# Assessing the Effect of Litter Size on Growth Pattern and Homeothermy Acquisition in the Pampas Mice *Akodon azarae* (Rodentia, Muridae)

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**ABSTRACT:** We evaluated in pups of *Akodon azarae* both the growth pattern from birth to 48 days of age and the effect of litter size on growth pattern and homeothermy acquisition from birth to weaning age. Individual pups gained weight as expected by a Gompertz growth pattern. Until weaning, litter size affected both the slope of the relationship between body temperature and age and the rate of growth of pups. Pups from small litters increased both body temperature and weight until body temperature and body weight of adults at higher rates than those from large litters.
**KEY WORDS:** Growth, Homeothermy, Thermoregulation, Body Temperature, Gompertz, von Bertalanffy, Logistic, Lineal, *Akodon azarae*.

**RESUMEN**: En este trabajo se evaluó en *Akodon azarae*, el patrón de crecimiento en crías desde el nacimiento hasta los 48 días de edad y el efecto del tamaño de la camada sobre la tasa de crecimiento y la capacidad termorregulatoria desde el nacimiento hasta el destete. Los individuos de *A. azarae* aumentaron de peso según lo esperado por el modelo de Gompertz. El tamaño de la camada afectó tanto la tasa de adquisición de la homeothermia en las crías hasta el destete como su tasa de crecimiento.

## INTRODUCTION

Growth pattern and homeothermy acquisition are important determinants of mammalian offspring survival (McClure and Randolph, 1980). The analysis of growth curves is of analytic value because they estimate important attributes such as growth rate, maximum size and age at maximum growth (Moscarella 2001).

Evolutionary ecologists agree that accelerating growth to maturity is among the most effective ways of increasing fitness (Koteja 2000). However, both growth as well as homeothermy development can be delayed by a limited energy intake by pups. During lactation, milk is the unique food source brought to the young, and restriction on lactation, such as limited amounts of food or water, or a poor ability of mothers to provide milk (Rogowitz and McClure, 1995) would affect pups' development. Regarding food availability, a high number of pups per litter would affect energy intake in each pup, because rates of milk production will reach a maximum value at which high milk demands will not stimulate a proportional increase in milk production. Therefore, large litters will produce a decrease in the quantity and/or quality of milk per pup, affecting rate of growth and delaying both acquisition of thermoregulatory capacity and maternal independence.

*Akodon azarae* is one of the most abundant rodent species inhabiting agro-ecosystem of the Pampas region in Central Argentina (Bilenca *et al.* 1992). At 15 day of age, young of *A. azarae* started with exploratory behavior, and independence from mother is evident after the fourth week after birth (Suarez, 1996). At this time, thermal independence is needed to ensure survival and dispersion. When individuals become adults they have a mean body weight and temperature of 24 g and 36°C respectively. In a previous work, Antinuchi and Busch (2001) characterized *A. azarae* pups as altricial and reported that litter size affected body weight when pups were 10 days old. However, no studies on both growth pattern and effect of litter size on growth and homeothermy acquisition throughout lactation had been performed in *A. azarae* pups.

In this study we assessed the growth pattern from birth to 48 days of age in *Akodon azarae* pups and the influence of litter size on both, growth pattern and homeothermy acquisition until weaning age.

## MATERIALS AND METHODS

Young used in this study (45 pups and 15 litters) were laboratory-born individuals. Mothers were either wild-born or first generations of wild-born individuals, captured using Sherman live traps, at Necochea, Buenos Aires Province, Argentina ("Pampeana" biogeographic province, 38° 29' S, 58° 50' W).

Animals were housed individually in animal cages (0.3 x 0.22 x 0.15 m). Wood shavings and cotton for nesting were provided in the cages. All animals were maintained at constant photoperiod (LD: 10:14), ambient temperature ranged from 19 to 25°C and relative humidity ranged from 50% to 70%. Animals were fed *ad libitum* with pellet (Nutrimento®, Moisture: 12.5%. Composition in %: minimum protein: 23.7; minimum fat: 3.5; maximum fiber: 3.7; maximum ashes: 8.4; average calcium: 1.4; phosphorous: 1; lysine: 1.5). Tap water was provided *ad libitum*.

Couples of mice were arranged in cages (0.6 x 0.3 x 0.2 m) for reproduction in the condition mentioned above. Animals were daily checked until pups were born. At that time, the male was separated from the female and neonates, and the females and each neonate were daily weighed (nearest 0.01 g). Young mice were maintained with their mother until 18 days of age, at that time young were disposed individually in animal cages.

To evaluate homeothermy acquisition, all pups were placed in a temperature-regulated chamber. Body temperature of pups was measured as pharyngeal temperature, with an YSI probe model 93k73545-402 connected to a thermistor Cole-Parmer instrument Company model 8402-10 from birth to weaning age. After and before measuring Tb, pups were kept in an incubator at 15°C for 20 min.

Litter size and body weight at birth were recorded for all litters. Since body mass is a reasonable measure of body sizes when small mammals are growing, we used body mass as a measure of growth (Iskjaer, *et al.*, 1989). Rate of pups' growth before weaning was calculated from data, as the slope of the lineal relationship between body weight and age.

To characterize pups' body weight development, data of pups' body weight from birth to 48 days old (when young reach adult body weight) were fitted to four growth model hypothesis with Pop-tools (a macro-complements for Microsoft Excel downloaded from http://www.cse.csiro.au/CDG/poptools/).

Lineal

$$W(t) = kt + b$$

Gompertz

$$W(t) = Ae^{-e^{-k(t-i)}}$$

Logistic

$$W(t) = A(e^{-k(t-i)}+1)^{-1}$$

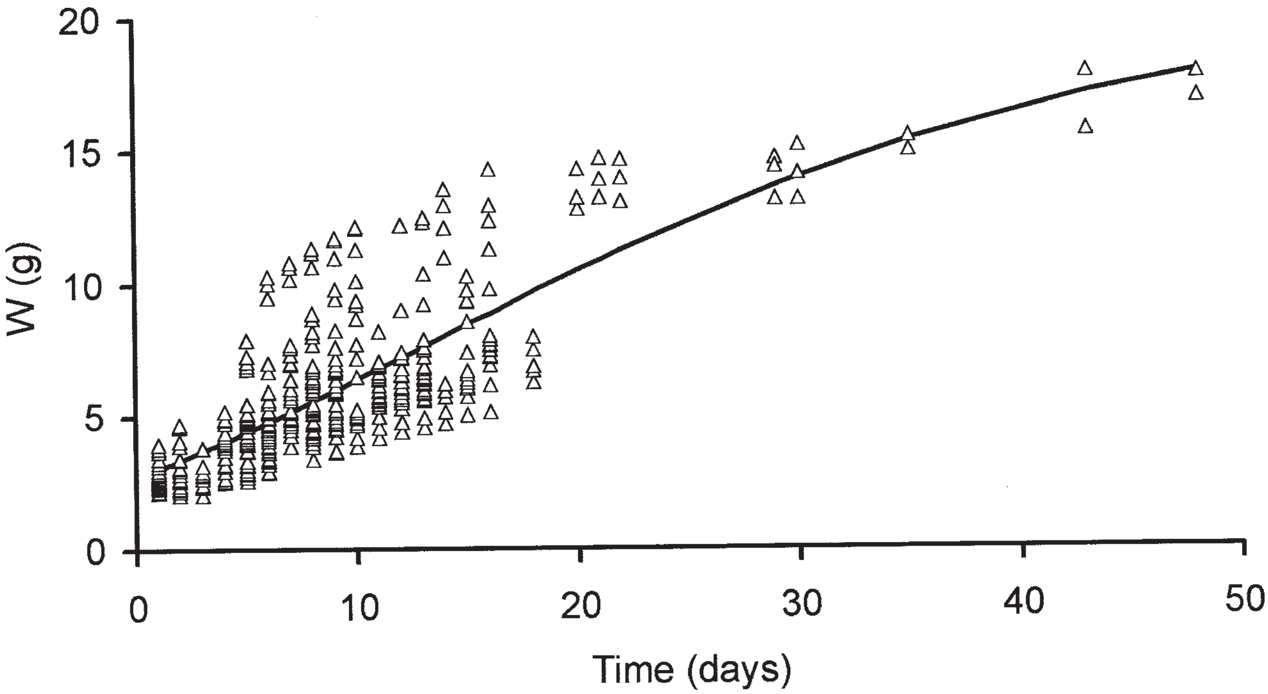


FIGURE 1: Relationship between body weight and age from birth to 48 days of age in *Akodon azarae* pups. Total number of individuals at t0 = 45. Number of young used to fit to growth models decreased after weaning because some of them were used in others studies on energetics.

von Bertalanffy

$$W(t) = A(1-(1/3e^{-k(t-t_1)}))^3$$

where W(t) = body weight (g) at time t (days); A = asymptotic value of body weight (g); k = growth constant (days $^{-1}$); i = age at the inflection point (days); $t_i$ = the age at zero body weight (days); b = interception (g).

The number of young used to fit to growth models decreased after weaning because some post weaning young were used in others studies on energetics.

*Statistics*

In all cases, we tested homogeneity of variances / covariances and normality. To mitigate the potential effect of low number of litters obtained in this study, data were grouped into two categories for comparisons. Because of Bilenca *et al.* (1994) reported a mean litter size of 5 pups per litter, data were grouped for analysis in small (< 5 pups) and large litter size (5 ≥ pups).

Lineal regression analysis was used to compare both body weight and body temperature between pups from small and large litters until weaning age.

*Growth models*

Bootstrap iteration (Pop-tools macro-complements for Microsoft Excel) was used to obtain standard deviation of growth equation parameter. Akaike Information Criterion (AIC, Hilborn and Mangel, 1997) was calculated and used to select the growth model that showed the best fit to the data.

*Body weight and growth of pups*

A t-test was used either to evaluate the null hypothesis of no differences in body weight between

TABLE 1

Parameter of Logistic, von Bertalanffy, Linear and Gompertz growth equations.
A=asymptotic body weight value (g); k=growth constant (days $^{-1}$); i=age at the inflection point (days); $t_i$=the age at zero body weight (days); b=y intercept (g) and AIC= Akaike Information Criterion.
Data are represented as the mean ± standard error (SE)

| Parameter | Gompertz | von Bertalanffy | Logistic | Linear |
|---|---|---|---|---|
| A | 20.88 ± 1..75 | 40.53 ± 13.47 | 18.14 ± 1.02 | – |
| k | 0.05 ± 0.005 | 0.012 ± 0.005 | 0.099 ± 0.007 | 0.37 ± 0.013 |
| i | 13.11 ± 1.7 | – | 16.35 ± 1.29 | – |
| $t_i$ | – | -5.10 ± 0.88 | – | – |
| b | – | – | – | 2.7 ± 0.15 |
| AIC | 1790.97 | 1791.54 | 1792.04 | 1795.75 |

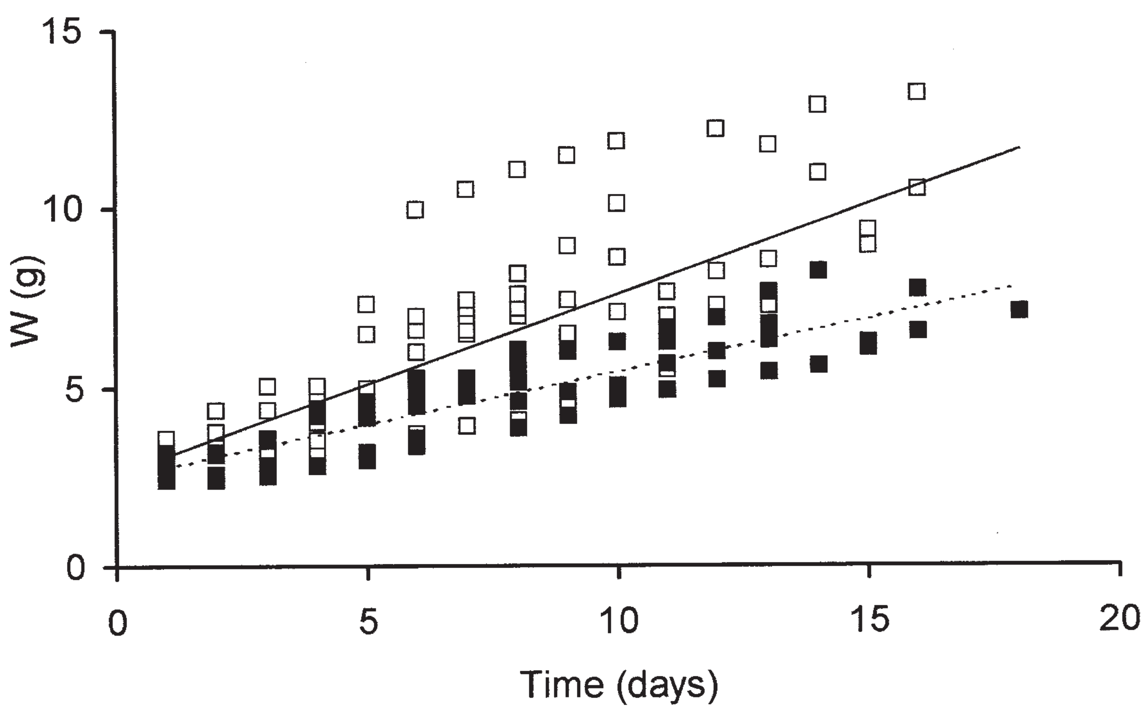


Figure 2: Relationship between age and body weight until weaning age in *Akodon azarae* pups from small and large litters. Empty squares represents pups from small litters (n = 21) and filled squares pups from large litters (n = 24). Solid line represents the regression line for pups from small litters. Dotted line represents the regression line for pups from large litters.

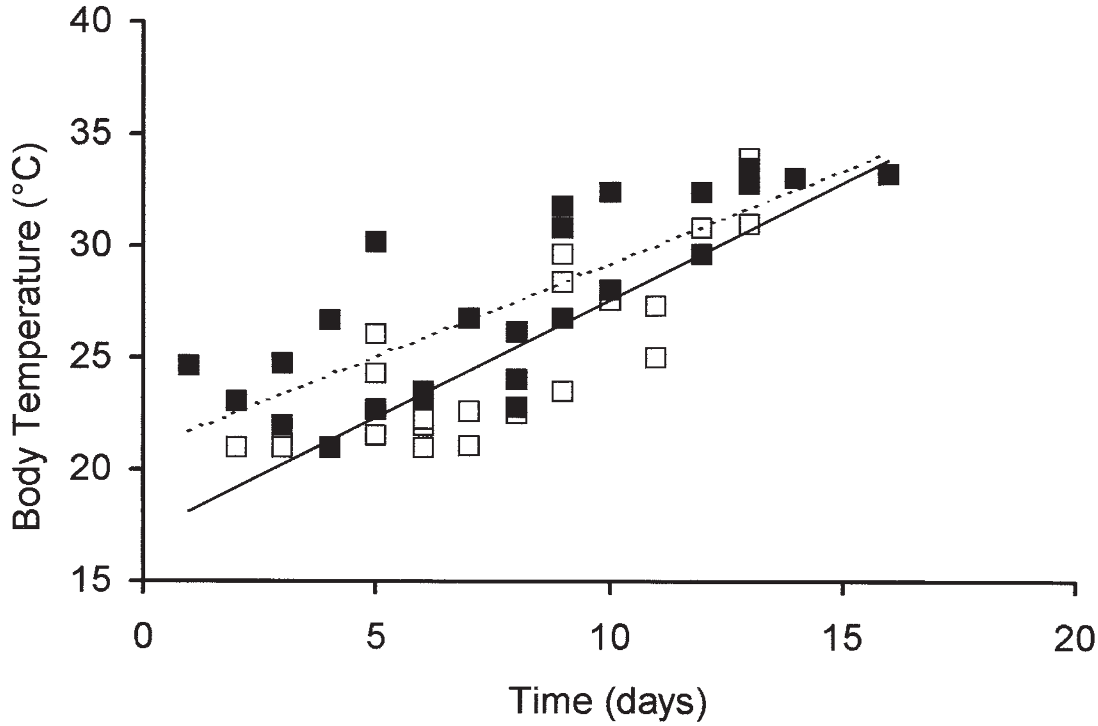


Figure 3: Relationship between age and body temperatures until weaning age in *Akodon azarae* pups from small and large litters. Empty squares represents pups from small litters (n = 21) and filled squares pups from large litters (n = 24). Solid line represents the regression line for pups from small litters. Dotted line represents the regression line for pups from large litters.

pups from small and large litters as well as to test the null hypothesis of no differences in the rate of growth between pups from small and large litters. A Pearson correlation test was used to evaluate the null hypothesis of no relationship among body weight at birth and litter size.

### *Thermoregulatory development*

A t-test was used to evaluate the null hypothesis of no differences in body temperature at birth between pups from litters with small and large number of pups. A t-test was used to evaluate the null hypotheses of no differences between slopes of lineal regression equations (Neter *et al.*, 1990).

## RESULTS

### *Growth models*

When data where fitted to Gompertz growth model, AIC was lower than either, Logistic, Lineal or von Bertalanffy models (Table 1, Figure 1):

$$W(t) = 20.88 {*} e^{-0.05(t-13.11)}$$

### *Body weight and growth of pups*

Because in *A. azarae* both the extent of weaning age is about 15 days (Antinuchi and Busch 2001) and the relationship between body weight and age was lineal until weaning age (see age at the inflection point in Gompertz equation) we used lineal regression analysis to compare rate of growth between pups from small and large litters.

Mean litter size was 4.10 ± 2.42 (range 1 – 7, median = 4, n = 15). The mean of pups' body weigh at birth was 2.83 ± 0.45 g (n = 45). Differences were detected in body weight at birth between individuals from small (Ws) and large litters (Wl); (Ws = 3.40 ± 0.50 g, n = 21; Wl = 2.69 ± 0.33 g, n = 24; $t_{crit}$ = 2.45; $t_{obs}$ = 3.26, P < 0.01). Body weight of pups at birth ranged between 2.19 g and 3.99 g and was inversely correlated with litter size (F = 9.12; d.f. = 1; P < 0.01; $R^2$ = 0.21; n = 45). Differences in growth were detected between pups from small and large litters (slope for small litters = 0.52 ± 0.043 g/day; slope for large litters = 0.29 ± 0.021 g/day, n = 45, tobs = 10.95, p < 0.01, Figure 2)

*Thermoregulatory development*

Statistical differences were detected in the slope of the relationship between body temperature and age between pups from small and large litters (slope small = 1.04 ± 0.13, slope large = 0.83 ± 0.13; t - test, $t_{crit}$ = 2.01; $t_{obs}$ = 8.23; n (s) = 21; n (l) = 24; P < 0.01). The relationship between Tb (Figure 3) and age was:

Tb small = 1.04t + 17.12 (F = 65.45; d. f. = 1; P < 0.00; $R^2$ = 0.73)

Tb large = 0.83t + 20.87 (F = 40.58; d. f. = 1; P < 0.00; $R^2$ = 0.62)

Where Tb is the body temperature (°C) and t is time (days of age). After weaning, Tb of individuals exposed at different ambient temperatures was relatively constant.

## DISCUSSION

Moscarella *et al.* (2001) suggested that altricial or precocial pup's development strategies could be considered as a trait to choose an appropriate growth model. They discussed that altricial species should follow a logistic way of growth whereas very precocious species should follow von Bertalanffy's way of growth. On the other hand, Gompertz's equation would fit intermediate cases. Our data suggested that, despite its altricial way of development (Antinuchi and Busch, 2001), individuals of *A. azarae* gained weight as expected by a Gompertz's growth equation and did not growth as other Cricetidae rodents, like *Oryzomis albigualaris* and *Bolomys temchucky*, which do it in a "Logistic" manner (Moscarella *et al.*, 2001). These differences could be attributed to the fact that Moscarella *et al.* (2001) did not use a statistical criterion as AIC to select the growth model showing the best fit to your data. The fit to Gompertz's equation of *Akodon azarae* data has additional support, since the inflection point, which represents the age of pups in which rate of growth decreases, was in accordance with observed weaning age of pups (Antinuchi and Busch 2001). Similar to other rodents (see Rogowitz and McClure, 1995), litter size had effect on both body weight and growth development, since pups from litters with a large number of individuals were both lighter and smaller than pups from small litters at birth.

Pre-weaning pups of *Akodon azarae* showed a weakly thermoregulatory capacity when they were younger than 10 days old (Antinuchi and Busch, 2001). Moreover, Antinuchi and Busch (2001) reported that grouping had no effect on maintenance of body temperatures when grouped and solitary nestlings from litters with the same number of individuals were compared. In this study, we found that litter size affected the rate at which homeothermy is reached in pups of *Akodon azarae*. Pups from small litters showed higher rates of body temperature development to adult body temperature than those from large litters.

Because of milk was the unique energy supply of pups during this study and food was provided to mothers *ad libitum*, limited milk production seemed to be the main factor responsible for the observed differences in body growth and homeothermy development between small and large litters. In nature, differences in growth and homeothermy acquisition between pups from small and large litters would improve fitness of former pups. Pups from small litters would reach adult body temperature, body weight and maternal independence faster than pups from large litters, increasing their chances to survive and reproduce in this way. On the other hand, a disproportionate low number of pups per litter would produce a decrease in fitness. In accordance, although in *Akodon azarae* the number of pups per litter varies among 1 and 10 (Suarez, 1996), Bilenca *et al.* (1994) reported a mean of 5 pups per litter.

Maternal constraint on offspring's growth in large litters not solely has been observed in *A. azarae*, but also in other species such as *Sigmodon hispidus, Spermophilus saturus, Mus musculus and Onychomys leucogaster* (Rogowitz and McClure, 1995, Sikes, 1995).

Further studies on milk quality and energy contents in relation to litter size and diet of *Akodon azarae* mothers will allow a better understanding of the relationship among physiological plasticity, life history traits and growth pattern.

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