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RESEARCH ARTICLE

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Reproductive potential of a vole pest (*Arvicola scherman*) in Spanish apple orchards

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Abstract

Fossorial water voles, *Arvicola scherman*, feed on tree roots causing important damages in European apple orchards. Since the intensity of crop damage produced by rodents ultimately depends on their inherent capacity to increase their population, the main goal of this study was to determine the reproductive potential of the subspecies *A. scherman cantabriae* in apple orchards from Asturias (NW Spain), where voles breed over the whole year. Our results were compared with those reported for the subspecies *A. scherman monticola* from the Spanish Pyrenees (where reproduction ceases in winter). Sexual characteristics, body condition, relative age class and number of embryos were recorded from 422 females caught in apple orchards along two years. We found pregnant females all along the year, which were able to produce a high number of litters per year (7.30) although litter size was relatively moderate (first year: 3.87 embryos/female; second year: 3.63 embryos/females). The potential number of pups per female and year (first year: 28.25; second year: 26.50) was substantially higher than that reported for Pyrenean voles, what is probably related with differences in the length of the breeding season and in life histories between subspecies. In our population, the number of implanted embryos correlated positively with the body condition of the mother. Our results reveal that management efforts should not be seasonal as they used to be so far and invite to explore the physiological consequences of management practices.

Additional keywords: *Arvicola scherman cantabriae*; body condition; litter size; reproduction; rodent pest; fossorial water voles.

Abbreviations used: BCI (body condition index); CM (carcass mass); HBL (head and body length).

Authors' contributions: MM and JV conceived and designed research. AS and MM conducted sampling. AS performed the necropsies and the study of specimens, and statistically analysed the data. AS, MM and JV wrote the manuscript.

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Introduction

The montane water vole, *Arvicola scherman* (formerly fossorial form of *Arvicola terrestris*; for taxonomic consideration see Musser & Carleton, 2005), is one of the most harmful rodent species in the European farmlands (Walther *et al*., 2008; Blant *et al*., 2009; Delattre & Giraudoux, 2009; Miñarro *et al*., 2012). These voles live underground, dig extensive burrow systems and feed preferably on roots, bulbs and tubers (Airoldi, 1976). In fruit crops, fossorial water voles feed on bark and roots and may kill the trees (Meylan, 1977; Walther *et al*., 2008). Additionally, injuries to the root system make the trees less productive and more susceptible to falling down by winds or high fruit load due to deficient anchorage. Its occurrence in crops can cause important economic losses, being estimated in German orchards between 50 and 40,000 €/ha/year (Walther *et al*., 2008).

In the Iberian Peninsula two forms of this species are currently recognised (Ventura & Gosálbez, 1989; Musser & Carleton, 2005): *A. scherman monticola*, which occurs in the Pyrenees, and *A. scherman cantabriae*, distributed along the Cantabrian region (NW Spain) and in the northern tip of Portugal. These taxa are geographically isolated and show significant morphological differences, being *cantabriae* 40% lighter (Ventura, 1993). *Arvicola scherman* is officially considered a pest in Spain and preventive actions to reduce its population density have been recommended (BOE, 2008). In Asturias (NW Spain) this species has become one of the main causes of economical loss in apple orchards (Miñarro *et al*., 2012), a crop that in 2010 occupied 10,324 ha in this region (INDUROT, 2010) supposing the 32.4% of the total planted area intended to this crop in Spain (31,812 ha), whose national production, for example in 2013, reached 546,400 tons (Faostat, http://faostat.fao.org).

The effect of seasonal environmental factors, such as the decrease of temperature or food availability, can vary with altitude, which in turn affects rodent reproduction at population scale (Dunmire, 1960; Bronson, 1979; Murie *et al*., 1980; Zammuto & Millar, 1985; Hille & Rödel, 2014). Until now, studies about reproduction of *A. scherman* populations have been conducted above 400 m a.s.l., where all of them showed a delimited breeding season (Airoldi, 1978; Morel, 1981; Pascal, 1981; Ventura & Gosálbez, 1990a; Ventura *et al*., 1991). However, populations located in apple orchards below 270 m a.s.l. in Asturias breed continuously throughout the year (unpublished own data). Furthermore, it must be kept in mind that favourable habitats with plenty of food, such as crops, may allow rodents to invest their extra energy in increasing the reproductive effort (Doonan & Slade, 1995; Koskela *et al*., 1998; Díaz & Alonso, 2003; Eifler *et al*., 2003). Particularly, pregnant females of *A. scherman cantabriae* were detected over the whole year and, consequently, the recruitment of young specimens was continuous (unpublished own data). The optimal investment hypothesis predicts that litter size produced by a female should give the best reproductive success in a particular environment (Mappes *et al*., 1995). Therefore, reproductive potential among populations of *A. scherman* could vary through phenotypic plasticity or microevolution driven by the corresponding environmental conditions (Williams *et al*., 2014).

It is worth noting that in both, semiaquatic (*A. amphibius*; see Musser & Carleton, 2005) and fossorial water voles, the number of implanted embryos is positively correlated with the body dimensions of the mother (van Wijngaarden, 1954; Pelikán, 1972; Wieland, 1973; Ventura & Gosálbez, 1990b). In that sense, the reproductive potential is closely related to the body condition in semiaquatic water vole populations, which reflects energy provisions (Evsikov *et al*., 2008). Likewise, a good body condition in pregnant females leads to a higher number of ovulated oocytes and implanted embryos, and decreases the risk of pregnancy failure (Evsikov *et al*., 2008; Nazarova & Evsikov, 2008; Yuzhik *et al*., 2015). Furthermore, mother body condition in *A. amphibius* has also effects on the reproductive success, life span, body mass (Nazarova & Evsikov, 2008) and maturation of young (Yakovleva *et al*., 1997). Conversely, a worsening of the body condition might lead to lower fitness in females (Evsikov *et al*., 2008).

Management of rodent pests requires effective, specific, environmentally benign, economically feasible and socially acceptable approaches (Jacob, 2013). Thus, and especially in organic farming, there is a need for environmentally sustainable strategies, avoiding the use of rodenticides which generate genetic resistance in target species and are a risk to non-target wildlife (Rattner *et al*., 2014). Anticoagulant rodenticides have been routinely applied in France to control fossorial water vole populations since the 1990s (Defaut *et al*., 2009) with negative effects on predators (Coeurdassier *et al*., 2014) and scavengers (Montaz *et al*., 2014). Enhancing the implementation and success of sustainable control methods entail a deep knowledge of relevant aspects of the pest biology and ecology (Delattre & Giraudoux, 2009; Jacob, 2013; Ranchelli *et al*., 2016).

The reproductive characteristics of fossorial water voles in the Iberian Peninsula are known exclusively from studies on a population from the Aran Valley placed at about 900 m a.s.l. in the Spanish Pyrenees (Ventura & Gosálbez, 1990a, b). Thus, regarding the lack of information on this subject on *A. scherman* from NW Spain and given the pest condition of the species in this geographic area, the main aim of this study was to determine the reproductive potential of fossorial water voles in the Cantabrian region (*A. scherman cantabriae*), specifically in apple orchards located at low altitude in Asturias. Since body size and the length of the breeding season differ substantially between Pyrenean and Asturian populations (Ventura & Gosálbez, 1989, 1990a; unpublished own data), differences in the reproduction potential between those populations can be expected. Thus, a further goal of the present research was to elucidate this question by comparing our results on *A. scherman cantabriae* with those previously reported for *A. scherman monticola.* The information obtained in this study could be useful to design effective control plans for fossorial water vole populations both in our study area and in others with similar environmental characteristics.

Material and methods

Study site

The study was conducted in ten experimental and commercial apple orchards throughout two years (first year: February 2011-January 2012; second year: February 2012-January 2013). These orchards are separated from each other by 20.5 km as maximum and are located in two municipalities of the autonomous community of Asturias (NW Spain): Villaviciosa (43º 38’ 55” N, 5º 26’ 08” W) and Nava (43º 21’ 31” N, 5º 30’ 29” W). Their surface ranged from 1 to 6 ha and their altitude from 3 to 270 m a.s.l. Apples in Asturias are mainly produced in an area characterized by an irregular topography of smooth hills and valleys and distributed in a mosaic landscape of small agricultural plots separated by hedgerows and woodlands. The studied area has a temperate hyperoceanic climate (Rivas-Martínez & Rivas-Sáenz, 2015) with abundant rainfall spread evenly along the year, and mild temperatures even in winter with low risk of frost and snowfall. The relatively high rainfall and fertile soils of Asturian meadows favor the establishment of evergreen and dense grass coverage in orchards all the year around (Díaz-González & Fernández-Prieto, 2005; Miñarro, 2012).

Specimen and data collection

A total of 422 female water voles were analysed. Each month, one or more orchards, depending on orchard surface and vole density, were visited for trapping. Individuals were captured with snap traps (Topcat® Andermatt Biocontrol, Switzerland) placed in galleries. Traps were activated during the day and checked twice per day for five days maximum. Shortly after capture the specimens were cryopreserved to -20°C until the necropsy. The recommendation of the Directive of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes (EU, 2010) was followed during the field work.

For each specimen, head and body length (HBL) and carcass mass (CM; body mass of the animal without neither the skin nor the thoracic and abdominal organs) were recorded using a digital calliper to the nearest 0.01 mm and a precision scale to the nearest 0.5 g, respectively. The body length of each embryo and the uterus of implantation were also registered. Litter size was evaluated according to the number of embryos implanted in uteri, discarding those showing macroscopic sign of resorption. Considering HBL range (1.4 - 38.1 mm) and mean value (12.5 mm) obtained in all embryos analysed, pregnant females whose embryos showed a HBL average lower than 6 mm were considered females in an early pregnancy state. The number of placental scars in each uterus was recorded.

The main criterion used to establish the sexual maturity state was the presence/absence of *corpora lutea*. Ovaries were extirpated and preserved in 70% ethanol until the analysis was conducted. Four longitudinal histological sections (sections of 5 µm thick separated between them 150 µm) were performed in the left ovary to obtain a whole representation of this organ. Sections were stained with hematoxylin-eosin. Other sexual parameters considered in each specimen were the following: development and vascularisation degree of the uteri, and presence and number of placental scars and/or embryos (Ventura & Gosálbez, 1990b). The sexual maturity states were the following: immature, specimens without *corpora lutea*, uteri poorly developed and vascularised, without placental scars; mature, individuals with *corpora lutea*, uteri completely developed and well or scarcely vascularised, and showing placental scars and/or embryos.

Specimens were distributed into six classes of relative age (0-V) according to the following criteria (for details see Ventura & Gosálbez, 1992): moulting stage, sturdiness of the mastoid process and the condylar process, the separation of angular process from the ascending branch of the mandible, and the values of the interorbital crest index, which relates the breadth of interorbital crests and the rostral length. The approximate age intervals corresponding to these relative age classes are the following (see Ventura & Gosálbez, 1992, and references therein): class 0, 0-3 weeks; class I, 3-6 weeks; class II, 6-10 weeks; class III, 10-14 weeks; class IV, specimens older than 14 weeks that have not overwintered; class V, specimens that have overwintered at least once. To assess differences on CM and body condition index (see below) between relative age classes, a random selection of non-pregnant females from relative age classes IV and V was used in an attempt to balance the number of analysed specimens in each age class.

Data analyses

The pregnancy frequency (F) gives information about how many times a female may become pregnant and was evaluated according to the method described by Emlen & Davis (1948): $F = (T/V) \cdot R$; where T = duration of the study (days), V = number of days in which pregnancy is visible (16 days: see Ventura & Gosálbez, 1989 and references therein) and R = ratio of visible pregnant females in relation to the total number of matures ones.

Mann-Whitney U-tests were used to assess interannual differences in litter size and differences in embryo implantation in each uterus. Differences in litter size and the number of placental scars among relative age classes were assessed by Kruskal-Wallis tests. Then, Mann-Whitney U-tests with Bonferroni adjustment for multiple tests (α = 0.05/number of pairwise comparisons) were used to establish differences among relative age classes.

The individual body condition index (BCI) was calculated as the difference between measured CM and the theoretically expected CM, which was obtained from the corresponding regressions against HBL after logarithmic transformation of the data of both variables (Evsikov *et al*., 2008). Females with visible signs of gestation were not taken into account to construct the regression equations. One-way ANOVA tests was used to assess differences in CM among relative age classes. This same test was also used to assess differences between years in BCI of mature non-pregnant and pregnant females. General linear models (GLM) were used to assess differences in BCI between years and relative age classes of non-pregnant and pregnant females. Tukey HSD tests were used to establish differences in BCI and CM among relative age classes. Changes in BCI of mature non-pregnant females along time were assessed by a Spearman correlation. This method was also used to determine relationships between pairs of the following variables: CM, BCI, litter size, number of placental scars and age class. Statistical analyses were performed with SPSS v22.0 (IBM Corp., Armonk, NY, 2013).

Results

Pregnant females

Out of the 422 females analysed, 101 females (32.0 % of total mature females; n = 316) were pregnant; 55 of them were obtained during the first year and 46 during the second one (Table 1). Pregnant females were found in all months excepting January 2013. The youngest pregnant females corresponded to age class III and were detected in all seasons (Table 1).

Litter size

The total number of implanted embryos was 380, distributed in litters formed by 1-9 embryos (Table 2). The most frequent litter size was formed by four (35.6 %, n = 36) and three (33.7 %, n = 34) embryos, and the less frequent by nine (0.9 %, n = 1). The mean (± SE)

Table 1. Number and percentage of pregnant females respect to the total number of mature females according to the relative age class and the month of capture in *Arvicola scherman cantabriae*.

Year	Month	Age class			Total	N matures	% pregnants
		III	IV	V			
1st 2011	Feb		1	1	2	9	22.2
	Mar		1	1	2	19	10.5
	Apr	1	2	1	4	9	44.4
	May	3	4	2	9	20	45.0
	Jun	1	1	4	6	12	50.0
	Jul		1	2	3	13	23.1
	Aug	2	2	2	6	12	50.0
	Sep		3	1	4	16	25.0
	Oct		3	3	6	14	42.9
	Nov	3	2	1	6	15	40.0
	Dec		2		2	22	9.1
	Jan	1	2	2	5	12	41.7
2nd 2012	Feb			1	1	11	9.1
	Mar	1	2	2	5	13	38.5
	Apr	1	1		2	12	16.7
	May		4	4	8	14	57.1
	Jun	1	1	6	8	15	53.3
	Jul			2	2	11	18.2
	Aug		1	3	4	13	30.8
	Sep		1		1	10	10.0
	Oct		2		2	11	18.2
	Nov	1	5	2	8	14	57.1
	Dec	2	2	1	5	9	55.6
2013	Jan				0	9	0
Total		17	43	41	101	315	32.1

Table 2. Litter size and number of individuals with resorbed embryos along the sampling period in *Arvicola scherman cantabriae.*

Year	Month	N	Litter size											Cases of resorbed embryos
			1	2	3	4	5	6	7	8	9	Mean	SE	
1st	Feb	2		1	1							2.50	0.50	
	Mar	2					2					5.00		
	Apr	4			1	1		2				4.75	0.75	
	May	9		1	1	4	2				1	4.44	0.65	1
	Jun	6		1	3	1	1					3.33	0.42	1
	Jul	3			1	1		1				4.33	0.88	
	Aug	6			3	2	1					3.67	0.33	1
	Sep	4	1			1	1	1				4.00	1.08	
	Oct	6			1	3	2					4.17	0.31	1
	Nov	6	1		4		1					3.00	0.52	1
	Dec	2				1	1					4.50	0.50	1
	Jan	5			4	1						3.20	0.20	1
2nd	Feb	1			1							3.00		
	Mar	5			1	1	3					4.40	0.40	1
	Apr	2				2						4.00		
	May	8			1	4	2	1				4.38	0.32	
	Jun	8		1	3	4						3.38	0.26	2
	Jul	2				2						4.00		
	Aug	4			2	2						3.50	0.29	
	Sep	1			1							3.00		
	Oct	2			1	1						3.50	0.50	
	Nov	8	1	1	2	4						3.13	0.40	
	Dec	5		1	3	1						3.00	0.32	1
	Jan	0												
Total		101	3	6	34	36	16	5			1	3.76	0.12	11
%		100	3.0	5.9	33.7	35.6	15.8	5.0			0.9			10.9

number of implanted embryos per female was 3.76 (± 0.12). There were no inter-annual significant differences in the litter size (Fig. 1) (first year: 3.87 ± 0.18 (SE); second year: 3.63 ± 0.14 (SE); U = 1165.0; $p = 0.475$).

The mean number of embryos in the right uterus (2.11 ± 0.11) was significantly higher than the number in the left one (1.66 ± 0.10) (U = 3031.5; $p < 0.01$). Considering pregnancy states, females in early pregnancy state (n = 27) did not show significant differences in the average of embryos implanted in each uterus (right uterus: 1.92 ± 0.21; left uterus: 1.89 ± 0.21;U = 371.0; $p = 0.720$), whereas females in advanced pregnancy (n = 64) showed significant differences in this variable (right uterus: 2.16 ± 0.13 (SE); left uterus: 1.48 ± 0.11 (SE); U = 1260.0; $p < 0.001$).

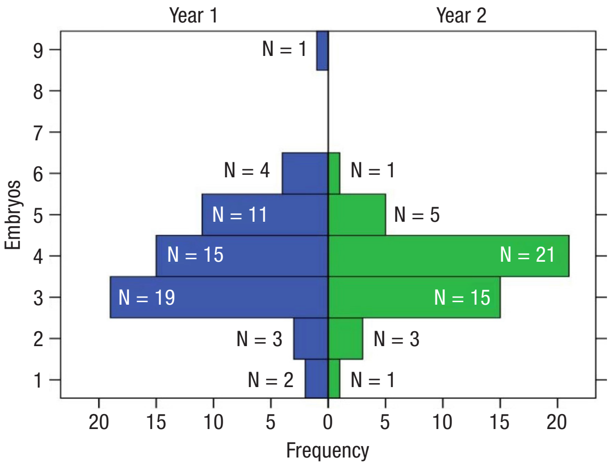

Figure 1. Frequency of litter size per year in the sample of *Arvicola scherman cantabriae* analysed.

Macroscopic signs of embryonic resorption were observed in 11 females in advanced pregnancy (10.9%) (Table 2). Some resorption signs were observed in 12 embryos (3.1%), in litter sizes of three (n = 4), four (n = 4) or five embryos (n = 4). Moreover, there were 14 litters with unilateral implantations (14.1%); 9 of these litters were found in the right uterus (R: right, L: left; 4R-0L, n = 2; 3R-0L, n = 3; 2R-0L, n = 2; 1R-0L, n = 2) and 5 in the left one (0R-3L, n = 4; 0R-1L, n = 1).

Litter size correlated positively with CM ($r_s = 0.294$; $p < 0.01$) (Fig. 2a) and increased with age ($r_s = 0.217$; $p < 0.05$), although it was not possible to establish differences among classes (H' = 4.881, $p = 0.087$)

Table 3. Carcass mass (CM, g), litter size and number of placental scars according to relative age classes in *Arvicola scherman cantabriae*. For each variable, significant differences among relative age classes are indicated by different letters.

Age class	N	CM			Litter size						Placental scars					
		Mean	SE		N	Mean	SE	Min	Max		N	Mean	SE	Min	Max	
III	51	31.2	1.1	a	17	3.3	0.2	1	5	a	13	3.5	0.6	1	9	a
IV	92	40.8	0.6	b	43	3.7	0.2	1	6	a	56	5.3	0.4	1	14	a
V	93	45.7	0.7	c	41	4.1	0.2	2	9	a	72	7.6	0.4	1	15	b

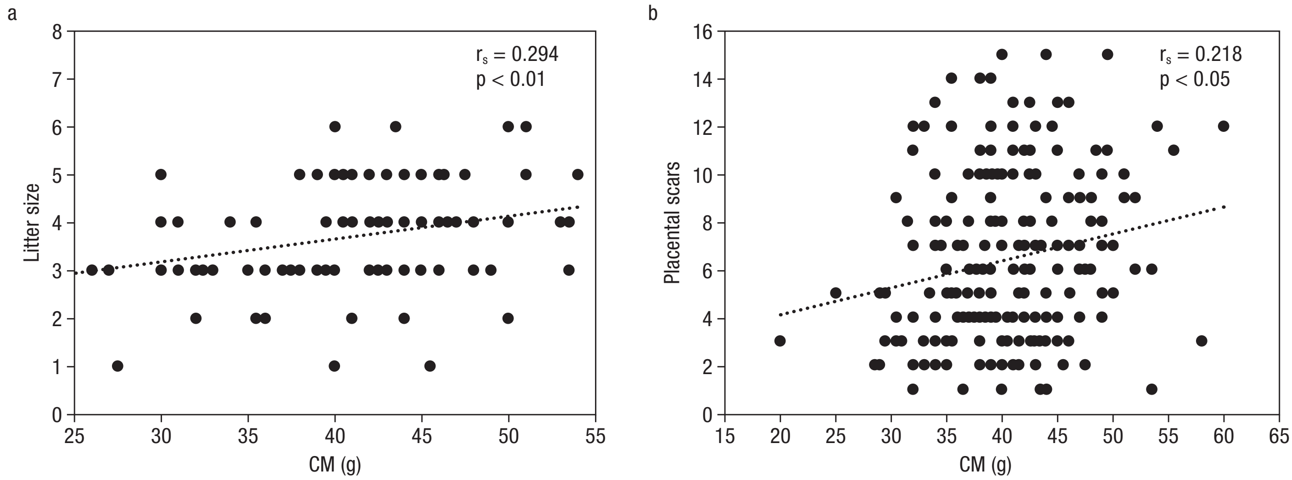

Figure 2. Correlations between carcass mass (CM) and litter size (a) and between CM and placental scars (b) in mature females of *Arvicola scherman cantabriae.*

(Table 3). It is worth mentioning that the only pregnant female with 9 embryos showed an extremely low CM value (-0.0294). So, we considered this individual as outlier and therefore it was discarded in the regression analysis. The number of placental scars per female ranged from 1 to 15, being four (13.8%) and three (12.0%) the most frequent values; this variable increased significantly with both relative age ($r_s = 0.439$; $p < 0.001$) (Table 3) and CM ($r_s = 0.218$; $p < 0.05$) (Fig. 2b).

Potential fecundity

The ratio of visible pregnant females for the first year of the study was 0.32 (55 out of 173 mature females). Accordingly, the potential number of litters per mature female during the breeding season was 7.30 (F = (365/16)·0.32). The average litter size was 3.87 and the potential number of offspring by female was 28.25 (3.87·7.30). For the second year, the ratio of visible pregnant females was also 0.32 (46/142) and the potential number of litters was also 7.30. However, the average litter size was 3.63, and hence the potential number of offspring by female was slightly lower (26.50).

Body condition index

The BCI of non-pregnant females did not vary significantly with the relative age ($F_{5,\,214} = 1.661$; $p = 0.145$) neither between years ($F_{1,\,214} = 0.057$; $p = 0.811$). The result of the interaction of both factors showed that differences in BCI among age classes depend on the year ($F_{5,\,214} = 2.364$; $p < 0.05$) (Table 4). Moreover, in pregnant females there were no significant differences in BCI among relative age classes ($F_{2,\,94} = 2.582$; $p = 0.081$) neither between years ($F_{1,\,94} = 0.193$; $p = 0.662$). Nevertheless, the interaction between relative age class and year in pregnant females showed that differences in BCI among age classes do not depend on the year ($F_{2,\,94} = 0.444$; $p = 0.643$) (Table 4). A significant positive correlation between BCI and litter size was observed ($r_s = 0.230$; $p < 0.05$) (Fig. 3).

Considering only mature specimens, mean values of BCI of non-pregnant females were significantly higher in the first year than in the second one ($F_{1,\,211} = 6.257$; $p < 0.05$); no significant inter-annual differences were found for pregnant females ($F_{1,\,98} = 0.006$; $p = 0.938$) (Table 4). Moreover, BCI was significantly higher in pregnant females respect to non-pregnant ones in the second year ($F_{1,\,137} = 9.726$; $p < 0.01$); whereas no significant differences between these groups were found

Table 4. Body condition index (BCI) of non-pregnant and pregnant females for each age class and for mature females in *Arvicola scherman cantabriae*. Significant differences in BCI among relative age classes for pregnant and non-pregnant females are indicated by different lower case letters. Significant differences in BCI between years in mature non-pregnant and pregnant females are indicated by different capital letters.

Age class	Year	Non-pregnant females						Pregnant females					
		N	Mean	SE	Min	Max		N	Mean	SE	Min	Max	
0	1	7	-0.0584	0.0258	-0.1909	0.0251	a						
I		33	-0.0004	0.0120	-0.2124	0.1177	ab						
II		16	-0.0141	0.0113	-0.1181	0.0422	ab						
III		19	0.0157	0.0140	-0.1138	0.1367	b	11	0.0151	0.0109	-0.0448	0.0727	a
IV		20	0.0123	0.0099	-0.0546	0.0920	b	24	0.0268	0.0088	-0.0701	0.1054	a
V		20	0.0194	0.0115	-0.0995	0.1079	b	20	0.0144	0.0105	-0.1044	0.0896	a
0	2	2	0.0644	0.0133	0.0511	0.0776	a						
I		7	-0.0337	0.0133	-0.0995	0.0007	a						
II		28	-0.0222	0.0081	-0.1277	0.0945	a						
III		15	-0.0041	0.0099	-0.0922	0.0631	a	6	-0.0012	0.0090	-0.0240	0.0268	a
IV		28	-0.0141	0.0098	-0.1245	0.1568	a	19	0.0332	0.0115	-0.0582	0.1423	a
V		31	-0.0015	0.0103	-0.1577	0.0935	a	20	0.0124	0.0053	-0.0281	0.0508	a
Matures	1	118	0.0106	0.0047	-0.1803	0.1615	A	55	0.0199	0.0058	-0.1044	0.1054	A
	2	96	-0.0068	0.0051	-0.1577	0.1568	B	46	0.0193	0.0058	-0.0582	0.1423	A

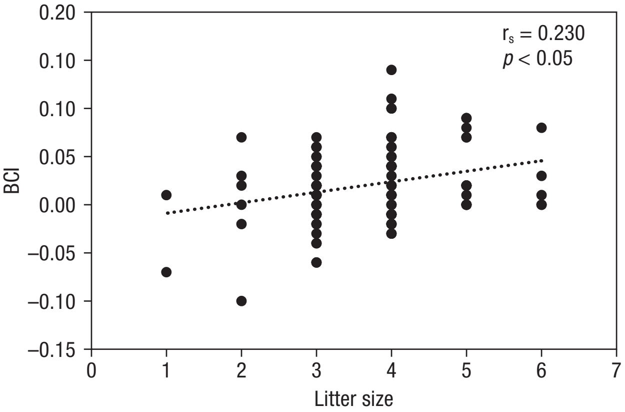

Figure 3. Correlation between body condition index (BCI) and litter size in *Arvicola scherman cantabriae.*

in the first year ($F_{1,172} = 1.380$; $p = 0.242$) (Table 4). In mature non-pregnant females BCI diminished along the sampling period ($r_s = -0.197$; $p < 0.05$) (Fig. 4).

Discussion

General results of this study reveal that *A. scherman cantabriae* inhabiting Asturian apple orchards shows higher reproductive potential than Pyrenean fossorial water voles. Specifically, during the study period each female was able to produce 7.3 litters in a year, a value that to our knowledge is the highest one reported for *A. scherman* and also in the closely related species *A. amphibius* (2.0 - 7.1 litters/year) (van Wijngaarden, 1954; Tupikova & Švecov, 1956; Hamar & Marin, 1962; Kminiak, 1968; Pelikán, 1972; Wieland, 1973; Ventura & Gosálbez, 1990b). On the contrary, the average litter size we found in *A. scherman cantabriae* (3.76 embryos per female) was among the smallest values reported for *A. scherman* and also for *A. amphibius* (Perry, 1943; Van Wijngaarden, 1954; Maksimov, 1959; Kminiak, 1968; Panteleyev, 1968; Stoddart, 1971; Pelikán, 1972; Wieland, 1973; Kratochvil, 1974; Blake, 1982; Evsikov *et al.*, 1989; Ventura & Gosálbez, 1990b). As a result of these values, the potential number of offspring per female and year reached 28.25 in Cantabrian voles, which is higher than the value indicated for *A. scherman monticola* (23.5) (Ventura & Gosálbez, 1990b). Therefore, populations of *A. scherman* can show different reproductive potential in the Iberian Peninsula depending, at least partially, on the habitat, which might ultimately determine their role as agricultural pest.

The length of the breeding season is a critical factor in determining litter size in small mammals: reproduction can be extended throughout the year at lower elevations, whereas in higher elevations larger litters are needed to sustain an equal level of reproduction during a shorter breeding season (Dunmire, 1960; Smith & McGinnis, 1968; Spencer & Steinhoff, 1968; Innes, 1978; Tkadlec & Zejda, 1998). Specifically in *Arvicola,* this pattern was found in semiaquatic water voles (Kratochvil, 1974). In that sense, apple orchards in Asturias are located in a temperate hyperoceanic climate (Rivas-Martínez & Rivas-Sáenz, 2015), which implies benign weather condition that allows fossorial water voles to breed continuously along the year (unpublished own data), whereas the breeding season of *A. scherman* is limited to the period March-November

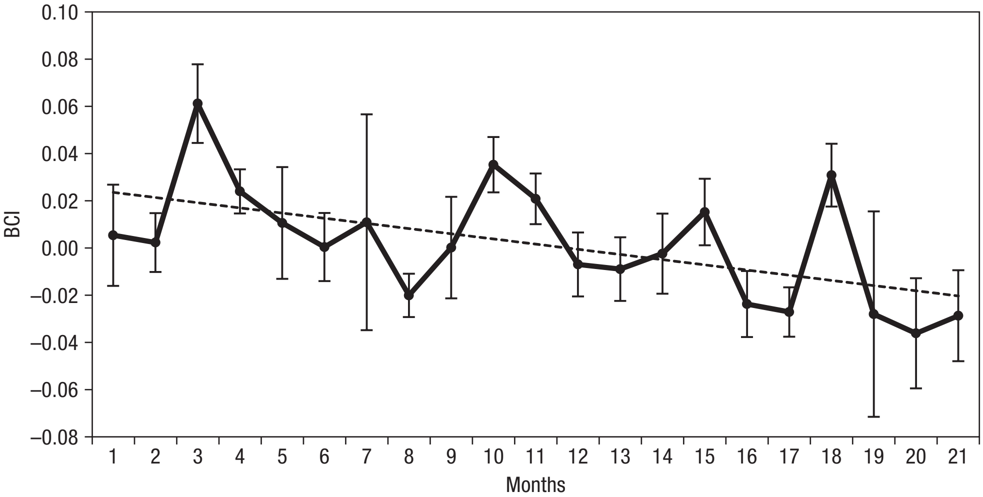

Figure 4. Body condition index (BCI) (mean ± SE) of mature non-pregnant females in *Arvicola scherman cantabriae* throughout the sampling period.

in the Pyrenees (Ventura & Gosálbez, 1990a). These differences between Spanish populations can be explained by an adaptation to seasonal changes of each geographic zone, as occur in other rodent species (Hansen *et al.*, 1999; Solonen, 2006). Although differences in litter size between the Cantabrian and Pyrenean populations can be related with an altitudinal effect, intrinsic factors could also be involved. For example, differences in ovulation rate between wild populations of the house mouse (*Mus musculus*) are mainly determined by body size, whereas the influence of extrinsic factors is low (Jacob *et al.*, 2007). Therefore, a smaller litter size in the small-sized *A. scherman cantabriae* (Ventura & Gosálbez, 1989; Ventura, 1993) cannot be discarded as an own characteristic of the subspecies. Additionally, we found in our sample that the body size of the mother was significantly correlated with the number of implanted embryos, result that is concordant with that reported for *A. scherman monticola* (Ventura & Gosálbez, 1990b). Thus, an intrapopulation effect of the relationship between body size and litter size can be added to that found between these variables in an evolutionary context.

As expected and also as observed in *A. scherman monticola* (Ventura & Gosálbez, 1990b), the number of placental scars increased with age in our population. Differences in the range of this variable between Cantabrian (1-15) and Pyrenean (2-20; Ventura & Gosálbez, 1990b) *A. scherman* are probably due to differences, at a population level, in the relationship between the number of reproductive events and litter size, and also to differences in the persistence of placental scars.

Pregnant females with macroscopic signs of resorption were also found in other populations of both *A. scherman* and *A. amphibius* (van Wijngaarden, 1954; Pelikán, 1972; Wieland, 1973; Ventura & Gosálbez, 1990b). In our sample, females in early pregnancy had similar embryonic implantation in each uterus, which suggests a balanced ovulation in both ovaries. Nevertheless, a significantly larger number of embryos implanted in the right uterus was observed for the total pregnant females, which might be due to embryo resorption. Indeed, an unequal uteri-functioning has been well described formerly in mouse and rat (Wiebold & Becker, 1987 and references therein). Moreover, Isakova *et al.* (2012) found in *A. amphibius* that an important percentage of embryos suffer a resorption in the uteri (16.7%) caused probably by mutation processes.

Body condition (relationship between the carcass mass and body length) indicates energy provision and plays an important role in the reproductive potential. Specifically, in *A. amphibius* it has been indicated that a good body condition in pregnant females leads to higher number of ovulated oocytes and implanted embryos, and decreases the risk of pregnancy failure (Evsikov *et al.*, 2008; Nazarova & Evsikov, 2008; Yuzhik *et al.*, 2015). This seems to agree with our results in *A. scherman cantabriae* regarding the increase of litter size with the BCI of the mother. Although a clear correlation between the number of implanted embryos and the body mass of the mother has also been reported in both *A. scherman* and *A. amphibius* (Ventura & Gosálbez, 1990b and references therein), our data suggest ultimately that the BCI is the key. Therefore, the body condition of the mother seems to be one of the main factors involved in the variation of the reproductive potential in Cantabrian voles.

The succession of monthly samplings during our two-year study involved a population control by removing specimens of both sexes from the burrow. Since the burrow system of fossorial water voles is generally inhabited by a breeding couple with their offspring (Airoldi, 1976, 1978; Morel, 1981; unpublished own data) and its maintenance and the storage of food are performed by the couple (Airoldi, 1976), the removal of the male mate might suppose a handicap for the female. In this situation, the female might face the maintenance of the burrow and the acquisition of food by its own until de arrival of a new partner which in turns can induce a decrease of the body condition, as we observed in mature non-pregnant females along this study period. Ultimately, this fact might have negative consequences on the reproductive success as it has been reported for several arvicoline species (Ostfeld, 1985).

As conclusions, the reproductive potential of *A. scherman cantabriae* in areas of NW Spain located at low altitude and with a temperate hyperoceanic climate is relatively high in comparison with that of fossorial water voles from the Pyrenees. Specifically, mild temperatures and enough resource availability along the whole year in our study area allow fossorial water voles to breed continuously and to perform a greater number of reproductive events per year than *A. scherman monticola*. Conversely, the favourable environmental conditions in the Pyrenean meadows are restricted to a part of the year, what leads voles to concentrate its energy in fewer but larger litters. Therefore, the smaller litter size in *A. scherman cantabriae* seems to fit with a different reproductive pattern, in which the variation in this parameter within the population may be due, at least in part, to the body condition of the mother.

Our results show that a female can produce almost 30 new specimens distributed along the whole year, what means a continuous and considerably high recruitment of juvenile specimens to the population. Such a situation suggests that management efforts should not be seasonal as they used to be so far, as it was generally assumed that reproduction stopped in winter. The recommended strategy was to increase control activity in winter in order to remove adults before the hypothetical breeding season, which was expected to start in spring. However, our results showing a continuous breeding season suggest extending these practices along the whole year. We do not mean to increase the effort (especially in the case of rodenticides given the associated environmental issues) but just that control practices could have a similar effect with independence of the time of the year. Also important to avoid secondary effects on non-target animals, surface signs of activity of *A. scherman* should be distinguished from those of *Talpa occidentalis*, an innocuous species inhabiting also apple orchards (Miñarro *et al*., 2012). For the same reason, alternative strategies, such as fences to avoid invasions from dispersal juvenile specimens from nearby source demes (Saucy & Schneiter, 1998; Saucy, 2002; Walther & Fuelling, 2010) should be also maintained all the year. The effectiveness of other sustainable control management strategies, such as frequent mowing (Morilhat *et al*., 2007; Jacob, 2008), livestock grazing (Defaut *et al*., 2009) or the use of repellents (Fischer *et al*., 2013) should be explored in our particular case. As mentioned for *A. amphibius*, mother body condition might also have other physiological consequences in the offspring, affecting the reproductive success, life span, body mass and maturation of young (Yakovleva *et al*., 1997; Nazarova & Evsikov, 2008). Since population density and the subsequent damage to crops could be ultimately proportional to the population growth, which in turn depends on the reproductive effort (Ostfeld, 1985; Cerqueira *et al*., 2006), further studies focused on the physiological consequences of management practices (those creating a more disadvantageous environment; Morilhat *et al*., 2007; Jacob, 2008; Defaut*et al*., 2009) will be welcome to unravel their effect on the reproductive fitness in *A. scherman*, and to evaluate their feasibility as alternative control strategies.

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