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Original investigation

Comparative short-term variation of urine concentration among three sigmodontine rodent species from contrasting habitats

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ABSTRACT

We compared urine concentration capacity among three phylogenetically closed sigmodontine rodent species that occur in two distinct habitats: *Necromys lasiurus* (xeric), *Akodon cursor* and *Akodon lindberghi* (mesic). Water conservation ability was evaluated in 30 h-trials, with urine collected every 6 h. A control (I- water and food *ad libitum*) and a test (II- food and water deprivation) were conducted for each species. Body mass loss (BML), urine volume (UV), urine relative volume (UVr) and urine concentration (UC) were compared between experiments I and II for each species and among species. Changes in urine osmolality between experiments were modeled using exponential and sine functions. Circadian variation of urine concentration was obtained by sine functions fit to the experiments I. Best fit was achieved for sine functions with an accumulation term for experiments II, showing that even in stressful conditions the circadian variation is still present. Highest UC and lowest BML were obtained from *Necromys lasiurus* individuals, which achieved them at faster rates. The lowest UC with highest BML were obtained for *A. cursor* individuals at slower rates and values than *A. lindberghi*, which were intermediate. *Necromys lasiurus* is more adapted for xeric habitats achieving higher urine concentration in shorter time and faster rate than species of genus *Akodon*.

Introduction

The capacity to produce high concentrated urine and decrease water loss, is one of the most important physiological factors determining the survival of a small mammals in habitats with low water availability (Al-Kahtani et al., 2004; Bozinovic et al., 2003, 2007a; Cortes et al., 1994; Favaroni-Mendes et al., 2004; Gallardo et al., 2005; Schmidt-Nielsen, 1996). Water balance among mammals from mesic and transitional regions are less studied. Therefore, studying the urine concentrating ability of small mammal species can help to understand the patterns and processes that shape their geographical distribution and habitat differences.

The urinary concentrating ability of rodent species from xeric regions of Australian, North America and Asia were already studied, and their capacity to produce highly concentrated urine demonstrated (Kan and Degen, 1988; Simon and Nevo, 1990; Webb, 1978; Yahav et al. 1990) Whether South American rodents, particularly sigmodontine rodents, presents similar metabolic adaptations for efficient water conservation in xeric conditions remains an open question. For some simodontinae and echymidae species, occurrence in these habitats has been mostly attributed to behavioral and ecological traits rather than physiological mechanisms (Mares, 1977a; McNab, 1982; Meserve, 1978; Ribeiro et al., 2004). However, recent studies involving rodents from Argentine and Chilean xeric regions concluded that these rodents "possess biological systems that act at different levels of biological integration (*e.g.* from behavior to molecular biology) to generate mechanisms of water conservation which are just as extraordinary as those of the "classical" desert rodents found in different xeric areas around the world" (Al-Kahtani et al., 2004; Bozinovic et al., 2007a; Tirado et al., 2008).

Water conservation mechanisms of small mammal species inhabiting brazilian xeric region are still poorly studied and researches suggest that brazilian rodents of xeric and humid environments lack physiological mechanisms to cope with water stress (Cerqueira et al., 2003; Mares, 1977a, 1977b, Mares et al., 1985; Meserve, 1978; Favaroni-Mendes et al., 2004; Fonseca and

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Cerqueira, 1991; Mares et al., 1985, Ribeiro et al., 2004; Streilein, 1982). However, it is possible that this apparent lack of physiological adaptations is a result of insufficient data and comparative studies among species from habitats differing in water availability (Bozinovic and Gallardo, 2006; Carvalhães et al., 2015; McNab, 1982). Favaroni-Mendes et al. (2004) showed that two populations of the same Echimyidae rodent species living in different climatic conditions in Brazil differ in their response to water and food deprivation. Therefore, it is possible that more subtle adaptations to conserve water than the extraordinary adaptive mechanisms of desert rodents play a role in the geographical distribution of Neotropical rodents.

Akodon cursor (Winge, 1887), *Akodon lindberghi* (Hershkovitz, 1990) and *Necromys lasiurus* (Lund, 1840) are closely related sigmodontine rodent species (Gonçalves et al., 2007; Patton et al., 1989; Silva et al., 2006). They are found in sympatry in many Brazilian localities. *Akodon cursor* and *N. lasiurus* are widely distributed throughout South America. *Akodon cursor* is commonly found in mesic areas (Geise, 1995), whereas *N. lasiurus* occurs in open dry areas regions of Caatinga, Cerrados and Amazonian savannas (De Oliveira et al., 1988; Dietz, 1983; De-Lima-Francisco et al., 1995; Becker et al., 2007) The latter is found in early succession stage microhabitats, which have drier micro-climatic conditions during dry periods (De-Lima-Francisco et al., 1995; Engel and e Mello, 1993; Streilein, 1982). *Akodon lindberghi* was first described from the Cerrado of Brasília, East-Central Brazil (Nowak 1999; Wilson and Reeder 1993). However, it seems to occur in habitats intermediary between Cerrado (dry) and Atlantic Forest (moist) (Geise et al., 1996; Geise et al., 2001). These three species have to withstand seasonal variations in water supply, *Necromys lasiurus* suffering more severe drought periods than those of *Akodon* species.

The habitat differences discussed above led us to hypothesize that *A. cursor*, *A. lindberghi* and *N. lasiurus* differ physiologically in their water conservation ability. Here, we compared their capacity to conserve water and try to model the temporal variation in the urinary concentration to understand how species achieves this urine concentration. Urinary concentration experiments were generally conducted taking one urine sample after a period (generally 12–24 h) of water deprivation (Antinuchi and Busch, 1999; Cerqueira et al., 2003; Diaz and Ojeda, 1999; Fonseca and Cerqueira, 1991; Ntshotsho et al., 2004). Therefore, they do not take in account possible short-term temporal variation on urine concentration. We try to study this using a using a new approach that can evaluate the short-term urine concentration and other classical parameters that are related with urine concentration ability like body mass loss and urine volume on situations of water deprivation. We expect that *N. lasiurus*, the broader geographical range species inhabiting habitats of Caatinga and Cerrado (more xeric), will differ from *Akodon* species, the species with more limited geographical range inhabiting more mesic habitats.

Material and methods

Specimens and animal housing

Individuals were collected from the field or were born in the laboratory. Specimens of *Akodon cursor* (n = 10, 5 females and 5 males) and *Akodon lindberghi* (n = 10, 4 females and 6 males) were collected at Simão Pereira, Minas Gerais -Brazil (21°58′S/43°19′W). *Necromys lasiurus* (n = 8, 3 females and 5 males) were captured at Cavalcante, Chapada dos Veadeiros, Brazil (13°47′52.0"S/47°54′00"W). The colonies were housed at the Laboratório de Ecologia de Vertebrados – UFRJ in a room with mean temperature 21.4 ± 1.68 °C and mean relative humidity 76.54 ± 10.22%, natural photoperiod of Rio de Janeiro state (Brazil). Mean body mass were 65.18 g (dp = 14.74) for *N. lasiurus*; 71.62 g (dp = 26.10) for *A. cursor*, and 22.93 g (dp = 4.8) for *A. lindberghi*. In order to avoid animal suffering, our procedures are in accord to guidelines of the American Society of Mammalogists for research with wild mammals (ASM, 2011), being approved by UFRJ Ethical Committee for Animal Use (License 01200.001568/2013-87).

A nutritional and balanced diet made with cropped food items was elaborated for each species based on the results of an alimentary preference test, conducted just after individuals arrived at the laboratory (Finotti, 2003; Périssé et al., 1989). The diet was tested comparing mean body mass variation in relation to initial weight for 10 days. Body mass loss was calculated as the proportion of mass lost since the first day, when the weight was considered to be at 100%. The results indicated that the diets were balanced: *A. cursor* 106.12% ± 3.26; *A. lindberghi* 103.00% ± 3.52; and *N. lasiurus* 100.85% ± 4.05.

Urine concentration experiment

Experiments were conducted in a room with natural photoperiod, 21.28 °C (dp = 1.90) of temperature and 73.34% (dp = 8.17) of relative humidity. Temperature and humidity were monitored every 30 min by data loggers (U14L series, ONSET HOBO). We tested the influence of these variables on urine excretion and concentration using a multiple regression technique (Zar, 1996). The mean temperature and humidity values of each six hour period were calculated and correlated with the mean urine concentration values obtained for the individuals at the end of the period. Variation in room temperature and relative humidity did not influence neither urine volume (UV) ($r^2 = 0.004$, $p = 0.92$ and $r^2 = 0.06$, $p = 0.86$, for *A. cursor*; $r^2 = 0.05$, $p = 0.46$ and $r^2 = 0.14$, $p = 0.24$ for *A. lindberghi* and $r^2 = 0{,}08$, $p = 0.89$ and $r^2 = 0.01$, $p = 0.95$ for *N. lasiurus*), nor urine concentration (UC) ($r^2 = 0.20$, $p = 0.76$ and $r^2 = 0.15$, $p = 0.67$, for *A. cursor*, $r^2 = 0.0002$, $p = 0.92$ and $r^2 = 0.09$, $p = 0.57$ for *A. lindberghi* and $r^2 = 0{,}08$, $p = 0.89$ and $r^2 = 0.15$, $p = 0.38$ for *N. lasiurus*).

To collect urine, individuals were placed inside metabolic cages with stainless steel mesh floors, which allowed urine to fall through into a glass collection dish containing hydrated mineral oil to prevent urine evaporation. Feces were retained by a stainless sieve, located just below the floor to prevent contamination of urine samples.

Each individual was weighted and placed inside metabolic cages at 10 a.m. for acclimation. Urine excreted until noon was discarded. Urine was collected during 24 h, with intervals of six hours, beginning at 06:00 p.m. Urine volume was measured using micropipettes 10–100 μl (Labnet International Inc.). We checked urine retention in funnels and noted that it was negligible compared to total urine excreted, thus it was not measured.

Urine samples were stored in 2 ml Eppendorf vials, sealed with plastic film to prevent evaporation, and kept at −20 °C until analysis. Osmolality was measured using a freezing point osmometer (Osmomat 030–Gonotec – resolution: 1.0 mOsmol/Kg). During the entire collection period, the health of experimental animals was checked. When the experiment was over, the animals were weighted again and placed inside plastic cages with water and food *ad libitum*.

Two types of experiments were performed. In the experiment I (control experiment), water was given *ad libitum* and a balanced diet was supplied during all 30 h experiment period. In the experiment II (test experiment), animals were deprived from water and food during 30 h experiment period.

The following parameters were analyzed: Body mass at the beginning and at the end of the experiment, Body mass loss (BML), Urine Volume (UV) (ml day^{-1}), Relative Urine volume (UVr) (μl h^{-1} . 100 g^{-1}) and Urine Concentration (UC) (mOsmol/Kg). Body Mass Loss was calculated as the proportion of mass lost between