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Body condition variation of wild rabbit population in the north-east Mediterranean island of Lemnos – Greece

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Abstract. European wild rabbit (*Oryctolagus cuniculus*) is a widely distributed species with a multiple role in both environmental sustainability and local economy. In the current study we examine the body condition of the species in relation to habitat type, age, sex and seasonality on the island of Lemnos. Body condition was assessed based on a visual estimation of the amount of abdominal fat. A high intra-annual variation in the body condition of the species has been observed, which is further affected by sex and age. The species appears to conserve and maintain high levels of energy reserves for two main reasons. The first is to ensure a better reproductive success, as expressed by the relatively high amount of stored fat reserves at the onset of breeding season, and their depletion during it. The second is to ensure survival during periods where the shortage of food creates a rather hostile environment, as expressed by the increased level of reserves during summer and their dramatic decrease from late summer until autumn. The temporal fluctuation in energy reserves differs between male and female rabbits, reflecting their temporally different energy demands. Further, differences in body conditions were observed between juvenile and adult individuals, reflecting their different needs in terms of body growth and maintenance. Our results could offer important insights for the development of a time specific management plan and measures to ensure either the control of the population or its conservation.

Key words: European wild rabbit, fat storage, energy reserves, Mediterranean environment, management

Introduction

The wild rabbit (*Oryctolagus cuniculus*) is a widely distributed small-sized mammal with a great ecological adaptability in varying environments and habitat types (Flux 1994). It constitutes a key prey species for predators and an important game (Delibes-Mateos et al. 2007, 2008), while it contributes to the increased heterogeneity and biodiversity of Mediterranean pastures (Gálvez-Bravo et al. 2011). However, in many Mediterranean islands it is considered an invader and pest (Courchamp et al. 2003), competing with local wild fauna and livestock, causing destruction in soils and cultivations, and negatively affecting local biodiversity (Lees & Bell 2008).

On the island of Lemnos, rabbits were probably introduced since the antiquity by Phoenicians and/

or Romans (Gibb 1990, Flux 1994), and no conflicts between them and the local economic activities, such as agriculture, were recorded until the 1990s, when a rabbit population increase was observed (Kontsiotis et al. 2013a). After continuous allegations by local farmers, the Hellenic Organization of Agricultural Insurances (ELGA) launched a compensation scheme in 2007 for wild rabbit damages to crops (ELGA 2007). The increased need for management of wild rabbits on Lemnos and in most parts of its range, requires the in depth knowledge of its physiology and ecology.

Information for demographic process and animal-habitat interactions can arise from the assessment of animal's body condition (Huot et al. 1995, Gaston et al. 2001), providing an important tool in the designing and implementation of management plans.

In wild fauna, fat storage is usually used as a body condition index (Blem 1990, Batzli & Esseks 1992) and it is considered an important determinant of fitness, behaviour, vigour, survival and reproductive success (Hodges et al. 2006, Peig & Green 2009, Cabezas et al. 2011). It constitutes a fundamental source of endogenous energy for many mammals and birds (Blem 1990, Robbins 1993). Boos et al. (2005) demonstrated that reproductive success, intra-specific competition and other aspects of wild rabbit's population dynamics are linked to its body condition. Rödel et al. (2004) suggested that body condition of sub-adult individuals determines to a large degree the likelihood of their overwintering survival.

Body condition can be affected by many environmental factors including rainfall amount and ambient temperature (Seltmann et al. 2009), landscape fragmentation (Hewison et al. 2009), soil quality (Klein & Strandgaard 1972) and topography (Mysterud et al. 2001). Subsequently, habitat selection can largely determine the body condition of terrestrial mammals (Fisher et al. 2011).

Endogenous factors such as sex and age have also been reported to affect body condition. In female mammals body condition seems to notably affect reproductive success; e.g. both litter size and neonatal mass increase with body condition (Atkinson & Ramsay 1995, Dobson & Michener 1995, Wauters & Dhondt 1995). Prendergast & Jensen (2012) suggested that body fat decreases as number of carried embryos increases in muskrat (*Ondatra zibethicus*), demonstrating the importance of fat reserves in reproduction, especially for mammals producing multiple litters annually. For wild rabbits in particular, Rödel et al. (2005) suggested that a good body condition is essential for the onset of breeding. Boyd & Myhill (1987) observed a reduction in body fat reserves during reproduction for both sexes, while similar results were reported by Parer & Libke (1991) during breeding and lactation for females. Furthermore, Boussès & Chapuis (1998) documented the high energetic cost of increasing testes size and production of androgens by males.

According to Boss et al. (2005), age may significantly affect body mass lipids. Rödel & von Holst (2008) suggested that sub-adult wild rabbits have a lower body mass than adults and a subsequent disadvantage in surviving under adverse environmental conditions. Furthermore, Wallage-Drees & Deinum (1986) suggested that high body mass is related to high fat reserves, and as a result significant differences in body condition are expected between individuals of different age.

Despite the high importance of body condition in population dynamics there is still limited information on its annual variation, especially in insular and seasonal environments. Furthermore, there is still a discrepancy over the differences in body condition between sexes, across their annual cycle (Boyd & Myhill 1987, Parer & Libke 1991). The aim of the current study was to investigate the variation in body condition in relation to habitat, season, sex and age in the wild rabbit population of a typical Mediterranean island. These factors are expected to have a significant impact on body condition due to the induced differences in food availability, reproductive status, nutritional demands and growth tradeoffs. Our results are expected to provide useful insights for the sound management of the species.

Material and Methods

Study area

The study was conducted on Lemnos island (475.6 km²) (39°46'-40°02' N, 25°02'-25°26' E), in the north Aegean Sea. The island is dominated by plain areas, and only on its western part low hills of up to 430 m a.s.l. can be found. The climate is typical Mediterranean, with very hot and dry summers and mild winter (average annual temperature is 15.9 °C and average annual precipitation is 474 mm).

The main habitat types dominating the island are phrygana and agricultural cultivations. Phrygana are composed primarily by *Sarcopoterium spinosum*, *Thymus capitatus*, *Centaurea spinosa*, *Genista acanthoclada* and *Anthyllis hermannia* while agricultural crops are mainly cereals and vineyards. In order to study the annual variation in body condition one representative site of 100 ha for each habitat type was selected. Each site was located in the center of a wider area covered by the same habitat type in order to avoid population interactions, while the horizontal distance between them was 13 km. The location of each site was selected based on the internal homogeneity as well as on the homogeneity of the surrounding area. The agricultural site was located in the central and the phryganic on the southeastern part of the island (Fig. 1).

Methods

Data collection was carried out between February 2007 and January 2008. The study was performed by postmortem analysis on 360 wild rabbits (15 samples per month per habitat) of which 175 were males and 185 females. Spotlight shooting was the method employed for sample collection, after acquiring the necessary authorization by the Hellenic Ministry of Rural Development and Food. Only individuals with

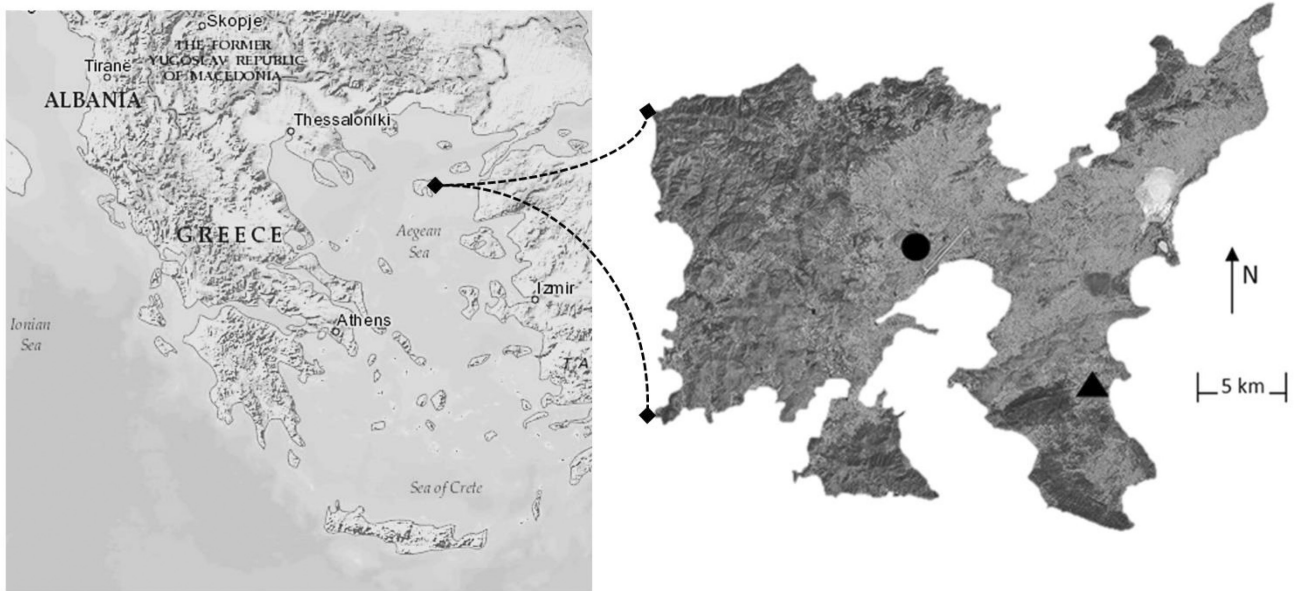


Fig. 1. Orientation map of the Lemnos island and location of studied habitats. Circle indicates the agricultural site and triangle indicates the phryganic site.

a, visually assessed, large body size (≥ 900 g), were targeted avoiding the just emergent. Immediately after shooting, each wild rabbit was placed in a plastic bag, in order to avoid any water loss due to bleeding and dehydration (Boos et al. 2005). Within three hours of their collection, rabbits were weighed (intact body mass, ± 1 g), and their body length, from the nose to the last caudal vertebra (± 1 mm), was measured. Eyes were removed and placed in 10 % formalin for at least 48 h while the rest of the body was frozen at -18 °C. The eye lenses were then removed and oven-dried at 85 °C for 72 h and weighed to the nearest milligram. The mean weight for each pair of lenses was calculated and used to determine the individual's age using the Myers & Gilbert (1968) formula: age (days) = $-57 + 181.4/\log_e [314/\text{lens wt (mg)}]$. Rabbits were classified into two age classes, namely juveniles (below four months old) and adults. The 24 individuals that had damaged eye lens, due to shooting, were classified based on their weight, with those above 1200 g classified as adults and the rest as juveniles (King et al. 1983). After thawing, wild rabbits were sexed optically and their body condition was estimated according to their abdominal fat on a 4-scale classification (Fraser 1988): 1st class: no abdominal fat, 2nd class: restricted fat around the kidneys, 3rd class: modest fat around the kidneys which spreads several cm along the abdominal wall, 4th class: large amounts of fat which almost covers the kidneys and expands to thick layers around the stomach and small intestines. The reproductive status of males was assessed based on the position (abdominal or scrotal) and weight of

testes. The testes were excised, separated from the epididymides and weighted at the nearest 0.01 g. Individuals with at least one testis in the scrotal position or those with testes weight exceeding 2 g were considered as fertile (Bell 1986, Vandewalle 1989, Parer & Libke 1991, Boussès & Chapuis 1998). Female reproductive status was determined by evidence of pregnancy and/or lactation (Fraser 1988, Gonçalves et al. 2002). Pregnancy was assessed by the presence of implanted embryos or the presence of placental scars, while lactation was assessed by inspecting the mammary gland development. We conducted seasonal vegetation inventories in each habitat type (agricultural and phryganic) in order to quantify the availability of green vegetation, which constitutes the main food source. Vegetation cover was estimated using line-point intercepts (Cook & Stubbendieck 1986, Elzinga et al. 1998) along five, randomly located, 25 m linear transects on each site, and expressed as percentage cover (%). Points were spaced at 0.25 m apart within line transects, and a total of 500 points were sampled per season and habitat type.

Statistical analysis

Differences in body condition between sexes, habitat types and among the four seasons (spring: March-May, summer: June-August, autumn: September-November and winter: from December to February), on adult wild rabbits only, were tested using hierarchical log-linear model analysis. The number of variables included in the analysis was restricted due to the sample size limitation of log-linear analysis (Knoke &

Burke 1980). Log-linear analysis was also employed in order to identify differences in body condition between adult and juveniles and between the two habitat types. The variable season was not included in this case because the sample size was smaller as a result of our attempt to include an equal number of adults and juveniles per month. In both cases, the full model showed a good fit to the data and the technique of backward elimination was followed until the final model was selected (Sokal & Rohlf 1995). Finally, we employed the non-parametric Friedman's statistical test to detect differences in cover of green vegetation among seasons (Friedman 1937), while the Wilcoxon test (Wilcoxon 1945), was employed for post-hoc comparisons between the four seasons.

Results

Among the 360 collected individuals, 72 were juveniles and 288 were adults. Most juveniles were collected in spring and summer, 22 and 35 respectively, while 12 and three were collected in autumn and winter respectively.

According to their body condition, most wild rabbits of the total sample were mounted in the 1st class (45.3 %), which followed in descending order by the 2nd (29.2 %), 3rd (14.4 %) and 4th class (11.1 %).

For the adult wild rabbits, the hierarchical log-linear analysis of the full model included the four body condition classes as dependent variable and the four seasons, two habitat types, two sexes and their interactions as independent variables. The full model showed a good fit to the data ($G^2 = 7.071$, $df = 9$, $P = 0.630$), while following the technique of backward elimination the final model revealed that the variation in body condition can be explained by the interaction of season and sex only ($G^2 = 33.112$, $df = 32$, $P = 0.413$; Fig. 2).

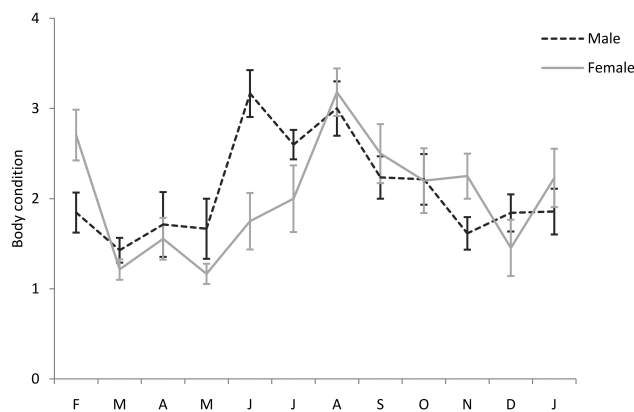


Fig. 2. Monthly fluctuation of the body condition (Mean ± SE) of adult male and female wild rabbits.

In the second analysis, where differences in body condition between adults and juveniles in the two habitat types were tested, the full model showed a good fit to the data ($G^2 = 0.269$, $df = 3$, $P = 0.966$), and after backward elimination, body condition was found to significantly differ between juveniles and adults, independently of the habitat type ($G^2 = 5.887$, $df = 8$, $P = 0.660$; Fig. 3).

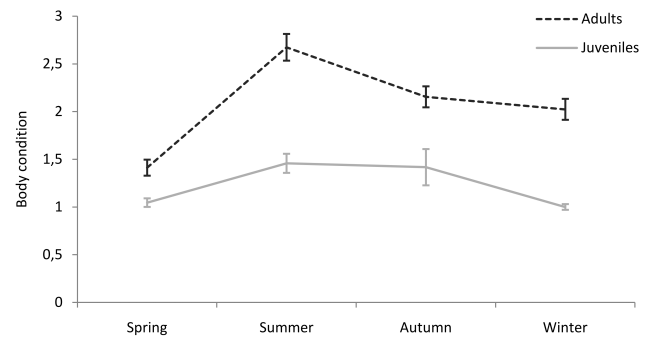


Fig. 3. Seasonal fluctuation of the body condition (Mean ± SE) of adult and juvenile wild rabbits.

Since habitat type was not found to significantly affect body condition, the analysis of reproductive status and green vegetation cover was not done separately for each habitat. Testes size was found to vary considerably across the annual cycle, maximizing in winter, with testes weight reaching almost 3 g, and minimizing during summer with testes weight of less than 1 g. The reproductive period begins in December and ends in June (Fig. 4), with the main reproductive period falling between February and May. Lactating period, on the other hand, begins in January and ends in August.

Significant differences between seasons were found in the pattern of green vegetation cover ($P < 0.001$; Fig. 5). Green vegetation maximizes in spring, exceeding a

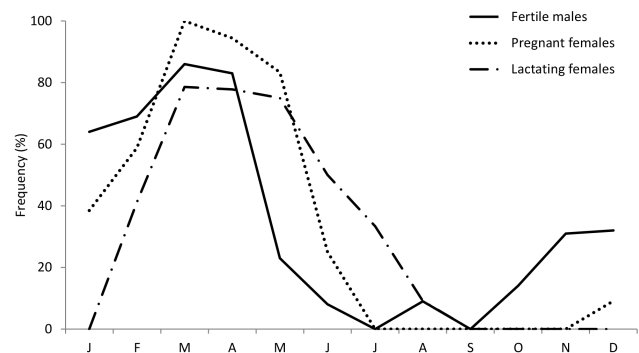


Fig. 4. Frequency of pregnancy and lactation of female, and fertility of male wild rabbits from both habitat types.

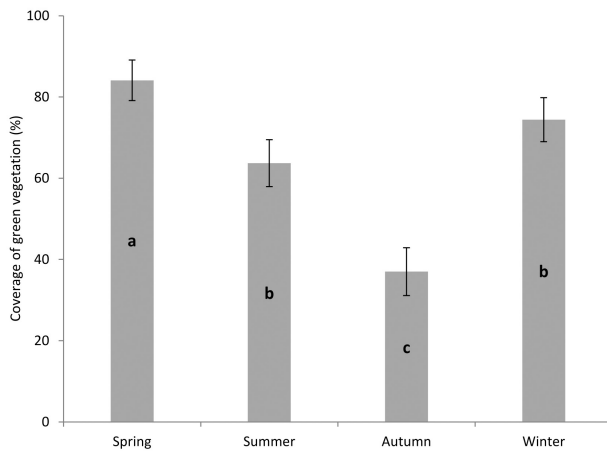


Fig. 5. Seasonal percentage covers of green vegetation (Mean \pm SE). Different letters inside the columns indicates statistically significant differences ($P < 0.05$).

cover of 80 %, and it drops significantly from summer to autumn where it falls below 40 % cover. In winter it recovers as a result of the onset of wet season from late October.

Discussion

The results presented above conform to our hypothesis that body condition varies seasonally, between sexes and between age classes. This is probably due to differences in energy needs between individuals of different age and sex and the seasonal differences in the availability of external energy sources. The absence of a habitat effect on body condition was rather unexpected but it could be explained by the dietary flexibility of the species and its ability to exploit a wide range of food resources (Rödel 2005). Since the viability of a population depends on successful reproduction and survival during harsh conditions, a successful survival strategy should ensure a body condition suitable to overcome those critical parts of a life cycle.

Body condition exhibits a high seasonal fluctuation, with a significantly different pattern between sexes. It is at its lowest level from March to May, for both sexes, which falls within the main reproductive period. Boyd & Myhill (1987), studying wild rabbits population in UK, reported the same findings demonstrating a reduction of the fat reserves during the breeding season, while in females the steepest decline occurs when exhibit greater productivity.

From May, male rabbits begin to recover the lost body fat by exploiting the available food resources and they reach the optimum body condition by June, remaining high until August. The maintenance of high levels of reserved fat during the summer period, where available food resources are decreasing, is attributed

to the fact that during this period they are decreasing the size of testes, dropping their metabolic rate and avoiding energy demanding activities such as sexual displays and competitive behavior (Slater 1978, Clutton-Brock & Albon 1979, Vehrencamps et al. 1989). Maintaining high levels of reserved fat during this period is critical, both to ensure survival during the harsh, in terms of food availability, conditions in late summer and autumn, as well as to ensure a better chance of success in the next breeding season (Boussès & Chapuis 1998).

The process of recovering the lost fat reserves in female rabbits, on the other hand, is much slower. Female rabbits consume reserved fat both during pregnancy and lactation (Bonanno et al. 2008, Brecchia et al. 2008), which is completed by June for the great majority of the individuals. Soon after, they start recovering their energy reserves (Dal Bosco et al. 2007), reaching the optimum body condition by August. Although from early summer food availability begins to be decreasing, the dietary flexibility of the species and the ability to browse the most qualitative food resources (Rödel 2005) allows it to restore fat reserves during summer. Armitage (1998), studying yellow-bellied marmots (*Marmota flaviventris*), reported similar differences in the recovery of fat reserves between males and females, attributing it to the higher energetic stress of the latter due to lactation. From late summer (August) the body condition of both sexes drops dramatically until November in males and until December in females. Late summer and early-mid autumn coincides with the period of the lowest availability of food resources, which forces the rabbits to utilize part of the reserved fat. When wet season begins at mid-late October, male rabbits exploit the onset of the new vegetative period and begin to restore some of the fat reserves, preparing themselves for the following breeding season. However, despite the tendency to restore lost fat reserves, their body condition does not dramatically improve. This is probably due to the fact that during late autumn and winter males consume large amounts of energy in competitive activities and physiological changes, such as increasing testes size and spermatogenesis (Gonçalves et al. 2002).

Female rabbits, however, appear to have low fat reserves in December, although they can also exploit the onset of the new vegetative period. This can possibly be attributed to the fact that some of the sampled females may have got pregnant and given birth during late October and November. The phenomenon of a breeding season soon after the first

autumn rains has also been observed by Gonçalves et al. (2002). In the current study this phenomenon is further confirmed by the presence of juveniles in the sample collected in January (10 %). Apparently the sampling intensity was not high enough to allow collection of pregnant females during October and November which would validate our assumption. In February, female rabbits appear to have restored the energy reserves and they are ready for entering the main breeding season. At this stage females store much higher amounts of fat than males, since a better physical condition ensures both a higher hierarchical ranking in their society and better chances of survival for their off-springs (von Holst et al. 2002).

Differences in body condition were also found between adults and juveniles. Similarly to the results reported by Wallage-Drees & Deinum (1986), our findings show that juveniles store much smaller amounts of fat than adults throughout the year. Juvenile and adult wild rabbits have different nutritional and energy requirements, since adults need them for maintenance and reproduction, whereas juveniles invest the acquired nutrients and energy in their body growth (Arenz & Leger 2000). In addition, young rabbits exhibit a higher temperature regulation costs (Rödel 2005), while their displacement by dominant males from the colony areas (von Holst et al. 1999) increases their social stress, leading to additional energy requirements (Rödel 2005). Consequently, juveniles appear to have limited amounts of excess energy to be stored as fat.

The understanding of the physiological status of a population along the annual cycle provides useful insights when management measures are planned. Apparently, the relatively poor body condition observed during autumn would make the population

much more vulnerable to measures related to population control, such as poisoned bait, capture and hunting. Although the effect of hunting was not explicitly studied in the current study we tend to agree with the findings by Angulo & Villafuerte (2003) who suggest that autumn hunting may have a higher impact on population numbers. The imperative need for food, in order to survive during periods of poor food availability, and the reduced fat reserves would increase the consumption of bait and increase the vulnerability to hunting as a result of a prolonged browsing time and extended browsing area (Newman 2007). On the other hand, when population conservation is aimed, then measures such as food provision or habitat improvement would be more effective during periods with poor physiological condition or when they need to build up body reserves in periods of food scarcity. The lack of fat reserves in this lean mammal could threaten its survival (Boos et al. 2005), especially in periods of extremely low food availability (Kontsiotis et al. 2013b). However, further research is needed to determine the optimum time of applying population management measures, since their effectiveness is affected by the population structure, the rabbit's seasonal behavior patterns, the hunting habits etc. (Daly 1980, Williams et al. 1995, Calvete et al. 2005).

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