

Article

Sex and season explain spleen weight variation in the Egyptian mongoose

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Abstract

The Egyptian mongoose (*Herpestes ichneumon* Linnaeus, 1758) is a medium-sized carnivore that experienced remarkable geographic expansion over the last 3 decades in the Iberian Peninsula. In this study, we investigated the association of species-related and abiotic factors with spleen weight (as a proxy for immunocompetence) in the species. We assessed the relationship of body condition, sex, age, season, and environmental conditions with spleen weight established for 508 hunted specimens. Our results indicate that the effects of sex and season outweigh those of all other variables, including body condition. Spleen weight is higher in males than in females, and heavier spleens are more likely to be found in spring, coinciding with the highest period of investment in reproduction due to mating, gestation, birth, and lactation. Coupled with the absence of an effect of body condition, our findings suggest that spleen weight variation in this species is mostly influenced by life-history traits linked to reproduction, rather than overall energy availability, winter immunoenhancement, or energy partitioning effects, and prompt further research focusing on this topic.

Key words: body condition, carnivore, *Herpestes ichneumon*, Iberian Peninsula, mongoose, spleen weight.

The vertebrate immune system must respond to antigenic challenges that irreversibly affect life-history traits and energy allocation trade-offs (Schulte-Hostedde and Gooderham 2011). Since it directly influences survival, indicators of immunocompetence may help to disentangle the factors underlying species success under different

environmental scenarios (Vicente et al. 2007). Immunocompetence can be affected by seasonal variations or by sex or indeed by interactions between these 2 factors. Despite this fact, to date, studies focusing on immune competence that use spleen weight as an indicator lack samples covering all seasons of the year (e.g., Corbin et al.

2008; Schulte-Hostedde and Elsasser 2011) or from both sexes (e.g., Schulte-Hostedde and Elsasser 2011). In order to investigate the factors that influence variance in immunocompetence within a species, samples covering a broad range of environmental conditions, demographic groups, and all periods of the life-cycle are necessary. The Egyptian mongoose *Herpestes ichneumon* is an appropriate model for such a study because it has colonized mainland Portugal over the past 30 years (Barros and Fonseca 2011), currently inhabiting a diverse array of habitats representing all environmental conditions available to the species. Furthermore, legal hunting generates a large number of specimens year-round of both sexes and all ages from the entire distributional range.

The spleen is the primordial secondary lymphoid organ in mammals, playing a key role in immune defense (Corbin et al. 2008). It is functionally and histologically divided into red pulp and white pulp (Mebius and Kraal 2005). The red pulp ensures blood filtration, removal of effete erythrocytes, regeneration of free ferrous iron, and pathogen clearance (Mebius and Kraal 2005). The white pulp houses lymphoid components under resting conditions, such as T cells, B cells, and antigen-presenting cells (Mebius and Kraal 2005). Maintaining the immune system is energetically costly, and individuals with better body condition are likely to have a greater capacity for the production and storage of lymphocytes (Ponlet et al. 2011; Schulte-Hostedde and Elsasser 2011), which may translate into heavier spleens. Spleen weight is considered a reliable proxy of individual immunocompetence, notwithstanding its other functions (Hosken and O'Shea 2001; Corbin et al. 2008; Hadidi et al. 2008; Navarro-Gonzalez et al. 2011; Manjerovic and Waterman 2012). At the intraspecific level, larger spleens may reflect greater investment in immunity by healthy individuals with better body condition (Møller et al. 1998b), whereas smaller spleens suggest the opposite (Diez-León et al. 2013). However, the use of spleen weight to evaluate within-species immunocompetence has limitations. Sudden increases in spleen weight due to intense physical exertion and stress (Corbin et al. 2008) or ongoing infection might bias estimates relating to immune capacity (Goüy de Bellocq et al. 2007). The relative amount of red blood cells stored in the mammalian spleen is known to vary depending on stress, exercise, or hemorrhagic trauma (Brendolan et al. 2007). Alternatively, enlargement of the spleen may reflect an immune response to parasitism (Nunn 2002; Goüy de Bellocq et al. 2007; Corbin et al. 2008), or inflammation due to pathological changes (Møller et al. 1998a). Finally, in addition to a dependence on body condition, spleen weight may also be influenced by energy partitioning and trade-offs between reproduction and growth (Vicente et al. 2007). Therefore, simultaneous consideration of indicators of overall health and energetic state (e.g., body condition), as well as information on season, sex, age, and environmental conditions can help overcome these limitations, facilitating use of spleen weight as a proxy of immunocompetence.

Body condition also can be altered by environmental conditions or individual traits such as sex or age (Toïgo et al. 2006). Body condition refers to the amount of energy reserves (such as fat and protein) that an animal possesses (Perez-Orella and Schulte-Hostedde 2005; Schulte-Hostedde et al. 2005), and it represents the energetic state of an animal (Schulte-Hostedde et al. 2001). Body condition scores have been developed as bioindicators of overall health or the physical quality of individual specimens (Peig and Green 2009). Several studies have assessed the relationship between body condition of mammals and a range of ecological parameters (Green 2001), including animal density and fecundity (Stewart et al. 2005), the effect of parturition and weight of litter (Dobson and Michener

1995), stress (Young and Monfort 2009), ectoparasitism (Perez-Orella and Schulte-Hostedde 2005), and prey selection (Pierce et al. 2000). Regarding its relationship with immunocompetence, body condition has been proposed to influence spleen weight through overall energy availability (Ponlet et al. 2011; Schulte-Hostedde and Elsasser 2011) and energy partitioning effects (Vicente et al. 2007). Therefore, body condition can be used as an indicator controlling for energetic state and overall health when studying spleen weight as a proxy of immune competence.

In this work, we set out to understand spleen weight variation in a sample of free-ranging Egyptian mongoose. If increased spleen weight is simply a result of the availability of energy to invest in immunity, we expected it to be primarily associated with higher body condition scores. Alternatively, spleen weight variation may result from energy partitioning effects and trade-offs between key biological functions, in which case we expected to find decreased spleen weight during periods of maximum investment in reproduction or growth. However, if spleen weight variation results from more specific or complex interactions, we might find it associated with one or more demographic, environmental, or life-history variables. Finally, if increased spleen weight is the result of an immune response to parasitism, the cost of that response might be reflected in body condition, with higher spleen weight values associated with poorer body condition scores.

Materials and Methods

Study area

Wild Egyptian mongoose specimens were collected from 13 of the 18 districts of continental Portugal. Geographic origin of the specimens was attributed to either north or south of the Tagus River, given the marked differences in bioclimatic, biogeographic, and anthropogenic pressures previously observed between both regions (Bandeira et al. 2016, 2018). The Tagus River was previously considered a geographical barrier for the species, since the distribution of the Egyptian mongoose was more concentrated in the south until 3 decades ago (Borralho et al. 1996; Barros and Fonseca 2011). The vegetation of the southern region is mainly characterized by evergreen *Quercus*, whereas in the north monoculture plantations of *Eucalyptus* sp. have largely replaced *Pinus pinaster* and native deciduous trees (Alves et al. 2009). The northern region generally presents lower temperatures and higher levels of rainfall compared with the southern region (Hijmans et al. 2005). Furthermore, human pressure is lower in the south, having fewer urbanized areas, a lower population density, a less extensive road network, and fewer fragmented habitats (Alves et al. 2009; European Commission 2015; IGP 2015). Also, there are fewer mountainous ridges and a less extensive hydrographic network south of the Tagus River (SNIRH 2015).

Sampling procedures

Sampling took place between January 2008 and December 2014. Capture date was classified as winter (January–March), spring (April–June), summer (July–September), or autumn (October–December). Specimens were obtained from hunting activities (box-trapped under legal game management actions aimed at controlling predator densities), according to legal requirements and under license from competent authorities [Instituto da Conservação da Natureza e das Florestas (ICNF)]. A total of 508 Egyptian mongooses were included in this study, 266 females and 242 males.

Table 1. Numbers of Egyptian mongoose samples obtained for each region, North and South of the Tagus River, together with information on age cohort and sex

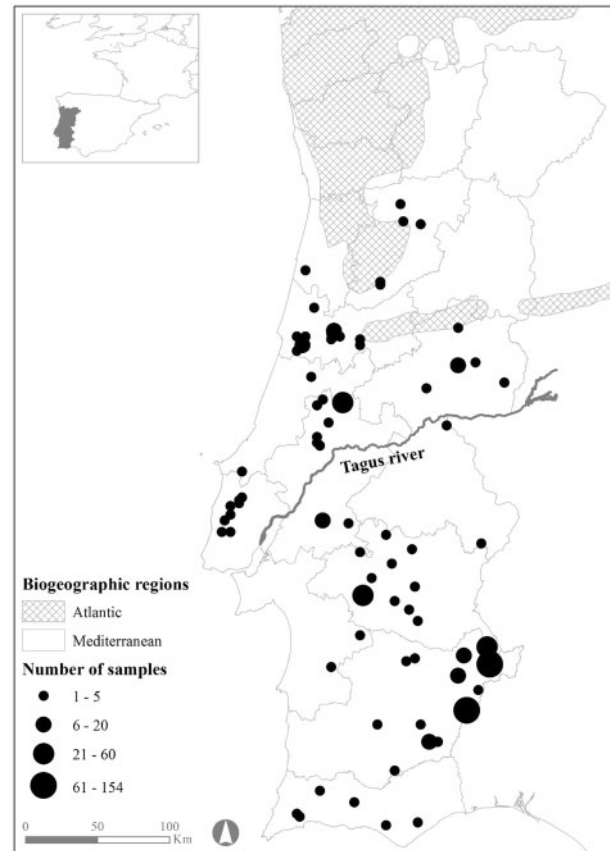
Age	Sex	Region	
		North	South
Adult	Female	41	107
	Male	35	80
Sub-adult	Female	12	33
	Male	16	23
Juvenile II	Female	4	34
	Male	9	47
Juvenile I	Female	4	31
	Male	9	23
Total	Female	61	205
	Male	69	173

There were 263 adults, 84 sub-adults, 94 type II juveniles, and 67 type I juveniles (Table 1, see definitions below). Age was determined by dentition according to [Bandeira et al. \(2016\)](#). Each specimen was assigned to 1 of 4 age cohorts: adults over 1 year of age, sub-adults between 9 and 12 months, type II juveniles between 5.5 and 9 months, and type I juveniles between 2.5 and 5.5 months of age. Regarding geographic origin, 130 specimens came from north of the Tagus River and 378 from the south (Figure 1 and Table 1). Carcasses were labeled with collection date and location and stored at -20°C until processed. In the laboratory, samples were thawed, sexed, weighed, measured, and dissected. Spleens were collected and weighed separately. Six biometric measurements ([Bandeira et al. 2016](#)) were taken: snout–tail length (terminal hairs not included); right hind leg length; right hind foot length; shoulder height; neck perimeter; and head width. Only specimens for which age determination was possible and that possessed an intact spleen were assessed. Pregnant females were excluded to avoid bias from body weight and body condition variables.

Environmental variables

Based on ecological requirements and physiological characteristics of Egyptian mongoose, we selected 5 environmental variables that could directly or indirectly influence body condition and spleen weight for modeling (e.g., [Ben-Yaacov and Yom-Tov 1983](#); [Delibes et al. 1984](#); [Palomares and Delibes 1990, 1991a](#); [Palomares 1993b](#); [Barros et al. 2015](#); [Bandeira et al. 2016, 2018](#)). Landscape structure can influence physical condition due to differences in the amount of suitable habitat, prey availability, and its impact on the movement and dispersal of mongooses ([Palomares and Delibes 1993a](#); [Palomares 1994](#); [Bandeira et al. 2018](#)). Habitat change in recent decades seems to have favored the expansion of mongoose populations into new territories ([Barros et al. 2015](#)). Anthropogenic factors are a ubiquitous influence on ecosystems and, together with natural barriers, act as constraints on mongoose expansion, thereby shaping this species' distribution ([Barros et al. 2015](#)). We included anthropogenic factors in our study to assess possible associations with physical condition and immunocompetence, which we expected to be better in areas with low human pressure.

Each variable was represented by mean values in 2×2 km grid cells, considering the critical home area of the Egyptian mongoose ([Palomares and Delibes 1991b](#)). Nine variables (urban, rice fields, agro-forestry, shrubs, inland water bodies, vineyards and orchards, coniferous forest, broadleaved and mixed forests, and agricultural

**Figure 1.** Locations and sample sizes of Egyptian mongoose studied from districts in Portugal.

areas) representing habitat structure were retrieved from a [Corine Land Cover \(2006\)](#) dataset with a spatial resolution of 250 m and converted in a single categorical variable represented by the most abundant habitat type in each grid cell. Hydrographic data were obtained from the Sistema Nacional de Informação de Recursos Hídricos ([SNIRH 2015](#)). The degree of anthropogenic pressure was represented by 2 variables: population density [derived from the [European Commission \(2015\)](#)] and extent of the road network ([IGP 2015](#)). Geographic position of collected samples was reported in terms of latitude and longitude. Abundances of Egyptian mongoose (number of animals/400 ha) based on the number of animals hunted in each area and during the month, where and when each Egyptian mongoose sampled was collected, were established according to annual hunting yields (ICNF, unpublished data).

Statistical procedures

All variables were tested for normality with Kolmogorov–Smirnov tests (with Lilliefors correction for the significance level) ([Zar 1999](#)).

The principal component analysis (PCA) enables a single estimate of body size on the first component, based on the covariance matrix of various measures. Body size was calculated by combining specimen weight and the 6 biometric measurements into a single value through a PCA, using all variables with loadings >0.70 . The PCA was performed in STATISTICA version 7.1 ([Stat Soft Inc., 2005](#)). Spleen weight was corrected for body weight and is reported as spleen weight (in grams) per 100 g of total specimen mass (adjusted spleen weight). Body condition was scored according to the Scaled Mass Index ([Peig and Green 2009, 2010](#)).

Table 2. Model selection, using AICc, for the effect of age, season, sex, and their interactions, together with region, Egyptian mongoose abundance, habitat, extent of river network, and body condition (Scaled Mass Index), on adjusted spleen weight (expressed as g/100 g body weight) of the Egyptian mongoose in Portugal

Models	df	AICc	Δ AICc	AICc weight	R ²
Season + sex	7	-142.27	0.00	0.67	0.28
Season + region + sex	8	-139.89	2.38	0.21	0.20
Egyptian mongoose abundance + sex	5	-135.78	6.49	0.03	0.26
Season	6	-135.69	6.58	0.03	0.25
Sex	4	-135.12	7.15	0.02	0.17
Season + Egyptian mongoose abundance + sex	8	-135.01	7.26	0.02	0.31
Season + region	7	-134.16	8.11	0.01	0.17
Region + sex	5	-133.48	8.79	0.01	0.06
Season + region + Egyptian mongoose abundance + sex	9	-132.08	10.20	0.00	0.24
Region + Egyptian mongoose abundance + sex	6	-131.99	10.28	0.00	0.20
Season + Egyptian mongoose abundance	7	-129.10	13.17	0.00	0.28
Egyptian mongoose abundance	4	-128.95	13.32	0.00	0.23
Season + region + Egyptian mongoose abundance	8	-126.91	15.36	0.00	0.21
(Null)	3	-126.76	15.51	0.00	0.13
Region	4	-126.32	15.95	0.00	0.03
Season + sex + season * sex	10	-126.27	16.00	0.00	0.28
Region + Egyptian mongoose abundance	5	-125.76	16.51	0.00	0.17
Season + sex + scaled mass index	8	-125.50	16.77	0.00	0.25
Season + region + sex + season * sex	11	-124.32	17.95	0.00	0.21
Season + region + sex + scaled mass index	9	-124.21	18.06	0.00	0.13
Season + age + sex	10	-122.46	19.81	0.00	0.29
Age + sex	7	-122.44	19.83	0.00	0.22

Notes: Models considered as explanatory are in bold. Δ AICc is the difference between the AIC yielded by each model and the lowest AICc (considered the best model); df, degrees of freedom.

All explanatory variables were checked for collinearity using variance inflation factors (VIFs) (Zuur et al. 2009). A cut-off value of 2.5 was used to drop collinear variables. The predictor with the highest VIF value was removed in a stepwise procedure, removing one variable at a time, recalculating the VIF values, until all the remaining predictors had a $VIF \leq 2.5$ (Zuur et al. 2009). Only those that presented $VIF \leq 2.5$ were retained for model construction. Models explaining variation in spleen weight or body condition included the discrete variables region, habitat, sex, age and season and the interactions season \times sex, season \times age, and sex \times age, as well as the continuous variables Egyptian mongoose abundance, population density, extents of the road and river network, and body size, that were not excluded by VIF analysis. Body condition score was used as an explanatory variable in our model construction for spleen weight, and vice versa. For mixed modeling, we used Gaussian distribution, identity link function, and district (first-level administrative subdivision of mainland Portugal, compartmentalized based on history, common land use, and related issues) as a random factor to control for non-independence of samples from the same area.

Selection of models explaining either spleen weight or body condition variation was performed separately according to the procedure described in Zuur et al. (2009), whereby a ranking was made of all possible models using the Akaike Information Criterion (AICc) (Burnham and Anderson 2002) and only those with Δ AICc values < 2 were considered explanatory. The residual patterns were checked.

All statistical analyses were performed in R (version 2.13.2) using the packages *lme4* (Bates et al. 2014) and *MuMIn* for multimodel selection and model averaging approaches (Barton 2012).

Results

The PCA of specimen body size explained 74.6% of the variance in this parameter, with an eigenvalue of 5.220.

Two variables of anthropogenic factors (population density and extent of road network) and the variable body size were excluded from the initial set of predictors for adjusted spleen weight, as well as for body condition model construction, to avoid multicollinearity (Tables A1 and A2). Only one model (season + sex) with Δ AICc < 2 (AICc = -142.27, df = 507) could be considered explanatory (Tables 2 and 3). Overall, males presented higher adjusted spleen weight than females, and spleens were heaviest in spring, followed by winter and autumn, and they were lightest in summer (Table 3, see details in Figures A1 and A2). Despite the fact that the interaction sex \times season was not explanatory in our model, an analysis of the means of spleen weight for each sex-season group revealed different seasonal trends between males and females (Figure A3). In males, adjusted spleen weight was highest in winter and decreased in spring, reaching its lowest value in summer, before increasing again in autumn. However, in females, adjusted spleen weight reached its highest values in spring and remained low in the other 3 seasons of the year.

For body condition analyses, only one model with Δ AICc < 2 (AICc = 7089.62, df = 507) was considered explanatory (Table 4). It included the variables: spleen weight + season + age + sex + season \times age + season \times sex + age \times sex + habitat + region + Egyptian mongoose abundance (Tables 4 and 5). Males presented higher body condition scores than females (Table 5). Juvenile type I Egyptian mongooses had higher body condition scores compared with adults, sub-adults, and juveniles type II, in descending order, respectively (Table 5). Animals collected in autumn presented the highest body condition scores, followed by those collected in winter,

spring, and summer (Table 5). Specimens with heavier spleens presented higher body condition scores (Table 5). Mongooses captured from places with lower abundances of conspecifics had higher body condition, as well as those from the south (Table 5). Specimens collected from vineyards and orchards, rice fields, and urban habitats had the highest body condition scores (Table 5). Some selected factors are graphically presented in Figures A4–A6.

Discussion

Based on a large sample of free-ranging Egyptian mongoose of both sexes and all ages, sampled from throughout Portugal and year-round, our study shows that sex and season explain the variation in adjusted spleen weight. We found no evidence for an effect of body condition score on spleen weight in the explanatory model, contradicting the hypothesis that spleen weight depends primarily on the availability of energy to invest in immune function (Ponlet et al. 2011; Schulte-Hostedde and Elsasser 2011). In fact, our results suggest the opposite causal relationship, with spleen weight appearing as one of the factors in the model explaining the variation in body condition score. The directionality apparent from these results suggests that even though higher body condition scores may not necessarily imply larger spleens and a greater ability to invest energy in

immunity, larger spleens may indicate more competent immune systems, allowing animals to attain better body condition scores.

In general, the adjusted spleen weight of male mongooses is significantly higher than that of females, contrasting with results for red deer *Cervus elaphus* in which spleen weight adjusted for body size does not differ between sexes (Corbin et al. 2008). Our data also show that adjusted spleen weight varies across seasons. In spring, our Egyptian mongoose specimens presented the highest spleen weights, with a slight decrease in values during winter, and an abrupt decrease in summer, followed by a rise again in autumn. To our knowledge, this is the first time that year-round spleen weight measurements have been presented for a wild carnivore species. A study on wild male American mink *Neovison vison* (Persson et al. 2011) and a report focusing on wild boar *Sus scrofa* (Fernández-Llario et al. 2004) showed that animal spleens were heaviest during winter, but these studies only compared data from 3 and 2 seasons, respectively. Two hypotheses are offered in previous studies to explain seasonal variation in indicators of immunity in vertebrates (Martin et al. 2008). The first is the winter immunoenhancement hypothesis, whereby animals up-regulate their immune activity as a response to changes in photoperiod, to compensate for the immunosuppressive effects of winter temperatures and resource scarcity (Sinclair and Lochmiller 2000). The second is the trade-off hypothesis according to which the high cost of immune activity is incompatible with other costly physiological activities that occur at certain times of the year, such as reproduction (Vicente et al. 2007). Deeper analysis is necessary to determine which mechanism underlies the influence of sex and season on spleen weight in this Egyptian mongoose population. Since an increase in spleen weight in spring is apparently incompatible with the winter immunoenhancement hypothesis for seasonal variation in immunity (Martin et al. 2008), we speculate that different reproductive strategies, sex-specific behavior, and physiological variations may also be linked to spleen weight variation, especially since the Egyptian mongoose is a polygynic species (Palomares 1993a), exhibiting differential investment in

Table 3. Effects of the model considered as explanatory for adjusted spleen weight (expressed as g/100 g body weight) of the Egyptian mongoose in Portugal

Variables		Estimate	Standard error	t-Value
Intercept		0.328	0.023	14.197
Sex	Male	0.054	0.015	3.712
Season	Spring	0.081	0.020	4.079
	Summer	−0.026	0.020	−1.285
	Winter	0.071	0.023	3.142

Table 4. Model selection, using AICc, for the effect of age, season, sex, and their interactions, together with region, Egyptian mongoose abundance, habitat, extent of river network, and adjusted spleen weight (expressed as g/100 g body weight), on body condition (Scaled Mass Index) of the Egyptian mongoose in Portugal

Models	df	AICc	ΔAICc	AICc weight	R ²
Spleen weight + season + habitat + age + region + Egyptian mongoose abundance + sex + season×age + season×sex + age×sex	34	7,089.62	0.00	0.88	0.11
Spleen weight + season + habitat + age + region + sex + season×age + season×sex + age×sex	33	7,094.83	5.21	0.07	0.11
Spleen weight + season + habitat + age + region + river network + Egyptian mongoose abundance + sex + season×age + season×sex + age×sex	35	7,096.02	6.40	0.04	0.11
Spleen weight + season + habitat + age + Egyptian mongoose abundance + sex + season×age + season×sex + age×sex	33	7,097.90	8.28	0.01	0.12
Spleen weight + season + habitat + age + region + river network + sex + season×age + season×sex + age×sex	34	7,101.54	11.92	0.00	0.11
Season + habitat + age + region + Egyptian mongoose abundance + sex + season×age + season×sex + age×sex	33	7,103.01	13.39	0.00	0.12
Spleen weight + season + habitat + age + sex + season×age + season×sex + age×sex	32	7,103.15	13.53	0.00	0.12
Spleen weight + season + habitat + age + river network + Egyptian mongoose abundance + sex + season×age + season×sex + age×sex	34	7,104.00	14.39	0.00	0.11
Season + habitat + age + region + sex + season×age + season×sex + age×sex	32	7,108.72	19.10	0.00	0.12
Season + habitat + age + region + river network + Egyptian mongoose abundance + sex + season×age + season×sex + age×sex	34	7,109.47	19.85	0.00	0.11
Spleen weight + season + habitat + age + river network + sex + season×age + season×sex + age×sex	33	7,109.59	19.97	0.00	0.11

Notes: Models considered as explanatory are in bold. ΔAICc is the difference between the AIC yielded by each model and the lowest AICc (considered the best model); df, degrees of freedom.

Table 5. Effects of the model considered as explanatory for body condition (Scaled Mass Index) of the Egyptian mongoose in Portugal

Variables	Estimate	Standard error	t-Value	
Intercept	1,878.46	80.44	23.351	
Sex	Male	59.80	76.10	0.786
Age	Juvenile 1	3.96	128.17	0.031
	Juvenile 2	-226.44	86.41	-2.621
	Sub-adult	-70.07	91.39	-0.767
Season	Spring	-113.97	74.15	-1.537
	Summer	-187.89	76.52	-2.455
	Winter	-111.04	81.22	-1.367
Region	South	53.18	57.99	0.917
Spleen weight		181.37	79.61	2.278
Egyptian mongoose abundance		-10.09	8.44	-1.195
Habitat	Agro-forestry	41.35	61.20	0.676
	Broadleaved and mixed forests	-5.81	42.05	-0.138
	Rice fields	80.84	254.03	0.318
	Shrubs	9.47	82.46	0.115
	Urban	61.44	122.44	0.502
	Vineyards and orchards	115.56	170.09	0.679
Sex×age	Male×Juvenile I	3.28	104.63	0.031
	Male×Juvenile II	-49.27	94.62	-0.521
	Male×Sub-adult	-96.32	93.66	-1.028
Sex×season	Male×Spring	-93.12	91.93	-1.013
	Male×Summer	-109.86	90.44	-1.215
	Male×Winter	71.71	99.91	0.718
Season×age	Spring×Juvenile I	30.38	158.76	0.191
	Spring×Juvenile II	304.12	133.21	2.283
	Spring×Sub-adult	156.35	126.01	1.241
	Summer×Juvenile I	115.86	137.33	0.844
	Summer×Juvenile II	261.95	107.34	2.440
	Summer×Sub-adult	-129.61	172.67	-0.751
	Winter×Juvenile I	-320.24	276.42	-1.159
	Winter×Juvenile II	236.69	198.20	1.194
	Winter×Sub-adult	32.04	112.64	0.284

reproduction and sex-skewed survival (Palomares 1993a; Palomares and Delibes 1993b).

According to a study on the reproductive parameters of the Egyptian mongoose in Spain, courtship and mating begin in winter and extend almost to the end of spring (Palomares and Delibes 1992). During the breeding season, males engage in physical aggression to defend their territory, to gain access to females, to mate, and to compete for prey (Palomares 1991, 1993a). Therefore, male investment in immune function may decrease during the reproductive period, since energetic resources should be directed primarily toward reproduction and less toward immune responses (Zuk and Stoehr 2002; Stoehr and Kokko 2006). However, our results do not support this trade-off hypothesis because spleen weight in our sample of males actually tends toward its highest levels in winter. In Spain, pregnant Egyptian mongooses are observed mostly in March and April (Palomares and Delibes 1992). We also observed this pattern in our sample, with 80% of pregnancies detected in the 3 spring months (Bandeira et al., unpublished data). The energetic cost of gestation, birth, and lactation (Gittleman and Thompson 1988; Speakman 2008), coupled with the increased difficulty in obtaining food and decreased foraging time (Bandeira et al. 2018), is expected to result in a trade-off between reproduction and immunity that is more pronounced in spring for Egyptian mongoose females. However, trends in spleen weight data from our female specimens

showed a peak in spring, thus again contradicting the reproductive trade-off scenario.

Animals are expected to have larger spleens during active infection due to increased lymphocyte production and/or pathological inflammation (Møller et al. 1998a) or parasitism (Nunn 2002; Göuy de Bellocq et al. 2007; Corbin et al. 2008), so we must consider a third hypothesis, whereby these seasonal differences in spleen weight can be influenced by parasitism. Additionally, pathogen infection and parasite burden may be more pronounced in males of polygynous species (Moore and Wilson 2002; Perez-Orella and Schulte-Hostedde 2005), which has been attributed to sexual selection, competition, and larger home ranges (Zuk 1990). Unfortunately, the lack of measures of diversity and abundance of parasites in our study is a major limitation to investigate this effect. However, costly immune responses to parasitism and high parasite burdens are often negatively correlated with body condition (e.g., Irvine et al. 2006; Davidson et al. 2015; Taylor et al. 2018). Therefore, the fact that in our sample the peak in spleen weight coincides with peaks in body condition for both sexes apparently conflicts with a parasite-driven variation in spleen weight, and highlights the need for further research.

In summary, body condition does not predict spleen weight variation in this Egyptian mongoose population, which instead is primarily explained by sex and season. Moreover, spleen weight peaks in spring and coincides with the period of maximum reproductive investment for the species, thus contradicting the winter immunoenhancement and reproductive trade-off hypotheses. Nevertheless, the coincidence of the peaks in spleen weight trends with the period of maximum investment in reproduction for each sex suggests that spleen weight variation is closely related to the species' reproductive biology. This relationship warrants further research to establish if it is a consequence of reproductive behavior, a biologically timed investment to prepare for a challenging period in terms of immunity or simply a result of seasonal host–pathogen dynamics.

Author Contributions

V.B., E.V., and C.F. conceived and designed the experiments. V.B. and A.A. performed the experiments. V.B. and E.V. analyzed the data and performed the statistical analyses. J.C. generated maps and provided ecological, habitat, and climatic data. V.B., E.V., A.A., M.V.C., and C.F. wrote the manuscript. All authors contributed to the development of ideas and approved the final version of the manuscript.

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Appendix

Table A1. VIFs for the full set of explanatory variables for adjusted spleen weight model construction

Variable		VIF ¹	VIF ²	VIF ³	VIF ⁴
Season	Spring	1.84	1.84	1.83	1.82
	Summer	1.82	1.82	1.82	1.81
	Winter	1.63	1.63	1.61	1.60
Sex		1.11	1.11	1.11	1.06
Age	Juvenile 1	3.32	3.32	3.32	1.26
	Juvenile 2	1.78	1.77	1.77	1.31
	Sub-adult	1.31	1.31	1.29	1.19
Region		1.97	1.80	1.75	1.76
Egyptian mongoose abundance		1.44	1.44	1.43	1.42
Habitat	Agro-forestry	1.57	1.55	1.53	1.51
	Broadleaved and mixed forests	1.87	1.82	1.65	1.65
	Rice fields	1.04	1.03	1.03	1.03
	Shrubs	1.15	1.13	1.12	1.13
	Urban	5.07	3.12	1.17	1.17
	Vineyards and orchards	1.13	1.12	1.11	1.11
Population density		14.66	—	—	—
Road network		12.07	4.57	—	—
River network		1.92	1.92	1.62	1.63
Body size		3.26	3.24	3.24	—
Body condition		1.10	1.09	1.09	1.08

Note: Variables removed are in bold.

Table A2. VIFs for the full set of explanatory variables for body condition model construction

Variable		VIF ¹	VIF ²	VIF ³	VIF ⁴
Season	Spring	1.85	1.84	1.83	1.82
	Summer	1.78	1.78	1.78	1.77
	Winter	1.65	1.65	1.63	1.62
Sex		1.14	1.13	1.13	1.09
Age	Juvenile 1	3.30	3.29	3.29	1.25
	Juvenile 2	1.78	1.77	1.77	1.30
	Sub-adult	1.31	1.31	1.29	1.19
Region		2.00	1.83	1.78	1.79
Egyptian mongoose abundance		1.46	1.45	1.44	1.44
Habitat	Agro-forestry	1.57	1.56	1.54	1.52
	Broadleaved and mixed forests	1.87	1.82	1.65	1.65
	Rice fields	1.04	1.03	1.03	1.03
	Shrubs	1.17	1.15	1.14	1.15
	Urban	5.06	3.12	1.18	1.17
	Vineyards and orchards	1.14	1.12	1.11	1.11
Population density		14.57	—	—	—
Road network		12.04	4.57	—	—
River network		1.90	1.90	1.60	1.61
Body size		3.25	3.23	3.22	—
Adjusted spleen weight		1.18	1.18	1.18	1.17

Note: Variables removed are in bold.

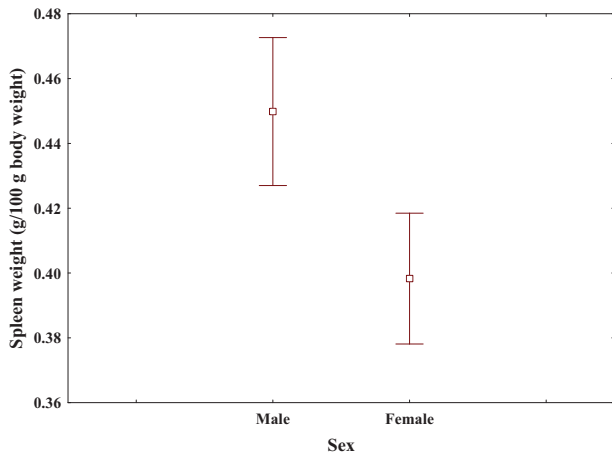


Figure A1. Means of Egyptian mongoose adjusted spleen weight (expressed as g/100 g body weight) observed for both sexes. Vertical bars denote 95% confidence intervals.

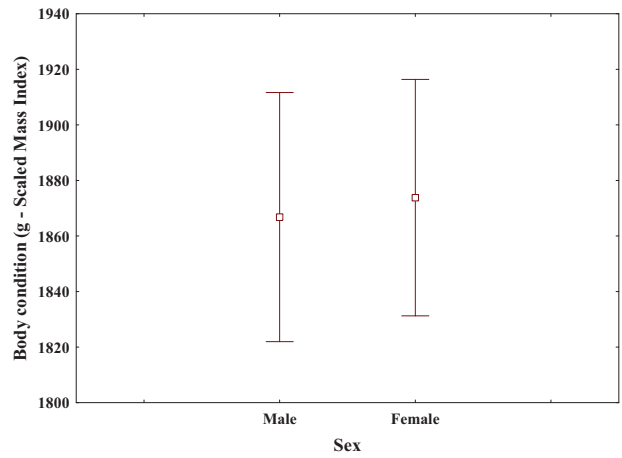


Figure A4. Means of Egyptian mongoose body condition [Scaled Mass Index—predicted body mass (in grams) for an individual standardized to linear body measurement] observed for both sexes. Vertical bars denote 95% confidence intervals.

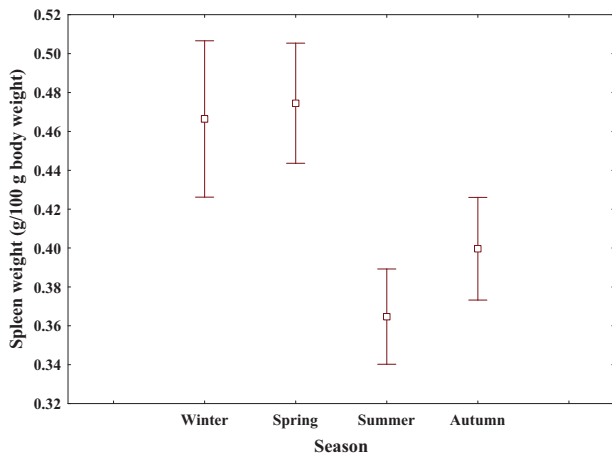


Figure A2. Means of Egyptian mongoose adjusted spleen weight (expressed as g/100 g body weight) observed for each season. Vertical bars denote 95% confidence intervals.

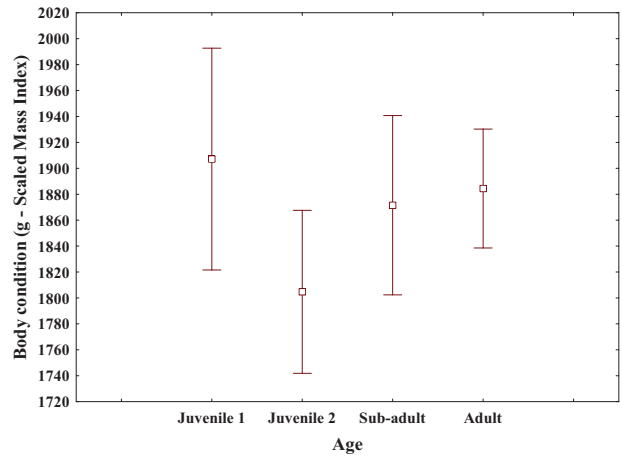


Figure A5. Means of Egyptian mongoose body condition [Scaled Mass Index—predicted body mass (in grams) for an individual standardized to linear body measurement] observed for each age cohort. Vertical bars denote 95% confidence intervals.

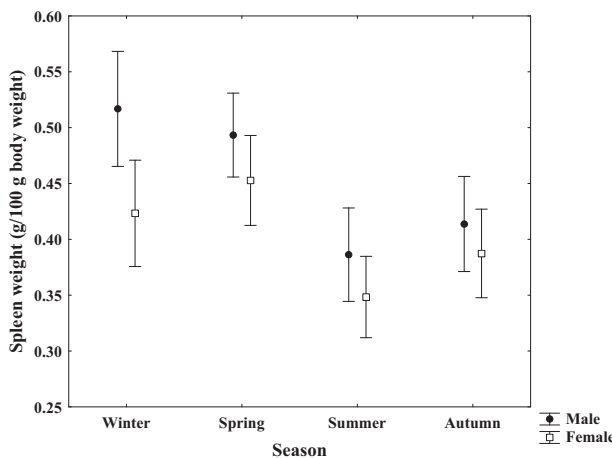


Figure A3. Means of Egyptian mongoose adjusted spleen weight (expressed as g/100 g body weight) observed for each sex and season. Vertical bars denote 95% confidence intervals.

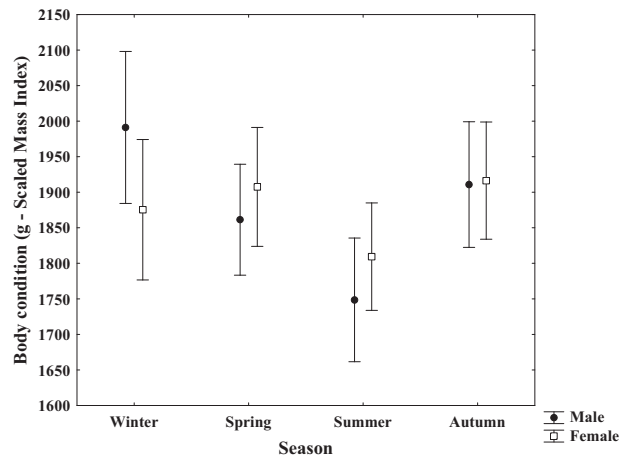


Figure A6. Means of Egyptian mongoose body condition [Scaled Mass Index—predicted body mass (in grams) for an individual standardized to linear body measurement] observed for each sex and season. Vertical bars denote 95% confidence intervals.