WATER CONSERVATION ABILITY OF THREE SPECIES OF THE GENUS *THRICHOMYS* (RODENTIA, HYSTRICOMORPHA).

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ABSTRACT

We tested the water conservation ability of three species of the genus *Trichomys* that occur in localities with very different climatic regimes, *T. fosteri* (Pantanal), *T. aff . laurentius* (Cerrado) and *T. laurentius* (Caatinga). Individuals were submitted to laboratory urinary concentrating experiments using two treatments: one where food and water *ad libitum* was offered (control experiment - I) and the other of food and water deprivation (test experiment - II). Experiments were conducted during 24 hours and urine volume was noted and collected every 6 hours. We compared the differences in body mass loss, urine excretion and concentration between treatments for each species and between species for the test experiment (II). The patterns of temporal variation in urine concentration were also analyzed during the experiments. Species showed significant differences in urine volume and urine concentration but not in body mass loss when experiments I and II were compared. *T. fosteri* showed significantly higher body mass loss and urine volume compared to the other species in experiment II, suggesting that this species is less efficient in conserving body water. However, no significant differences in the concentration of urine were found. All species presented low mean urine concentration values (*T. aff . laurentius* = 1210.02±498 mOsmol/kg , *T. laurentius*= 1328.68±662.68 mOsmol/kg , *T fosteri*= 1301±541 mOsmol/kg) compared to other South American rodents. However, subtle differences on *Thrichomys* species water conservation ability are important to habitat and geographical differentiation. The temporal patterns of urine concentration from experiments I and II are very similar for all species, changes in the pattern of urine concentration over time were not observed in experiment II, as was found in other studies. This may be evidence that these species are capable of undergoing periods higher than 24 hours of food and water deprivation without major changes in urine concentration.

Keywords: water conservation, body mass loss, urine concentration, Semi-arid and Adaptation.

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INTRODUCTION

Species of the genus *Thrichomys* (Trouessart, 1880) have a complex taxonomic history. It was believed to be a monospecific genus (*T. apereoides* (Lund, 1839)) but in the 50's and 60's Moojen (1952), Vieira (1955) and Cabrera (1961) described some subspecies of *T. apereoides* and named the genus *Cercomys*. More recent studies based on geometric morphometric (Bandouk & Reis 1995, Reis *et al.* 2002a, 2002b, Pessoa *et al.* 2004, Neves & Pessôa 2011), chromosomal (Bonvicino *et al.*, 2002, Pessoa *et al.* 2004), molecular (Braggio & Bonvicino 2004) and bionomic (Teixeira *et al.* 2005) characters demonstrated differences between geographically separated populations and currently *Thrichomys* is considered a complex of 8 species (Nascimento *et al.* 2013). These species are distributed along the open dry and semi-arid vegetational formations of Cerrado, Pantanal and Caatinga, spanning a diagonal from eastern Brazil to Paraguay (Favaroni-Mendes *et al.* 2004).

Among these species, *T. aff. laurentius*, *T. laurentius* and *T. fosteri* have quite distinct geographical distribution and habitat characteristics. *T. laurentius* occurs in the semi-arid areas (Caatinga), associated with mesic refuges and rocky habitats (slopes of mountain chains and "lajedos" formations), where they find suitable microhabitat with more moderate temperature and humidity levels. *T.aff laurentius* can be found throughout the Brazilian northeast, above the São. Francisco river, in open shrub areas. *T. fosteri* is found in open areas of Pantanal in Mato Grosso do Sul state, where there is marked seasonality in water availability (Nascimento *et al.* 2013). It habitats vary from open vegetation areas and grasslands with isolated trees to typical Cerrado formations, with high tree density and forest islands (Streilen 1982b; Basile, 2003).

The ability to conserve body water is one of the most important physiological strategies that enable the habitation of xeric environments by small mammals and can contribute to explaining the differences in habitat and geographical distribution (Prosser 1973, Mares 1977a, 1977b, Kan and Degen 1988, Beauchat 1990, Beauchat 1996, Schmidt-Nielsen 1996, Ivanova *et al*. 2000). This characteristic has been studied in small mammals that inhabit xeric environments in South America (Cortés *et al* 1990, Cortés *et al.* 1994, Díaz & Ojeda 1999, Shanas *et al.* 2003, Al-Kahtani *et al.* 2004, Bozinovic & Gallardo 2006, Díaz *et al.* 2006) but

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there are few of these studies with Brazilian species and its importance on their evolution and geographical distribution (Meserve 1978, Streilein 1982a, Mares *et al.* 1985, Fonseca & Cerqueira 1991, Cerqueira *et al.* 2003, Favaroni-Mendes *et al.* 2004, Ribeiro *et al.* 2004, Finotti *et al.* submitted).

Semi-arid Brazilian (Caatinga) species were supposed to lack water conservation abilities (Mares *et al.* 1981, Ribeiro *et al.* 2004). In fact, mean urine osmolality found for species captured at Caatinga localities were usually lower (2193 mOsmol for *T. inermis*, 2649 mOsmol.kg^{-1} for *Oligoryzomys nigripes* and 2450 mOsmol.kg^{-1} for *Necromys lasiurus*) comparing to other South American desert rodents (3300-4500 mOsmol.kg^{-1}) (Cortes *et al.* 1998, Bozinovic 1995, Favaroni-Mendes *et al.*, 2004, Ribeiro *et al.* 2004, Finotti *et al.* submitted) and North American and Australian desert species (*Notomys alexis* – 9.370 mOsmol/Kg, *Dipodomys merriane* – 5.500 mOsmol/Kg and *Jaculus jacullus* - 6.500 mOsmol/Kg) (MacMillen & Lee 1967).

However, data on the ability to survive on dry seeds for long periods and on intraespecific differences on body mass loss and urine volume decrease for *Thrichomys* species can be an indicative of some degree of adaptation to arid conditions and, even being small, they can be significant for habitat and geographical differentiation. Streilein (1982b) found that individuals of *T. apereoides* can stay up to 18 days under water deprivation feeding only on dry grain and losing only 12% of their body mass. Favaroni-Mendes *et al.* (2004) compared the ingestive balance of two Caatinga populations of *T. apereoides* (now *T. inermis* (Nascimento *et al.* 2013)) with different rainfall regimes, in 18h food and water *ad libitum* and water and food deprivation trials. They found that individuals of the more mesic area have higher body mass, water intake and urine volume excretion than individuals from more xeric ones but differences on urine concentration were not found. This lack of high urine osmolality values are generally viewed as a consequence of the low evolutionary time that these species had to adapt to arid conditions (Mares *et al.* 1977, Streilein 1982b, Ribeiro *et al.* 2004).

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Finotti *et al.* (submitted), analyzing the daily variation of urine excretion and concentration of sigmodontine rodents (mean weight from 22.93 to 65.18g) under food and water deprivation conditions, found that urine concentration starts to increase after 18h and reach their maximum at 24 to 30h. So, it is possible that *Thrichomys* species are not achieving their maximum concentration capacities and greater time periods of water deprivation are needed. Moreover, they also observed a daily urine concentration variation at normal conditions that still occurs when animals are water deprived. Urine osmolality at periods of day where individuals are inactive were higher than that excreted on periods of activity.

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Here we analyzed the differences on body mass loss, urine excretion and concentration between three *Thrichomys* species, *T. fosteri*, *T. aff. laurentius* and *T. laurentius*, using water deprivation laboratory experiment. We expect that they could have different abilities to cope with water deprivation as they occur at localities with very different aridity conditions. We also analyzed daily temporal variation urine concentration during food and water *ad libitum* and deprivation conditions.

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MATHERIALS AND METHODS

Individuals were collected at localities with different climatic regimes (Table 1). We used for the experiments thirty (30) individuals of *T. laurentius* captured at a locality near PARNA Serra da Capivara, southeast of Piauí state; seventeen individuals (17) of *T. fosteri*, collected at Fazenda Rio Negro, municipality of Aquidauna / MS (20°28'29 "S / 55°47'10" W) (Pantanal) and sixteen (16) individuals of *T. aff. laurentius* in the municipality of Caetité / BA (14°03'45"S - 42°29'10" W) (transition caatinga/cerrado).

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TABLE 1

Animals were brought to the Vertebrate Laboratory - UFRJ where they were placed in plastic cages and maintained under room standard laboratory conditions of temperature (22 - 27°C), humidity (50-70%) and natural photoperiod. A hydric and nutritionally diet was prepared and tested for each species (Perissé *et al.* 1989).

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Urinary concentration experiment

We used the methodology developed by Fonseca & Cerqueira (1991) and Finotti *et al.* (submitted). The animals were weighed and placed in metabolic cages with collectors containing mineral oil (Nujol). The animals were weighed and placed in metabolic cages at the beginning of the experiment. Urine was collected and its volume was registered every 6 hours in 24 hours trials. Collected urine was stored in a freezer at -4°C and urinary osmolality was measured with a freezing point osmometer (Osmomat 030 - Gonotec ®).

Animals were visually checked for health conditions during every urine collection period. If some change in an individual's movements and behavior was noted, it was immediately removed from the metabolic cages and put back into a plastic cage with water and food available *ad libitum*. The urine excreted at the first 3 hours was discarded to prevent possible changes in urine volume and concentrations due to stress, this period was considered the period of acclimation to metabolic cages (Finotti *et al.* submitted). After 24 hours of experiment, the animals were removed from the metabolic cages, weighed again and placed in plastic cages with food and water available *ad libitum*.

Each individual was subjected to two types of experiment, namely: Control Experiment (I) – where individuals have access to food and water *ad libitum* during a 24 hours period; Test Experiment (II) – where individuals are food and water deprived during a 24 hours period.

Data were tested for normality using Komolgorov-Smirnov (D) tests. The body mass loss (BML), the mean urine volume (MUV) (the sum of urine excreted in all collection periods by an individual in each type of experiment) and mean urine osmolality (MUO) were compared between gender and no significant differences were found, so data were grouped. The BML was calculated as the ratio between the difference in final body mass and the initial body mass. BML was converted for the arcsine square root. BML, MUV and MUO were compared between experiments for each species using Repeated Measures ANOVA and between species in experiment II by Analysis of Variance (ANOVA) and Tukey (HSD) test as *post hoc* test. Urine volume (MUV) and urine osmolality (MUO) were correlated by linear regression for each species for the two experiments (Zar 1996).

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Individuals of *T. fosteri* have significantly greater body mass (346.83±251.76) than individuals of other species (n = 3, H = 4.14, p = 0.0009), being about 1.3 times greater than those of *T. aff. laurentius* (272.95 ± 16.53) (z = 2.98, p <0.01), and about 0.8 times greater than *T. laurentius* (mean = 278.02 ± 22.86) (z = 3.62, P <0.01). As total volume of urine excretion was significantly correlated with body mass (n = 122, F = 38.38, r ² = 0:23) at test experiment (II), this volume was divided by body mass (urine (ml)/body mass (g)) and transformed to the square root of the arcsine to be compared between species.

Urine osmolality temporal variation for each species at each one of the experimental types (I and II) was analyzed by testing the differences between the experimental hours using Repeated Measures ANOVA. All data was analyzed using the Statistica-software (StatSoft Inc. Statistica 7.0.).

RESULTS

None of the species showed significant body mass loss between experiments I and II (Wilks lambda = 0.70, F(40) = 0.30, p = 0.59 for *T. aff laurentius*; Wilks lambda = 0.53, F(62) = 2.53, p = 0.12 for *T. laurentius*; Wilks lambda = 0.84, F(34) = 0.93, p = 0.34 for *T. fosteri*). MUV was significantly lower at experiment II for the three species (Table 2) (Wilks lambda = 15.5, F(137) = 21.29, p = 0.0001 for *T. aff. laurentius;* Wilks lambda = 13.9, F(164) = 18.37, p = 0.00003 for *T. laurentius* and Wilks lambda = 10.4, F(86) = 4.78, p = 0.03 for *T. fosteri*) and MUO were significantly greater at experiment II for the three species (Wilks lambda = 12, F(83) = 8.81, p = 0.004 for *T. aff. Laurentius*; Wilks lambda = 17.3, F(161) = 22.03, p=0.00001 for *T. laurentius* and Wilks lambda = 8.89, F(132) = 6.75, p = 0.002 for *T fosteri*). Urine volume and concentration, presented a significant negative relationship for all species at both experiments (Figure 1). All the variables showed a high intraspecific variation.

FIGURE 1

Comparing the species between experiment II, *T. fosteri* showed significantly higher BML (11.99 ± 7.81%) compared to *T. aff. laurentius* (8.48 ± 8.98%) and *T. laurentius* (6.64% ± 3.70) (n = 139, F = 9.71, p = 0.0001) and higher MUV (n = 65, M = 7.95, p = 0,02) when compared with *T.*

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laurentius (p = 0.014) but not in relation to *T. aff. laurentius* (p = 0.70) (Table 2), these two latter species did not differ either. No significant differences were found in MUO between species (Table 3) (n = 174, M = 0,52, p = 0,59). MUO varied from 1210 to 1328 MOsmol/l and some individuals reached values up to 3226 MOsmol/l.

TABLE 2

Significant differences were also not found for the temporal variation of urine excreted at each time period and the pattern found for each species between experiments was the same (For *T.aff. laurentius:* Wilks lambda = 0,72, F(58) = 1,71, p = 0,13; for *T. fosteri*:Wilks lambda = 0,82, F(6,120) = 2,14, p = 0,054, and for *T. laurentius*:, Wilks lambda = 0,93, F(132) = 0,81, p = 0,56) (Figure 2).

FIGURE 2

DISCUSSION

Mean values found in this study for all three species are lower to those found for other South American species, including the congeneric species *T. inermis* that reached 2193MOsmol in 18h of water and food deprivation (Favaroni-Mendes *et al.* 2004). However, some individuals reached values as high as 3226 mOsm/l and the individuals seem not to be hydric stressed as they did not present any change in the pattern of urine concentration temporal variation comparing control and experiment trials as found for other south American rodent species (Finotti *et al.* submitted). Additionally, species did not present differences in BML and urine concentration between the experiments. These facts can be an indicative that the experimental time period was not sufficient to induce hydric stress. It is possible that water and food deprivation periods greater than 24h hours are needed to induce an adequate response in these species.

Although values found here are not conclusive regarding the three species urine concentration abilities, it is representative of the great physiological variability that is found for other South American small rodents (Al-katahni *et al.* 2004, Bozinovic et al. 2006, Bozinovic & Gallardo, 2006). Considering the three species studied here, some evidences indicates that *Thrichomys* species may have important differences on their capacity to conserve water and this can be related to their

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geographical occurrence. The species of more mesic habitats, *T. fosteri*, seems to be the species with the lowest ability to conserve water. It lost more body mass and was less capable of reducing urine excreted in the same time period. On the contrary, *T. laurentius*, the species from a Caatinga locality seems to have more ability as it presents less BML and MUV than *T. fosteri*. *T. aff. laurentius* presents an intermediate pattern. The three species presented decreased urine excretion and increased urine osmolality in response to water and food deprivation but this did not represent a significant body mass loss. Although not significant, BML between experiments was high (17 to 22%) when compared to the data reported by Favaroni-Mendes *et al.* (2004) of BML from 3.5 to 4.4% for two Caatinga populations of *T. inermis*. The MUV decreased from 45 to 68% at experiment II and MUO increased from 30 to 38%, values slightly different than those found for *T. inermis* (Favaroni-Mendes et al. 2004).

Differences in body mass are also indicative of the differences discussed above and are in accordance with the hypothesis that body mass reduces with aridity (Degen 1997). The species of a more mesic locality (*T. fosteri*) showed a greater body mass than the others that inhabit more xeric habitats. Furthermore, individual body mass values of *T. fosteri* are similar to those of *T. apereoides* from a more mesic population (310±30g) and the values found here for *T. aff. laurentius* and *T. laurentius* are lower than these values but much higher than that found for *T. apereoides* of more xeric populations (145±16g) (Favaroni-Mendes et al. 2004). So, besides low values of urine osmolality have been found, subtle differences can be important to the species geographical differentiation (MacMillen 1983, Shanas *et.al.* 2003).

Our data also go against the hypotheses of Mares *et al.* (1981) and Streilein (1982b) and are indicative of selective forces acting on the great intra- and interspecific variability found for *Thrichomys* species on a set of characteristics that go from body mass to urine concentration physiology. We reinforces the necessity to analyze not only the urine concentration capacity but also the physiological mechanisms in action when animals are water deprived (urine concentration temporal variation, Finotti *et al.* (submitted)) at a diversity of organizational levels (Bozinovic &

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Gallardo 2006) to understand all the physiological diversity found in South American small mammals species.

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Table 1- State/biome, mean temperature (ºC) / standard deviation (SD), annual precipitation (mm) / standard deviation (SD) and aridity index (AI) of the localities where each species was collected.

Specie	State/biome	T(ºC)/SD	Prec. (mm)/SD	AI	Climatic class
Thrichomys aff. laurentius	BA/Cerrado	22-25/2,1	1300-1700/283mm	0.56	Dry sub-humid
Thrichomys laurentius	PI/Caatinga	25-29/2,8	300-800/353,5mm	0.32	Semi-Arid
Thrichomys fosteri	MS/Pantanal	22,5–26,5/2,8	1000-1400/283mm	0.84	Humid

Excluído: A

[A34] Comentário: This is not mean temperature.

[A35] Comentário: This is variation in annual precipitation.

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TABLE 2- Mean/Standard Deviation (SD), median, and amplitude of BML (% of body weight), MUV (mg/ml) and MUO (MOsmol/l) at the two experiments (I e II) for the three species.

		BML (%)			MUV (mg/ml)			MUO (MOsmol/l)		
	Experiment	**mean/SD**	**median**	**amplitude**	**mean/SD**	**median**	**amplitude**	**mean/SD**	**median**	**amplitude**
T.aff laurentius	I	7.74±6.36	6.91	1.89-34.42	0.016±0.011	0.016	0-0.037	769.66±379.73	724.41	213-1907
	II	9.23±11.13	6.71	4.21-57.09	0.010±0.007	0.009	0-0.021	1226.14±608.03	1171	99-2291
T.laurentius	I	5.80±2.44	5.93	1.01-10.17	0.021±0.015	0.016	0-0.055	854.11±412.88	800.50	166 5-2846
	II	7.42±4.48	7.44	1.80-23.15	0.09±0.006	0.007	0.002-0.019	1210.02±498	1210.50	243-3226
T. fosteri	I	10.72±8.64	9.40	2.47-31.77	0.033±0.018	0.029	0.003-0.076	904.52±658.49	691	194 66-1919
	II	13.26±6.9	7.35	4.09-24.17	0.015±0.008	0.016	0.002-0.027	1328.68±662.9	1201	223-3055

[A36] Comentário: Theses values correspond to which time frame? In the middle of the experiment, after 24h of experiment? Explain!

[A37] Comentário: Define abbreviations here.

Excluído: (m)

Excluído: (med)

Excluído: ,

[A38] Comentário: What's the time unit? Min? Hour? Day? The way the data are presented it is not possible to compare them with other published data.

[A39] Comentário: Really? Nearly 60% BML? How does this translate to a 0.021ml/mg MUV?

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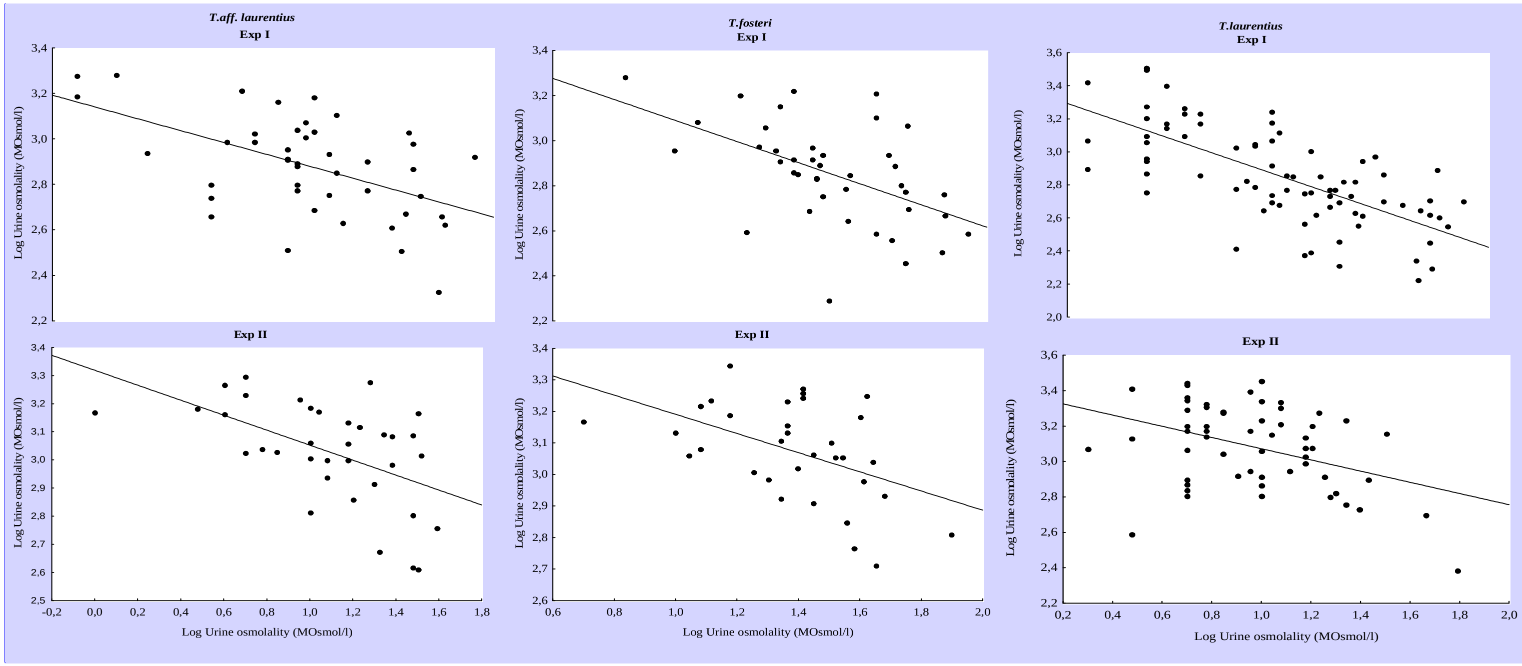


Figure 1 – Linear regression between Log Urine volume and Log Urine Osmolality for each species at each experiment (I and II). For *T. aff. laurentius* (n = 44; ExpI: r^2=-0.28, β=-0.53, F=16.48, p=0.0003, ExpII: r^2=-0.16, β=-0.53, F=6.37, p=0.0164), for *T.fosteri* (n=41, ExpI: r^2=-27, β=-0.59, F=14.08, p=0.0006,

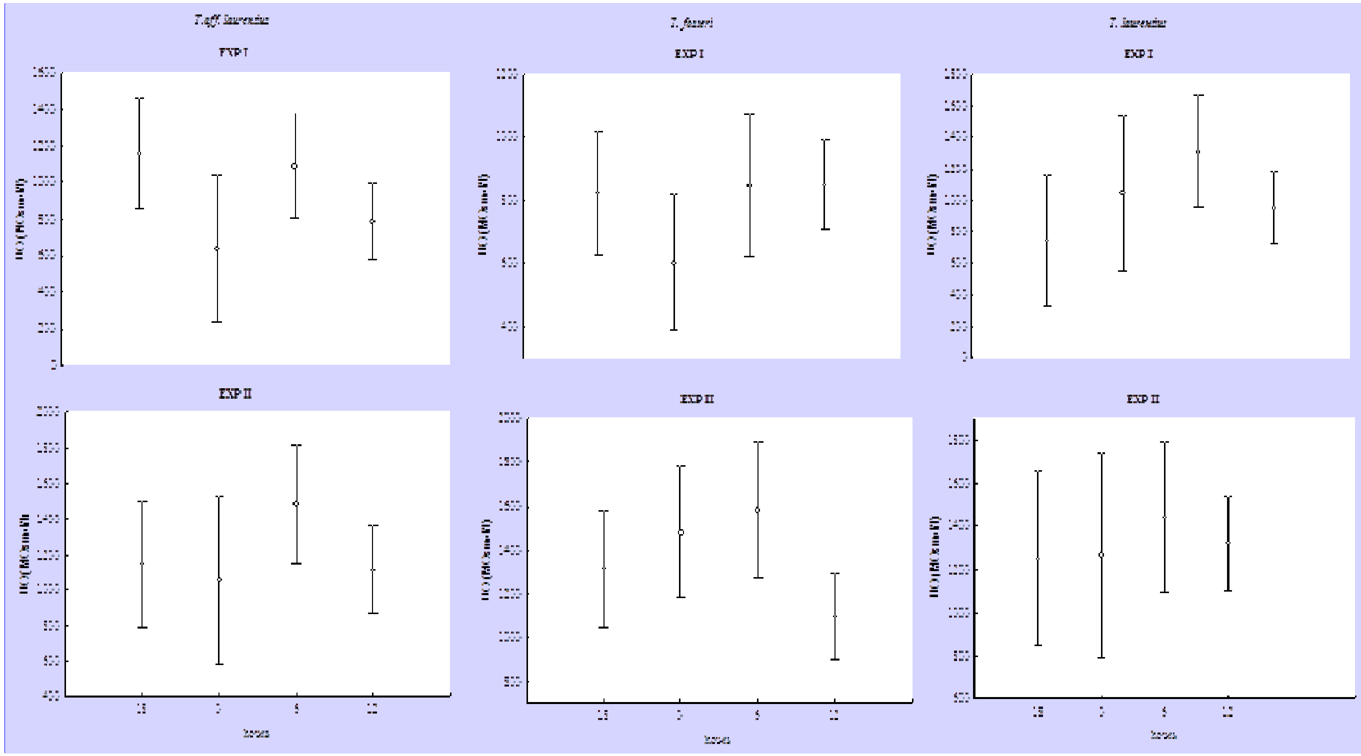
[A40] Comentário: Apparently the authors are plotting Log Urine Osmolality against Log Urine Osmolality, which does not make sense.

[A41] Comentário: This figure is not discussed at all in the discussion. Whats the physiological significance of correlating urine volume (assuming its plotted on the x-axis) and urine concentration? If the authors do not discuss these data, they do not need to present them at all.

Excluído: concentration

Excluído:

ExpII: r^2=-0.22, β=-0.48, F=9.10, p=0.0051) and for *T.laurentius* (n=79, ExpI: r^2=-0.48, β=-0.40, F=10.98, p=0.0000, ExpII: r^2=-0.16, β=-0.69, F=71.89, p=0.0016).



[A42] Comentário: The y-axis have to be on the same scale for better comparison. maximum y-axis values range from 1200 up to 2000, which makes a visual comparison of the results impossible.

Figure 2- Temporal variation of urine osmolality at *ad libitum* food and water experiments (I) and water and food deprivation experiments (II) for the three *Thrichomys* species. Points represent means and bars represent confidence limits within 95%.

[A43] Comentário: What do the hours mean? 0 = after 6h of experiment? Why not 0, 6, 12, 18?

[A44] Comentário: Why not join data from EXPI and EXP2 into one graph using different symbols for better visual comparison?

Excluído:

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Excluído: *i*

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Página 7: [1] [A17] Comentário **Autor**

This should be discussed intesively in the discussion, because some animals apparently lost 30-57% body mass during 24h under both experimental treatments (I and II), which is quite amazing since normal mammals possess around 75% body water, meaning that some animals lost quite na enourmas amount of water, both extracelular and intracelular.

Página 7: [2] [A18] Comentário **Autor**

Looking at the standard deviation given, I find it highly doubtful to find higly significant diferences (p = 0.0001)between the species.

Página 7: [3] [A19] Comentário **Autor**

There are other values given in Tabe 2 regarding BML of the three species.

Página 8: [4] [A24] Comentário **Autor**

In the results sections the author listed significant MVU and MUO differnces in species between Exp I and Exp II as well as between T. fosteri and the other two species. Please verify your conlclusion or be more specific what you are discussing: EXP I versus EXP II or species comparison during EXP II, etc..

Página 8: [5] [A25] Comentário **Autor**

Body mass loss maximum between 30 and 57%? This represents to me a very large BML that sould be addressed in the discussion.

Página 8: [6] [A26] Comentário **Autor**

Why not conclusive?