

## Zoological techniques parameters of *Proechimys guyannensis* (Rodentia, Echimydae) bred in captivity

### Parâmetros zootécnicos de *Proechimys guyannensis* (Rodentia, Echimydae) criados em cativeiro

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#### ABSTRACT

The rodents *Proechimys guyannensis* have led a great interest because they are considered as hosts to several pathogens causing zoological diseases. Therefore, it is important to know their biological and physiological parameters. The age at first cubs delivery and the average of birth interval per female were evaluated. The estimated age at puberty was 83 days. The number of pups born per delivery did not differ significantly when associated with the female mating age. However, an increase in the average mortality rate correlated with the increase in delivery number per female was detected. There is no statistical difference neither in the litter size in different seasons of the year, nor in the body weight between sexes for all ages. Differences were found in the daily water consumption and in the feed intake in animals with approximately 100 days of age, perhaps owing to the increased need for energy intake due to sexual activity or even because they had not reached adult weight yet. The parameters assessed in the present study are of relevance for maintaining this species in captivity as a laboratory animal.

**Keywords.** *Proechimys guyannensis*, rodents, spiny rat, reproductive performance, physiological parameters.

#### RESUMO

Roedores da espécie *Proechimys guyannensis* têm motivado grande interesse por serem frequentemente considerados como hospedeiros de diversos agentes patogênicos causadores de doenças. É importante estabelecer seus parâmetros biológicos e fisiológicos; neste contexto, foram avaliadas a idade ao primeiro parto e a média de intervalo de natalidade por fêmea. A idade à puberdade foi estimada em 83 dias. O número de filhotes nascidos por parto não diferiu significativamente com a idade das fêmeas ao acasalarem. Observou-se aumento da taxa média de mortalidade de acordo com o aumento do número de partos por fêmea. Não houve diferença estatística no tamanho da ninhada em diferentes estações do ano; tampouco houve diferença de peso entre os sexos. Foram detectadas diferenças no consumo diário de água e no consumo de ração em animais com menos e mais de 100 dias de idade, talvez em função de aumento da necessidade de consumo de energia pela atividade sexual ou mesmo por ainda não terem alcançado o peso corporal de adulto. Os parâmetros apresentados são relevantes para a futura manutenção desta espécie em cativeiro como animal de laboratório.

**Palavras-chave.** *Proechimys guyannensis*, refinamento, roedores, rato sóia, desempenho reprodutivo, parâmetros fisiológicos.

## INTRODUCTION

Classically, the rodents of the genus *Proechimys* (Rodentia-Echimyidae) have a great diversity of species and wide geographical distribution in the Neotropics. These rodents have attracted great interest in the area of biology, and epidemiology of viral and tropical zoonotic diseases because they are often found as hosts to protozoa of the genus *Trypanosoma* and viruses<sup>1-3</sup>. It is also known that they are reservoir hosts for *Leishmania*<sup>4,5</sup>. In studies of epilepsy<sup>6-8</sup>, the *Proechimys* species were found to be resistant to this disease. There is a discussion about the karyotype of rodents of the specimen *Proechimys guyannensis* 2n=40 or 2n=46 (Ribeiro et al<sup>9</sup>, Silva et al<sup>10</sup>).

For experiments involving animals as mentioned above, it is necessary that their physiology is kept unchanged, with strict control of variation limits. This makes it important to know the values of different health, biological and physiological parameters used to measure homeostasis and modifications induced by pathological processes, as well as to evaluate the results obtained in the experimental procedures, which can be influenced by ambient conditions or infectious agents there by interfering with the sensitivity of the results.

It is known that the biological and physiological parameters may vary according to sex, and be influenced by age, diet, management and environment, among other factors.

This work was carried out to survey the reproductive performance and physiological parameters (water and feed consumption, body weight, onset of puberty, calving interval and gestation length) of the *P. guyannensis* species. The objective was to standardize this biological species for further study of their biology and behavior as host and/or reservoir and animal model of infectious diseases affecting humans; since no work specifically with this species has been published so far in the literature (<http://www.ncbi.nlm.nih.gov/pubmed>).

The development of an animal model such as that proposed in this study can be considered as a measure of refinement. If the knowledge of the biology of the same is taken into account it may result in a decrease in the number of animals of other species used in some researches that die because of their high sensitivity and in which the *Proechimys* are not so sensitive.

## MATERIAL AND METHODS

### Animals

The animals were obtained from the municipality of Cachoeira do Arari in the state of Pará, latitude 01° 00' 36.00" S, longitude 048° 57' 36.00" W. Permission to use these animals for scientific purposes came from the Ministry of Environment through the Chico Mendes Institute for Biodiversity Conservation - ICMBio and Authorization System and Information on Biodiversity - SISBIO (Number:24065-1) as well as approval by the Ethics Committee of the Federal University of São João del Rei, MG, Brazil. They were kept in quarantine for a period of 90 days and not having any clinical symptoms, were therefore considered healthy. The specimens were identified, according to Silva et al<sup>10</sup>.

These animals from *P. guyannensis* species were used to establish a foundation colony at the Federal University of São João del Rei to survey performance biology and behavior parameters. All animals used in this article were considered genetically wild since they were taken directly from the Amazon forest. Since there was no way of knowing the age of the animals, they were mated and their offspring, male and female, were followed to record the information presented in this article. They remained in an experimental laboratory of which the microbiological status was conventional (microbiological status not specified).

They are not nervous animals but in the beginning they did jump or scramble away if given a chance, also their claws are very sharp and even with an experienced handler the tail may autotomize. As a result the daily working procedure was to handle the animals using a tube constructed from a transparent plastic bottle whose bottom was closed with transparent cellophane. This worked so well and produced so little stress in the animals that some of them even adapted sufficiently so that today they transfer from one cage to another without it being necessary to touch them.

Zootechnical control was performed with daily notes in individual performance records of dates of birth, weaning, mating, calving, number and sex of pups per litter in order to permit calculation of the onset of puberty, the interval among births, the duration of pregnancy, and periods of reproductive cycles. Unlike the conditions suggested by Weir<sup>11</sup>, the test animals reproduced in monogamous pairs, because it was realized in the beginning that when

the male was with more than one female, some of the females delayed their delivery, probably for fear of losing their young.

### **Recordings were performed from August 2010 to August 2012.**

Earlier, in August 2010, there were 5 pairs of adult wild-caught animals that in the beginning were randomly mated and at the end of the first year there were already a total of 20 pairs in reproduction with their records being kept for evaluation at a later date. Results were expressed as means with ranges of measurements performed on each of the 20 pairs and their offspring during these two years.

Unlike the conditions cited by Weir<sup>11</sup>, the test animals were raised in special boxes (cages for rats produced from polypropylene and measuring 41 x 34 x 16 cm), with wood shavings. Each box contained a mating pair and their offspring or at most a group of four animals of the same sex when they were not mated. They were maintained in a controlled environment and each box was exchanged and cleaned at intervals of one week. This interval was determined because the animals kept their environment organized in the sense that their excreta was made on only one side of the box.

Throughout the experiment, animals were kept and monitored under standard laboratory conditions: light-dark cycle of 12 hours with fluorescent lighting c. 300 Lux at ceiling height from 06:00 h to 18:00 h, temperature regulated at  $20 \pm 2$  °C, relative humidity  $55 \pm 15$  % not regulated,  $20 \pm 5$  air changes per hour.

The animals were kept on standard animal bedding (softwood), with access to food (laboratory animal diet for mice and rats Nuvital®, Nuvilab CR-1 Autoclavable, Colombo, Paraná, Brazil) and drinking water (mains water, untreated) *ad libitum*. Pups were separated from their parents at the age of 40 days and were housed with same sex littermates, with a maximum of four animals per box.

Daily food and water intake was recorded for each animal. The feed intake was estimated by the difference between feed and left overs in feeders during the experimental period. Individual feeders and feed pellets were used, being weighed on digital scales. Water intake was recorded daily, using individual drinkers volumetrically graduated. This experimental phase was performed using individual cages for the animals.

Measures to protect the microbiological status of

the animals were primary barriers (personal protective equipment) and secondary barriers (building closed, air conditioning, exhaust air) that were regarded as vital elements of microbiological containment measures. Therefore the animals were free from ecto and endoparasites (coprological analysis), and changing parameters of hemogram (leukocytes, neutrophils and eosinophils) (Kaminsky et al<sup>12</sup>).

There was no period of conditioning since the records were taken during husbandry conditions.

### **Statistical procedures**

Descriptive statistics for the reproductive and productive variables were made.

The calving interval was evaluated by the Mann-Whitney test because this characteristic did not show a normal distribution for its sampling errors (D'Agostino K<sup>2</sup> test -  $p < 0.05$ ). On the other hand, the age at first calving was evaluated by an unpaired "t test" because this characteristic did show a normal distribution for its sampling errors (D'Agostino K<sup>2</sup> test -  $p > 0.05$ ).

The age of puberty was estimated according to a mathematical model. The mortality rate from birth to weaning in accordance with the birth order was evaluated by linear regression. Litter size was evaluated to check the reproductive potential of the captive animal model. These variables were evaluated using analysis of variance in the Kruskal-Wallis test followed by Dunn's *posthoc* pairwise comparison for further analysis, since these variables did not show normal distribution for their sampling errors (D'Agostino K<sup>2</sup> test -  $p < 0.05$ ).

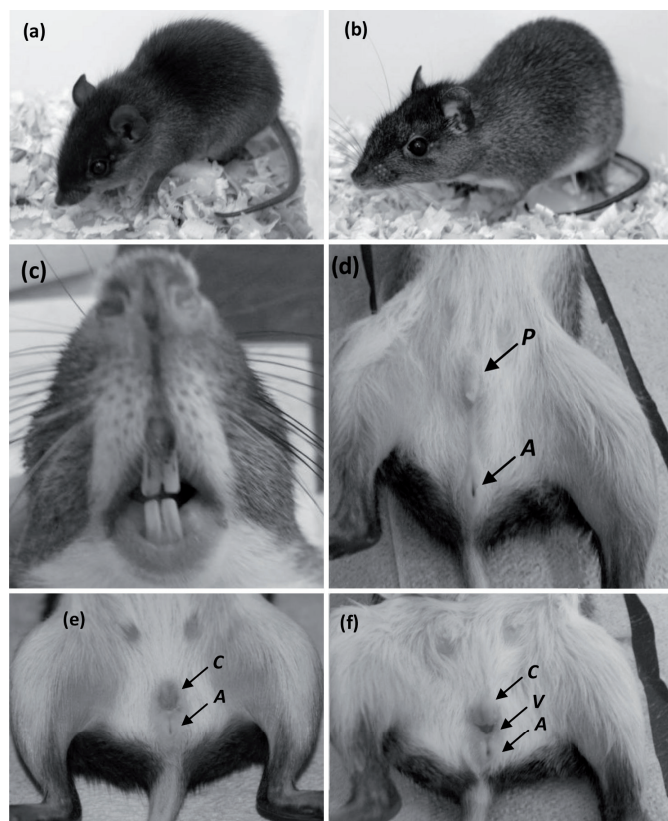
The weight of the animals, separated by sex, was evaluated by nonlinear regression.

Feed intake and water was evaluated according to the unpaired "t test" since the sample error showed not normal distribution (D'Agostino K<sup>2</sup> test -  $p > 0.05$ ).

## **RESULTS AND DISCUSSION**

The *P. guyannensis*, like the casiragua used in the study of Weir<sup>11</sup>, resemble the laboratory rat at first sight (Figure 1a and b). However, the pelage is spiny, they have anagouti coloring from a yellowish-brown to red, the ears are prominent but small, the incisor teeth are slightly orange (Figure 1c); just like those of the casiragua. The eyes are large and it was observed that they stay protuberant when the animals are afraid. The naked, scaly tail readily autotomizes at the base or between the

5th and 6th caudal vertebrae and does not regenerate. When disturbed they release a moaning which rises like before an attack by another animal. These characteristics were also seen by Weir<sup>11</sup> as well as Silva et al<sup>10</sup>.



**Figure 1.** (a) female with approximately two month old, (b) adult female, (c) the teeth from adult *P. guyanensis*. The external genitalia of *Proechimys guyanensis*: (d) male; note the separation of the urinary system (P) and anal apertures (A); (e) female; during dioestrus and pregnancy there is no opening between the clitoris (C) and the anus (A); (f) Female; the vaginal membrane (V) is perforate at oestrus and parturition

### Reproductive characteristics

The vaginal closure membrane and its features are found in other hystricomorphs so far examined, as related by Weir<sup>11</sup>. Females of *P. guyanensis* have a vaginal closure membrane which is perforated only at oestrus and parturition as can be seen in Figure 1 (e and f) while the male has a greater distance between the urinary system and the anal region (Figure 1d).

Descriptive statistics (mean ± standard error) were conducted to evaluate the characteristics of age at first calving and calving interval for females mated at more (233.7 ± 22.63; 65.73 ± 2.88; respectively) or less (208.92 ± 20.19; 66.45 ± 3.85, respectively) than 70

days old and did not differ statistically by the unpaired t test and Mann-Whitney test, respectively. The calving interval was very close to that presented by Weir<sup>11</sup> of 62.6 days for the *Proechimys guairae*.

An estimate was made forage at puberty using the following formula:

$$PA = AFC_{min} - ABI_{min}$$

Where PA= puberty age;  $AFC_{min}$  = minimum age at first calving (131 days old);  $ABI_{min}$  = minimum average calving interval (48 days). By this formula a possible puberty age of 83 days was determined.

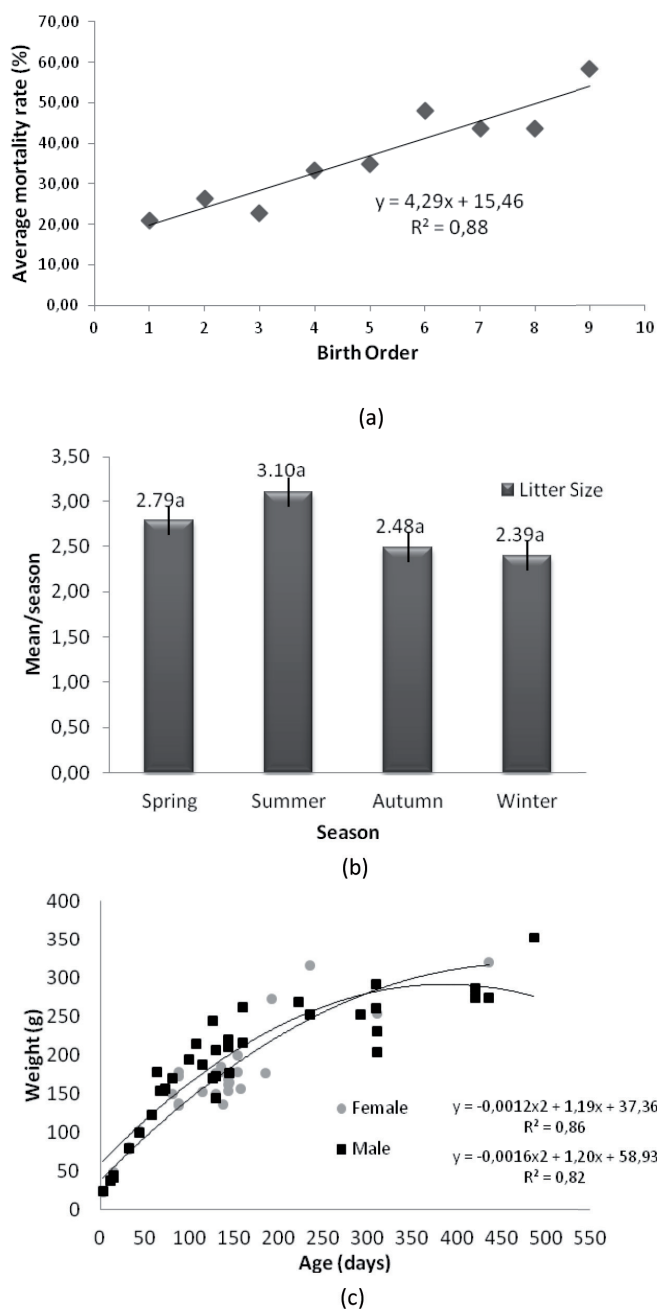
The average number of pups born (2.0, 3.0, 3.0, 3.0, 2.0, 3.0, 3.0, 3.5, 3.0, 4.0) per birth order (from 1 to 10, respectively) in a total of 102 calvings, did not differ significantly between birth orders (Kruskal-Wallis's anova followed by post hoc Dunn's test,  $p > 0.05$ ) and this result was independent of whether the females were mated at more or less than 70 days old. However, as can be seen in Figure 2a, there is an increase in the average mortality rate with the increase of birth order. From these data it can be concluded that, despite there being no change in average number of pups born per birth, it is inappropriate to stay in a bioterium with animals above the 6<sup>th</sup> delivery since the pup mortality rate approaches 50 % above this birth order. Furthermore, Figure 2a shows a linear regression analysis where it can be observed that there is a moderate correlation between birth order and the average mortality rate from birth to weaning, with Spearman correlation coefficients,  $r = 0.378$  and  $p = 0.00009$ , between the two variables.

As previously performed, a nonparametric analysis of variance (Kruskal-Wallis) followed by Dunn's posthoc pairwise comparison of the litter size according to season of calving was made, and it was found that in the captive state there is no significant difference in the litter size in each season although no trend in a greater number of births between the spring and summer was seen (Figure 2b).

### Growth characteristics

Concerning the evaluation of growth, after applying the test of normality ( $p > 0.05$ ) a non linear regression analysis was performed as shown in Figure 2c. Through regression analysis it can be estimated that at 83 days, puberty beginning, the females are around 143.79 g and the males 169.74 g.

As for growth evaluation (weight) no statistical difference between sexes for all ages was found (Figure 2c).



**Figure 2.** (a) Linear regression of average mortality rate, from birth to weaning, by birth order. (b) Litter size. Means that do share a common superscript letter do not differ significantly ( $P>0.05$ ) in the Kruskal-Wallis's anova and post hoc Dunn's test. (c) Nonlinear regression of weight by age of the specie *Proechimys guyannensis*

### Eating habits

The evaluation of eating habits was made by water consumption and feed intake. The characteristics were tested by normality ( $p>0.05$ ) and a parametric

analysis of variance followed by an unpaired "t test". The average daily water consumption and feed intake from the animals showed a significant difference ( $p=0.0084$  and  $p=0.0037$ , respectively) in consumption when animals with ( $28.07 \pm 1.08^a$ ;  $15.15 \pm 0.79^a$ ) and more ( $33.98 \pm 1.43^b$ ;  $19.03 \pm 0.81^b$ ) than 100 days age are compared by the unpaired t test, this is probably due to the need for increased fluid and energy intake due to growth and sexual activity.

Although the weights of the species *P. guyannensis* apparently are smaller than the *P. guairae*, species studied by Weir<sup>11</sup>, they present the same pattern with respect to males presenting higher weights than females. In their article they found at 100 days of age, males ( $302 \pm 7$  g) were significantly ( $p<0.001$ ) heavier than females ( $237 \pm 6$  g).

Finally with the constant increase of human pressure on the natural environment, wild animals like *P. guyannensis* have adapted to anthropic activity, sharing space with man when searching for food and shelter. Thus, it is essential that the natural conditions of these species are known in terms of their physiological parameters and reproductive conditions, in order to promote a balance between these species and their environment.

### CONCLUSION

There was great success in the breeding colony maintained in captivity with a good response in reproduction, growth and behavior aspects, with very low mortality rates in adult animals, showing that the animals were in healthy conditions for breeding in captivity.

Since there is little knowledge about this species, the parameters presented are relevant to future creations as a laboratory animal.

With the recorded data it was determined that the nursing period should not exceed 50 days, so there is no overlap of births and the mother can have a rest interval between one birth and the next.

Knowing the puberty age and the average number of pups per litter is of vital importance when planning the number of matings to meet the demands of the animals when being used for research purposes.

The information on weight and consumption of adult animals will be valid when planning future requirements for creation or use in research.

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