Relationship between habitat, densities and metabolic profile in brown hares (*Lepus europaeus* Pallas)

Gisella Paci[1], Antonio Lavazza[2], Marco Ferretti[3], Marco Bagliacca[1]

[1] Dipartimento di Produzioni Animali. Università di Pisa, Italy
[2] Istituto Sperimentale della Lombardia ed Emilia Romagna "B. Umbertini". Brescia, Italy
[3] Ambito Territoriale di Caccia Firenze 5. Firenze, Italy

Corresponding author: Prof. Gisella Paci. Dipartimento di Produzioni Animali. Università di Pisa. Viale delle Piagge 2, 56124 Pisa, Italy - Tel. +39 050 2216903 - Fax: +39 050 2216901 - Email: gpaci@vet.unipi.it

ABSTRACT

Some habitat traits and haematic parameters were studied to understand the relationships between the hare densities, habitat characteristics and physiological and nutritional condition of the animals.

A total of 33 protected areas, reserved for wild game reproduction, located in the Province of Florence (Central Italy), were monitored during a 2-year period. In each protected area the hares were submitted to census. The habitat features of the protected areas were studied and the following parameters were categorised: altitude; cleared-land/total-land ratio; main exposure; main ground composition; water availability; main slope; anthropogenic presence; predator presence; wooded borders; presence of trees and shrubs; surveillance against hunting; demographic predator control; kind of cultivation; unharvested crops for game. After the census the hares were captured for translocation outside in "free" hunting areas. During capture the hares were put in darkened, wooden capture-boxes and remained inside for a variable period of time (10min to 3h). A sample of 3 to 7 hares, captured per year and per each protected area, were removed from the boxes (physically restrained, with covered eyes) for blood sample collection, sex, age and live weight determination. The following analyses were performed on frozen plasma samples: ALanine aminoTransferase (ALT), ASpartate aminoTransferase (AST), glucose, cholesterol, Blood Urea Nitrogen (BUN), Ca, P, Mg, Na, K, and Cl concentrations. The relationship between hare density and habitat characteristics was analysed by single regressions analysis. Then the habitat characteristics were subjected to multivariate analysis in relationship to hare body condition. The haematic parameters were analysed by least square means considering habitat traits, animal density, age and sex, as main categorical factors, interaction sex*age, and "pregnant and non-reproducing" nested within sex.

Results showed that the highest density and best body conditions can be found in highlands, open fields with low tree presence and wooded borders, medium mixture soils, scarce predator presence and limited anthropogenic presence and with abundant water availability and shrubbiness. The study of the absolute values of metabolic profile, indicator of the physiological and nutritional condition of the reared animals, did not show any nutritional winter deficiency in wild hares and, as census data, should be repeated for several years since, probably, only their variations can be used as indicators of preliminary problems.

Key words: Lepus europaeus Pallas, Habitat, Density, Blood samples.

RIASSUNTO
CARATTERISTICHE AMBIENTALI, DENSITÀ DI POPOLAZIONE E PROFILO METABOLICO NELLA LEPRE (*LEPUS EUROPAEUS* PALLAS)

È stato effettuato uno studio per conoscere le relazioni tra alcune caratteristiche ambientali, la densità di popolazione ed i parametri ematici nella lepre.

A tale scopo, 33 aree protette (Zone di Ripopolamento e Cattura) della provincia di Firenze sono state sottoposte a monitoraggio ambientale e censimento delle lepri.

Per ogni area sono stati effettuati i seguenti rilievi ambientali: altitudine; rapporto tra aree aperte e area totale; esposizione prevalente; natura del suolo; dotazioni idriche naturali; acclività; antropizzazione; presenza predatori; adiacenze boschive perimetrali; presenza di alberi e cespugli; controllo caccia di frodo; controllo predatori; coltivazioni; colture a perdere. Dopo il censimento tutte le lepri catturate sono state traslocate in zone libere di caccia.

Un totale di 172 soggetti è stato sottoposto ai seguenti rilievi: prelievo ematico dalla vena auricolare per le analisi del glucosio, colesterolo, urea, AST, ALT, Ca, P, Mg, Na, K e Cl, determinazione dell'età, del sesso e del peso vivo. Le relazioni fra densità di popolazione e caratteristiche ambientali sono state analizzate singolarmente, quindi tutte le caratteristiche ambientali sono state sottoposte al processo di selezione "stepwise" in funzione del peso corporeo rilevato nelle lepri. I dati ematici sono stati analizzati mediante il metodo dei minimi quadrati considerando le caratteristiche ambientali, la densità censita, l'età ed il sesso degli animali come fonti di variazione categoriche, l'interazione sesso* età e lo stato fisiologico delle femmine "nested" nel sesso femminile.

I risultati hanno evidenziato la presenza di una più alta densità di popolazione e le migliori condizioni corporee nelle zone collinari caratterizzate da aree aperte con scarsi boschetti e confini boscosi, con suolo di medio impasto, scarsa presenza di predatori, limitata antropizzazione e abbondante disponibilità idrica naturale e zone ricche di cespugli adatti al rifugio. Lo studio dei valori assoluti del profilo metabolico, indicatori dello stato fisiologico e nutritivo degli animali allevati, non ha evidenziato alcuna carenza nutrizionale negli animali selvatici nel periodo invernale considerato. Presumibilmente, come i dati dei censimenti, devono essere ripetuti per più anni dal momento che è la variazione ad essere utile come indicatore di stato e non il valore assoluto.

Parole chiave: Lepus europaeus *Pallas, Habitat, Densità, Parametri ematici.*

Introduction

Hare populations, like other wild animal populations all over the world, are known to undergo cyclical changes in density.

Researchers study the possible causes of a decrease in hare populations and the best way to establish optimum density in each area (Pandini *et al.*, 1998; Eskens *et al.*, 1999; Nyenhuis, 1999; Panek and Kamieniarz, 1999; Vaughan *et al.*, 2003). However, the studies involved in investigating the causes of mortality can be problematic, since different factors may be considered responsible (Pandini *et al.*, 1998; Vaughan *et al.*, 2003).

Type of crop production (intensive or extensive), the land and its geographical features, ground cover (particularly the ratio between fields, arable land, grassland, woods and shrubs), plant species, size of areas with woods and shrubs, predator and human presence, natural and artificial barriers (rivers and roads) and hunting activity, may all greatly affect the presence and density of hares (Eskens *et al.*, 1999; Nyenhuis, 1999; Panek and Kamieniarz, 1999) and their mortality rate.

Although a general decline in hare densities has been reported throughout most parts of Italy in the last few decades, natural densities are usually temporarily improved by the release of non-native animals (Paci and Bagliacca, 2003). However, few wild animals are translocated from habitats similar to that of the release, while

most hares are released from captivity or imported from other countries. This method often produces poor results (Paci and Bagliacca, 2003) and should be abandoned as soon as possible. For this reason, an increase in protected areas and/or an increase in the hare populations in protected areas is useful to increase the availability of animals for translocation programs.

Understanding the relationship between the physiological and nutritional condition of the hares and the habitat and population densities may aid in management of the protected no hunting areas; for this reason some habitat traits and haematic parameters were studied.

Material and methods

The study was carried out in 33 protected areas for wild game reproduction of the province of Florence over two years.

Total area was 18,587ha, average ha per area=588±238.9, covering from 10°50' to 11°40' East, 43°20' to 43°50' North terrestrial coordinates (Figure 1).

The following habitat traits were categorised (by Archview GIS 3.1 on the digitalised maps of the official wildlife management plan of Tuscany): altitude (<400 m and >400m); cleared-land/total-land ratio (expressed in percentage as cleared land/cleared land + woodland); main exposure (North, East, North-West, South-East, South-West); main ground composition (clay and rocky, medium mixture, deep and fertile); water availability (abundant, scarce); main slope (plain, slight slope, steep slope); anthropogenic presence (limited, middling, considerable); predator presence (scarce, normal, excessive); wooded borders (none, up to 60% of the border); presence of trees and shrubs (few, limited to country roads, abundant); surveillance (none, regular presence of game wardens in the area);

Figure 1.   Location of the study areas.
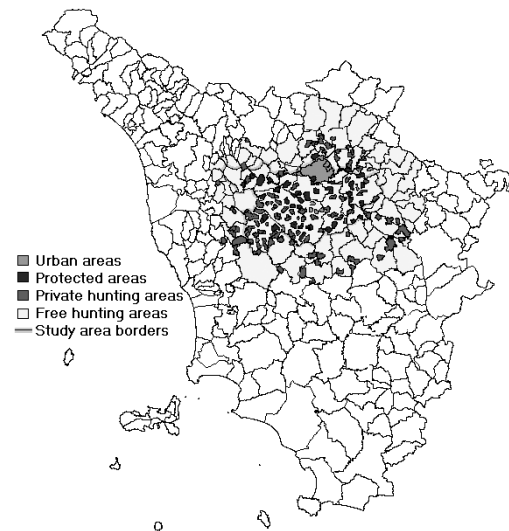
demographic predator control (fox and crow capture, no control); type of crops (pasture, rotated cereals, mono-cultivation, orchards such as olive, apple or vineyards); unharvested crops for game (cultivated or uncultivated). In each area the hares were submitted to census.

Hare census was estimated by night-driven transects. A single transect covering 15.6%±6.38 of the cleared-land habitat, largely representative of each area, was driven along a predetermined route in each protected area. Two nights were spent on each transect in order to maximise the number of hares observed. A 4-wheel-drive pick-up truck was used; this was fitted with a viewing deck that allowed one person to stand upright while the vehicle was in motion. The vehicle was driven at approximately 15km/hour and the fields along one side of the road were scanned with a 1,000,000-candle spotlight for the presence of hares. Hare sightings were marked approximately on the digitised maps during the drive. After the drive, the different vegetation types encountered and the borders

between the small fields, typical of the hilly Italian agricultural system, allowed categorisation of the collected positions (15m interval; 90m truncation) (Buckland *et al.*, 2001).

Censuses were carried out February through March and the same roads were followed each year. Each night, surveys commenced after dusk and continued as the road was driven for up to 5 hours (Langbein *et al.*, 1999). Collected data were used to generate population estimated densities and hare numbers using Distance 3.5 software (Thomas *et al.*, 1998).

Forty-five days after the census, 27.4%±21.4 of the hares present in each protected area was captured for translocation in "free" hunting areas.

The hares were captured by coursing with 3-4 dogs (greyhounds or lurchers). The different team of hunters released the dogs to find and drive into trammel nets any hare that was seen running. After capture all the hares were put in a darkened, wooden capture-boxes and remained inside for a variable period of time (10min to 3h). For blood sample collection 172 hares were removed from the boxes, physically restrained, their eyes immediately covered and blood collected within 1 to 2 minutes by auricular vein.

A sample of 3 to 7 captured animals from each protected area was weighed, checked for general health, sex and age (*radius* and *ulna* ossification stage, examined by palpation, Stroh's sign: Broekhuizen and Maaskamp, 1979; Suchentrunk *et al.*, 1991). In relationship to the presence of Stroh-tubercle the hares were consequently classified in young (<1 yr old) and adult (>1 yr old).

Samples (~1ml of plasma, Li-heparin) were frozen at -20°C and the following analyses were performed on plasma with AU400 Olympus using Olympus Diagnostics Kits:

- Glucose (GLU): colorimetric determination with oxidase-peroxidase;
- Cholesterol: enzymatic colorimetric determination;
- Blood Urea Nitrogen (BUN): enzymatic colorimetric method;
- Calcium (Ca): colorimetric determination with o-Cresolphthalein;
- Phosphorus (P): colorimetric determination with phosphomolibdate;
- Magnesium (Mg): colorimetric determination with Xylidine-Blue;
- Sodium (Na): ISE method;
- Potassium (K): ISE method;
- Clorine (Cl): ISE method;
- ALanine aminoTransferase (ALT): kinetic UV test IFCC;
- ASpartate aminoTransferase (AST): kinetic UV test IFCC.

The relationship between hare density and habitat characteristics was analysed by single regressions analysis. Then the habitat characteristics were subjected to multivariate analysis in relationship to hare body condition to select (through Stepwise, Probability to Enter 0.25, Probability to Leave 0.10) the habitat traits with the most important effect on the hare body condition. The haematic parameters were analysed by least square means considering habitat traits, animal density, age and sex, as main categorical factors, interaction sex*age, and "pregnant and non-reproducing" nested within sex. Minimum statistical differences were studied using Bonferroni confidence intervals (SAS, 2002).

Results and discussion

Habitat characteristics and hare density
The effects of habitat characteristics that showed significant differences on hare density are reported in Figures 2 and 3. Surveillance, demographic predator control, kind of cultivation, and unharvested

Figure 2. Effect of habitat characteristics on hare density.
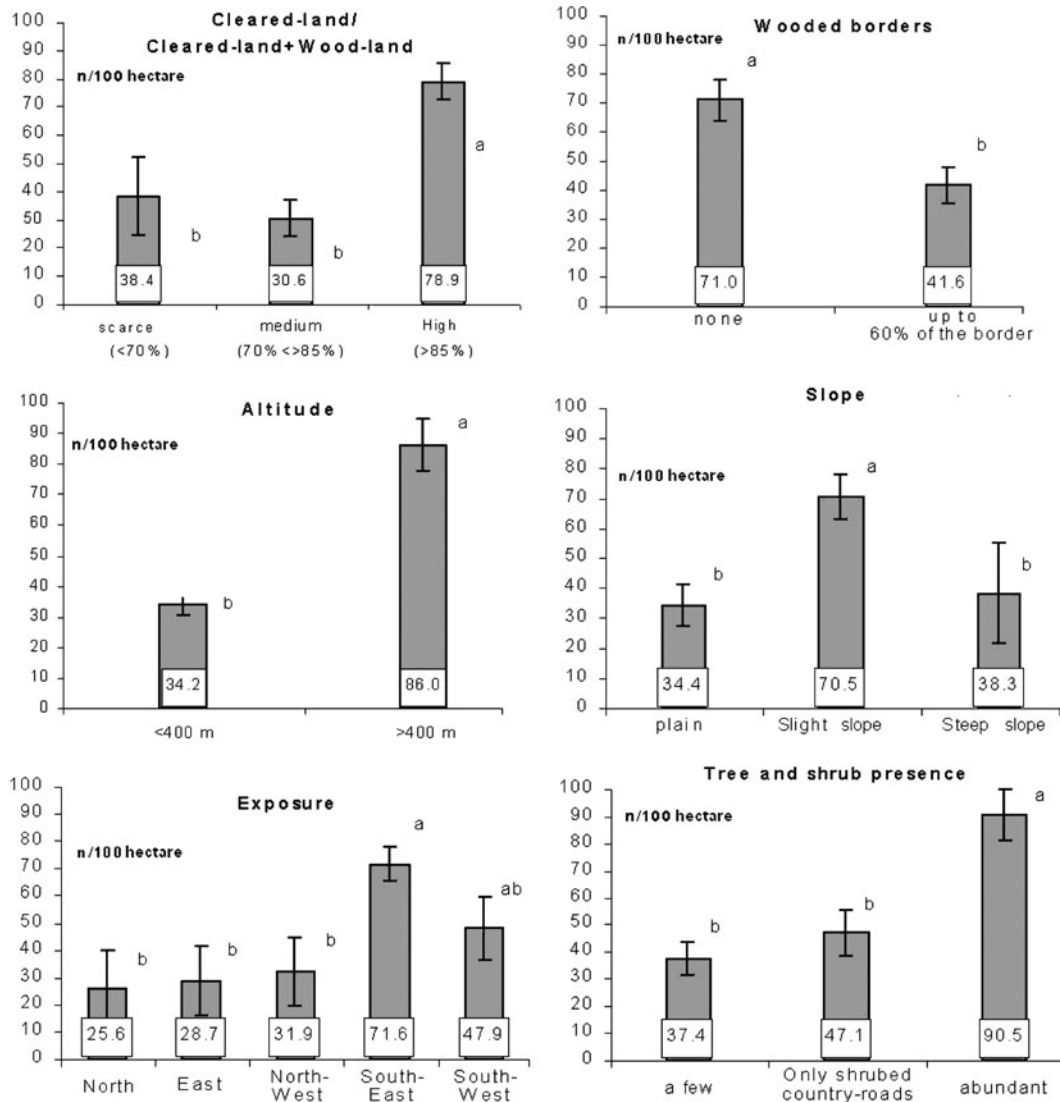
Note: Bars bearing different letters differ for P<0.05.

crops for game did not seem affect hare densities. The areas with the highest proportion of cleared land (>85%) showed the highest densities of hares (n=78.9/100ha, P<0.05), while the areas with a medium or scarce proportion of cleared land showed the lowest densities (n=30.6/100ha and n=38.4/100ha, P<0.05). The highest presence of hares was also observed in the protected areas characterised by absence of woods near the borders (n=41.6/100 ha with wooded borders vs n=71.0/100ha with no

Figure 3.　Effect of habitat characteristics on hare density.
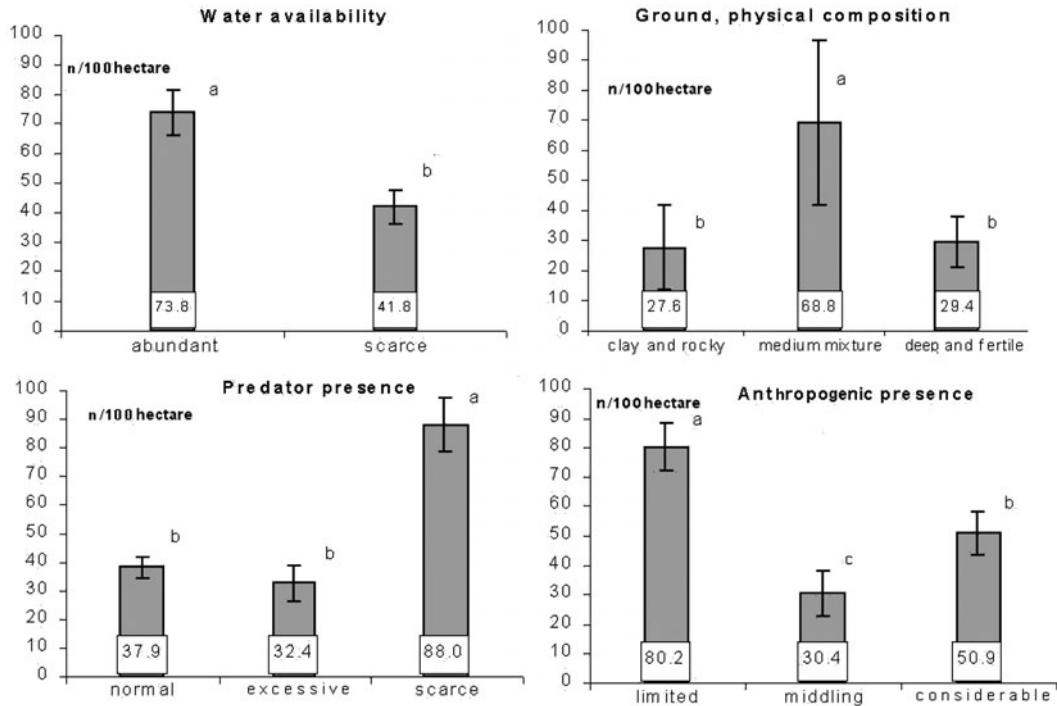
Note: Bars bearing different letters differ for P<0.05.

woods along the borders, P<0.05).

The higher densities of hares were linked with highlands (n=86/100 ha in highlands vs n=34/100ha in plains, P<0.05), slight slopes (n=70.5/100ha in slight slopes, vs n=38.3/100 ha vs n=34.4/100ha, on steep slopes and plains respectively, P<0.05), south-east exposure (n=71.6/100ha vs an average of n=33.5/100ha of the other exposures, P<0.05) and high presence of trees and shrubs (n=90.5/100ha vs an average of n=42.3/100ha of the others, P<0.05). The density of hares increased in habitats with abundant water (n=3.8/100ha vs n=41.8/100ha, P<0.05), with medium mixture soils (n=68.8/100ha vs an average of n=28.5/100ha, P<0.05), scarce predator presence (n=88.0/100 ha vs an average of n=35.2/100ha of the other conditions,

P<0.05) and low anthropogenic presence (n=80.2/100ha vs n=50.9/100ha vs n=30.4/100ha in considerable and middling conditions, respectively, P<0.05).

Our results confirmed the typical choices of the hares. As previously observed by other authors (Vaughan *et al.*, 2003), hares prefer to live in habitats with fields, where improved grass and arable land are present. The improved density of the hares when woodland is reduced is probably due to a decrease in predator pressure. In fact, predators, such as foxes, generally prefer areas with considerable woodland, since these habitats provide a natural refuge where they are less discernible (Cavallini and Lovari, 1994; Reynolds and Tapper, 1995).

The suitability of cleared land for the

hares has been related to the fact that the hares use woodland to shelter only during the day (Tapper and Barnes, 1986) but may also avoid it (Marboutin and Aebischer, 1996) and generally few hares can be found in areas with a lot of woodland (Panek and Kamiemiarz, 1999).

The higher hare densities found in highlands with slight slopes and south-east exposures, soil of medium mixture, few trees but many shrubs may be explained by conditions that support a favourable improvement of refuges. The relationship between altitude and hare density may be related to the fact that, in Italy, highlands are generally less intensively cultivated than plains. Intensive agriculture negatively affects hare population densities. In fact, the intensification of agriculture (which reduces the diurnal refuge areas and the availability of different grass species), the increased mechanisation (which directly increases mortality, especially of leverets), and the loss of habitat diversity, are reported to be the principal causes of the decline of hare density (Dingerkus and Montgomery, 2002; Vaughan et al., 2003).

Our results also confirmed that anthro-pogenic presence negatively affects the hare densities (Nyenhuis, 1999). The presence of small enclosures around many small country-houses probably also implies an increase in cats and dogs, the new hare predators linked to the human presence (Boitani and Fabbri, 1982; Liberg, 1984; Woods et al., 2003).

The effect of the presence of small ponds, streams or pools may be useful in sustaining the green vegetation during the summer season. The direct effect as source of free water is in fact very improbable, since the hares' diet is naturally rich in water grasses and its free water requirements are consequently very low (Mussa et al., 1986; Spagnesi and Trocchi, 1992).

Habitat characteristics
and body conditions

The relationships between habitat traits and live weights (Table 1), were analysed by multiple regression model, submitted to stepwise selection, included 6 variables in the final model (5 regression coefficients were statistically significant). The final multivariate regression model explained 21% of the total variance.

Table 1. Multiple regression model between the body conditions of captured hares and the variables measured in 33 protected areas.

Entered parameters	Regression coefficient of estimated values	SE	F Ratio	P>F
Intercept	3365	78.27	43	<.0001
Altitude {<400m}	-200	38.17	-5.25	<.0001
Ground {clay and rocky - medium mixture - deep and fertile}	-43	44.86	-0.97	0.332
Ground {medium mixture - deep and fertile}	-87	31.60	-2.76	0.006
Wooded borders {None}	-135	48.57	-2.8	0.006
Cleared-land/Cleared-land+Wood-land {scarce - high - average}	-188	46.96	-4.02	<.0001
Cleared-land/Cleared-land+Wood-land {high - average}	-72	29.28	-2.48	0.014

The selected regression coefficients were: altitude for which altitude <400m negatively affected body condition (b=-200g); type of ground for which deep and fertile was the best ground followed by medium mixture and, last, clay and rocky ground; wooded borders for which absence of wooded borders negatively affected body conditions (b=-135g); cleared -land/cleared-land + woodland for which the medium incidence of cleared land was the condition linked with the best body conditions followed by high incidence and, last, scarce incidence.

Live weights and metabolic profile in relation to sex, age, population density and physiological status of females

The females did not show any significant differences for reproductive condition. Consequently, the data of pregnant and non reproducing females were analysed together. The effects of sex* age, population density on live weights and metabolic profile are shown in Table 2. Regarding the interaction sex by age, adult females showed higher body weights than adult males, young males and females (3825g vs 3588g, 3406g, 3590g, respectively, P<0.05). As concerns the population density, heavier hares were observed in conditions of high or low densities (3659g and 3712g, respectively), while in conditions of medium density, weights were lighter (3435g, P<0.05). Considering the metabolic profile, the sex*age interaction only demonstrated differences in the glucose level which was significantly lower in adults with respect to young females (12.0 vs 13.1mmol/l; P<0.05). Regarding the population density effect, only Calcium content was influenced (P<0.05) indicating higher Ca concentration in conditions of higher density (3.1mmol/l) with respect to lower and medium densities (2.9 and 2.9mmol/l, respectively).

Age and sex, as is well known, affect the size and live weights of the animals. Our results showed few differences between the live weights of the adult males, young males and females. The reduced differences were probably due to the capture period (allowed only after the end of the hunting season, in January/February). In this period the growth of the hares born early in the previous year is rather complete, Stroh's sign is not yet discernible and a mistaken evaluation of the age regularly occurs in the hares born during the first months of the previous year (Suchentrunk *et al.*, 1991). The well known difference between the body weight of the females and the males (Spagnesi and Trocchi, 1992) appeared particularly high in the adults, while in the young animals the weights were similar. The presence of some hares, already pregnant at this time, and also two lactating hares, whose capture means the death of the leverets, should suggest to the legislative authorities the need to anticipate the end of the hare hunting season so that translocations can be carried out in advance. Regarding the body weight no significant difference was observed for the physiological status, probably related to the reduced number of pregnant hares. It is interesting to note that heavy hares are present in both high and low-density conditions (estimated weights, adjusted for sex and age distribution). For this reason the only evaluation of the body weights, commonly used to check the status of the hare populations, cannot be used to evaluate habitat fitness. In fact the higher weights are probably related to greater food availability and better habitats in high density conditions but the high weights in low density conditions show that food availability may not be the limiting habitat factor in the hare population density.

The values of the whole haematic

Table 2. Live weight and metabolic profile (estimated values) in relation to sex, age, population density and physiological status

		Physiological status		Interaction sex by age				Main effect density (n/100ha)		
		Pregnant	Non-reproducing females	Adult females	Young females	Adult males	Young males	60-100 High	20-40 Medium	5-10 Low
	n.	8	76	38	46	45	43	36	116	20
Live weight	g	3774	3577	3825 [a]	3590 [b]	3588 [b]	3406 [b]	3659 [a]	3435 [b]	3712 [a]
	SE	139.4	41.8	64.0	57.5	55.9	59.9	61.9	31.8	85.2
Glucose	mmol/l	13.4	12.3	12.0 [b]	13.1 [a]	12.6 [ab]	13.0 [ab]	12.7	12.4	13.0
	SE	0.98	0.32	0.5	0.4	0.4	0.4	0.4	0.2	0.6
Cholesterol	mmol/l	0.33	0.39	0.40	0.37	0.39	0.41	0.39	0.39	0.39
	SE	0.053	0.017	0.027	0.024	0.024	0.025	0.025	0.014	0.035
BUN	mmol/l	21.2	20.3	19.5	20.0	19.4	19.8	18.9	20.8	19.3
	SE	2.03	0.66	1.00	0.88	0.85	0.92	0.92	0.51	1.25
AST	U/l	136	175	149	188	181	165	138	177	197
	SE	40.5	12.9	18.2	15.3	15.3	15.9	16.0	9.1	22.3
ALT	U/l	104	74	92	67	62	69	72	70	75
	SE	24.0	7.9	10.5	9.2	9.1	9.6	9.7	5.4	13.2
Na	mmol/l	139.7	135.5	136.2	135.3	136.9	137.0	137.6	136.6	134.8
	SE	2.30	0.76	1.01	0.88	0.83	0.92	1.03	0.50	1.16
K	mmol/l	4.1	4.7	4.7	4.6	4.7	4.4	4.6	4.6	4.8
	SE	0.42	0.14	0.21	0.18	0.18	0.19	0.22	0.11	0.25
Ca	mmol/l	3.1	2.9	3.0	3.0	3.0	3.0	3.1 [a]	2.9 [b]	2.9 [b]
	SE	0.10	0.04	0.05	0.04	0.04	0.05	0.05	0.03	0.06
P	mmol/l	1.2	1.2	1.2	1.4	1.3	1.3	1.2	1.2	1.5
	SE	0.19	0.06	0.09	0.08	0.08	0.09	0.09	0.05	0.11
Mg	mmol/l	1.1	1.3	1.3	1.3	1.2	1.3	1.3	1.2	1.2
	SE	0.15	0.05	0.07	0.06	0.06	0.06	0.07	0.04	0.08
Cl	mmol/l	102.1	105.6	104.1	106.5	104.5	104.0	104.0	104.5	105.8
	SE	2.58	0.85	1.16	1.01	0.96	1.07	1.18	0.58	1.34

Different superscripts within the same row indicate statistically significant differences, P<0.05.
BUN = Blood Urea Nitrogen; AST = ASpartate amino Transferase; ALT = ALanine amino Transferase; Na = Sodium ; K = Potassium ; Ca = Calcium; P = Phosphorus; Mg = Magnesium; Cl = Chloride; ha=hectare.

parameters, except AST, were included in a physiological range, and the observed variations between individuals did not show trends in deficient or surplus conditions in relation to density. The availability of energy and protein in winter grasses seem sufficient to satisfy the requirements of the resident hares, as demonstrated by the glucose and cholesterol levels. The lower glucose levels observed in the adult females may be related to a higher degree of stress suffered by some animals (Paci *et al.*, 2006). Also, plasma urea (BUN) was always compatible with subjects in good nutritional condition. It is a fundamental parameter to allow good weight gain, hints at protein insufficiency at low levels, and is an index of protein excess, renal problems and/or dehydration at high levels.

Regarding AST, after capture some hares presented no physiological values, probably due to the capture itself (in fact the values of ALT were always within the normal range) (Paci *et al.*, 2006). In addition to capturing stress, AST also increased in relation to habitat pollutants, toxic substances, and very high parasite infestations. In any case, high AST values found in captured hares are a sure index of a pathologic condition and/or muscle tissue damage.

As regards the plasma's mineral content, no reduction in the presence of K linked to Na saving, was observed in any sample (as a possible consequence of the start of water retention). Thus no metabolic alterations or unbalanced absorption, indirectly or directly related to pathologies, were observed. However, higher Ca values, observed in protected areas characterised by high-density conditions, might be due to a more precocious start of the reproduction season in these areas. In fact, Ca levels are affected not only by food availability of the element but also by the presence of two hormones, Parathyroid and Calcitonin hormone (PTH and CT), and one vitamin (vitamin D) whose concentration and activity are greatly affected by the physiological status of the hares (non reproducing *vs* reproducing).

Data from the haematic parameters, using the live weights as a covariate are reported in Table 3. Only the plasma glucose concentration was significantly affected by the hare's age - lower in adult hares than in young hares (12.1 *vs* 12.9mmol/l; P<0.05). The lower glucose level in the adults may be related to a normal trend but also to possible different running times before the capture of the adults with respect to the young hares since running and escape attempts during capture cause a considerable depletion of carbohydrate resources (Bateson and Bradshaw, 1997).

A significant relationship between live weights and haematic parameters was only observed for two plasma components (BUN and K), but the values of the regression coefficient were very low (b=-0.00714 and b=-0.00065, respectively; P<0.05). In wild animals a relationship between live weights and metabolic profiles can be observed during starvation by lack of food availability and can cause thinness. Our results seem to confirm the sufficient food availability in every protected area, at least during the winter, the season of capture.

Live weight and metabolic profile related to the habitat traits

The effects of the habitat traits on the live weight and haematic parameters are reported in Tables 4 and 5. Results (Table 4) showed that the body weight was significantly affected by the ratio between Cleared-land and Cleared-land + Wood-land (3266g with reduced open countryside *vs* 3565g with 70-85% of open countryside, P<0.05), altitude (3729g in highlands *vs* 3452 in plains, P<0.05), exposure (higher

Table 3. Metabolic profile related to the hare's sex and age (covariate for the live weight).

		Main effect Sex		Main effect Age		Regression coefficient for live weight
		Females	Males	Adult	Young	
	n.	84	88	83	89	172
Glucose	mmol/l	12.3	12.7	12.1[b]	12.9[a]	0.01307
	SE	0.28	0.27	0.28	0.27	0.00905
Cholesterol	mmol/l	0.39	0.40	0.39	0.39	-0.00044
	SE	0.017	0.016	0.017	0.016	0.00116
BUN	mmol/l	20.6	19.9	20.2	20.3	-0.00714 *
	SE	0.61	0.59	0.61	0.59	0.00306
AST	U/l	153	167	160	159	-0.00632
	SE	8.7	8.3	8.6	8.2	0.01595
ALT	U/l	60	59	60	60	-0.00085
	SE	3.1	2.9	3.0	2.9	0.00553
Na	mmol/l	136.0	137.0	136.8	136.2	-0.00023
	SE	0.63	0.60	0.62	0.60	0.00111
K	mmol/l	4.7	4.5	4.7	4.4	-0.00065 *
	SE	0.13	0.12	0.13	0.12	0.00023
Ca	mmol/l	3.0	3.0	3.0	2.9	-0.00008
	SE	0.03	0.03	0.03	0.03	0.00023
P	mmol/l	1.2	1.2	1.2	1.3	-0.00042
	SE	0.06	0.06	0.06	0.06	0.00033
Mg	mmol/l	1.2	1.2	1.3	1.2	0.00006
	SE	0.04	0.04	0.04	0.04	0.00019
Cl	mmol/l	105.0	104.2	104.0	105.2	0.00093
	SE	0.72	0.68	0.71	0.68	0.00127

Different superscripts within the same row indicate statistically significant differences, P<0.05.
BUN = Blood Urea Nitrogen; AST = ASpartate amino Transferase; ALT = ALanine amino Transferase;
Na = Sodium ; K = Potassium; Ca = Calcium; P = Phosphorus; Mg = Magnesium; Cl = Chloride.

live weights in hills with eastern and southeastern exposure, 3636g and 3557g, respectively, and lighter weights in areas with northern exposure, 3266g, P<0.05), predator abundance (heavier hares – 3725g - in conditions of scarce predator presence, P<0.05), and anthropogenic presence (heavier hares-3644g - in condition of limited anthropogenic presence, P<0.05), see Table 4.

As regards the energetic profile and the metabolic profiles (estimated values adjusted for sex, age and live weight effects), the glucose levels were higher in areas without wooded borders (13.2mmol/l *vs* 12.0mmol/l; P<0.05) and with scarce predator presence respect to excessive predator presence (14.3 *vs* 11.9mmol/l; P<0.05).

Cholesterol levels were found to be higher in areas with scarce water availability (0.41mmol/l *vs* 0.35mmol/l, P<0.05). BUN

Table 4 - Live weight, energetic and metabolic profile of the hares related to habitat traits (value adjusted for effects of sex, age and live weight).

		Cleared-land/Cleared-land + Wood-land			Altitude		North	Exposure					Slope		Water availability	
		<70% Scarce	70%-85% Medium	>85% High	<400m	>400m		East	North-West	South-East	South-West	Plain	Slight slope	Steep slope	Scarce	Abundant
Areas	n.	4	15	14	30	3	5	4	5	15	4	14	16	3	23	10
Hares	"	14	74	84	143	29	18	21	22	87	24	71	92	9	115	57
Live weight	g	3266[b]	3565[a]	3488[ab]	3452[a]	3729[b]	3266[b]	3636[a]	3406[ab]	3557[a]	3425[ab]	3547	3493	3289	3502	3495
	SE	93.1	44.0	42.1	31.3	68.6	92.4	82.2	80.6	40.3	74.8	45.5	40.7	105.7	36.8	49.1
Glucose	mmol/l	11.5	12.5	12.6	12.6	12.0	12.8	12.4	12.8	12.2	13.0	12.6	12.3	12.9	12.4	12.7
	SE	0.69	0.29	0.28	0.21	0.48	0.61	0.56	0.54	0.27	0.52	0.30	0.26	0.85	0.24	0.34
Cholesterol	mmol/l	0.42	0.41	0.37	0.39	0.42	0.44	0.33	0.37	0.40	0.42	0.37	0.41	0.39	0.41[a]	0.35[b]
	SE	0.040	0.018	0.017	0.013	0.029	0.036	0.032	0.032	0.016	0.031	0.018	0.016	0.051	0.014	0.020
BUN	mmol/l	20.3	20.7	19.9	20.9[a]	17.3[b]	19.8	20.4	18.4	21.1	19.0	20.9	19.8	19.0	20.3	20.1
	SE	1.5	0.7	0.6	0.5	1.0	1.3	1.2	1.2	0.6	1.1	0.7	0.6	1.8	0.5	0.7
AST	U/l	178	158	158	170	117	219	154	195	149	130	157	162	157	166	148
	SE	20.5	9.0	8.5	6.3	13.5	17.2	14.6	16.5	7.8	15.5	9.7	8.1	25.8	7.2	10.2
ALT	U/l	72	71	72	75	53	82	56	88	67	80	85	62	61	77	59
	SE	15.1	6.9	6.5	4.9	11.0	13.6	12.2	12.4	6.3	11.9	7.0	6.0	18.9	5.3	7.9

		Ground			Tree and shrub presence			Wooded borders		Predator presence		Anthropogenic presence			
		Clay and rocky	Medium mixture	Deep and fertile	A few	Shrubs, country-roads	Abundant	None	Till 60% of the border	Scarce	Normal	Excessive	Limited	Middling	Notable
Areas	n.	2	21	10	18	7	8	14	19	3	22	8	9	11	13
Hares	"	15	110	47	88	42	38	71	97	20	113	39	54	51	67
Live weight	g	3436	3479	3570	3537	3379	3530	3495	3493	3725[a]	3470[b]	3481[ab]	3644[a]	3439[b]	3432[b]
	SE	89.9	37.5	55.6	41.5	57.5	61.9	45.9	39.4	88.8	35.3	62.1	51.6	51.2	46.8
Glucose	mmol/l	13.1	12.8	11.6	12.4	12.4	12.8	13.2[a]	12.0[b]	14.3[a]	12.4[ab]	11.9[b]	12.3	12.7	12.5
	SE	0.65	0.24	0.36	0.27	0.40	0.42	0.30	0.25	0.55	0.23	0.39	0.35	0.36	0.31
Cholesterol	mmol/l	0.31	0.41	0.39	0.40	0.41	0.37	0.37	0.42	0.41	0.39	0.41	0.41	0.40	0.37
	SE	0.040	0.015	0.022	0.016	0.024	0.025	0.018	0.015	0.035	0.014	0.025	0.021	0.021	0.019
BUN	mmol/l	20.5	20.1	20.6	20.7	19.5	20.4	19.9	20.5	19.8	20.7	19.2	20.2[ab]	21.9[a]	19.0[b]
	SE	1.4	0.5	0.8	0.6	0.9	0.9	0.7	0.6	1.2	0.5	0.9	0.8	0.7	0.7
AST	U/l	159	156	170	168	148	155	163	157	127	169	152	151	167	161
	SE	19.7	7.5	11.5	8.3	12.3	12.7	9.4	7.9	17.0	7.3	11.8	10.9	10.8	9.6
ALT	U/l	59.5	70.6	77.0	74.8	68.4	66.0	61.8	57.7	79.9	72.2	65.2	150.9	167.0	161.5
	SE	15.45	5.59	8.60	6.32	9.21	9.68	3.27	2.71	13.58	5.51	9.18	10.87	10.81	9.57

Different superscripts within the same row indicate statistically significant differences, $P<0.05$.

BUN = Blood Urea Nitrogen; AST = ASpartate amino Transferase; ALT = ALanine amino Transferase; Na = Sodium ; K = Potassium; Ca = Calcium; P = Phosphorus; Mg = Magnesium; Cl = Chloride.

Table 5 - Electrolyte profile of the hares related to the habitat traits (value adjusted for effects of sex, age and live weight).

Areas		Cleared-land/Cleared-land + Wood-land			Altitude		Exposure						Slope		Water availability	
		<70% Scarce	70%-85% Medium	>85% High	<400m	>400m	North	East	North-West	South-East	South-West	Plain	Slight slope	Steep slope	Scarce	Abundant
Hares	n.	4	15	14	30	3	5	4	5	15	4	14	16	3	23	10
	"	17	65	68	134	16	18	18	24	73	17	64	73	13	99	51
Na	mmol/l	138.5	135.9	137.2	136.7	135.6	136.8	135.6	136.6	135.9	140.4	137.7	135.9	138.1	136.6	137.3
	SE	1.36	0.68	0.65	0.45	1.31	1.20	1.19	1.03	0.59	1.24	0.69	0.62	1.73	0.53	0.80
K	mmol/l	5.0	4.6	4.4	4.4 [b]	5.5 [a]	4.6	4.8	4.6	4.6	4.1	4.3	4.8	3.9	4.6	4.5
	SE	0.26	0.13	0.13	0.09	0.24	0.24	0.25	0.22	0.13	0.25	0.13	0.12	0.33	0.10	0.16
Ca	mmol/l	2.7 [c]	2.9 [b]	3.0 [a]	2.9	3.1	2.8	3.0	3.0	3.0	3.0	3.0	2.9	3.1	2.9	3.0
	SE	0.06	0.03	0.03	0.02	0.06	0.06	0.06	0.05	0.03	0.06	0.03	0.03	0.09	0.03	0.04
P	mmol/l	1.3	1.1	1.3	1.2 [b]	1.5 [a]	1.3	1.3	1.3	1.2	1.0	1.3 [a]	1.2 [ab]	0.9 [b]	1.2	1.2
	SE	0.12	0.06	0.06	0.04	0.12	0.11	0.11	0.10	0.06	0.11	0.06	0.06	0.15	0.05	0.07
Mg	mmol/l	1.4	1.2	1.3	1.2	1.3	1.1	1.2	1.2	1.3	1.2	1.3	1.2	1.2	1.2	1.3
	SE	0.09	0.05	0.04	0.03	0.09	0.08	0.08	0.07	0.04	0.08	0.05	0.04	0.12	0.04	0.05
Cl	mmol/l	101.1 [b]	105.5 [a]	105.1 [a]	104.9	104.1	104.5	105.2	106.4	105.2	101.4	104.5	105.3	103.5	104.9	104.6
	SE	1.55	0.77	0.74	0.55	1.52	1.41	1.45	1.29	0.74	1.46	0.81	0.72	2.02	0.62	0.93

Areas		Ground			Tree and shrub presence			Wooded borders		Predator presence		Anthropogenic presence			
		Clay and rocky	Medium mixture	Deep and fertile	A few	Shrubs, country-roads	Abundant	None	Till 60% of the border	Scarce	Normal	Excessive	Limited	Middling	Notable
Hares	n.	2	21	10	18	7	8	14	19	3	22	8	9	11	13
	"	13	91	46	74	28	33	56	79	15	93	31	44	49	57
Na	mmol/l	139.0	136.2	137.6	136.2	137.9	137.2	138.2 [a]	135.8 [b]	136.6	136.5	137.9	136.2	136.9	137.2
	SE	1.61	0.56	0.82	0.62	1.00	0.92	0.69	0.59	1.35	0.54	0.94	0.85	0.80	0.71
K	mmol/l	3.8 [b]	4.7 [a]	4.4 [ab]	4.5	4.6	4.6	4.3 [b]	4.7 [a]	4.9	4.6	4.3	4.4	4.7	4.5
	SE	0.31	0.11	0.16	0.12	0.20	0.18	0.14	0.12	0.26	0.11	0.18	0.17	0.16	0.14
Ca	mmol/l	3.1 [a]	3.0 [a]	2.8 [b]	2.9	2.9	3.0	3.0	3.0	3.0	2.9	2.9	3.0	3.0	2.9
	SE	0.08	0.03	0.04	0.03	0.05	0.04	0.03	0.03	0.07	0.03	0.05	0.04	0.04	0.04
P	mmol/l	1.4	1.2	1.3	1.1 [b]	1.4 [a]	1.3 [ab]	1.3	1.2	1.1	1.3	1.0	1.1	1.3	1.3
	SE	0.14	0.05	0.07	0.05	0.08	0.08	0.06	0.05	0.12	0.05	0.08	0.08	0.07	0.06
Mg	mmol/l	1.1	1.3	1.2	1.2 [b]	1.2 [b]	1.4 [a]	1.3	1.2	1.3	1.3	1.2	1.2	1.3	1.2
	SE	0.11	0.04	0.05	0.04	0.07	0.06	0.05	0.04	0.09	0.04	0.06	0.06	0.05	0.05
Cl	mmol/l	105.1	105.5	103.5	105.2	104.1	104.3	104.4	105.0	106.0	105.1	103.6	105.3	104.5	104.7
	SE	1.86	0.65	0.95	0.71	1.14	1.04	0.80	0.68	1.56	0.63	1.08	0.98	0.92	0.82

Different superscripts within the same row indicate statistically significant differences, P<0.05.

BUN = Blood Urea Nitrogen; AST = ASpartate amino Transferase; ALT = ALanine amino Transferase; Na = Sodium ; K = Potassium; Ca = Calcium; P = Phosphorus; Mg = Magnesium; Cl = Chloride.

was significantly affected by altitude (lowland 20.9mmol/l *vs* highland 17.3mmol/l, P<0.05) and anthropogenic presence (middling 21.9mmol/l *vs* notable, 19.0mmol/l, P<0.05). ALT and AST did not present any variation in relation to the considered factors but some hares (n. 9=5.2%), showing non-physiologic AST values (Tataruch *et al.*, 1991; Paci *et al.*, 2006), were excluded before the analysis.

The electrolytic profile of the captured hares differed as a function of the habitat traits of provenance (Table 5). Na was higher in areas without wooded borders (138mmol/l, P<0.05); K was higher in highlands, >400m, (5.5mmol/l, P<0.05), in medium-mixture soils (4.7mmol/l, P<0.05), and in areas bordered by woods (4.7mmol/l, P<0.05); Ca was higher in land with high open fields (3.7mmol/l, P<0.05), and in clay and rocky ground (3.1mmol/l, P<0.05); P was higher in highlands (1.5mmol/l, P<0,05), in plain areas (1.3mmol/l, P<0.05), and in areas with shrubs and country roads (1.4mmol/l, P<0.05); Mg was higher in areas with abundant trees and shrubs (1.4mmol/l, P<0.05); Cl was higher in areas with medium and high open fields (105.5 and 105.1mmol/l, respectively; P<0.05).

Most of these differences might be explained by the different grasses, pastures, and crops in the various protected areas. Possible treatments used in intensive agriculture (e.g. fertilizers), different exposure and characteristics of the habitat can modify the variety of plants and the composition of grasses, herbs and arable crops and, consequently, the metabolic profile of the animals that eat those plants. In any case, the variations in the haematic parameters, always within the physiological range, do not seem to be linked to variations in population productivity (measured by hare density) and are probably due to numerous other factors, unidentified and/or "confused" within the identified categories.

Conclusions

Monitoring the protected areas proved to be useful in identifying the characteristics that the habitat must possess in order to be suitable for the hares. The results confirmed some information and presented others suggestions concerning the preferences and requirements of wild hares in the Mediterranean areas.

High densities and good body conditions are linked to highlands characterised by open fields with low tree presence and wooded borders, medium mixture soils, normal or scarce predator presence, limited anthropogenic presence and plentiful water and shrub growth.

The study of the metabolic profile, a good indicator of the physiological and nutritional condition of the reared animals, did not show any nutritional deficiency in the wild hares during the winter season, confirming that winter food availability is probably not a limiting factor. Body weight, easy to test and commonly used as an indicator of the nutritional status and, consequently, the status of the populations, cannot be used as a general indicator of the hares' condition, but its continuous use will most likely be useful for noticing changes. Like census data, these types need to be repeated for several years, since their absolute values are not conclusive. However, the observed differences from one year to another can be used as indicators of preliminary problems.

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