

1 **When the tap runs dry: The physiological effects of acute experimental**  
2 **dehydration in *Peromyscus eremicus***

3

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9

10 **Abstract**

11 Desert organisms have evolved physiological, biochemical, and genomic mechanisms

12 to survive the extreme aridity of desert environments. Studying desert-adapted species

13 provides a unique opportunity to investigate the survival strategies employed by

14 organisms in some of the harshest habitats on Earth. Two of the primary challenges

15 faced in desert environments are maintaining water balance and thermoregulation. We

16 collected data in a simulated desert environment and a captive colony of cactus mice

17 (*Peromyscus eremicus*) and used lab-based experiments with real time physiological

18 measurements to characterize the response to water-deprivation. Mice without access

19 to water had significantly lower energy expenditures and in turn, reduced water loss

20 compared to mice with access to water after the first 24 hours of the experiment.

21 Additionally, we observed significant weight loss likely related to dehydration-associated

22 anorexia a response to limit fluid loss by reducing waste and the solute load as well as

23 allowing water reabsorption from the kidneys and gastrointestinal tract. Finally, we

24 observed body temperature correlated with sex, with males without access to water  
25 maintaining body temperature when compared to hydrated males while body  
26 temperature decreased for females without access to water compared to hydrated,  
27 suggesting daily torpor in females.

28

## 29 **Keywords**

30 energy expenditure, *Peromyscus*, physiology, respirometry, total water loss,  
31 dehydration

32

## 33 **Introduction**

34 Water is arguably the single most important factor for life on Earth and in organisms,  
35 water is stored in intracellular and extracellular spaces (Fitzsimons 1963). Dehydration  
36 occurs where there is a decrease in extracellular fluid volume caused when the loss is  
37 outpaced by fluid intake and metabolic water production, leading to a negative fluid  
38 balance and increased serum osmolality (Thomas et al. 2008). Regardless of the  
39 habitat, animals must regulate body fluids to protect against or cope with dehydration  
40 (Takei 2015). Mammals have developed many different mechanisms for body fluid  
41 regulation (Christian and Matson 1978; Frank 1988; Jirimutu et al. 2012; Marra et al.  
42 2014; Yang et al. 2016; Kordonowy et al. 2017) and these mechanisms could aid in  
43 survival given the most well-supported climate change scenarios predict increased  
44 aridity (Mirzabaev et al., 2019).

45 Climate change is rapidly reshaping habitats globally and is predicted to continue  
46 (Hughes 2000; Parmesan and Yohe 2003; Parmesan 2006), modifying selective

47 pressures for many populations (Hochachka and Somero 2002; Pörtner 2002; Pörtner  
48 and Farrell 2008). Understanding environmental tolerance and the capacity for  
49 adaptation in one species can provide insight into the potential for similar species to  
50 respond to increasingly extreme climatic patterns which are likely to affect many  
51 habitats. In recent years, many habitats have recorded some of the hottest  
52 temperatures to date (IPCC 2019; Stillman 2019), resulting in environmental thresholds  
53 that may exceed organismal tolerance. Furthermore, climate change is increasing  
54 global desertification rates, increasing water stress among wildlife (Loarie et al. 2009;  
55 Mirzabaev et al. 2019). To maintain viable populations, organisms must survive and  
56 successfully reproduce under climate warming and aridification by either using existing  
57 phenotypes and phenotypic plasticity, rapid evolution, or geographic range and  
58 phenological shifts (Hofmann and Todgham 2010; Hoffmann and Sgrò 2011; Brown et  
59 al. 2016). Despite climate change altering habitats and impacting populations, habitat  
60 distributions for rodents have remained remarkably stable over the last century of  
61 climate change, largely due to behavioral changes (Pardi et al. 2020; Riddell et al.  
62 2021).

63 Mice of the genus *Peromyscus* have the widest distributions of any North  
64 American mammal and have unparalleled habitat diversity (Bedford and Hoekstra  
65 2015). Several members of the genus, including the cactus mouse (*Peromyscus*  
66 *eremicus*) are native inhabitants of the arid deserts in southwest North America (Murie  
67 1961; Pavlik 2008). Past studies have shown that cactus mice are extremely efficient at  
68 water retention (Kordonowy et al. 2017), have limited tissue damage when dehydrated  
69 (MacManes 2017), the slowest metabolism amongst the *Peromyscus* species (Mueller

70 and Diamond 2001), have a suite of genomic adaptations (MacManes 2017; Tigano et  
71 al. 2020; Colella et al. 2021a), but lack the kidney modification present in kangaroo rats  
72 (Dewey et al. 1966; MacManes 2017). Furthermore, animals of this genus can be held  
73 in captivity (Crossland et al., 2014), have extensive genomic resources (Colella et al.,  
74 2021a; Tigano et al., 2020), and a wealth of samples collected historically and  
75 contemporaneously in natural history museums (Pergams and Lawler, 2009). Together,  
76 these features make the cactus mouse ideal for investigating water economy.

77 Desert habitats are characterized by such an extreme lack of precipitation which  
78 exerts a controlling effect on biological processes (Rocha et al., 2021). Daily  
79 temperatures in the Sonoran Desert can fluctuate by as much as 30-50 °C (Reid 1987;  
80 Sheppard et al. 2002). During the summer months, temperatures can reach upwards of  
81 50 °C during the day, while at night they may drop to as low as 15 °C (Coppernoll-  
82 Houston and Potter 2018). In the winter, daytime temperatures are typically between 20  
83 – 30°C, while nighttime temperatures can drop to near freezing (Boyd Deep Canyon  
84 Desert Research Center). Daily rainfall in the Sonoran Desert is relatively rare, with  
85 most areas receiving less than a centimeter of rain per year (Boyd Deep Canyon Desert  
86 Research Center). Organisms that are adapted to live in desert habitats must manage  
87 their water budgets over long dry and hot periods of time.

88 Here we expand on the long history of studies of organismal water management  
89 in desert taxa (Albright et al., 2017; Blumstein et al., 2022; Bradford, 1974; Cortés et al.,  
90 2000; Frank, 1988; Hayes et al., 1998; Kordonowy et al., 2017; MacMillen, 1983;  
91 Schmidt-Nielsen and Schmidt-Nielsen, 1952; Schmidt-Nielsen and Schmidt-Nielsen,  
92 1952) to assess the physiological response to water deprivation in a hot and dry

93 environment. To accomplish this, we compared multiple physiological responses, rate of  
94 water loss, energy expenditure, respiratory quotient, a suite of electrolytes, body weight,  
95 and body temperature, for mice with and without access to water for 72 hours to  
96 understand how animals survive the extreme heat and aridity of deserts and further  
97 characterize *P. eremicus*' response to water deprivation.

98

## 99 **Methods**

### 100 *Animal Care and Experimental Model*

101 All animals used in this study were captive born, sexually mature, non-reproductive  
102 healthy adult male and female *P. eremicus*. Individuals were descended from wild  
103 caught animals from a dry-desert population in Arizona and maintained at the University  
104 of South Carolina Peromyscus Genetic Stock Center (Columbia, South Carolina, USA).  
105 Animal care procedures were approved by the University of New Hampshire Institutional  
106 Animal Care and Use Committee under protocol number 210602 and followed  
107 guidelines established by the American Society of Mammalogists (Sikes and the Animal  
108 Care and Use Committee of the American Society of Mammalogists, 2016). Mice were  
109 housed in a large walk-in environmental chamber designed to simulate the temperature,  
110 humidity, and photoperiod of their native desert environment (Kordonowy et al. 2017;  
111 Colella et al. 2021b; Blumstein et al. 2022). The daytime (light) phase lasted for 12  
112 hours (08:00 to 20:00) at a room temperature of 32°C and 10% RH followed by a one-  
113 hour transition period to the nighttime (dark) phase which lasted for 9 hours (21:00 to  
114 06:00) at a room temperature of 24°C and 25% RH. To complete the cycle a two-hour  
115 transition period occurs to return the room to light phase conditions (Kordonowy et al.

116 2017; Colella et al. 2021b; Blumstein et al. 2022). Mice were provided a standard diet  
117 and fed *ad libitum* (LabDiet® 5015\*, 26.101% fat, 19.752% protein, 54.148%  
118 carbohydrates, energy 15.02 kJ/g, food quotient [FQ] 0.89).

119 Prior to experimental conditions mice were weighed (rounded to the nearest  
120 tenth of a gram) on a digital scale. A temperature-sensing passive integrated  
121 transponder (PIT) tag (BioThermo13, accuracy  $\pm 0.02^{\circ}\text{C}$ , BioMark®, Boise, ID, USA)  
122 was implanted subdermally between the shoulders of each rodent using a tag injector  
123 (Biomark® MK10). Animals were then allowed to recover individually in an experimental  
124 chamber for one week of observation before the experiments were started. Body  
125 temperature was recorded at noon and midnight via a Biomark® HPR Plus reader and  
126 weight was measured every noon over the duration of the experiment. A randomly  
127 selected set of animals were assigned to the two water treatment groups (n=9 of each  
128 treatment, female mice with water, female mice without water, male mice with water,  
129 and male mice without water, total n=36). At the start of the experiment (day 0, time 0,  
130 10:00), water was removed from three of the chambers corresponding to those animals  
131 in the dehydration group. No mortality occurred during these experiments. Three days  
132 later, at the conclusion of the experiment, mice were euthanized via isoflurane overdose  
133 and decapitation, and we collected 120  $\mu\text{l}$  of trunk blood for serum electrolyte  
134 measurement using an Abaxis i-STAT® Alinity machine. Using i-STAT CHEM8+  
135 cartridges (Abbott Park, IL, USA, Abbott Point of Care Inc), we measured the  
136 concentration of sodium (Na, mmol/L), potassium (K, mmol/L), blood urea nitrogen  
137 (BUN, mmol/L), hematocrit (Hct, % PCV), ionized calcium (iCa, mmol/L), glucose (Glu,  
138 mmol/L), osmolality (mmol/L), hemoglobin (Hb, g/dl), chlorine (Cl, mEq/L), total CO<sub>2</sub>

139 (TCO<sub>2</sub>, mmol/L), and Anion gap (AnGap, mEq/L). Using Na, Glu, and BUN, we  
140 calculated serum osmolality. The experimental setup was repeated six times, three  
141 male batches and three female batches.

142

### 143 *Metabolic phenotyping*

144 During the experiment mice were exposed to either water deprivation or normal  
145 conditions for three continuous days while being housed in transparent 9.5L  
146 respirometry chambers with dried cellulose-based bedding. Air was continuously pulled  
147 from the chambers using a pull flow-through respirometry system from Sable Systems  
148 International (SSI) starting with SS-4 Sub-Sampler Pumps, one for each chamber, at a  
149 rate of 1600 ml min<sup>-1</sup> (96 l h<sup>-1</sup>). The SSI MUXSCAN was used to multiplexed air  
150 streams, measuring each chamber 120s approximately twice every hour. Finally, the  
151 Field Metabolic System (FMS, zeroed and spanned between each 72-hour experiment  
152 using dry gas with known concentrations of CO<sub>2</sub> and O<sub>2</sub>) sub-sampled the airstream at  
153 250 ml min<sup>-1</sup> and measured water vapor, CO<sub>2</sub>, and O<sub>2</sub> with no scrubbing.

154

### 155 *Calculations and Statistical Analysis*

156 We analyzed our data using methods fully described in Colella et al. (2021b) and  
157 Blumstein et al. (2022). Rates of CO<sub>2</sub> production, O<sub>2</sub> consumption, and water loss were  
158 calculated using equations 10.6, 10.5, and 10.9, respectively, from Lighton (2018).  
159 Respiratory quotient (RQ, the ratio of VCO<sub>2</sub> to VO<sub>2</sub>) and Energy expenditure (EE) kJ hr<sup>-1</sup>  
160 was calculated as in Lighton (2018, eq. 9.15). All downstream statistical analyses were  
161 conducted in R v 4.0.3 (R Core Team 2020). The R package mgcv::gamm was used

162 and included the fixed effects; access to water and sex, and interacting nonlinear  
163 smoothing regression terms with pairwise fixed effect combinations as interactions; time  
164 in days and diurnal cycle (Lin and Zhang 1999; Wood 2017) and visualized using *gratia*  
165 (Simpson 2023). Experimental batches and the mice nested within the experimental  
166 batch were used as random effects to ensure we were not violating the assumption of  
167 independence. This allows us to explain the average differences between groups of  
168 mice instead of explaining differences between individual mice. To test for statistical  
169 significant ( $p < 0.05$ ) differences in electrolytes after the treatments were applied and for  
170 each time point weight and body temperature were collected we used a student's two-  
171 tailed t-test (`stats::t.test`) between the sexes for each experimental group.

172

## 173 **Results**

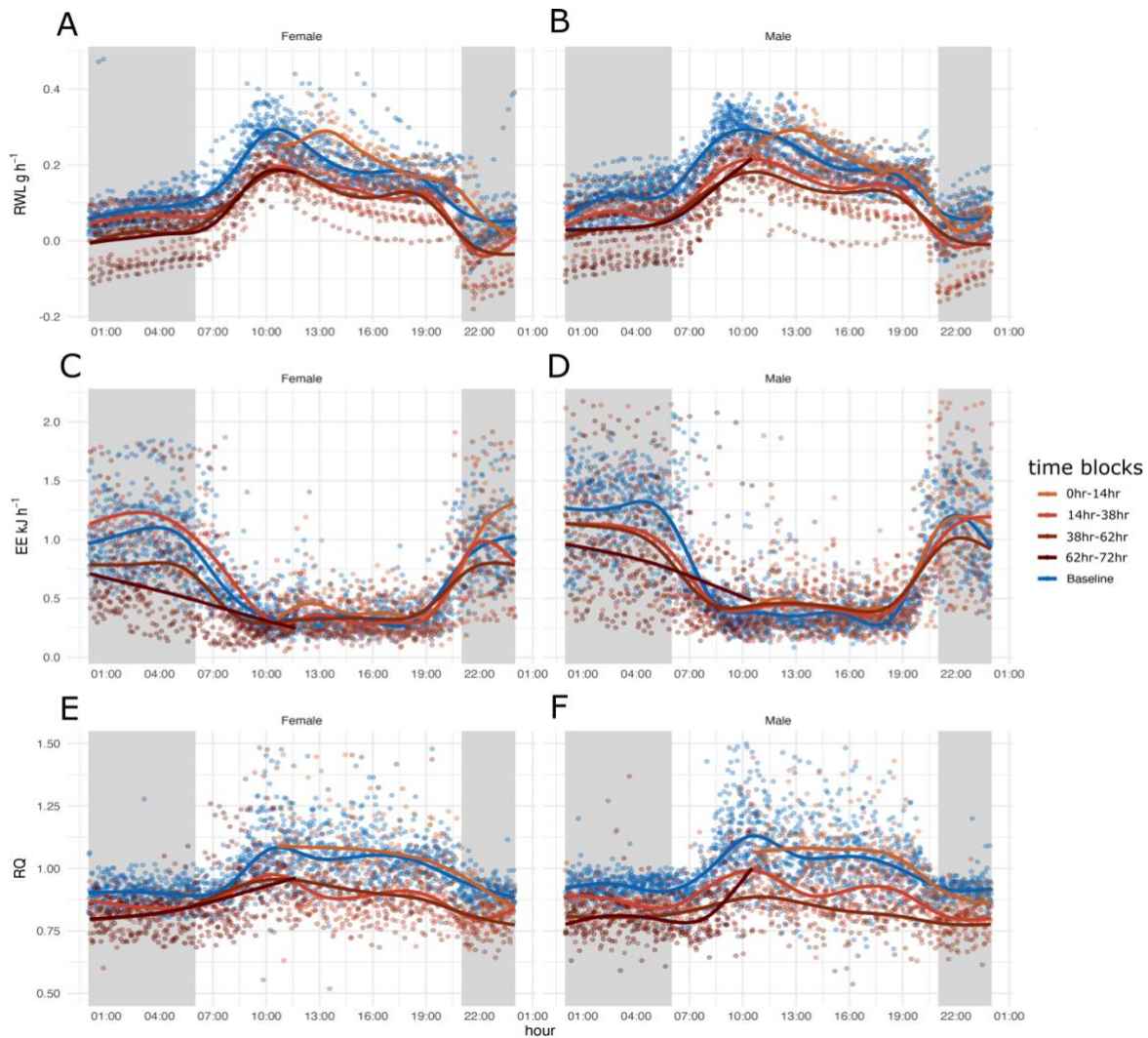
### 174 *Rate of Water Loss*

175 Both experimental groups, water access and water deprivation, had diurnal patterning of  
176 rate of water loss (RWL) with the highest occurring during the light phase and lowest  
177 during the dark phase (Figure 1A and 1 B). Each day of the experiment had similar  
178 patterns regardless of the treatment however, RWL was higher in males without access  
179 to water and for females lower or similar to the groups with water *ad lib* during day one  
180 of the experiment. For days two and three both males and females without access to  
181 water had lower RWL (Figure 1A and 1B).

182 Generalized additive modeling (GAM) analysis explained 77.2% of the deviance  
183 in RWL (Supplemental Figure 1, Supplemental Table 1). Significant predictors of RWL  
184 included sex ( $p < 2^{-16}$ ) and water access ( $p < 2^{-16}$ ) but not sex by water access ( $p =$



185 0.13). All treatment combination splines were significant (Supplemental Table 1. For  
186 both males and females without access to water, the curves for time in days by 24-hour  
187 cycle were very complex, oscillating with the light dark cycle and decreasing over time  
188 (Figure 1A and 1B). The curves for time in days for males and females with access to  
189 water oscillated with the light dark cycle as well (Figure 1A and 1B). Generally, mice  
190 without water had higher RWL during the first 24 hours and lower RWL for the  
191 remainder of the experiment based on GAM analysis and visualization (Supplemental  
192 Figure 1, Supplemental Table 1, Figure 1A and 1B). When comparing the four curves  
193 (males without water, males with water, females without water, females with water),  
194 RWL was similar to mice with access to water converged during the light to dark  
195 transition phases, with the exception of the first transition (Supplemental Figure 1).  
196



197

198 **Figure 1**

199 72 hours of respirometry data collection split by sex for 18 adult males and 18 adult  
200 females plotted in a 24-hour window to display circadian patterns for each group:  
201 Baseline measurements of mice with water access (blue) and measurements of mice  
202 without access to water for one, two, three, and four days (four shades of brown).  
203 Shaded sections in gray indicate the dark phase when animals are active, and  
204 unshaded blocks indicate light phase when animals are inactive. **A and B)** 24-hour rate  
205 of water loss (RWL, H<sub>2</sub>O g hr<sup>-1</sup>) **C and D)** energy expenditure (EE kJ hr<sup>-1</sup>), and **E and F)**  
206 respiratory quotient (RQ), for females (A, C, E) and males (B, D, F).

207

## 208 *Energy Expenditure*

209 Males and females in both experimental groups, water access and water deprivation,  
210 show diurnal patterning, with the highest EE occurring during the dark (active) phase and  
211 the lowest EE occurring during the light (inactive) phase (Figure 1C and 1D). Each day  
212 of the experiment for males and females with and without water has a similar pattern,  
213 elevated during the dark phase, and reduced during the light phase, regardless of the  
214 treatment.

215         During the first 24 hours, EE was highest for females without access compared  
216 to males without access to water and all mice with access to water. EE decreased over  
217 the subsequent 48 hours for mice without access to water with females without access  
218 to water having the lowest EE compared to males without access to water and all mice  
219 with access to water during the dark phase of days two and three of the experiment  
220 (Supplemental Figure 2, Supplemental Table 2, Figure 1C and 1D). Mice were manually  
221 weighted at 12:00 every day, resulting in a transient increase of EE at that time (Figure  
222 1C and 1D). The GAM analysis explained 61.4% of the deviance in EE with significant  
223 predictors being sex ( $p < 2^{-16}$ ), water access ( $p < 2^{-16}$ ), and sex \* water access ( $p =$   
224 0.0464). All treatment combination splines were significant (Supplemental Table 2).

225

## 226 *Respiratory Quotient*

227 RQ had diurnal patterning for both experimental groups and for both sexes (Figure 1E  
228 and 1F). RQ was highest during the light phases (Figure 1E and 1F) and lowest and  
229 comparable to the FQ during the dark phases (Figure 1E and 1F) 43.3% of the deviance

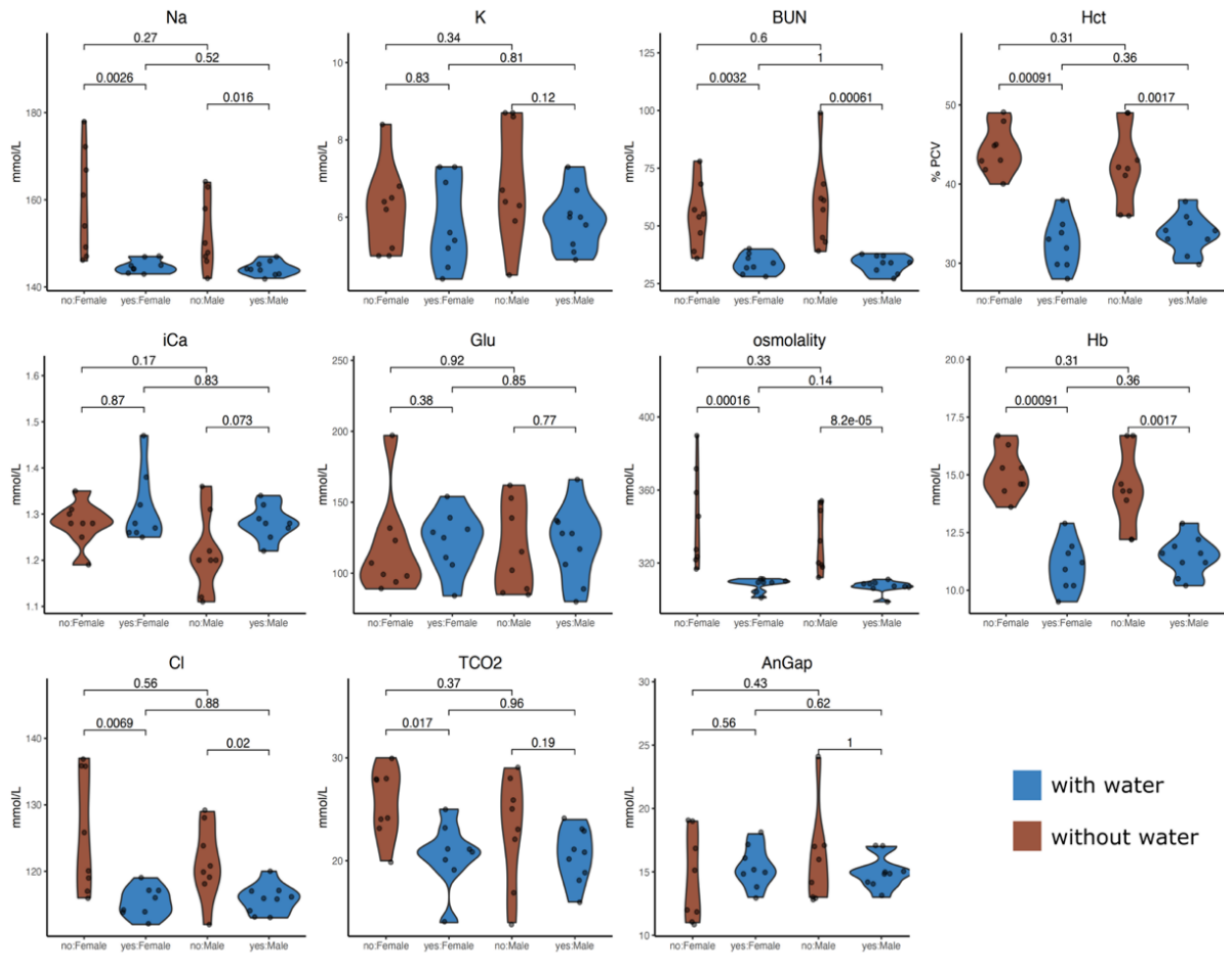
230 was explained in the GAM analysis with significant predictors being sex ( $p < 2^{-16}$ ), water  
231 access ( $p = 4.14^{-08}$ ), and the interaction between sex and access to water ( $p = 1.41^{-07}$ ).  
232 All treatment combination splines were significant (Supplemental table 3) and complex,  
233 oscillating with the light dark cycle (Figure 1E and 1F, Supplemental Figure 3). Males  
234 and females without water access had higher RQ compared to mice with water access  
235 during the first 24-hours based on GAM analysis and visualization (Supplemental Figure  
236 3, Supplemental Table 3). Interestingly, males without access to water had the lowest  
237 RQ of any group over the course of the entire experiment during the second dark phase  
238 and for the remainder of the experiment (Supplemental Figure 3).

239

#### 240 *Electrolytes, Weight, and Body Temperature*

241 Several electrolytes were significantly different when comparing males with and without  
242 access to water and females with and without access to water (male and female Na  $p =$   
243  $0.0016$  and  $p = 0.0026$  respectively, BUN  $p = 0.001/0.003$ , Hct  $p = 0.002/0.001$ ,  
244 osmolality  $p = 8.2^{-05}/0.0001$ , Cl  $p = 0.02/0.007$ , Hb  $p = 0.017/0.009$ , and TCO<sub>2</sub> female  $p$   
245  $= 0.017$ ) (Figure 2). No electrolytes were significantly different when comparing males to  
246 females for either water treatment (Figure 2).

247



248 **Figure 2**

249 Violin plots showing the distribution of serum electrolyte measurements (Na = sodium

250 (mmol/L), K = potassium (mmol/L), BUN = blood urea nitrogen (mmol/L), Hct =

251 hematocrit (% PCV), iCa = ionized calcium (mmol/L), Glu = glucose (mmol/L),

252 osmolality (mmol/L), Hb = hemoglobin (g/dl), Cl = chlorine (mEq/L), TCO<sub>2</sub> = total CO<sub>2</sub>

253 (mmol/L), and AnGap = Anion gap (mEq/L), for female and male *Peromyscus*

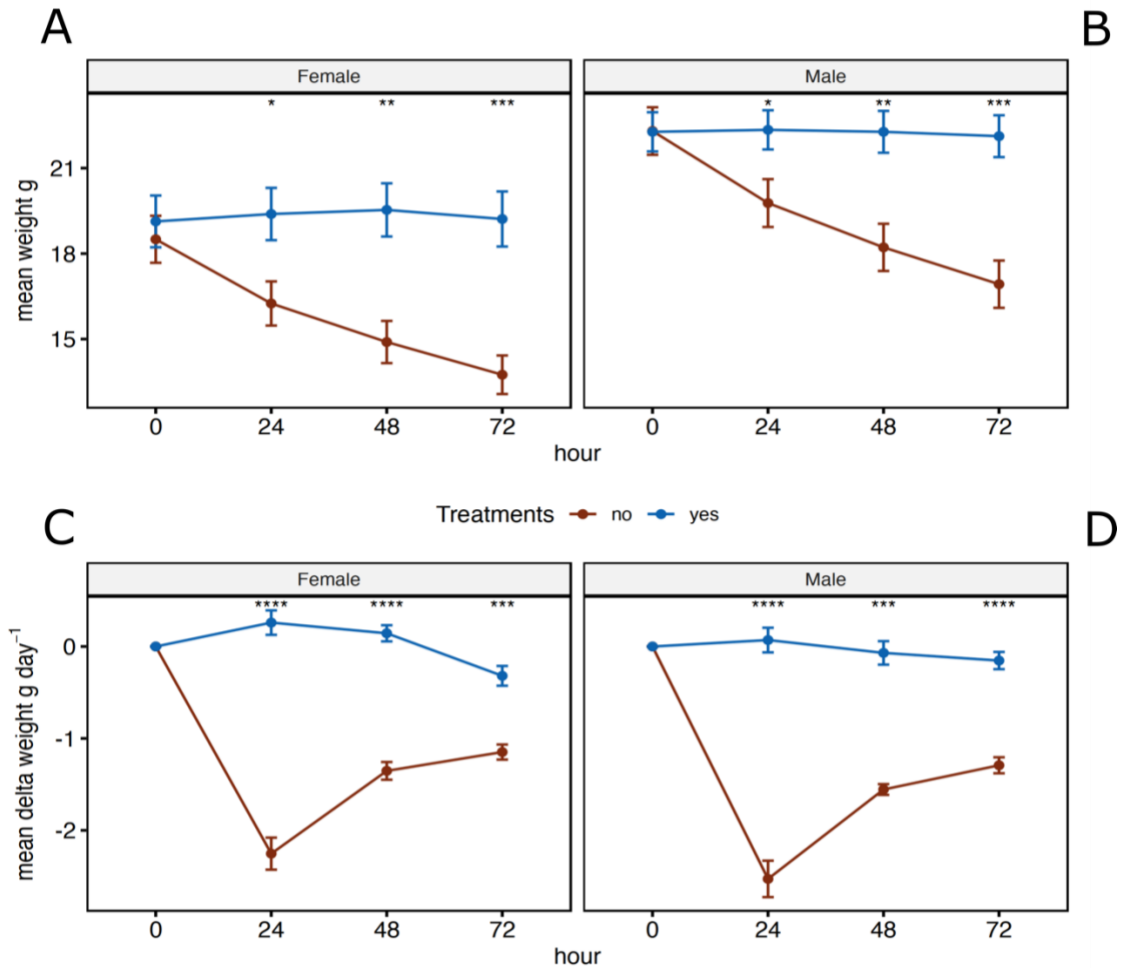
254 *eremicus* with (blue) or without (brown) access to water for 72 hours. Observations (n=9

255 of each treatment, total n=36) are represented by black dots. P-values from pairwise t-

256 tests are reported above the brackets.

257

258           While the weights of males and females were insignificant at the beginning of  
259 the experiment, both sexes lost weight over the course of the water deprivation  
260 experiment with the most weight loss occurring in the first 24 hours without water  
261 (Figure 3). When comparing males without access to water to males with water access  
262 and females without access to water to females with water access, mice without water  
263 weighed significantly less than mice with water at 24 hours ( $p = 0.024, 0.019$ ), 48 hours  
264 ( $p = 0.004, 0.002$ ) and 72 hours ( $p = 0.001, 0.005$ ) (Figure 3A and 3B). Only animals  
265 held without water lost weight (Figure 3C and 3D), and analysis of these changes were  
266 significantly different at all timepoints after water had been removed (24 hours,  $p = 4.1 \cdot$   
267  $10^{-5}, 4.1 \cdot 10^{-5}$ ), (48 hours,  $p = 0.001, 4.1 \cdot 10^{-5}$ ), and (72 hours,  $p = 4.1 \cdot 10^{-5}, 0.001$ ).



268

269 **Figure 3**

270 Mean weights (A and B) and mean delta weights (C and D) for female and male

271 *Peromyscus eremicus* with (blue) or without (brown) access to water every 24 hours

272 over the course of the 72-hour experiment. Error bars represent the standard error and \*

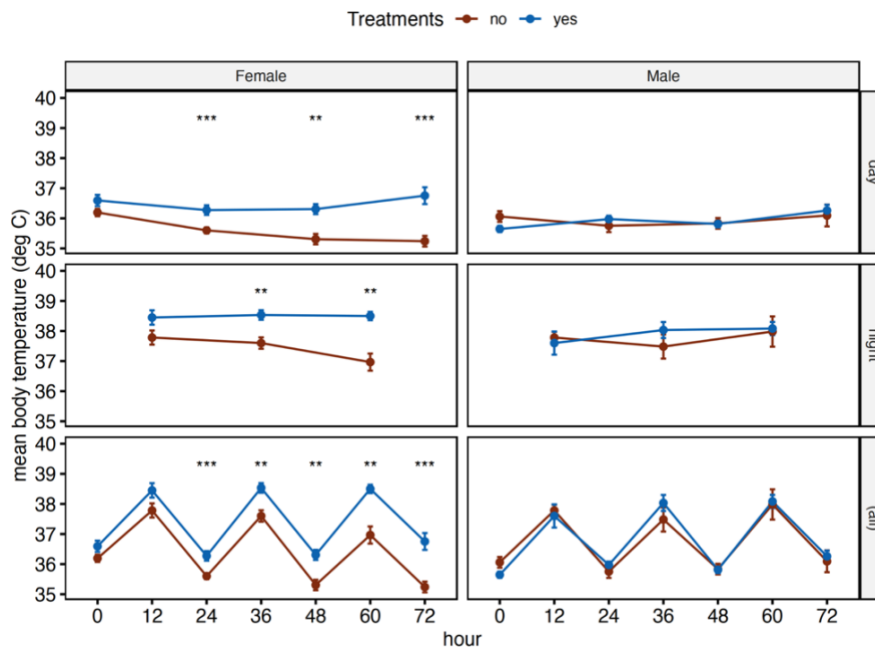
273 across the top denote statistical significance from t-tests between the two treatments, with

274 and without water, at each timepoint (\* p <= 0.05, \*\*: p <= 0.01, \*\*\*: p <= 0.001, \*\*\*\*: p

275 <= 0.0001).

276

277 Body temperature showed diurnal patterning with the highest body temperature  
278 during the dark (active) phase and the lowest during the light phase (Figure 4. For  
279 females, body temperature followed a similar pattern as described above and were  
280 significantly lower for mice without access to water at 24 hours ( $p = 0.001$ ), 36 hours ( $p$   
281  $= 0.005$ ), 48 hours ( $p = 0.001$ ), 60 hours ( $p = 0.002$ ), and 72 hours ( $p = 0.0003$ ) while  
282 males were not significantly different at any of the time points (Figure 4).



283 **Figure 4**  
284 Mean body temperatures for female and male *Peromyscus eremicus* with (blue) or  
285 without (brown) access to water every 12 hours over the course of the 72-hour  
286 experiment. The top row of graphs are measurements taken only during the light phase,  
287 middle row are measurements taken only during the dark phase, and bottom row  
288 represents all the measurements. Error bars represent the standard error and \* across  
289 the top denote statistical significance from t-tests between the two treatments, with and  
290 without water, at each timepoint (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq$   
291  $0.0001$ ).



292

## 293 **Discussion**

294           Physiological mechanisms can act as a buffer, expanding organismal tolerance  
295 to new or extreme environments (Bijlsma and Loeschcke, 2005; Gabriel, 2005; Lau et  
296 al., 2017; Lui et al., 2015; Wilson and Franklin, 2002), however, biochemical and  
297 physical constraints eventually limit physiological capacity (Campbell-Staton et al.,  
298 2021; Velotta and Cheviron, 2018; Velotta et al., 2018), and ultimately determine a  
299 population's persistence (Parmesan, 2006; Parmesan and Yohe, 2003; Van der Putten  
300 et al., 2010). In xeric habitats, further increased ambient temperature is compounded by  
301 reduced water availability, potentially affecting an animal's ability to maintain  
302 homeostasis of body temperature and fluids (Reece et al., 2015). Given the current  
303 rapid pace of climate change (IPCC 2019), it is vital that we understand how species  
304 are responding to changes in their environment. Increased drought and changes in  
305 precipitation patterns are having several impacts on the availability of water, both in  
306 terms of availability, quantity, and quality (IPCC, 2019; Mirzabaev et al., 2019).

307           Organisms maintain water homeostasis in several ways, including seeking out  
308 sources of free-flowing water, preformed dietary water (Frank 1988; Orr et al. 2015)  
309 and/or water produced by metabolism. However, if adequate water is not acquired,  
310 dehydration can negatively affect an animal's ability to regulate its body temperature,  
311 impair its' cardiovascular function, and decrease perfusion to organ systems.  
312 Specifically, dehydration results in a decrease in blood volume and increase in  
313 osmolality, primarily driven by the increase in serum sodium levels (Leib et al., 2016;  
314 Thornton, 2010). As a result, several neurohormonal systems are activated to maintain

315 blood pressure to perfuse tissues appropriately (Kaufmann et al., 2020). Water is  
316 recovered in the gastrointestinal tract (Thiagarajah and Verkman, 2018) and in the  
317 kidneys it is reabsorbed from the tubule system back into the blood stream (Fuller et al.,  
318 2020; Kortenoeven and Fenton, 2014). In severe cases, dehydration can lead to organ  
319 failure and death.

320 We explored the relationships and tradeoffs between thermoregulation,  
321 osmoregulation, and energy expenditure, of desert adapted mice without access to  
322 drinking water for three days while housed in an environmental chamber that simulated  
323 the desert environment. There are multiple avenues of water loss, including loss via  
324 urine and feces, as well as via cutaneous evaporation and respiration, and the  
325 measurements presented here represent their sum. We observed that when water was  
326 removed, energy expenditure and evaporative water loss are reduced in both sexes  
327 (presumably to conserve body water) at the expense of homeothermy, resulting in lower  
328 core body temperature in females but not in males. Though it may save water and/or  
329 energy, these physiological shifts could ultimately increase the risk of mortality and  
330 decrease fitness if water continues to be unavailable for extended periods of time.

331

### 332 *Weight loss and water deprivation*

333 Our study targeted responses to water deprivation, investigating how physiological  
334 variables changed in response to dehydration throughout the course of the experiment.  
335 We found that in response to water deprivation, cactus mouse phenotypic responses  
336 changed rapidly. During the first 24-hours of the water-deprivation experiment both  
337 males and females increased EE, resulting in an increase in RWL, and a significant

338 decrease in body weight. The reasons behind this dramatic shift are unclear but may be  
339 a result of 1) a behavioral response related to searching for drinking water and or 2)  
340 suppression of food intake as suggested by pilot studies.

341 The relationship between eating and drinking has been extensively studied  
342 (Fitzsimons and Le Magnen, 1969; Kissileff, 1969; Smith, 2000; Watts, 1998; Zorrilla et  
343 al., 2005) and it has been documented that dehydration-anorexia that is an adaptive  
344 response to limit fluid loss (Watts and Boyle, 2010), as typically the processing of food  
345 requires the use of water. Previous studies have demonstrated that dehydrated animals  
346 with *ad lib* food match the same attributes of food restricted animals, such as  
347 expression of hypothalamic neuropeptide genes, leptin and insulin levels, and  
348 corticosterone concentrations (Watts et al., 1999). Furthermore, the reduction of food  
349 intake results in a series of adaptive responses that target GI function, allowing for the  
350 absorption of the osmotically sequestered water that is normally in the GI (Kutscher,  
351 1966; Lepkovsky et al., 1957; Schoorlemmer and Evered, 1993). Finally, reduced food  
352 intake also reduces the solute load (Rowland, 2007) and the need for removal of waste  
353 products via urinary water loss (Schoorlemmer and Evered, 1993). In the study  
354 discussed herein, several tissues, including the GI tract, were removed at the  
355 conclusion of the experiment for future RNAseq analysis, and the GI tract was empty of  
356 food and feces (unpublished observations), suggesting that the intake of solid food had  
357 been decreased dramatically. As mentioned above, we saw a significant decrease in  
358 weight during the first 24 hours of the experiment, however, the RWL during the first 24  
359 hours is not enough to account for the weight loss, suggesting weight loss through other

360 means such as dehydration-anorexia (Armstrong et al., 1980; Hamilton and Flaherty,  
361 1973).

362 Previous studies have found that access to water (Hochman and Kotler 2006;  
363 Shrader et al. 2008; Levy et al. 2016) and specific dietary composition (Blumstein et al.,  
364 2022; Frank, 1988; Manlick et al., 2021; Orr et al., 2015; Schmidt-Nielsen, 1975;  
365 Schmidt-Nielsen and Adolph, 1964; Wolf and del Rio, 2003) strongly effects populations  
366 living in arid environments. These external factors influence species distributions  
367 (McKee et al. 2015), modifying foraging decisions (Gedir et al. 2016, 2020), and altering  
368 behavior and reproduction (Douglas 2001; McKinney et al. 2001; Cain et al. 2008). In  
369 the wild, cactus mice have been documented shifting diet seasonally, consuming  
370 arthropods during the winter (Hope and Parmenter, 2007), and transitioning to the  
371 consumption of cactus seeds and/or fruits during the summer (Hope and Parmenter,  
372 2007; Orr et al., 2015). In addition to preformed water, the composition of diet is also  
373 very important for *P. eremicus* as described in Blumstein et al. (2022). Specifically, mice  
374 fed a diet low in fat with *ad lib* water lost significantly more water and had electrolyte  
375 levels suggesting dehydration compared to mice fed a diet higher in fat, suggesting a  
376 limited capacity to tolerate water deprivation if optimal foods become less abundant  
377 (Blumstein et al. 2022). Furthermore, the temperatures required to balance evaporative  
378 water loss with metabolic water production on dry seed are much lower than what  
379 occurs during the summers in desert regions (MacMillen and Hinds 1983; Walsberg  
380 2000), suggesting that *P. eremicus* may not be able to survive on a only a dry diet,  
381 unlike the Heteromyids, which survive on dry diets alone (Frank, 1988; Schmidt-nielsen  
382 et al., 1948).

383 Consistent with predictions of altered physiology and behavior mediated by water  
384 restriction, we recorded a decrease in EE, RWL, body weight, and body temperature  
385 and a shift in serum electrolytes in water deprived *P. eremicus* during all three 24-hour  
386 time blocks. While males and females without access to water had different magnitudes  
387 of change in EE and RWL throughout the duration of the study, both metabolic rates  
388 and the rate at which water is lost decreased, similar to what has been recorded in other  
389 desert organisms (Schmidt-Nielsen et al. 1967; Taylor 1969; Finch and King 1982). EE  
390 and RWL are inherently related in animals as lower EE leads to lower water loss by  
391 decreasing the amount of dry air passing through the respiratory track (McFarlane and  
392 Howard 1972). Furthermore, catabolism of different diets vary in the amount of available  
393 energy (Sánchez-Peña et al., 2017), water potential, as well as their obligatory water  
394 loss (Schmidt-Nielsen, 1975). At lower humidity, oxidation of carbohydrates produces a  
395 net metabolic water gain while lipids and proteins result in water loss, mainly through  
396 urination which is required to remove products of their metabolism like urea.

397

### 398 *Sexual Dimorphism*

399 Interestingly, males and females responded differently to lack of water, with body  
400 temperature being the most notable difference. Females decreased their body  
401 temperature while males maintained their body temperature when compared to their  
402 hydrated counterparts. Whether this sexually dimorphic response is a strategy or  
403 consequence is an open-ended question that cannot be answered using the data  
404 presented here, this response may be the product of high costs of reproduction in  
405 females, but not males. Indeed, similar patterns of sexual dimorphism in response to

406 resource availability has been observed in other rodent species (Cranford 1977;  
407 Randolph et al. 1977; Murray and Smith 2012). Previous studies hypothesized that  
408 sexual dimorphism differences can be explained by differences in body size,  
409 metabolism, respiratory rate, or activity (Cryan and Wolf, 2003). While we do not have  
410 direct measurements of respiratory rate or activity, the production of CO<sub>2</sub> follows the  
411 patterns of EE, providing indirect yet strong evidence that respiratory and metabolic  
412 rates (EE) as well as activity are all sexually dimorphic, consistent with observations in  
413 humans (Glucksmann 1974; Mittendorfer 2005; Pomatto et al. 2018) and has also been  
414 observed in *P. eremicus* by McNab and Morrison (1963) and Colella et al. (2021b).

415         Male reproduction is mainly limited by access to females (Bateman 1948),  
416 therefore, torpor or estivation by males could reduce male reproductive success.  
417 Furthermore, sperm quantity and quality is dependent on body temperature (Moore,  
418 1926; Pérez-Crespo et al., 2008) and while typically resolved by externalizing the testes  
419 to the scrotum during excessive heat, a decrease in body temperature, as is seen in  
420 females (discussed below), could reduce sperm viability. Maintaining consistent body  
421 temperatures also allows for regular biological reactions, such as enzymatic processes  
422 and protein folding which have evolved to function best at a single temperature and can  
423 influence a series of functions not directly related to reproduction, such as growth rate,  
424 metabolic biorhythms, and environmental sensing (Glucksmann 1974; Hochachka and  
425 Somero 2002; McPherson and Chenoweth 2012; Calisi et al. 2018). Our data supports  
426 this as body temperature was unchanged for dehydrated males compared to their  
427 hydrated counterparts for the entire experiment, suggesting a maintenance of  
428 reproductive investment at the cost of long-term survival.

429 In contrast, female reproduction is primarily limited by their access to resources  
430 (Bateman 1948), in this case water. During the course of our study, female body  
431 temperature, EE, and RWL all decreased, suggesting torpor and or estivation,  
432 consistent with MacMillen (1983). Specifically, homeostatic responses such as adaptive  
433 heterothermy, a process which reduces evaporation by storing body heat, reduces the  
434 air to body temperature gradient thus decreasing inward heat flow, minimizes water loss  
435 from evaporative cooling (Schmidt-Nielsen et al. 1956; Schoen 1972; Taylor 1972; Cain  
436 et al. 2008), and in small endotherms with high surface area to volume ratios  
437 heterothermy can lead to substantial energy and water savings (Walsberg 2000;  
438 Speakman and Król 2010; Turbill and Stojanovski 2018). For females, reproductive  
439 demands are especially high, particularly during pregnancy and in lactating females (not  
440 measured in this study, Sorensen et al. 2005; Murray and Smith 2012), and minimizing  
441 energy costs or allocating pulses of resources to reproductive energy could increase  
442 reproductive success (Smith et al. 2014; Flores-Manzanero et al. 2019). While  
443 homeostatic responses are quite common among endotherms (Boyles et al. 2011,  
444 2013; Canale et al. 2012; McGuire et al. 2014; Dammhahn et al. 2017) and are  
445 essential for short term survival, they incur energetic, resource, and or fitness costs  
446 when the disturbance lasts longer than the homeostatic tolerance (Wingfield et al. 1992;  
447 Boonstra 2004; Canale et al. 2012; McGuire et al. 2014; Dammhahn et al. 2017).

448

#### 449 *Electrolytes*

450 In order to gain a deeper understanding of how water deprivation affects the  
451 physiological functioning of endotherms in desert environments, we collected serum

452 electrolyte data from both males and females with and without access to water at the  
453 end of the experimental period. Electrolytes are essential for all physiological functions,  
454 including regulating fluid balance, transmitting nerve impulses, and maintaining the acid-  
455 base balance (Hasona and Elsbali, 2016). Additionally, electrolyte levels can provide  
456 insight into an individual's overall metabolic state, renal function, and can be indicative  
457 of dehydration, kidney disease, or heart failure (Kutscher 1968; Chevront et al. 2010).

458         The kidney typically ensures that fluid and electrolyte balance remain within a  
459 narrow range, and this is conducive to efficient biochemical and physiological  
460 processes. Altered electrolytes, such as K, iCa, and Na, are associated with  
461 dehydration (Abubakar and Sule, 2010; Chevront et al., 2010; Kutscher, 1966), and  
462 may result in fatigue, cognitive dysfunction, and changes in osmotic pressure which may  
463 affect blood pressure. More severe electrolyte abnormalities may cause cardiac  
464 arrhythmias, and lead to death (Abubakar and Sule, 2010). We uncovered significant  
465 differences in electrolyte values between water treatments (Na, BUN, Hct, osmolality,  
466 Hb, Cl, and total CO<sub>2</sub>), suggestive of dehydration, but synthetic markers of renal  
467 function were unchanged. Together, supporting findings from (Kordonowy et al., 2017),  
468 this suggests that end-organ perfusion is maintained despite dehydration.

469         Despite being statistically insignificant, glucose trended downwards for males  
470 and females without water when compared to their hydrated counterparts. During  
471 fasting, blood glucose levels decrease due to a lack of glucose absorbed from the GI  
472 tract (Jensen et al., 2013). Previous studies have shown that the duration of fasting  
473 significantly affects blood glucose levels up to 72 hours, but after 72 hours there is no  
474 further decrease (Jensen et al., 2013). In humans, glucose concentrations are



475 maintained regardless of the duration of starvation (Watford, 2015). Initially,  
476 carbohydrates are depleted during the first 24 hours, however, during prolonged  
477 starvation gluconeogenesis provides glucose by breaking down skeletal muscle proteins  
478 (Watford, 2015). While the current study does not measure food intake, glucose is being  
479 maintained, suggesting they could be reducing in food intake consistent with other  
480 studies of dehydration-anorexia in rodents and or shift toward increased glycogenolysis  
481 and lipolysis to maintain glucose concentrations (Salter and Watts, 2003; Schoorlemmer  
482 and Evered, 2002; Watts and Boyle, 2010), meaning the liver is possibly serving as a  
483 buffer for blood glucose concentration.

484

## 485 **Conclusion**

486 The extreme aridity of desert environments plays a role in shaping biological processes  
487 however, the physiological mechanisms that allow animals to maintain salt and water  
488 homeostasis are still not well understood. Rapid climate change can challenge this  
489 tolerance. The cactus mouse (*Peromyscus eremicus*) is native to the arid deserts in  
490 southwest North America. Past studies have shown that cactus mice are highly adapted  
491 to desert conditions, with efficient water retention and dehydration tolerance. Therefore,  
492 cactus mice represent an interesting experimental model to examine physiological  
493 adaptations and thresholds.

494 In this study, we explore the physiological mechanisms that enable cactus mice  
495 to survive in desert habitats. By integrating laboratory-based experiments with long-term  
496 physiological data collected from a captive colony of cactus mice in a simulated desert  
497 environment, we investigate their response to water deprivation. Our findings reveal that

498 mice without access to water exhibit significantly lower energy expenditures, leading to  
499 reduced water loss compared to mice with access to water. Moreover, significant weight  
500 loss was observed during the first 24 hours, likely attributed to dehydration anorexia—  
501 an adaptive response aimed at limiting fluid loss by reducing waste and the solute load,  
502 while facilitating water reabsorption from the kidneys and gastrointestinal tract.  
503 Furthermore, our observations indicate a relationship between body temperature and  
504 sex. Males without access to water maintained their body temperature compared to  
505 hydrated males, while females without access to water experienced decreased body  
506 temperature, suggesting the occurrence of daily torpor in females as an adaptive  
507 response, likely related to reproductive investment.

508         By examining the physiological responses of water deprived *P. eremicus*, we  
509 gain valuable insights into how adaptations developed over long evolutionary  
510 timescales. Given the current global climate change and the escalating desertification  
511 trends, it becomes imperative to investigate the plasticity and mechanisms of response  
512 in desert-adapted species. Such investigations hold the potential to enhance our  
513 understanding of organismal responses to the increasingly unpredictable climatic  
514 conditions.

515

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523

## 524 **Author Contributions**

525 Conceptualization: M.D.M.; Methodology: D.M.B., M.D.M.; Formal analysis: D.M.B.,  
526 Investigation: D.M.B., Resources: M.D.M.; Writing - original draft: D.M.B.; Writing -  
527 review & editing: D.M.B., M.D.M.; Visualization: D.M.B.; Supervision: M.D.M.; Project  
528 administration: M.D.M.; Funding acquisition: M.D.M.

529

## 530 **Competing Interests**

531 No competing interests declared.

532

## 533 **Data Availability**

534 Macro processing files, processed respirometry data, and cage sampling scheme files  
535 are available on Zenodo: <https://zenodo.org/record/8091766>. All R scripts used in this  
536 project are available through GitHub at: [https://github.com/DaniBlumstein/dehy\\_phys](https://github.com/DaniBlumstein/dehy_phys).

537

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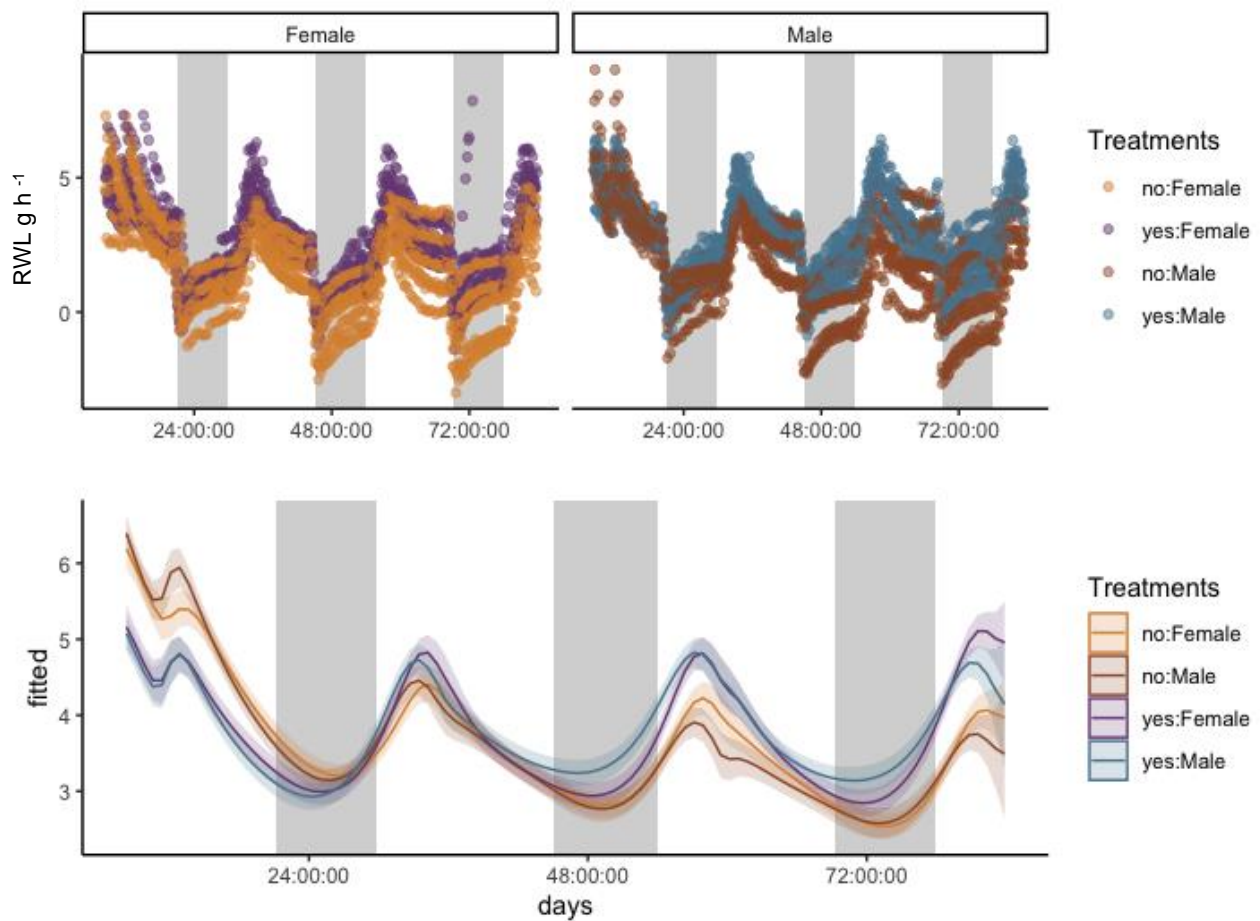
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- 744

746 **Supplemental**

747 **Figure 1**

748 Raw plotted data (top) and general additive mixed models (GAMM) graph (lower) for  
749 rate of water loss (RWL  $\text{g h}^{-1}$ ) for female and male *Peromyscus eremicus* with and  
750 without access to water. The smoothing curves for each response variable included two  
751 fixed effects; water treatment (yes vs no) and sex, two random effects; mouse  
752 identification number and date of data collection, and two regression terms: time in days  
753 and diurnal cycle. For the lower graph, the y-axis is the effect of the x-axis on RWL as  
754 estimated by a multivariable GAMM. Shaded areas are 95% confidence intervals.  
755



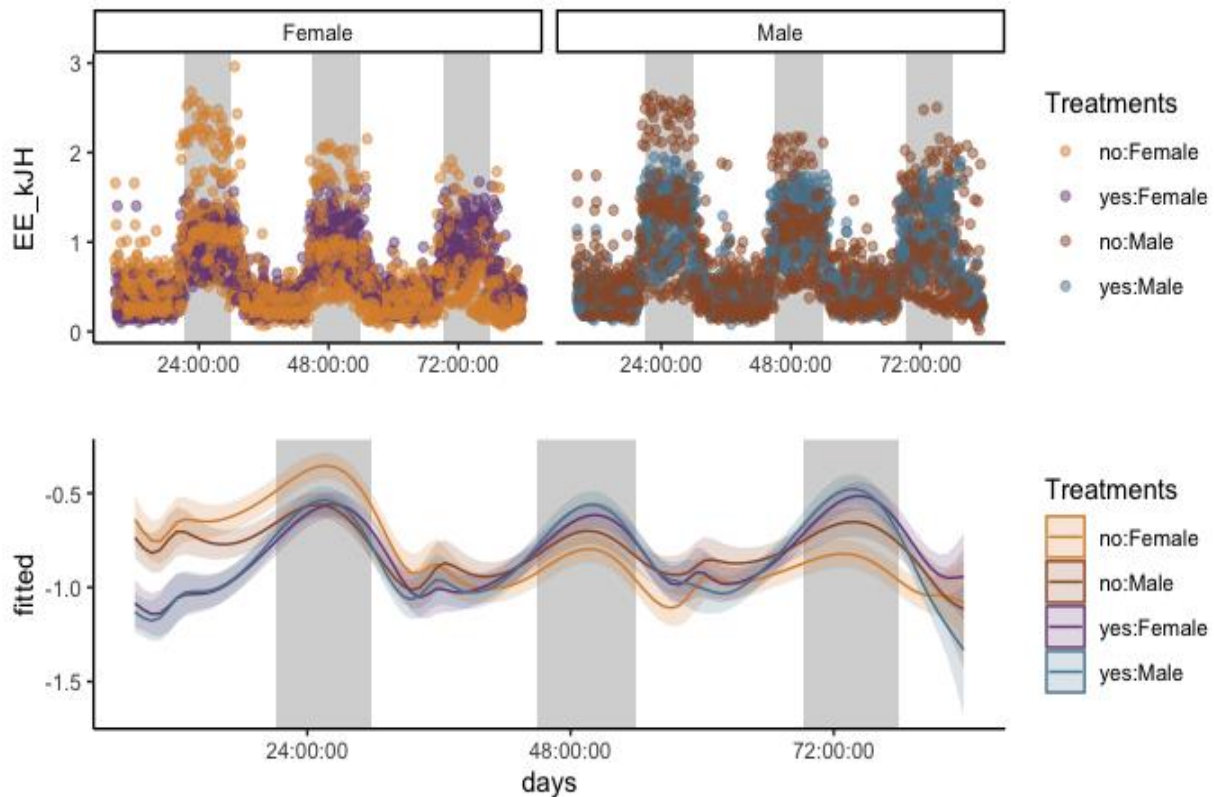
756

757



758 Figure 2

759 Raw plotted data (top) and general additive mixed models (GAMM) graph (lower) for  
760 energy expenditure (EE  $\text{kJ h}^{-1}$ ) for female and male *Peromyscus eremicus* with and  
761 without access to water. The smoothing curves for each response variable included two  
762 fixed effects; water treatment (yes vs no) and sex, two random effects; mouse  
763 identification number and date of data collection, and two regression terms: time in days  
764 and diurnal cycle. For the lower graph, the y-axis is the effect of the x-axis on EE as  
765 estimated by a multivariable GAMM. Shaded areas are 95% confidence intervals.  
766

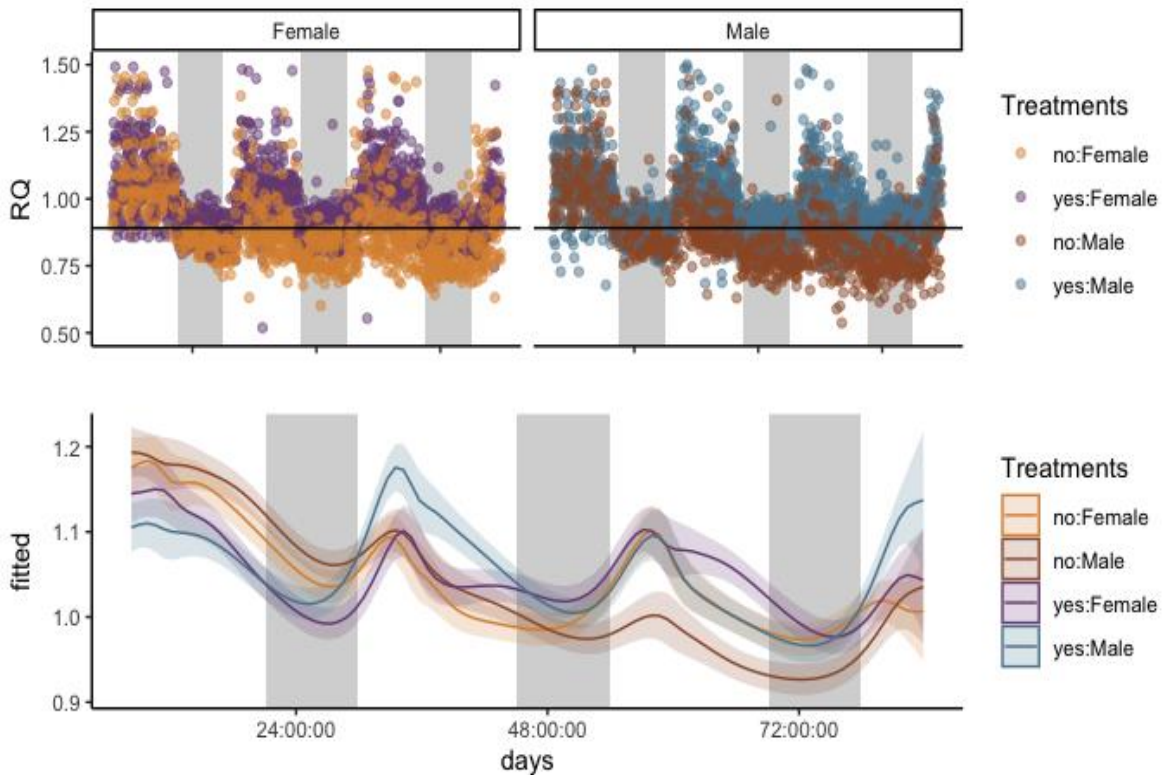


767

768

769 Figure 3

770 Raw plotted data (top) and general additive mixed models (GAMM) graph (lower) for  
771 Respiratory Quotient (RQ) for female and male *Peromyscus eremicus* with and without  
772 access to water. The smoothing curves for each response variable included two fixed  
773 effects; water treatment (yes vs no) and sex, two random effects; mouse identification  
774 number and date of data collection, and two regression terms: time in days and diurnal  
775 cycle. For the lower graph, the y-axis is the effect of the x-axis on RQ as estimated by a  
776 multivariable GAMM. Shaded areas are 95% confidence intervals.  
777



778

779

780 Table 1

781 Generalized additive mixed models (GAMM) stational model and results for rate of water  
782 loss (RWL, H<sub>2</sub>O g/hr<sup>-1</sup>).

783 Formula:

784 H2Omg ~ H2O \* Sex + s(days, time\_in\_D, by = tt, k = 70), data = dd, random =  
785 list(startexp = ~1, Animal\_ID = ~1|startexp), method="REML")

786

787 Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	1.67969	0.02029	82.775	<2e-16 ***
H2Oyes	0.83920	0.02874	29.195	<2e-16 ***
SexMale	0.27509	0.02878	9.557	<2e-16 ***
H2Oyes:SexMale	0.06247	0.04054	1.541	0.123

793 ---

794 Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

795

796 Approximate significance of smooth terms:

	edf	Ref.df	F	p-value
s(days,time_in_D):ttno:Female	56.81	71.02	67.57	<2e-16 ***
s(days,time_in_D):ttyes:Female	63.72	78.17	55.76	<2e-16 ***
s(days,time_in_D):ttno:Male	62.10	76.51	66.08	<2e-16 ***
s(days,time_in_D):ttyes:Male	62.61	77.02	58.38	<2e-16 ***

802 ---

803 Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

804

805 R-sq. (adj) = 0.764 Deviance explained = 77.3%

806 -REML = 8209.6 Scale est. = 0.65701 n = 6463

807

808 Table 2

809 Generalized additive mixed models (GAMM) stational model and results for  
810 measurements of energy expenditure (EE kJ/hr<sup>-1</sup>).

811 Formula:

812 log(EE\_kJH) ~ H2O \* Sex + s(days, time\_in\_D, by = tt, k = 70), data = dd, random =  
813 list(startexp = ~1, Animal\_ID = ~1|startexp), method="REML")

814 Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	-0.75378	0.01067	-70.658	< 2e-16 ***
H2Oyes	0.07460	0.01511	4.937	8.12e-07 ***
SexMale	0.21041	0.01511	13.925	< 2e-16 ***
H2Oyes:SexMale	-0.04238	0.02128	-1.992	0.0464 *

820 ---

821 Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

822

823 Approximate significance of smooth terms:

	edf	Ref.df	F	p-value
s(days,time_in_D):ttno:Female	43.79	53.83	42.03	<2e-16 ***
s(days,time_in_D):ttyes:Female	42.67	52.67	45.47	<2e-16 ***
s(days,time_in_D):ttno:Male	42.46	52.42	33.68	<2e-16 ***
s(days,time_in_D):ttyes:Male	46.23	56.15	53.83	<2e-16 ***

829 ---

830 Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
831  
832 R-sq.(adj) = 0.603 Deviance explained = 61.4%  
833 -REML = 3951.7 Scale est. = 0.18183 n = 6463  
834

### 835 Table 3

## 836 Generalized additive mixed models (GAMM) statical model and results for respiratory 837 quotient (RQ).

838 Formula:

839  $RQ \sim H2O * Sex + s(days, time\_in\_D, by = tt, k = 70), data = dd, random =$   
840  $list(startexp = \sim 1, Animal\_ID = \sim 1 | startexp), method = "REML")$

841

842 Parametric coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.911886	0.002997	304.246	< 2e-16 ***
H2Oyes	0.079386	0.004245	18.702	< 2e-16 ***
SexMale	-0.023301	0.004243	-5.492	4.14e-08 ***
H2Oyes:SexMale	0.031496	0.005976	5.271	1.41e-07 ***

848 ---

849 Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

850

851 Approximate significance of smooth terms:

	edf	Ref.df	F	p-value
s(days,time_in_D):ttno:Female	37.40	47.03	20.25	<2e-16 ***
s(days,time_in_D):ttyes:Female	37.59	47.22	17.27	<2e-16 ***
s(days,time_in_D):ttno:Male	29.41	37.68	27.09	<2e-16 ***
s(days,time_in_D):ttyes:Male	37.85	47.46	16.95	<2e-16 ***

857 ---

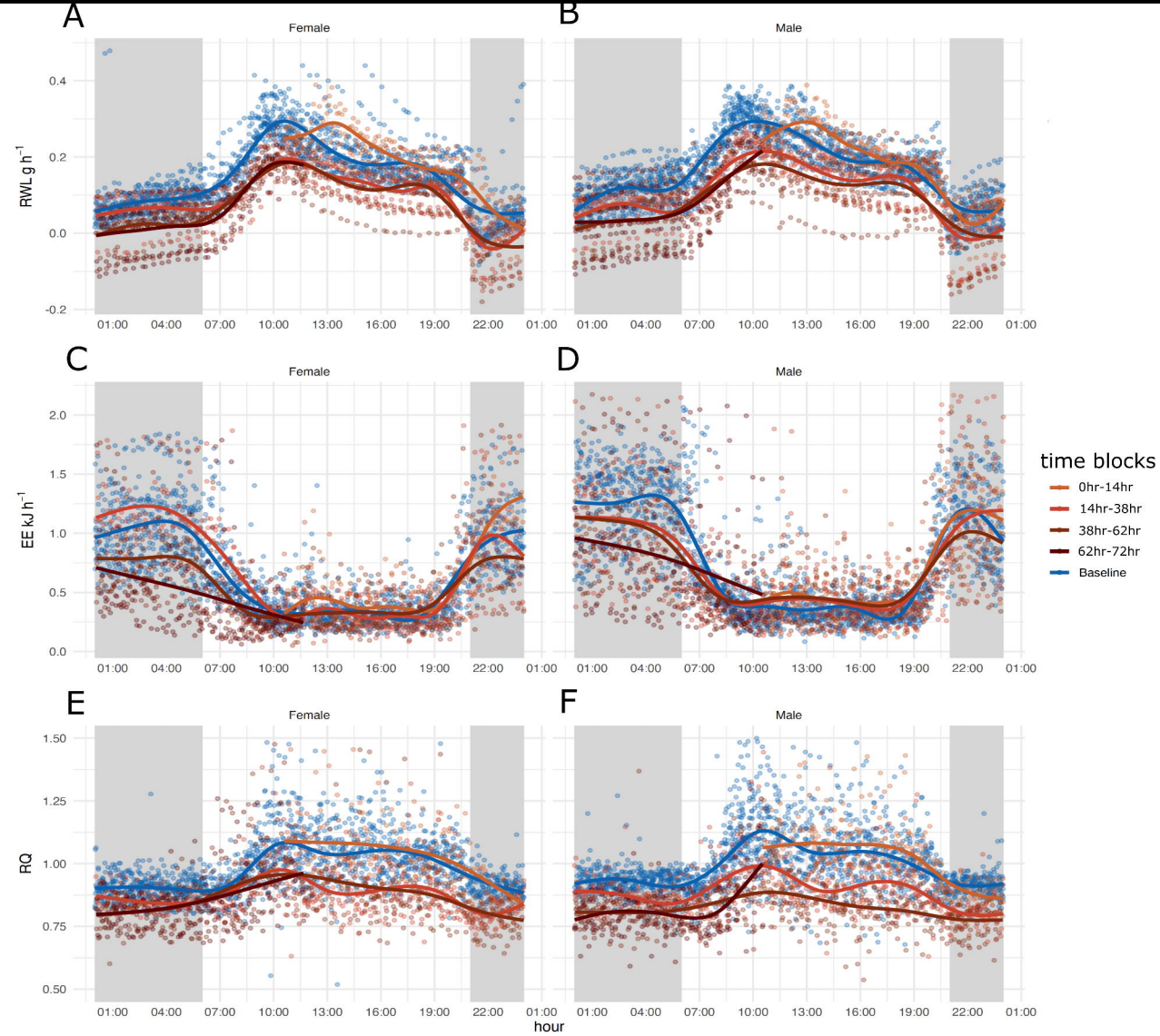
858 Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

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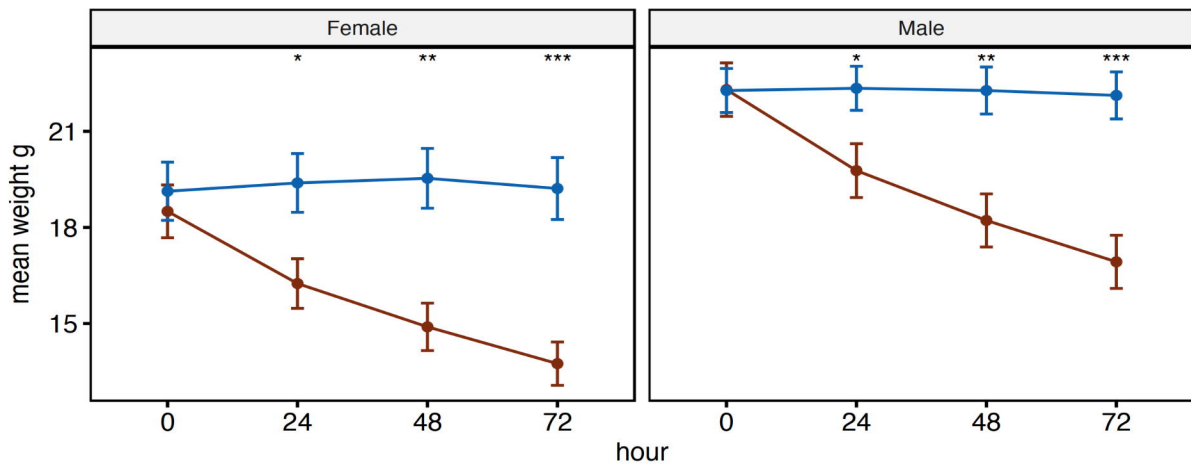
860 R-sq.(adj) = 0.42 Deviance explained = 43.3%

861 -REML = -4299.2 Scale est. = 0.014362 n = 6463

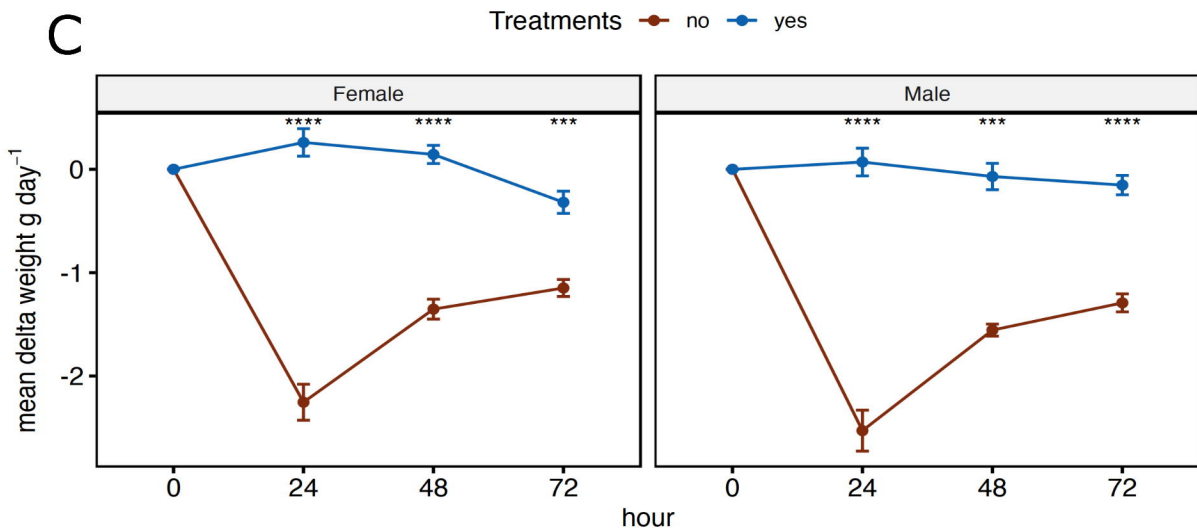
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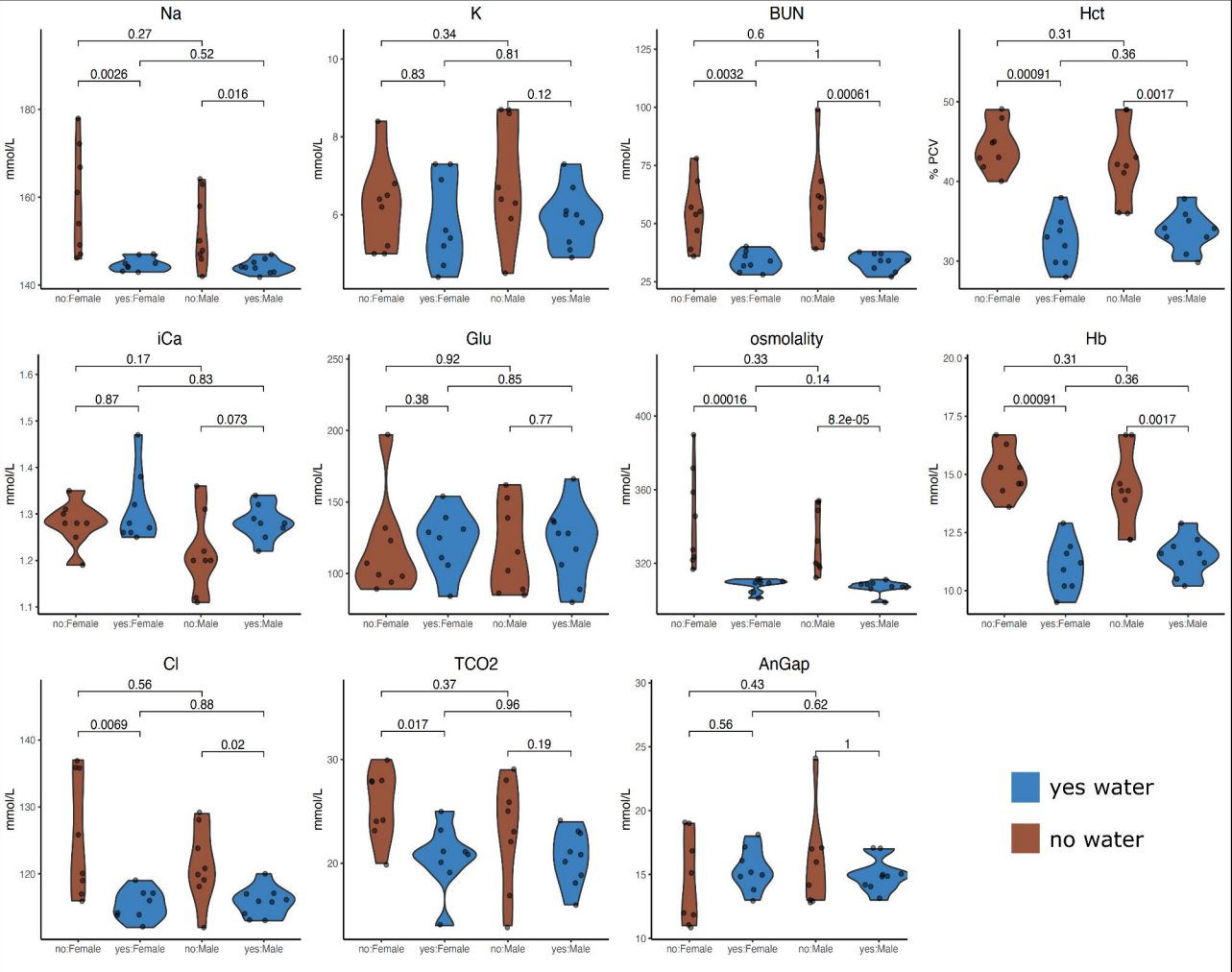


A



C





Treatments — no — yes

