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POST-WEANING GROWTH OF ENDEMIC IBERIAN WILD RABBIT SUBSPECIES, *ORYCTOLAGUS CUNICULUS ALGIRUS*, KEPT IN A SEMI-EXTENSIVE ENCLOSURE: IMPLICATIONS FOR MANAGEMENT AND CONSERVATION

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Abstract: Little is known about the endemic Iberian wild rabbit subspecies, *Oryctolagus cuniculus algirus*, despite its importance in Mediterranean ecosystems. An individual's physical condition is of undisputed importance when evaluating the quality of habitats or restocking enclosures to assess the corresponding population status. We analysed post-weaning body weight and growth of 351 individuals of the endemic *Oryctolagus cuniculus algirus* subspecies under semi-natural conditions in a breeding enclosure in central Portugal. From these data, we described and estimated growth rates for juvenile and adult stages, and 3 sigmoidal growth models were developed. Body weight showed a linear growth of 0.00765 kg/d until 0.6 kg, whereafter it began to decrease steeply until 1 kg (<0.005 kg/d between 0.6 and 0.7 kg, <0.003 kg/d between 0.7 and 0.9 kg, and <0.001 kg/d then on). An age prediction linear growth equation was estimated for individuals up to 0.6 kg. The von Bertalanffy model best described the observed body weight growth. Juvenile body weight growth of *Oryctolagus cuniculus algirus* is lower than that of the widespread *Oryctolagus cuniculus cuniculus*. Our data revealed that *Oryctolagus cuniculus algirus* seems to be smaller, grows less and for less time than *Oryctolagus cuniculus cuniculus*, according to the literature. Body weight of the studied individuals seems to be more similar to free-living than to cage-bred individuals, according to the literature. These results should provide important indicators to assist managers and conservationists in accessing habitat quality for wild rabbit populations.

Key Words: growth, weight, wild rabbit, *Oryctolagus cuniculus algirus*.

INTRODUCTION

The European wild rabbit (*Oryctolagus cuniculus* Linnaeus, 1758) is a keystone species in Mediterranean habitats (Delibes-Mateos *et al.* 2007; Gálvez *et al.*, 2008) and the main prey of the critically endangered Iberian lynx (*Lynx pardinus* Temminck, 1827) and Iberian imperial eagle (*Aquila adalberti* Brehm, 1861), as well as of other 27 species (Delibes and Hiraldo, 1981; Ferrer and Negro, 2004). *Oryctolagus cuniculus* also has economic and cultural importance in Mediterranean countries that have significant hunting activity (e.g. Spain and Portugal), mostly based on small game species (Virgós *et al.*, 2007; Sánchez-García *et al.*, 2012). Although it is also distributed in North Africa, as well as in Mediterranean and Atlantic islands, *Oryctolagus cuniculus algirus* (Loche, 1858) is considered endemic to the southwestern Iberian Peninsula, while *Oryctolagus cuniculus cuniculus* occupies the northeastern part of the peninsula and remainder sites where the species occurs (Branco *et al.*, 2000; Carneiro *et al.*, 2010). Their numbers have undergone a dramatic decrease in their native habitats over the past 50 yr (Ferreira and Delibes-Mateos, 2010; Guerrero-Casado *et al.*, 2013), mainly due to the widespread incidence of diseases (e.g. myxomatosis and rabbit hemorrhagic disease), but also as a consequence of habitat loss and fragmentation and

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from incorrect game practices (Delibes-Mateos *et al.*, 2009). Hunters and conservationists strive to restore previous population stocks, by habitat management and restoration measures that decrease the deleterious impact of disease on populations, as well as local rabbit breeding and reintroduction programs (Delibes-Mateos *et al.*, 2009; Ferreira *et al.*, 2013). Their efforts have unfortunately not been followed by an increase in rabbit population numbers, perhaps as a consequence of lack of funds to maintain habitat management, rise of mortality through new disease outbreaks and poor knowledge of the most suitable places to introduce and establish farmed animals (Ward, 2005; Dalton *et al.*, 2012; Ferreira, 2012; Guerrero-Casado *et al.*, 2013).

Only recently has conservation and ecology research been focusing on integrating physiological knowledge to assess biodiversity and ecosystem response to environmental change, the so-called conservation physiology (Cooke *et al.*, 2013). This discipline may help assess the population status of vulnerable species by measuring physiological characteristics, which has been considered a potentially more effective evaluation method than more commonly used techniques of species distribution estimation, e.g. population abundance and presence/absence (Horne, 1983; Cooke *et al.*, 2013). Weight, as it is easy to measure, has been widely used as a good indicator of the physiological status of individuals in a vast array of studies concerning different species (Bjorndal and Bolten, 1988; Rhind and Bradley, 2002; Johannesen and Andreassen, 2008; Zhao *et al.*, 2010; Janin *et al.*, 2011; Haigh *et al.*, 2012).

Previous studies involving body growth rate estimation in rabbits are mostly limited to Australia and New Zealand (Dunnet, 1956; Webb, 1993), where the *O. c. cuniculus* subspecies was introduced and has an extremely invasive behaviour (Gibb, 1990; Williams *et al.*, 1995). Furthermore, these studies have been restricted to the first months of life, where rabbit growth is considered linear and hence easier to estimate (Southern, 1940; Dunnet, 1956). In the Iberian region, only Soriguer (1980) studied the adult (>0.900 kg) body growth of a wild population of *O. c. algirus*, while a recent study also addressed this issue, but during the first 10 mo of age of caged rabbits (González-Redondo, 2013a). Consequently, little is known about one of the Iberian rabbit subspecies' body growth in semi-extensive enclosures, either in an earlier juvenile phase or in later adult stages. This lack of knowledge could be a setback to proper rabbit population estimates and habitat quality assessments, either of enclosures or of natural settings.

In this paper, we aim to describe the body growth and weight of a wild rabbit semi-captive population and estimate a body growth rate for *Oryctolagus cuniculus algirus*.

MATERIALS AND METHODS

Study Area

This study was conducted in a wild rabbit enclosure located in Fornos de Algodres, Guarda, Portugal (40°37'34"N, 7°32'44"W) belonging to a local hunting club (Clube de Caça e Pesca de Fornos de Algodres), over a 4-yr period between July 2006 and June 2010. Average 60-yr temperatures for the region varied between a minimum of 2.2°C in January and maximum of 29.2°C in July and August, while average annual precipitation was of 1170 mm. The enclosure had a total area of 2400 m^2, divided into 2 feeding sectors of 900 m^2 each and a shelter sector of 600 m^2. The first 6 breeding animals (3 males and 3 females) were introduced to the enclosure in April 2006, and belonged to a local wild population of *O. c. algirus*, confirmed by genetic and sanitary examinations (to screen for myxomatosis and rabbit hemorrhagic disease) performed by the Portuguese Forestry Authority (AFN), in accordance with Portuguese law (Decreto Lei nº 201/2005; Portaria nº 464/2001). Across the study duration, estimated population abundance between captures varied from 15 to 156 individuals, with a mean (±standard deviation [SD]) of 69 (±37).

Animals were given semi-natural conditions, where the shelter sector provided burrows for cover, whereas the feeding sectors yielded a rotational food supply, *i.e.*, only one feeding sector was connected with the shelter sector at a time. This rotation allowed for plants to grow back in the closed sector, while rabbits fed in the other. The feeding sector provided animals with plants commonly found on nearby agricultural lands (genera *Tricium*, *Avena*, *Zea*, *Vicia*, and *Trifolium*) and conditions similar to those encountered in the neighbouring habitat. Cultivated plants were irrigated throughout, so water availability was not a limitation. Food was provided *ad libitum*, with no direct limitation on the population feeding regime. The enclosure was surrounded by underground concrete walls, buried 1 m deep, and by 2.5 m tall steel fences. A net covered the entire area. Consequently, no predators were able to enter the enclosure.

Capture and Measurement Procedure

A capture-mark-recapture procedure (Southern, 1940) was implemented throughout the study, with monthly captures carried out in the feeding sectors. As the population lived in absolute freedom within the enclosure itself, the captures were constrained to animals that were active at the sampling day. Captures were made in the feeding sector, by closing access doors to the shelter sector the night before captures (doors would let animals reach the feeding sector, but would not let them return to the shelter sector). On the following morning, all animals present at the feeding sector were sampled. The rabbits were individually ear-tagged so that later identification would be possible. Body mass was measured using a portable digital hanging weight scale with 5 g accuracy. An estimated average of 39% (±26) of the population was captured on each sampling day (number of captured individuals ranged from 4 to 88).

Age Estimation

The exact age at which each animal was captured for the first time was virtually impossible to know, so estimation was mandatory. The growth rate of rabbits is described as slower in an early nestling, rising to a maximum at a post-weaning stage (Myers, 1964). Consequently, as no nestlings were measured, estimation was restricted to the post-weaning phase. This calculation was made according to the equation first used by Southern (1940) and revised by Dunnet (1956), assuming an age at weaning of 21 d (Myers 1958):

$$W=w_i+g(t-21)$$

where W is the weight (kg) at age t (d), w_i is the weight at weaning (kg), and g is the growth rate (kg/d). In the cited studies, both authors assumed weight at weaning as 0.200 kg. However, weight at weaning has been described as varying between 0.180 and 0.250 kg in works involving *O. c. cuniculus* (Webb, 1993). Accordingly, w_i was set at 0.180 kg, seeing that *O. c. algirus* is smaller (Gibb, 1990) and that 0.180 kg of weight at weaning was recorded in a field enclosure similar to the one described in the current work (Myers, 1958), also agreeing with weight at 21 d of age found for cage-bred kits of the *O. c. algirus* subspecies (González-Redondo, 2000).

Growth data for the previously mentioned individuals were fitted to the following 3 commonly used models (Zullinger *et al.*, 1984), using the Marquardt-Levenberg algorithm:

1 – The logistic equation $W=A\{1+\exp[-K(t-I)]\}^{-1}$;

2 – The Gompertz equation $W=A\exp\{-\exp[-K(t-I)]\}$; and

3 – The von Bertalanffy equation $W=A\{1-1/3\exp[-K(t-I)]\}^{3}$;

where A is the asymptotic value (kg), W is the body mass (kg) at age (t), K is the growth rate constant (kg/d), and I is the age at the inflection point (d).

Data Analysis

Growth rates were determined by subtracting individual weight measured in 2 consecutive captures. Nonetheless, as not all individuals were encountered on every capture occasion, it was mandatory to filter data, to reduce errors as a consequence of the high growth rates present in young individuals. As a result, individual weight data were constrained to captures less than 30 d apart, dropping from a total of 1164 observations to 306 filtered cases.

Growth rate was calculated for 0.100 kg class intervals, for a total of 14 classes, as no rabbit weighed less than 0.140 kg and more than 1.400 kg.

As most individuals had more than one measure, generalised linear mixed models (GLMMs) were used to cope with individual variance in the statistical analysis where group comparison was necessary (Zuur *et al.*, 2007). Each rabbit individual was consequently used as a random variable in weight and growth rate comparison between sexes, age, and weight classes.

Statistical analyses were performed using SPSS version 19 (SPSS, Chicago, IL, USA).

RESULTS

Filtered weights varied between 0.170 kg, in a juvenile female and 1.400 kg in an adult reproductive male. On average, individuals weighed (±SD) 0.716 kg (±0.299; n=306), with females weighing 0.727 kg (±0.314; n=149) and males 0.705 kg (±0.287; n=157). No statistical differences were found in weights across sexes (F=0.983; $P>0.05$). The highest growth record was found in a juvenile male, which had a 0.0171 kg/d weight increase in 21 d, growing from 0.310 to 0.670 kg in that time frame. Conversely, the lowest growth rate was encountered in an adult female, which decreased from 1.145 to 0.955 kg in 23 d, resulting in a 0.0083 kg/d decrease in weight.

No significant differences were found between the general male and female growth rates (0.0043±0.0039 *vs.* 0.0042±0.0042 kg/d; F=0.210, $P>0.05$), so the 2 sexes were plotted and analysed together. Significant differences were found in the overall growth rates (F=2373.026; $P<0.001$), so each successive weight class growth rate was tested for significance. Classes 1, 12, 13 and 14 were excluded from analysis due to the low number of observations (n<10). Significant differences were found between classes [5, 6] (F=17.309; $P<0.001$), [6, 7] (F=5.675; $P=0.021$) and [8, 9] (F=7.041; $P=0.010$) (Figure 1).

As no significant differences were found between the first 4 classes analysed (2, 3, 4 and, 5), we could assume that the growth up to 0.600 kg was linear, so henceforth the average growth for this period was considered equal among classes. Accordingly, average growth from 0.200 to 0.599 kg was of 0.00765 kg/d. This value was introduced in the linear growth equation mentioned in the methods section, and age (d) at first capture was estimated for all animals found weighing less than 0.600 kg at that time (n=835). Only 10 of the 351 tagged individuals weighed less than 0.180 kg, so we considered it reasonable to be assumed as the weight at weaning.

Weight was fitted to the 3 sigmoidal models, all providing high fitting values (Table 1). Nevertheless, the von Bertalanffy had the highest R-squared value ($R^2=0.945$), and was thus considered the model which best fitted the available data. This model predicted the values of 1.069 kg for the maximum weight, a growth rate of 0.01596 kg/d and an inflection point at day 38.57 (Figure 2).

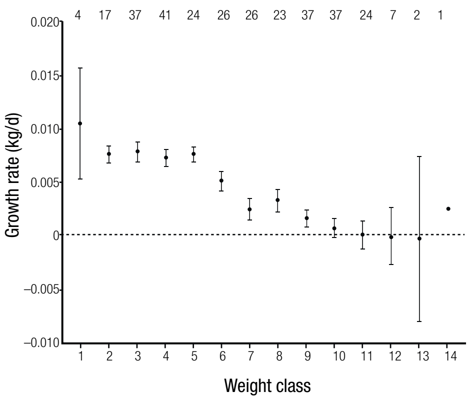

Figure 1: Average growth rates of *Oryctolagus cuniculus algirus* by 0.100 kg weight classes. The error bars represent 95% confidence intervals. Number of observations for each class is shown on top.

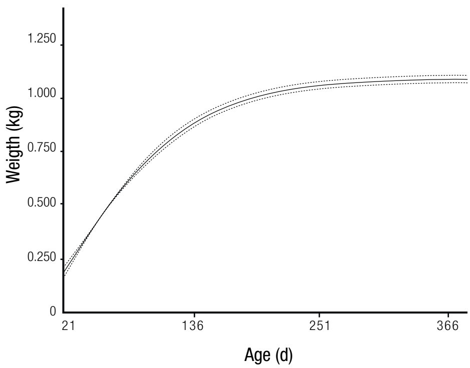

Figure 2: Estimated von Bertalanffy growth curve for the first year of *O. cuniculus algirus* life (dashed lines represent confidence intervals at 95%).

Table 1: Estimated parameters from the logistic, Gompertz and von Bertalanffy equations fitted for *Oryctolagus cuniculus algirus* growth, and respective 95% confidence intervals (CI). A is the asymptotic value (kg), K is the growth rate constant (kg/d), and I is the age at the inflection point (d). R-squared values for each model are also shown.

Model	Parameter	Weight±standard deviation	Lower CI	Upper CI
Logistic	A	1.049±0.005	1.038	1.060
	K	0.02617±0.00062	0.02495	0.02740
	I	69.69±0.77	68.15	71.18
	R^2	0.939		
Gompertz	A	1.063±0.006	1.052	1.074
	K	0.01835±0.00042	0.01751	0.01918
	I	48.92±0.63	47.68	50.17
	R^2	0.944		
von Bertalanffy	A	1.069±0.006	1.058	1.080
	K	0.01596±0.00037	0.01524	0.01668
	I	38.57±0.632	37.33	39.81
	R^2	0.945		

DISCUSSION

Oryctolagus cuniculus algirus has been described in the literature as smaller than *O. c. cuniculus* (Gibb, 1990; Villafuerte, 2007), with no distinct differences between sexes in *O. c. cuniculus* (Gibb *et al.*, 1985). Our study validates that the Iberian wild rabbit subspecies is indeed smaller than the widespread *O. c. cuniculus*. Similarly, males and females do not differ in weight. This is also in agreement with studies carried out both in wild and in caged *O. c. algirus* individuals (Soriguer, 1981; González-Redondo, 2013a).

Previous studies with free-living populations of *O. c. cuniculus* have shown an increase in weight during the first three years (or 36 months) of life, up to a maximum of about 1.800 kg in females (Gibb *et al.*, 1985). Their growth was referred as a linear 0.010 kg per day increase until about 0.900 kg (Southern, 1940; Dunnet, 1956; Williams *et al.*, 1995). These results differ from our study with the *O. c. algirus* subspecies, as no significant growth was recorded after the first year of life or 1.000 kg. Our studied rabbits also showed a slower daily linear growth rate during juvenile development when compared to the previously mentioned works. These studies also revealed that after the 0.900 kg threshold is attained, the growth rate starts to gradually diminish until the referred 1.800 kg. However, this lower growth rate is still fairly high – 0.087 kg/day in a free-living *O. c. cuniculus* population (Wood, 1980) –, when compared to the one described in the present work. The cited growth differences should be due to physiological differences between subspecies, as already described with maximum body weight (Gibb, 1990), sexual maturity (Villafuerte, 2007), and genetic analyses (Branco *et al.*, 2000). The same conclusions were found for cage-bred rabbits of *O. c. algirus* (González-Redondo 2013a).

We used three sigmoidal models (logistic, Gompertz and von Bertalanfy) to fit the observed weight values. From these, we considered the von Bertalanfy as the model that best fitted the data. The values predicted by the chosen model are within the average for those observed in the current study, as few individuals weighed more than the predicted asymptotic mass of 1.069 kg. Both the juvenile growth equation and the von Bertalanfy equation gave similar age values for the first 0.6 kg. Our results can be compared with those of Soriguer (1981) from a wild population, and González-Redondo (2013) with cage-bred rabbits, as being closer to the values obtain by the former. Although both studies used Gompertz equations to fit the rabbit growth, the assymptotic mass obtained by Soriguer 1981 (1.125 kg) is closer to the value predicted by the Gompertz model in our study (1.063 kg) than the same parameter in the González-Redondo (2013) study (0.894 kg). Moreover, the maximum weight of 1.400 kg obtained in our population is almost identical to the one referred by Soriguer (1981) (1.490 kg). The similarity of our values to those of a free-living population, and difference from those of cage-bred individuals, might reveal that our population lived in conditions similar to its natural habitat. At the same time, this result appears to indicate that semi-captive populations, while more difficult to manage, are a valid solution for repopulation programmes, as these animals have a similar body condition to those found in the wild. We can also admit that the equations derived by this study are of good biological value and might be used by stakeholders to estimate juvenile age and/or to assess individuals' general status.

Some of the rabbit population recovery procedures directly focus on restocking either by translocation or *in situ* breeding, also known as supportive breeding (Ryman and Laikre, 1991). These actions have already been described as potentially hazardous to the genetic variability present in the rabbit populations of the Iberian Peninsula, as hybridisation could occur (Delibes-Mateos *et al.*, 2008), although it does happen naturally along the overlap zone, which separates the two rabbit lineages (Branco *et al.* 2000; Geraldes *et al.* 2006). The ecological implication of such subspecies mixing is, and will probably continue to be, unknown, but as mentioned previously, it is widely recognised that significant body differences exist between *O. c. algirus* and *O. c. cuniculus*, again confirmed by the results shown in the current work. Furthermore, weight differences have been described as being of ecological importance in a wide variety of factors, as described in greater detail by Calder (1983), one of which concerns population density, as species with lower weight tend to have larger population densities than heavier ones (Greenwood *et al.*, 1996). Accordingly, caution is advised in performing restocking procedures, mainly when the introduced species or subspecies is different from that of native populations (Simberloff, 1996; Delibes-Mateos *et al.*, 2008).

Attention should also be focused on the inbreeding processes motivated by the enclosed facility where the population grew and on the few original breeding individuals (Ryman and Laikre, 1991; Laikre *et al.*, 2010). These problems are transversal to most repopulating programmes and body fitness conclusions drawn from these facilities should be addressed with care (Ryman and Laikre, 1991). Moreover, although conditions present at the enclosure were similar to the surrounding habitat, natural settings are always difficult to simulate in enclosures; for example, stress associated with predation, which might reduce fitness and general weight (Yom-Tov *et al.*, 2012), was never a problem at our site, as no predators were able to enter. Likewise, food availability in a very small enclosed area could pose a setback to maintaining individuals in a healthy environment, as the home range of free-living individuals of high density populations is wider ($\approx$ 7000 m^2 in Devillard *et al.*, 2008) than the 1300 m^2 available at all times in our enclosure. However, as a home range is assumed to include all the necessary requirements (such as food and shelter) for upkeep and reproduction (Devillard *et al.*, 2008), the enclosure provided all the requirements for a healthy wild rabbit population, as reproduction occurred throughout the study, and rabbit body condition was similar to that of a free-living population (Soriguer, 1981).

In conclusion, the results postulated by this study may provide a good indicator of the general individual status of rabbits captured both in similar enclosures and in the wild, and may provide stakeholders with important information regarding rabbit management and conservation.

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To my father (A. Ferreirra).

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