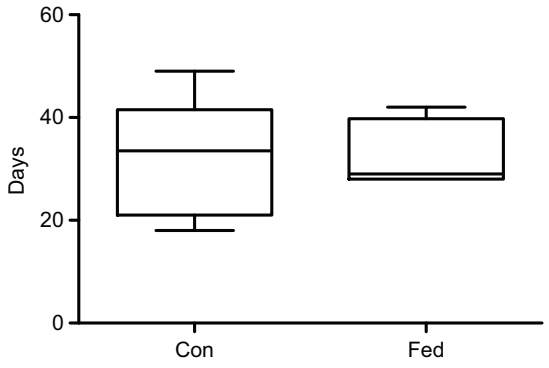

Fig. 2. The total number of days it took moderately dehydrated Gila monsters to reach an extreme dehydration state following a rat meal (Fed) or no meal (Con). Boxes represent quartiles and error bars 95% confidence intervals.

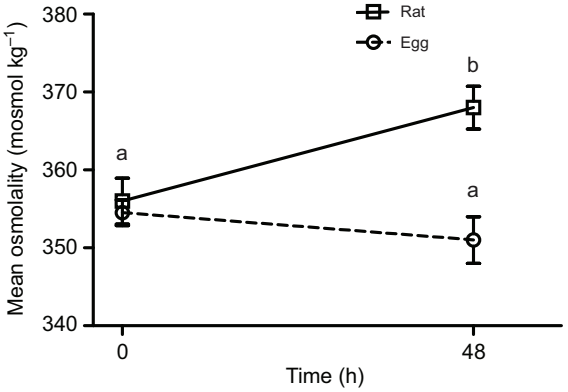

Fig. 3. Osmolality of extremely dehydrated animals before and 48 h after feeding either Egg or Rat. Rat-fed animals experienced a significant increase in osmolality while Egg-fed animals had no change in osmolality 48 h after feeding. Letters represent significant differences across sampling time points. Error bars are ±1 s.e.m.

Experiment 2

Both time and treatment had an effect on the osmolality of normosmotic Gila monsters (rmANOVA time: $F_{4,40}$=23.555, P<0.001; rmANOVA treatment: $F_{1,10}$=9.414, P=0.012, Fig. 4A). However, there was no interaction between time and treatment at this hydration state (rmANOVA time and treatment: $F_{4,40}$=2.230, P=0.083, Fig. 4A). *Post hoc* analysis revealed that 6 h post-feeding, the osmolality of normosmotic Gila monsters was elevated above baseline and remained elevated for the duration of the sampling period (6 days post-treatment). Additionally, animals in the Rats treatment had a significantly higher osmolality compared with Gila monsters in the Eggs treatment (Fig. 4A).

Time had a similar effect on osmolality of moderately dehydrated Gila monsters (rmANOVA time: $F_{4,32}$=6.602, P=0.001, Fig. 4B). However, there was no effect of treatment on osmolality at this hydration state, nor was there an interaction between time and treatment (rmANOVA treatment: $F_{1,8}$=0.025, P=0.88; rmANOVA time and treatment: $F_{4,32}$=2.521, P=0.06, Fig. 4B). *Post hoc* analysis showed that within 6 h of consuming a meal, plasma osmolality of moderately dehydrated animals in both treatment groups increased significantly above pre-feeding levels and remained at this elevated state for 24 h post-feeding before returning to pre-feeding levels 48 h and 6 days post-treatment (Fig. 4B).

As was the case with the previous two hydration states, time had an effect on osmolality of extremely dehydrated Gila monsters supplemented with an amount of free water similar to the amount of dietary water in their respective meal treatments (rmANOVA time: $F_{4,36}$=100.129, P<0.001, Fig. 5). As with the mildly dehydrated state, there was no effect of treatment on osmolality, nor was there an interaction between time and treatment, (rmANOVA treatment: $F_{1,9}$=2.618, P=0.14; rmANOVA time and treatment: $F_{4,36}$=0.689, P=0.60). *Post hoc* analysis revealed that within 6 h of free water supplementation, the plasma osmolality of extremely dehydrated Gila monsters in both treatment groups was significantly lower than

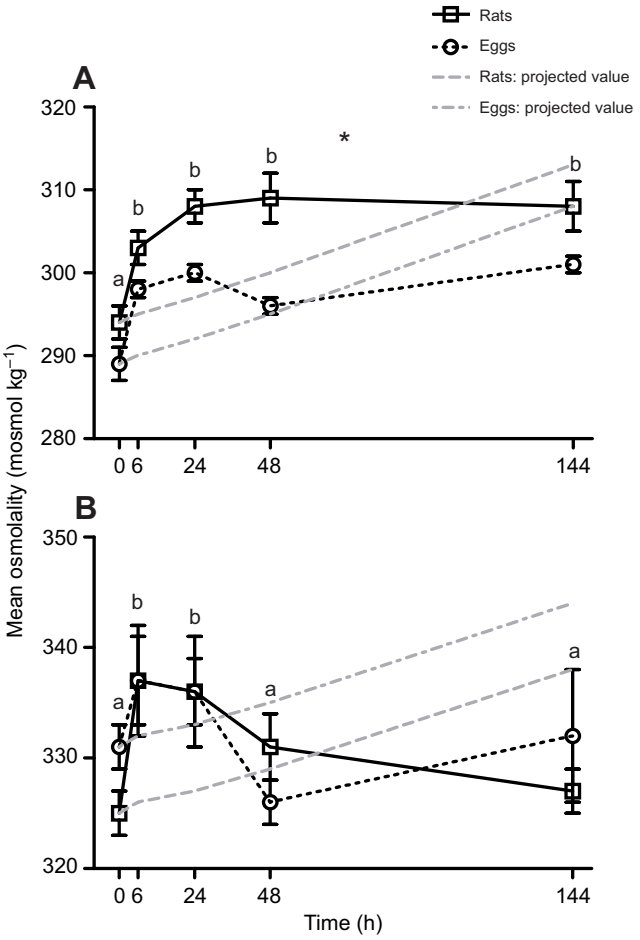

Fig. 4. Mean osmolality of (A) well-hydrated and (B) moderately dehydrated Gila monsters 0, 6, 24, 48 and 144 h (6 days) post-feeding with either Eggs or Rats. Light gray lines represent projected plasma osmolality values for animals in both treatment groups. These values were determined by using the rates of change in plasma osmolality determined in experiment 1 for Gila monsters in the Con treatment group. Letters represent significant differences across sampling time points. *Significant overall treatment effect. Error bars are ±1 s.e.m.

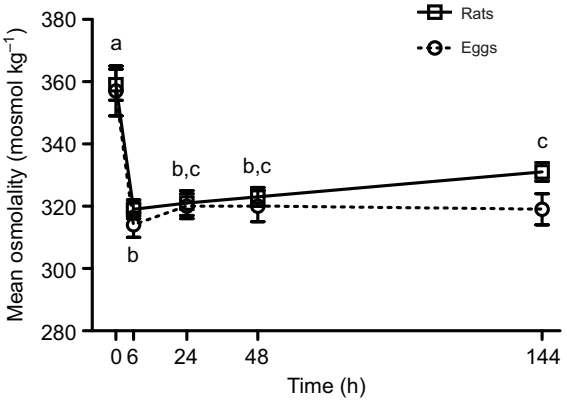

Fig. 5. Average osmolality of extremely dehydrated Gila monsters 0, 6, 24, 48 and 144 h (6 days) post-free water supplementation. There was a significant effect of time on osmolality, but there was no effect of treatment or an interaction between time and treatment. Letters represent significant differences across sampling time points. Error bars are ±1 s.e.m.

plasma osmolality prior to water supplementation, and it remained reduced throughout the sampling period (Fig. 5).

Four Gila monsters in the Eggs treatment and one in the Rats treatment exhibited clinical signs of dehydration (e.g. lethargy with reduced response to stimulation) before they reached the final meal treatment. Due to concerns for the well-being of the animals, we removed these animals from the study and provided them with water *ad libitum* for 3 h (binge drinking treatment). Due to this unanticipated response, our sample sizes for Eggs and Rats treatments at an extremely dehydrated state were unbalanced (N=2 for Eggs, N=5 for Rats) and too small to perform a valid statistical analysis.

Once animals either exhibited clinical signs of dehydration or had their final blood sample drawn while in the final feeding treatment, we examined the effect of a single binge drink on recovery from dehydration. We found that time and treatment independently had an effect on the osmolality of these extremely dehydrated Gila monsters (rmANOVA time: $F_{1,10}$=297.24, P<0.001; rmANOVA treatment: $F_{1,10}$=6.913, P=0.03, Fig. 6). There was no interaction between time and treatment (rmANOVA time and treatment: $F_{1,10}$=1.588, P=0.24) (Fig. 6). *Post hoc* analysis revealed that the osmolality of animals in both the Eggs and Rats groups had significantly decreased within 24 h of a binge drinking event (Fig. 6), similar to previous findings (Davis and DeNardo, 2007). Additionally, *post hoc* analysis revealed that the Rats group had a significantly higher mean osmolality relative to the Eggs group (Fig. 6). This result probably occurred because more animals in the Rats treatment group reached the extreme dehydration state for a second time without exhibiting clinical signs of dehydration, contrary to their Eggs treatment counterparts.

Overall, there was no difference in the number of days it took the Rats and Eggs treatment groups to reach an extreme dehydration state (Rats 41.4±2.5 days, Eggs 46.9±4.9 days, Mann–Whitney test: U=18.5, N_1=6, N_2=6, P>0.20, Fig. 7).

DISCUSSION

Meal consumption and water balance

The average time required for a Gila monster to reach an extreme state of dehydration was minimally affected by meal consumption (Fig. 2). Gila monsters given a single rodent meal took no longer to dehydrate (32.5 days) then did the unfed controls (32.5 days) or unfed Gila monsters with empty urinary bladders in a previous study (33.3 days) (Davis and DeNardo, 2007). Feeding multiple egg or rat meals to Gila monsters without access to free water did extend the time to dehydration (46.9 and 41.4 days, respectively). However, frequent meal consumption by Gila monsters is unlikely during the hot, dry season when few prey species are nesting. Thus from an ecological prospective, Gila monsters fall on the free water dependence side of the ‘continuum’ between free water independence and free water dependence (Gettinger, 1984).

Free water dependence is uncommon, particularly in xeric reptiles. Dietary and metabolically produced water completely satisfy the water requirements of the carnivorous heath monitor (*Varanus rosenbergi*) during the driest times of the year in southern Australia. Even during wetter portions of the year (i.e. spring and winter), these sources of water contribute 74 and 58%, respectively, of their water requirements (Green et al., 1991). Free-ranging, semiarid-dwelling goannas (*Varanus caudolineatus*) were also able to maintain water balance during the summer (in Western Australia)

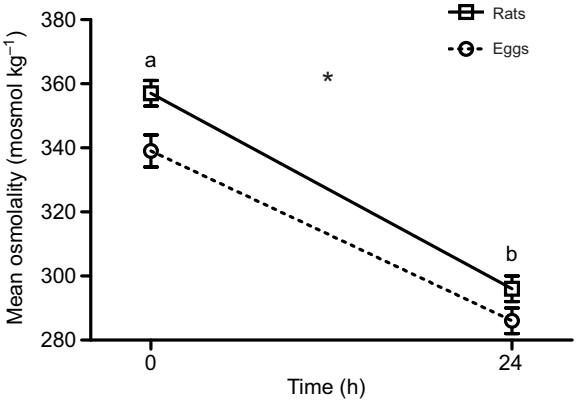

Fig. 6. Average osmolality of extremely dehydrated Gila monsters 0 and 24 h post-binge drinking (water provided *ad libitum* for 3 h). There was a significant effect of time and treatment; however, there was no interaction between time and treatment. *Overall treatment effect. Letters represent significant differences across sampling time points. Error bars are ±1 s.e.m.

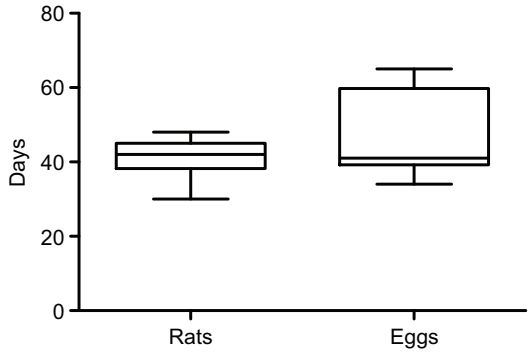

Fig. 7. Total number of days it took well-hydrated Gila monsters to reach an extreme dehydration state after two meals (Rats and Eggs), one at a well-hydrated and one at a moderately dehydrated state. Boxes represent quartiles and error bars 95% confidence intervals.

despite little to no free water being available (Thompson et al., 1997). Free-living Bibron's agama (*Agama impalearis*), an insectivorous ambush predator that inhabits the arid regions of North Africa, satisfy their water demands entirely through dietary water and metabolic water production (Znari and Nagy, 1997). Numerous herbivorous, xeric-dwelling reptiles are also able to maintain water balance without drinking free water, e.g. the Namibian sand dune lizard *Angolosaurus skoogi* (Nagy et al., 1991) and the desert iguana *Dipsosaurus dorsalis* (Minnich and Shoemaker, 1970). Given their low water demands, many xeric reptiles are capable of capitalizing on available dietary water to balance their water budgets without drinking free water.

Even among xeric arthropods and endotherms, reliance on free-standing water is atypical. Cooper (Cooper, 1985) examined the water balance of two species of free-ranging tenebrionid beetles, the desert stink beetle (*Eleodes armata*) and the death feigning beetle (*Cryptoglossa verrucosa*), which are sympatric with the Gila monster. Both are capable of satisfying most of their water requirements *via* dietary and metabolic water (Cooper, 1985). At the extreme for high energy systems, a variety of desert rodent species, such as Merriam's kangaroo rats (*Dipodomys merriami*), are capable of satisfying their water requirements without drinking free-standing water (Nagy and Gruchacz, 1994; Degen et al., 1997). This independence from needing free-standing water to balance water budgets is also present among larger vertebrates. For example, Ostrowski et al. (Ostrowski et al., 2002) examined the water intake of free-living oryxes (*Oryx leucoryx*) during the summer in the Arabian Desert, finding that 14.4% of total daily water influx rate was metabolically produced water, while the remainder of their total water influx was obtained *via* the plants they consume. Like the oryx, kit foxes (*Vulpes macrotis*) obtain sufficient water to maintain water balance from both metabolic water and their prey (Golightly and Ohmart, 1984).

Although many species are capable of obtaining sufficient water from both cellular respiration and food, it appears that Gila monsters are precluded from using these strategies to maintain water balance. Having a high mass specific metabolic rate allows many species to capitalize on metabolic water production, which can significantly contribute to water balance. However, the contribution of this endogenously produced source of water to water balance is less significant in: (1) larger vertebrates, because of their low mass specific metabolic rates, and (2) low energy systems that generally have lower overall metabolic rates when compared with high energy systems of comparable size. Gila monsters are medium-sized lizards that have very low mass-specific metabolic rates and spend the majority of their time inactive in refugia, particularly during the hot, dry season when the proportion of time spent in the burrow can exceed 90% (Beck, 2005; Davis and DeNardo, 2010). Thus the energetic efficiency of Gila monsters dictates that metabolic water production can only provide a minimal contribution to both short- and long-term water balance.

One key aspect of using dietary water to fulfil hydric needs is that animals often alter diet selection during dry periods. However, Gila monsters are nest specialists, and our data demonstrate little variation in water content among prey items as well as little variation in hydric benefits from consuming eggs *versus* juvenile rodents. These factors preclude Gila monsters from using a strategy many other species use to help maintain water balance.

However, Gila monsters are not alone in their inability to satisfy water requirements solely through dietary and endogenously produced sources. The xeric mottled rock rattlesnake (*Crotalus lepidus*) cannot fulfil water requirements *via* prey ingestion and metabolic water production alone, and is in negative water balance during the summer (Beaupre, 1996). Extensive work examining the physiological ecology of the desert tortoise, a species sympatric with the Gila monster, implies that green succulents are an important source of water for desert tortoises (Nagy and Medica, 1986; Peterson, 1996a; Henen et al., 1998). However, during the dry season, they consume dry plant matter that is osmotically stressful, requiring the tortoise to store excess ions and nitrogenous wastes in its bladder until they can be voided by drinking free-standing water that comes only with sufficient rainfall (Nagy and Medica, 1986; Peterson, 1996a; Peterson, 1996b; Henen et al., 1998). Recent evidence has also demonstrated that marine snakes, including sea kraits, sea snakes and file snakes, require fresh water to maintain water balance (Lillywhite and Ellis, 1994; Lillywhite et al., 2008; Lillywhite et al., 2012). Preliminary analysis of their water budgets indicates that dietary water is insufficient at correcting water balance (Lillywhite et al., 2008). Finally, the white-winged dove (*Zenaida asiatica*), another species sympatric with the Gila monster, relies heavily on saguaro cactus nectar and fruit throughout the summer, but they can also use free-standing water sources to maintain water balance as well (Wolf and Martinez del Rio, 2000).

Given their mobility, white-winged doves and many other birds can take advantage of free-standing water that may otherwise be inaccessible to less mobile species.

The hydric cost of digestion

Our prediction that ingestion of a meal would result in a short-term water cost at all hydration states and that such negative effects would be more substantial in rodent meals was partially supported. At normosmotic and moderately dehydrated states, animals in both treatment groups exhibited a significant elevation in plasma osmolality at 6 h post-feeding and it remained elevated for at least 24 h. At 48 h post-feeding, the osmolality of normosmotic animals remained elevated while the osmolality of moderately dehydrated animals returned to pre-feeding levels (Fig. 4A,B). Additionally, consumption of egg or rodent at an extremely dehydrated state failed to improve hydration state within 48 h post-feeding (Fig. 3), thus failing to support our prediction that meal consumption would improve but not fully rehydrate extremely dehydrated Gila monsters. These results were similar regardless of meal type. Only when osmolality was >350 mosmol kg^{-1} did meal type affect the acute hydration response. Rodent-fed animals exhibited an osmolality that was significantly higher 48 h post-feeding relative to baseline and when compared with the osmolality of animals 48 h post-egg consumption (Fig. 3). Additionally, the egg and rodent treatments failed to rehydrate Gila monsters to the extent of either a single binge drinking event (Davis and DeNardo, 2007) or to the extent that free water given in the amount equivalent to the dietary water did (Fig. 5).

The fact that, when given in equal volumes, free water provides greater hydric benefit than dietary water, suggests a substantial water cost associated with meal digestion. Post-prandial energetic costs associated with digestion have been well documented in a wide variety of taxa (Secor, 2009), with some of the most dramatic responses occurring in infrequently feeding snakes such as the Burmese python (*Python molurus bivittatus*), which shows up to a 44-fold increase in metabolism during digestion compared with its standard metabolic rate (SMR) (Secor and Diamond, 1997). Although Gila monsters are infrequent binge feeders similar to Burmese pythons, they exhibit a more modest 4.0- to 4.9-fold increase in metabolic rate relative to SMR (Christel et al., 2007). Although the post-prandial metabolic response of many organisms, including the Gila monster, has been well documented, to our knowledge there are no studies quantifying the hydric cost of digestion.

In support of a significant hydric cost to digestion, Gila monsters, regardless of hydration state, showed an initial increase in plasma osmolality shortly after feeding. While osmolality decreased from this peak with time, it never went below pre-feeding levels (Fig. 4A,B), as was seen when animals were given an equal volume of free water (Fig. 5) or allowed a single binge drink (Fig. 6). There are a number of possible mechanisms that might explain the post-prandial changes in plasma osmolality observed in Gila monsters. First, the rapid rise in plasma osmolality shortly after meal consumption might be driven primarily by the secretion of fluid into the lumen of the alimentary canal to aid in transport, digestion and absorption of nutrients *via* solvent drag. Additionally, evidence of post-prandial increases in: (1) the wet tissue mass of digestive and accessory organs and enterocyte volume (e.g. Starck and Beese, 2001; Cramp and Franklin, 2005; Lignot et al., 2005; Starck et al., 2007; Wood et al., 2007) and (2) blood flow to digestive organs (Starck and Wimmer, 2005), further demonstrate significant fluid investment into digestion. Hydric costs of digestion can also probably be attributed to the significant post-prandial increase in Gila monster metabolic rate (Christel et al., 2007), which would entail a concomitant increase in ventilatory water loss. Furthermore, hydric costs would be associated with eliminating meal-associated waste products in both the feces and urine. Clearly, given the void in our understanding of meal-associated hydric costs, further work is needed to quantify the relative importance of the various water-consuming aspects of digestion and how meal type might influence this balance.

One alternative explanation for increased plasma osmolality during digestion is the sudden increase in plasma constituents associated with nutrient absorption. However, while Gila monsters exhibit significant post-prandial increases in plasma glucose and triglyceride concentrations after feeding, these changes do not occur until 24 h after meal consumption and remain elevated for at least 72 h (Christel and DeNardo, 2007). This timeline does not reflect the pattern observed in plasma osmolality, where osmolality peaked at 6 h post-feeding, and typically returned to near-baseline levels at 48 h post-feeding (Fig. 4A,B). Although the plasma osmolality of normosmotic animals remained elevated throughout our sampling period, the lack of any clear trends between our results and the temporal variation in plasma nutrient concentrations reported by Christel and DeNardo (Christel and DeNardo, 2007) indicates that the mechanisms proposed above are more likely to be driving the observed post-prandial changes in plasma osmolality.

Meal consumption, hydration state and state-dependent foraging strategies

Survival depends on an organism using a suite of activities to fulfil multiple physiological demands, especially energetic and hydric demands. When to use each activity and the extent to which an activity is used depends on the integration of information regarding environmental conditions as well as the individual's physiological condition. Foraging represents a major activity of most organisms and many studies have demonstrated that animals optimize foraging so as to maximize benefits and/or mitigate costs, and that these decisions are often driven by the internal state of the organism (Clark, 1994; Nonacs, 2001), which is termed state-dependent foraging (SDF) (Nonacs, 2001). Foraging-induced costs must be paid with discrete currencies (e.g. energy, water) and while energetic costs tend to receive the most attention in studies of SDF strategies, in arid environments water costs might be more important. This is especially true if food acquisition does not significantly benefit water balance, as is the case in our study. Some foods contain little water (e.g. dry vegetation) or may have high water cost associated with meal processing. Although water availability and the contribution of various sources of water (free, dietary and metabolic) to water balance can affect foraging behavior (Kotler et al., 1998), there is, to the best of our knowledge, no work examining the interplay between an organism's hydration state, the contribution of various sources of water, and the use of SDF strategies in organisms. Our work has shown that in some situations there can be little hydric benefit to meal consumption. Coupled with observations that free-ranging Gila monsters dramatically reduce surface activity when osmotically stressed during the hot, dry summer (Davis and DeNardo, 2009), these studies indicate that foraging behavior can be directly impacted by an organism's hydration state and that foraging activity can come at a significant hydric cost to the organism regardless of foraging success. Given the impact that food consumption can have on energy and water balance, future studies examining foraging behavior in organisms should be expanded to

include an organism's hydration state and the hydric costs/benefits to foraging and meal acquisition. In doing so, we can build a more thorough understanding of the interplay between an organism's physiological condition and its foraging behavior.

LIST OF SYMBOLS AND ABBREVIATIONS

Con	treatment group in experiment 1 that did not receive a meal when moderately dehydrated
Egg	treatment group in experiment 1 that received a 60 g blended chicken egg meal when extremely dehydrated
Eggs	treatment group in experiment 2 that received a 60 g blended chicken egg meal or an amount of free water equivalent to that present in a 60 g blended chicken egg meal when normosmotic, moderately dehydrated and extremely dehydrated
Fed	treatment group in experiment 1 that did receive a 60 g rat meal when moderately dehydrated
Rat	treatment group in experiment 1 that received a 60 g rat meal when extremely dehydrated
Rats	treatment group in experiment 2 that received a 60 g rat meal or an amount of free water equivalent to that present in a 60 g rat meal when normosmotic, moderately dehydrated and extremely dehydrated
rmANOVA	repeated-measures analysis of variance
SDF	state-dependent foraging

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AUTHOR CONTRIBUTIONS

C.D.W. and D.F.D. made significant contributions to the conception, design and execution of the study, the interpretations of the findings, and the drafting and revising of the article. M.L.J. made significant contributions to the execution of the study.

COMPETING INTERESTS

No competing interests declared.

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