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Effects of high density on spacing behaviour and reproduction in *Akodon azarae*: A fencing experiment

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ABSTRACT

We studied the short term spacing behavioural responses of Pampean grassland mouse (*Akodon azarae*) with regard to population density in four 0.25 ha enclosures (two control and two experimental) in the 2011 breeding season. Based on the hypothesis that *A. azarae* breeding females exhibit spacing behaviour, and breeding males show a fusion spatial response, we tested the following predictions: (1) home range size and intrasexual overlap degree of females are independent of population density values; (2) at high population density, home range size of males decreases and the intrasexual home range overlap degree increases. To determine if female reproductive success decreases at high population density, we analyzed pregnancy rate, size and weight of litters, and period until fecundation in both low and high enclosure population density. We found that both males and females varied their home range size in relation to population density. Although male home ranges were always bigger than those of females in populations with high density, home range sizes of both sexes decreased. Females kept exclusive home ranges independent of density values meanwhile males decreased home range overlap in high breeding density populations. Although females produced litters of similar size in both treatments, weight of litter, pregnant rate and period until fecundation varied in relation to population density. Our results did not support the hypothesis that at high density females of *A. azarae* exhibit spacing behaviour neither that males exhibit a fusion spatial response.

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1. Introduction

Considering that space use is predicted to reflect a trade off between the costs and benefits of residing in a particular area (Davies and Houston, 1984), space use decisions and the resultant

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spacing patterns exhibited by organisms have profound ecological consequences, affecting individual fitness, population dynamics, and evolution of species (Madison, 1980). A common assumption from the point of view of cost-benefit is that conspecific competitor density increases the settling costs in a specific area, affecting space use of individuals (Moorhouse and Macdonald, 2008; Stradiotto et al., 2009; Sommaro et al., 2010; Eccard et al., 2011). The way individuals uses space can affect its survival, growth and reproductive success, and finally impact on the social organization of population (Stamps, 1990; Brant et al., 1998; Stamps and Krishnan, 2001; Aloise King, 2013).

Home range size and overlap degree could vary according to population density, and both depend to a great extent on the sex and reproductive conditions of individuals (Ochiai and Susaki, 2002; Steinmann et al., 2006a,b; Stradiotto et al., 2009; Sommaro et al., 2010). Home range size has often been considered to decrease when density increases while the amount of overlap among home ranges often increases with density (Ochiai and Susaki, 2002). According to Ims et al. (1993) this spacing pattern is known as fusion response. However, this relationship does not always appear to be the case (Bondrup-Nielsen, 1986a,b; Ostfeld, 1990; Ims et al., 1993). Thus, responses of the home range size and overlap to high population density are variable and flexible and depend both on sex and age of individuals as on the degree of habitat saturation and availability of vacant territories (Wolff et al., 1988, 2002; Lambin et al., 2001; Wolff, 2003; Moorhouse and Macdonald, 2005, 2008).

In small mammals, Madison (1980) and Bondrup-Nielsen (1985, 1986a,b) considered that individuals exhibit spacing behaviour when they maintain constant size home ranges and mutually exclusive territories, respect to those of conspecifics of same sex independently of population density values. Then, when all space is occupied by breeding conspecific these individuals may limit the size of the breeding population through spatial exclusion and/or by reproductive suppression of individuals of their same sex (Koskela et al., 1997, 1999; Getz et al., 2003; Wolff, 2007; Moorhouse and Macdonald, 2008; Eccard et al., 2011). In small rodents species, if breeding male or female establish territories, or home ranges with little or none intrasexual overlapping degree, the potential exists for spacing behaviour to limit population density (Bondrup-Nielsen, 1985, 1986a,b; Batzli and Henttonen, 1993; Wolff, 1993, 2003; Steinmann et al., 2009; Sommaro et al., 2010). According to Patterson (1980), Rodenhouse et al. (1997) and Eccard et al. (2011) this would occur through the suppression or delay of breeding activities of non-territory holders. It has been found that spacing behaviour limits the breeding density and reproductive output in *Microtus townsendii* (Taitt and Krebs, 1981, 1982), *Microtus pinetorum* (Brant et al., 1998), *Myodes glareolus* (Bondrup-Nielsen, 1985; Gipps et al., 1985; Oksanen et al., 2007; Eccard et al., 2011), and *Calomys musculinus* (Sommaro et al., 2010). Therefore, spacing behaviour and territoriality can act as a density regulation mechanism (Rodenhouse et al., 1997; Sommaro et al., 2010; Eccard et al., 2011). Territoriality of females may be an adaptation for food resource defense (Ostfeld, 1990; Blondel et al., 2009) or for nest site defense and intrasexual infanticide deterrence (Wolff, 1993; Wolff and Peterson, 1998; Steinmann et al., 2009), while males spatial avoidance would reflect competition among them for monopolizing the receptive females (Clutton-Brock, 1989; Schradin, 2004; Blondel et al., 2009; Bonatto et al., 2012). However, vole and mice vary to a large extent in the degree to which they maintain their territoriality: some species always defend territories, other species do so in high-density populations but not in low-density populations, while others do the opposite (Nelson, 1995a,b; Bond and Wolff, 1999; Steinmann et al., 2006a,b; Loughran, 2007; Sommaro et al., 2010; Bonatto et al., 2015).

Akodon azarae (Cricetidae: Sigmodontinae) has a polygynous mating system that operates through female defense (Bonatto et al., 2013), in which a minority of males controls or gains access to multiple females leaving other males without access to them. In semi-natural populations Bonatto et al. (2012) found that only 40% of adult males gained access to receptive females, so the average operational sex ratio of *A. azarae* was 0.59 (±0.14). During the breeding season males have larger home ranges than females (240 ± 104.2 and 160 ± 89.7 m^2, respectively) and show longer movement distances (Gomez et al., 2011; Bonatto et al., 2012). In a previous study, Bonatto et al. (2012) found that in enclosure populations with sex ratio unbiased, both breeding females and males of *A. azarae* are territorial. However, at high density of males, male home range size and intrasexual overlap degree increased meanwhile females remains the same (Bonatto et al., 2015). Even though we have studied many aspects of space use in *A. azarae* both in natural and enclosed populations (Priotto and Steinmann, 1999; Bonatto et al., 2012, 2015) we never tested the effect of breeding density on spacing behaviour. Thus, the goal of this research, based on the hypothesis that *A. azarae* females exhibit spacing behaviour and males show a fusion spatial response, is to test the following predictions: (1) home range size and intrasexual overlap degree of females are independent of population density values, (2) at high population density, home range size of males decreases and the intrasexual home range overlap degree increases. In addition, in order to determine if female reproductive success decreases at high population density we analyzed pregnancy rate, size and weight of litters, and period until fecundation in both low and high enclosure population density.

2. Material and methods

2.1. The study species

A. azarae (Pampean grassland mouse) is one of the most abundant rodent species in Pampean agrarian ecosystems of central Argentina (Gomez et al., 2011). This small rodent species (adult average weight 25 g) is an opportunistic omnivore (Suárez and Bonaventura, 2001) and shows continuous activity, being mainly active during daytime and crepuscular hours (Priotto and Polop, 1997). This species is found in a great variety of stable habitats with high gramineous cover, including natural pastures, road borders, borders between cultivated fields or pastures, and railway banks (Busch et al., 1997). These habitats are characterized by keeping remnant native flora and fauna (Busch et al., 1997). *A. azarae* is a good indicator of habitat quality owing to its preference for these relatively undisturbed habitats (Martinez et al., 2014; Coda et al., 2015). *A. azarae* populations turn over annually, and individual lifespan is about 12 months (Hodara et al., 2000). Breeding occurs mainly in spring and summer (September to April) with a marked annual variation in population abundance. Low population numbers occur in spring (September–December), at the beginning of breeding period (about 40 individuals/ha), reaching about 100 individuals/ha at the end of this period (March–April) (Priotto and Polop, 1997; Priotto and Steinmann, 1999; Gomez et al., 2011).

Both juveniles female and male of *A. azarae* reach sexual maturity between 52 and 60 days of age, with a mean weight close to 15.5 g in females and 16.5 g in males (Bonatto, 2013). *A. azarae* has a gestation length of 23 days, and each female can produce a maximum of 4 litters, with a mean of 4.6 pups per litter (Bonatto, 2013). In this species the offspring is cared exclusively by females (Suárez and Kravetz, 2001). Females show a high frequency of postpartum estrus (63%), which implies that a new pregnancy may overlap with the lactation of the previously produced litter

(Bonatto, 2013).

2.2. Study site

This study was carried out on the Espinal Reservation in the National University of Río Cuarto Campus (33° 07′ S, 64° 14′ W) in Córdoba province, Argentina, in four 0.25 ha (62.5 m × 40.0 m) field enclosures (control I and II, and experimental I and II) made of galvanized iron sheets extending 0.3 m underground and 0.7 m above ground. The enclosures functioned as independent populations. The enclosures were situated in a stable natural pasture interspersed with woody shrubs and herbaceous weedy species, and high percentage of gramineous cover, similar to natural habitats of *A. azarae*. A 1-m-wide grass strip was devegetated on the inner margin of each enclosure to establish a matrix.

2.3. Study design

At the beginning of September 2011, we collected *A. azarae* adults ($\geq$17 g) from an area located 30 km away from the study site, along road borders and borders between cultivated fields. Sixty sexual mature females were mated with 60 sexual mature males in opaque polycarbonate reproductive cages (29 × 18 × 18 cm) at the Ecology Population Researching Laboratory. The 60 couples were maintained on a 16:8 light/dark photoperiod. Rodent chow and water were provided *ad libitum*, and sunflower and maize seeds were provided as weekly supplements. Wood shavings were provided for bedding. Because in this study we wanted to investigate the effect of density on spacing behaviour and reproduction on a breeding population in the short term, and considering that Bonatto et al. (2012) and Bonatto (2013) found that *A. azarae* populations are biased towards females, we established enclosure populations with an operational sex ratio (OSR) male/female 1:3. Thus, in this study OSR value (0.6) is consistent with the polygynous mating system proposed for *A. azarae* by Bonatto et al. (2012, 2013). Thereby in this study we minimized unwanted effects arising from pre-mating intrasexual competition between males. In order to conform four founding enclosure populations, two with low density (control enclosures) and two with high density (experimental enclosures), in the middle of November 2011, 64 adult individuals with reproductive experience (24 males and 40 females that had fathered a litter) were chosen to be taken to the enclosures. Before they were placed into the enclosures all animals were sexed, weighed, and ear-tagged. Three males and five females were located into each control enclosure (mean density 32 mice/ha) and nine males and fifteen females were located into each experimental enclosure (mean density 96 mice/ha). Thus, throughout the study the population operational sex ratio (males/females) was 0.6. In order to allow that females establish their home ranges without interference from the males, the latter were released into the enclosures three days after the females.

In each enclosure, we set a capture-mark-recapture (CMR) grid of 6 × 10 traps with an interstation distance of 6 m. One live trap similar to a Sherman trap was placed at each station and baited with a mixture of peanut butter and cow fat. To identify animals unable to settle down within the enclosures during the study, 32 live traps were placed at 6-m intervals in each devegetated edge (matrix). We assumed that all individuals were able to settle within the enclosures as no animal was trapped in the devegetated edge during the study.

During December 2011, after a settlement period of 10 days, we perform a trapping session of ten successive days. Thus, males and females had ten consecutive days to become familiar to the traps and the enclosures, establish their respective home ranges, and mate without the interference of the capture device. In order to allow that captured individuals remain absent from the population the least amount of time, and also avoid exposure to high temperatures inside traps, traps were not open between 11.00 and 18.00. Thus, traps were checked and closed each morning, and reopened at sunset. Trapped animals were sexed and weighed. Body and tail length were also recorded. The reproductive condition was determined on the basis of external characters (for males, scrotal or abdominal testicles; for females, perforate or imperforate vagina, nipples visible or not, and evidence of pregnancy). We also recorded trap locations.

To estimate the home range configuration of each animal, all captures were plotted on graph paper for each trapping session following the boundary strip methods (Stickel, 1954). To estimate the home range size (m^2), we used the minimum convex polygon method (Jenrich and Turner, 1969) for its mathematical simplicity and convenience for comparison with previous studies. The number of captures for each estimated home range varied from 7 to 9. Home range overlaps were estimated for each individual by the method suggested by Batzli and Henttonen (1993). In this study, and following these authors and Priotto and Steinmann (1999), we assumed that a home range overlap degree equal or lower than 10% is indicative of territoriality.

Once the trapping session was ended all animals were taken to the laboratory. In order to study the reproductive variables, females were individually housed in the same condition described above and checked daily. We calculated the pregnancy rate (PR) as: number of pregnant females in the population/total number of females in the population. At birth the size (LS) and weight of litter (WL) were recorded. To estimate the time elapsed since the release of the males until fecundation (period until fecundation: PF) we calculated the difference between the pups date of birth registered in the laboratory and the average length of gestation (23 days). The animals were treated in humane manner according to the current Argentinean Laws (National Law 14346).

2.4. Data analysis

We used general linear model (LM) to examine the relationships between each response variable (home range size and home range overlap) and predictor variables. In the analysis of home range size, the predictor variables were sex (fixed factor with two levels: male, female) and density (fixed factor with two levels: control, experimental). In the analysis of home range overlap, the predictor variables were overlap type (fixed factor with three levels: male/male; female/female; male/female) and density. Due to the shortness of our study and the fact that all enclosures had similar vegetation, enclosures were considered homogeneous and data from both control and experimental enclosures were pooled. The Akaike Information Criterion with a correction for small sample sizes (AIC_c) was used as a measure of the fit of a model (the smaller the AIC_c, the better the fit). Model comparison was based on differences in AIC_c values (ΔAIC_c); when ΔAIC_c was greater than two units, the model with the lowest AIC_c was considered as a better statistical description of the process that generated the data. The best resulting models were analyzed using analysis of variance (ANOVA). Reproductive variables LS, WL and PF in relation to population density were analyzed using one-factor ANOVA tests. Pregnancy rate was analyzed using test for equality of proportions. Because equal variance is the most important assumption underlying ANOVA (Crawley, 2007), in all cases we checked this assumption by using Bartlett's test (Crawley, 2007). When this assumption could not be met, the variable was transformed. Statistical analyses were carried out using program R version 2.12.2 (R Development Core Team 2011, www.r-project.org).

3. Results

During this study, of the 64 ear-tagged *A. azarae* (24 males and 40 females) in the laboratory, 59 individuals were captured 505 times in all enclosures. In both low density enclosures (controls) we captured 2 and 3 males, and 4 and 5 females, whereas that in both high density enclosures (experimentals) 7 and 9 males, and 14 and 15 females, were captured. In the experimental enclosures, 65.4% of the females showed external signs of fights, while in the control enclosures none female presented injuries. After a settlement period of 10 days the average survival rate of males was 83% (±0.7) and 90% (±0.7) in control and experimental enclosures respectively, meanwhile average survival rate of females was 97% (±1.4) and 96% (±0.7) in control and experimental enclosures respectively. We assumed that all animals were able to settle within the enclosures because no animal was trapped three consecutive times in live traps placed in the devegetated edge areas.

During this study 49 home ranges of *A. azarae* were estimated (15 males, 34 females): 5 male and 8 female home ranges in control enclosures, and 10 male and 26 female home ranges in experimental enclosures. LM analysis based on ΔAIC_c revealed that sex and density were the most important factors affecting home range size in *A. azarae* (Table 1). The best model included the additive effect between sex and density, and this was the only model with substantial support. Although home range size of males were always larger than those of females ($F = 59.35$; $df = 1$; $P = 0.00$), both males and females had smaller home range size in experimental enclosures than in control enclosures ($F = 18.16$; $df = 1$; $P = 0.00$; Fig. 1). In populations with high density, home range sizes of both sexes were about 40% lower than those obtained in low density populations (Fig. 1).

LM analysis based on ΔAIC_c revealed that interaction between overlap type and density was the best model for home range overlap percentage variable (Table 1). Because assumption of homogeneity of residual variances could not be obtained, even when the data were transformed, ANOVA tests were not performed. We conducted a Mann–Whitney U-tests (One-tailed test, $a = 0.05$) in order to analyze percentage of each overlap type in relation to treatments (low and high population density). To control experimental-wise error rate, we used the Bonferroni correction for multiple tests. This analysis revealed that both male/male intrasexual and intersexual overlap values varied between experimental and control enclosures ($U = 3$; $N = 15$; $P = 0.00$ and $U = 7$; $N = 15$; $P = 0.03$, respectively). Male/male intrasexual and intersexual overlap values were lower in experimental enclosures than in control enclosures (Fig. 2). On the other hand female/female intrasexual overlap values were similar between treatments ($U = 97.50$; $N = 34$; $P = 0.81$), being lower than 10% in both control and experimental closures (Fig. 2).

In respect to reproductive variables, statistical analysis revealed that WL was affected by density ($F = 310.96$; $df = 1$; $P = 0.00$). Litters conceived in experimental enclosures were less heavy than those conceived in control enclosures (Table 2). To analyze PF we used a Mann–Whitney U-tests (One-tailed test, $a = 0.05$) because assumption of equal variance could not be obtained even when data were transformed. This analysis revealed that the difference in PF was statistically significant between experimental and control enclosures ($U = 98$; $N = 21$; $P = 0.00$). The time elapsed up fecundation observed in experimental enclosures tripled the value obtained in control enclosures (Table 2). Respect to LS statistical analysis revealed that there was no significant effect attributable to the density ($F = 0.03$; $df = 1$; $P = 0.87$). Litters showed similar sizes both in control as in experimental enclosures (Table 2). Finally, of the 34 females captured at the end of the study, 21 females were pregnant and gave birth in the laboratory (7 and 14 from control and experimental enclosures respectively). Although PR was 0.88 and 0.54 for control and experimental populations respectively, no significantly differences were found in pregnant rates between treatments ($X^2 = 1.68$; $df = 1$; $P = 0.19$).

4. Discussion

Space use of voles and mice can vary within a species due to differences in habitat quality, age of individuals, familiarity of breedings, and density (Pusenius and Viitala, 1993; Steinmann et al., 2006a,b; Stradiotto et al., 2009). Many studies at population level have revealed the impact of density on home range size and overlap (Loughran, 2007; Moorhouse and Macdonald, 2008; Eccard et al., 2011; Sommaro et al., 2010; Steinmann and Priotto, 2011a; Bonatto et al., 2015). According to Bondrup-Nielsen (1985) when at high density individuals maintain mutually exclusive home ranges of constant size, the number of home ranges within any patch plot will be equal to patch size divided by the average range size. Thus, when a patch becomes filled with territorial female home ranges, whose size do not vary with population density; females attempting to acquire breeding home ranges might disperse in search of available space elsewhere (Bujalska, 1973; Saitoh, 1981; Wolff et al., 1988), or postpone their reproduction (Faulkes et al., 1991). In the promiscuous species *C. musculinus*, female home range sizes remained similar irrespective of population density (Sommaro et al., 2010; Steinmann and Priotto, 2011b). These authors proposed that *C. musculinus* females exhibit spacing behaviour and that territorial females could have a role in regulating population abundances limiting the number of females that acquire breeding spaces. Similar results were found in experimental studies in promiscuous rodent species in which population density was manipulated (Nelson, 1995a,b; Bond and Wolff, 1999; Moorhouse and Macdonald, 2008).

Contrarily, in this experimental study we found that both *A. azarae* males and females varied their home range size in relation to population density: home range size of both sexes decreases when density increases. Although *A. azarae* females kept exclusive home ranges in both control and experimental enclosures, they decreased their home range size in high density populations. The higher number of female competitors would cause that maintaining large areas would become a very costly strategy, and thus their reduction would be more beneficial. Thus, females of this species would not be able to limit the size of the breeding female population by constraining other females from settling.

As regards *A. azarae* males in experimental populations they established smaller home ranges and greatly reduced the degree of intrasexual overlap. Thus, home ranges of *A. azarae* males

Table 1
Model selection based on AIC_c comparison of general linear model (LM) describing home range size and overlap degree of *Akodon azarae*. The best model is indicated in boldface type. Models are ordered by ΔAIC_c, only models with ≤4.

Response variable	Model	AIC_c	ΔAIC_c	Nº of parameters	Deviance
Home range size	Sex + Density	44.574	0	4	36.574
	Sex * Density	46.881	2.307	5	36.881
Home range overlap	Type overlap * Density	227.696	0	7	213.696

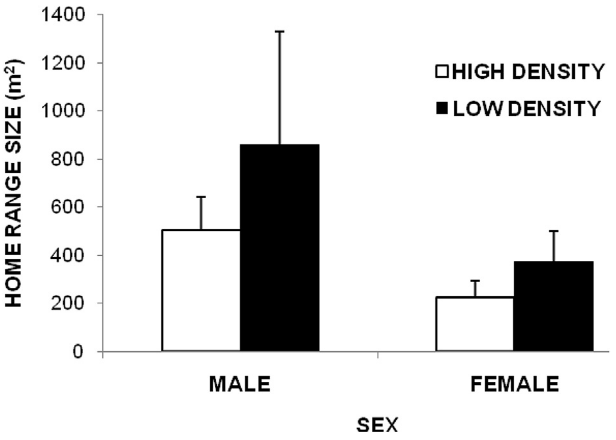

Fig. 1. Home range size (mean + SD) of *Akodon azarae* in relation to sex and density in population enclosures with sex ratio biased towards females. Low density: control enclosures; High density: experimental enclosures.

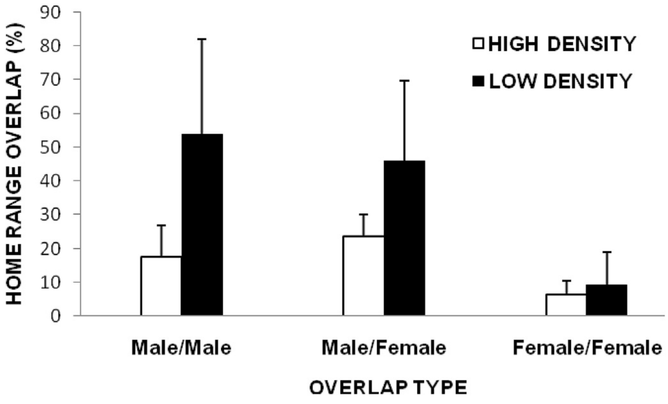

Fig. 2. Home range overlap (mean + SD) of *Akodon azarae* in relation to overlap type and density in population enclosures with sex ratio biased towards females. Low density: control enclosures; High density: experimental enclosures.

Table 2
Litter size (LS), weight of litters (WL) and period until fecundation (PF) (mean ± SD) of *Akodon azarae* in control and experimental enclosure with sex ratio biased towards females. **n**: Total number of breeding females analyzed.

Response variable	n	Control (low density)	Experimental (high density)
LS (Nº of litter)	21	4 ± 0.816	4.071 ± 0.997
WL (g)	21	4.051 ± 0.258	2.462 ± 0.158
PF (Day)	21	5.571 ± 3.599	16.214 ± 1.528

responded as elastic disc (Wilson, 1975) decreasing their size as density increases. Experimental studies with sex-ratio manipulations found a similar negative relationship between male home range size and population density in *Microtus canicaudus* (Bond and Wolff, 1999) and *Microtus agrestis* (Erlinge et al., 1990; Nelson, 1995a,b). Our results would indicate a lower movement rate of *A. azarae* males in populations with high density. In a study performed in linear habitats Gomez et al. (2011) found that at highest population densities movement distances of males diminished significantly. In voles and mice, during the breeding season space use of males is strongly affected by the search of mate, and female availability is a key resource in determining male intrasexual and intersexual overlap degree (Ylönen and Mappes, 1995; Fortier and Tamarin, 1998; Bond and Wolff, 1999; Steinmann et al., 2005, 2006b; Steinmann and Priotto, 2011; Bonatto et al., 2015). Until now, it was found that *A. azarae* males only show an increasing of their home range size and intra and intersexual overlap degree at high males density (Bonatto et al., 2015). In a previous study in *A. azarae* enclosure populations with operational sex ratio biased towards males and intermediate density value (48 mice/ha), Bonatto et al. (2015) suggest that males increased their displacements as a strategy of compensation of the few mating opportunities. However, this explanation also could be applied to our results in relation to space use of males in control populations; although in this study sexual rate was female biased, given the overall low density in control enclosures, receptive females constituted a relatively scarce and scattered resource (18 females/ha). Contrarily, in the experimental enclosures males would not have to increase their movements in order to search estrous females due to the high availability of breeding females (58 females/ha). Since the decrease of overlap degree of *A. azarae* males is a consequence of the reduction of their home range size (Bonatto, 2013), the lower intra and intersexual overlap would indicate a smaller movement rate of males in populations with high female availability. Besides, the lower intersexual overlap observed in this study in the experimental enclosures might reflect the most rewarding mating tactic: at high availability of females, males could have the chance of siring multiple litters mating at the edges of the home range of receptive females instead of wandering across their home range (Yoccoz et al., 2002; Mysterud et al., 2004, 2005; Waterman, 2007).

As mentioned above in this study, increasing the density has been found to reduce the size of reproductive females territories; reduced territory size may consequently decrease the amount of food resources and the number of secure nest sites within each territory, affecting female reproduction (Mappes et al., 1995; Koskela et al., 1997, 1999; Eccard et al., 2011). Morris (1985) proposed that a decrease in territory quality may restrict female reproductive effort (individual optimization hypothesis). Population density and other density-related factors such as availability of free breeding territories have been previously found to affect several traits in voles (e.g., sexual maturation, initiation of breeding, spacing behaviour, offspring growth) (Bujalska, 1985; Koskela et al., 1998, 1999; Prévot-Julliard et al., 1999; Eccard and Ylönen, 2001; Eccard et al., 2002, 2011). Therefore, the reasons contributing to the increased probability of cost of reproduction in high density populations are likely to be diverse. Our results showed that although *A. azarae* females produced litters of similar size in both treatments, at high population density each litter was composed by individuals with smaller body mass at birth. Thus, in this study the weight of the litter was more sensitive to variation in breeding density than litter size. Under spatial or temporal variation in environmental conditions, such as the seasonally changing densities, selection should favour variation in offspring phenotypes (Metcalfe and Monaghan, 2001; Ozanne and Hales, 2004). Besides its genetic background, body size at birth is a strongly maternally derived trait (Mappes et al., 1995; Koskela et al., 1999; Mappes and Koskela, 2004). Therefore, environmental factors affecting the mother's condition may have substantial influence on the body size at birth of her offspring (Oksanen et al., 2003, 2007). In relation to the others reproductive variables studied, pregnant rate and period until fecundation varied in relation to breeding population density. In high density while pregnant rate slightly decreased (38% less), the period elapsed up fecundation tripled the observed at low density. Many studies have reported that chemical signals such as urinary pheromones from females or social interactions among females are sufficient enough to inhibit reproduction between females that compete for limited resources (Schadler, 1990; Carter and Roberts, 1997; Solomon and French, 1997; Brant et al., 1998; Hurst and Beynon, 2004). This is consistent with other studies showing that females are reproductively suppressed or delayed by unrelated same-sex conspecifics (Schadler and Butterstein, 1979; Wasser and Barash, 1983; Insel et al., 1995; Solomon et al., 1996; Brant et al., 1998). These authors suggest that reproductive suppression or delay are the outcome of reproductive competition between females. At high density population *A. azarae* females would delay their reproduction as a consequence of an increase in competition between females in relation to a lower availability of free breeding territories. Because social interactions commonly include aggressive interactions, the presence of injuries in *A. azarae* females in high density population would support a behavioural mechanism of the reproductive delay more than chemical signals. In other small rodents species such as *Heterocephalus glaber* and *Peromyscus californicus*, suppressed reproduction between females also is mediated through direct physical interactions (Faulkes et al., 1991; Gubernick and Nordby, 1992; Faulkes and Abbott, 1993).

In summary, our results did not support the hypothesis that at high density population with sex ratio skewed towards females, *A. azarae* females exhibit spacing behaviour neither that males exhibit a fusion spatial response. Thus, in *A. azarae* enclosure populations the upper limit in population abundance would be reached gradually and mostly through a constraint in reproduction in females. Although the small number of replicates used in this study do not allow us to conclude for all *A. azarae* individuals throughout its range of distribution, our results provide valuable information about the effect of population density on spatial and reproductive responses in this species. Because these responses would have a great implication in the social organization of this species, future studies will be required to analyze its adaptive significance and demographic implications.

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