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Effects of habitat loss on tick load in central populations of the Eastern Green Lizard *Lacerta viridis* and its relationship with body condition and population density

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Abstract. Habitat loss can increase the susceptibility of individuals to parasitic infections, and hence, parasite load can serve as an early warning indicator of stress before the persistence of a population becomes threatened. In this study, we tested the effects of patch characteristics, isolation and landscape composition resulting from habitat loss on the tick load of individuals from central populations of the Eastern Green Lizard *Lacerta viridis*. We identified the spatial scale at which each landscape composition parameter has the strongest effect and evaluated its effects at this scale. Additionally, we tested the relationships between tick load and population density and body condition (BC) to understand possible mechanisms that determine tick loads in populations. We found that tick load was not affected by host population density. BC was not found to be affected by tick load, but BC did have a negative effect on lizards' tick loads. The proportion of habitat and cropland in the landscape and patch size had positive effects on tick loads, whereas the proportion of urbanized areas, isolation and perimeter/area ratio had negative effects. We discuss our finding in the context of how the landscape can affect tick populations and other potential hosts. We conclude that tick load can be a suitable early warning indicator of negative effects of habitat loss, reflecting the susceptibility of lizards to infestation. We suggest that this indicator be included in monitoring programs aiming at evaluating the status of populations of *L. viridis* in modified landscapes, and recommend that conservation measures be focused on the protection of habitat at broader scales to compensate negative effects of cropland and urbanized areas occurring at small scales.

Key words. Squamata, Lacertidae, *Lacerta viridis*, habitat loss, isolation, landscape composition, warning indicators, scale of effect, parasites, conservation physiology.

Introduction

Habitat loss is the main driver of biodiversity loss, and its effects have been mainly studied regarding changes in species distribution resulting from the extinction of local populations (Ellis et al. 2012, Maxwell et al. 2016). One of the main aims of conservation biology is to avoid the extinction of populations by identifying vulnerable populations before they irreversibly decline. Therefore, the identification of possible warning indicators of populations' vulnerability is of great importance for improving the implementation of monitoring programs in conservation.

Parasitism is a natural ecological regulator of animal host populations that has effects on population size and demography, reproduction, mating behaviour, morphology and survival of hosts (Poulin 1999, Møller 2006, Balenger & Zuk 2014, Sánchez et al. 2018). In anthropogenically modified landscapes, in which portions of habitat have been converted into other land cover types, individuals can be exposed to parasitic infestations to a greater extent (Faust et al. 2018). Therefore, in these landscapes, the effects of parasitic infestations have the potential to be stronger and detrimental to populations (Amo et al. 2007, Raharivololona & Ganzhorn 2009). Hence, parasite load has the potential to be useful as an early warning indicator of populations' vulnerability to the negative effects of habitat loss (Gillespie et al. 2005, Santos et al. 2018).

One of the main mechanisms through which an increased level of parasitism is associated to habitat loss is the direct bidirectional link it has with the physiological condition of hosts. On the one hand, hosts' body condition can be the factor initially being affected by habitat loss due to the reduced availability of resources, reduced habitat quality of the remaining habitat (e.g., due to edge effects), and increased competition amongst individuals (Zanette et al. 2000, Bucher & Entling 2011, Battles et al. 2013). This, in turn, can make individuals more vulnerable to parasitic infestations due to immunosuppression (Koski & Scott 2001, Sánchez et al. 2018). A higher investment of energy into immune defence is required, which consequently reduces mass or fat depositions, compromising body condition even more, and increases susceptibility to parasitic infestations (Bower et al. 2019). On the other hand, an increment in the burden of parasites within habitat patches, resulting from parasite-spillover effects at newly created ecotones or borders after land conversion (Faust et al. 2018, Borremans et al. 2019), can occur first; then, this can lead to a reduction in the body condition of hosts, which again would increase their susceptibility to infestations (Sánchez et al. 2018).

The relationship between habitat loss and parasitism can also be mediated by host population density, which can enhance transmission rates of parasites between individuals. This can occur at the initial stages after habitat loss, when population density transiently increases – a phenomenon known as crowding effect (Debinski & Holt 2000, Tischendorf et al. 2005, Krasnov et al. 2007) – or at later stages, after habitat reduction has occurred, if the response of the species to habitat loss still facilitates relatively high levels of population density (Krasnov et al. 2002, DeVore et al. 2020). Even in the case of parasites that are not transmitted by direct contact between individuals, like *Ixodes* spp. ticks, higher host density can lead to increased transmission rates due to elevated indirect contact rates amongst individuals that share a higher proportion of space and refuges (Ostfeld et al. 2006, Leu et al. 2010, Fenner et al. 2011).

Parasite load and prevalence has been shown to be sensitive to different parameters that characterize habitat loss. In lizards, it has been demonstrated to increase with decreasing habitat quality (Amo et al. 2007, Gwiazdowicz et al. 2020), and in small and medium-sized mammals, parasite prevalence and intensity was found to increase with fragmentation (Raharivololona & Ganzhorn 2009, van der Mescht 2011). Parameters related to landscape configuration and composition have been rarely tested, but the few studies in small mammals conducted to that effect have reported negative correlations between habitat availability in the landscape and parasite load and prevalence (Püttker et al. 2008, Thamm et al. 2009, Santos et al. 2018). However, although the effects of other land cover types on the distribution of parasites have been studied (Maupin et al. 1991, Talbot et al. 2019), it has not yet been tested how these land covers may affect host parasitism. Moreover, the spatial scale at which specific land cover types have the strongest ecological effects – scale of effect *sensu* Jackson & Fahrig (2012) – has been neglected in the research of parasitic infestations in transformed landscapes. This is despite the recognized importance of integrating different spatial scales to understand the effects of habitat loss (Garmendia et al. 2013, Remm et al. 2017, Prieto-Ramirez et al. 2020) and of the connection between processes occurring at landscape scale and the physiology of individuals (Ellis et al. 2012).

In this study, we evaluated the potential usefulness of tick loads as an early warning indicator of the vulnerability of populations to habitat loss and fragmentation. To accomplish this, we investigated the effects of patch characteristic variables, isolation, and landscape composition on the tick load (*Ixodes ricinus*) of individuals of *Lacerta viridis* inhabiting a fragmented landscape in central Bulgaria. Additionally, in order to gain insights into the possible underlying mechanisms influencing parasitism, we tested the relationship of parasite load with body condition and host population density.

Lacerta viridis is a generalist lizard species with a distribution range extending from northern Turkey to the central Czech Republic, with the historical core of its distribution range being located in Bulgaria (Marzahn et al. 2016). This species is listed in the European Habitats Directive (2007) under Annexure IV, which calls for strict protection of the species. Although at a national level, it is the most abundant lizard species in Bulgaria (Popgeorgiev & Mollov 2005), it does face shrinking habitats due to the expansion of land use for agriculture and urbanization in the central parts of the country, i.e., on the Thracian plateau (Mollov & Georgiev 2015). Earlier studies on lizards have been focused on the effects of habitat degradation and quality on parasite load (Amo et al. 2007, Pafilis et al. 2013, Carbayo et al. 2019), but the effects of landscape structure on parasite loads in lizards have been studied much less (Wu et al. 2019).

We predicted that parameters that increase resources at patch and landscape levels and ensure connectivity (patch area, habitat quality, and habitat availability in the landscape) have negative effects on the tick load on individuals of *L. viridis.* Conversely, parameters that reduce resource availability and connectivity (isolation, patch edge, and the proportion of cropland and urbanized areas in the landscape) were expected to increase tick load (Fischer & Lindenmayer 2007, Pardini et al. 2017, Hatfield et al. 2018). Additionally, we expected the amount of available habitat in the landscape, patch area, and habitat quality to reduce the effects of isolation and cropland and urbanized areas in the landscape on tick load, and edge effects to be intensified by non-habitat land covers surrounding patches (Hatfield et al. 2018). This work will contribute to the knowledge on the effects of habitat loss on the physiology of *L. viridis*, the interactions between ectoparasites and reptile host species, and the potential of applying a landscape perspective to investigate the physiological status of reptiles in modified landscapes. Moreover, it will support evidence-based decisions about the usefulness of tick load as an early warning indicator to be included in monitoring programs aiming at the protection of *L. viridis*.

Materials and methods

Study area

Our study was conducted in the surroundings of Plovdiv, Bulgaria, on the Thracian plateau. This is an alluvial plain dominated by the banks of the Maritza River and various tributaries. In this region, which forms part of the current and historical core of the distribution range of *L. viridis* (Marzahn et al. 2016), this lizard inhabits a broad range of natural and semi-natural habitat types with high vegetation structure, including river banks, shrublands and mesophilic mixed forest (Mollov 2011). Due to urban and agricultural expansion this species faces severe habitat loss in this region (Kambourova-Ivanova et al. 2012, Mollov & Georgiev 2015). Most of the former habitat is today configured in fragments of variable size that are separated from one another by a matrix of uninhabitable land covers. Based on other studies in the same area, the process of conversion and fragmentation of habitat started in the early 1930', intensifying during the 1990' (Mollov & Georgiev 2015), and the current landscape structure might have changed very little, if at all, during the last decade (Mollov & Velcheva 2010, Mollov 2011).

Study design and data collection

In the context of a broader study on occupancy and density in fragmented landscapes we analyzed 42 habitat patches (Prieto-Ramirez et al. 2018), and based on observations in these patches, six patches were selected to develop the present study; an additional patch was selected in a later survey (Fig. 1, see Appendix 1 for patch coordinates). The main criteria for this selection were: 1. patches with an encounter rate during the occupancy survey of ≥ 2 individuals per hour to ensure capturing individuals for the present study would be possible within a reasonable time period; 2. selected patches should cover the ranges of patch area and isolation in the study region; and 3. se-

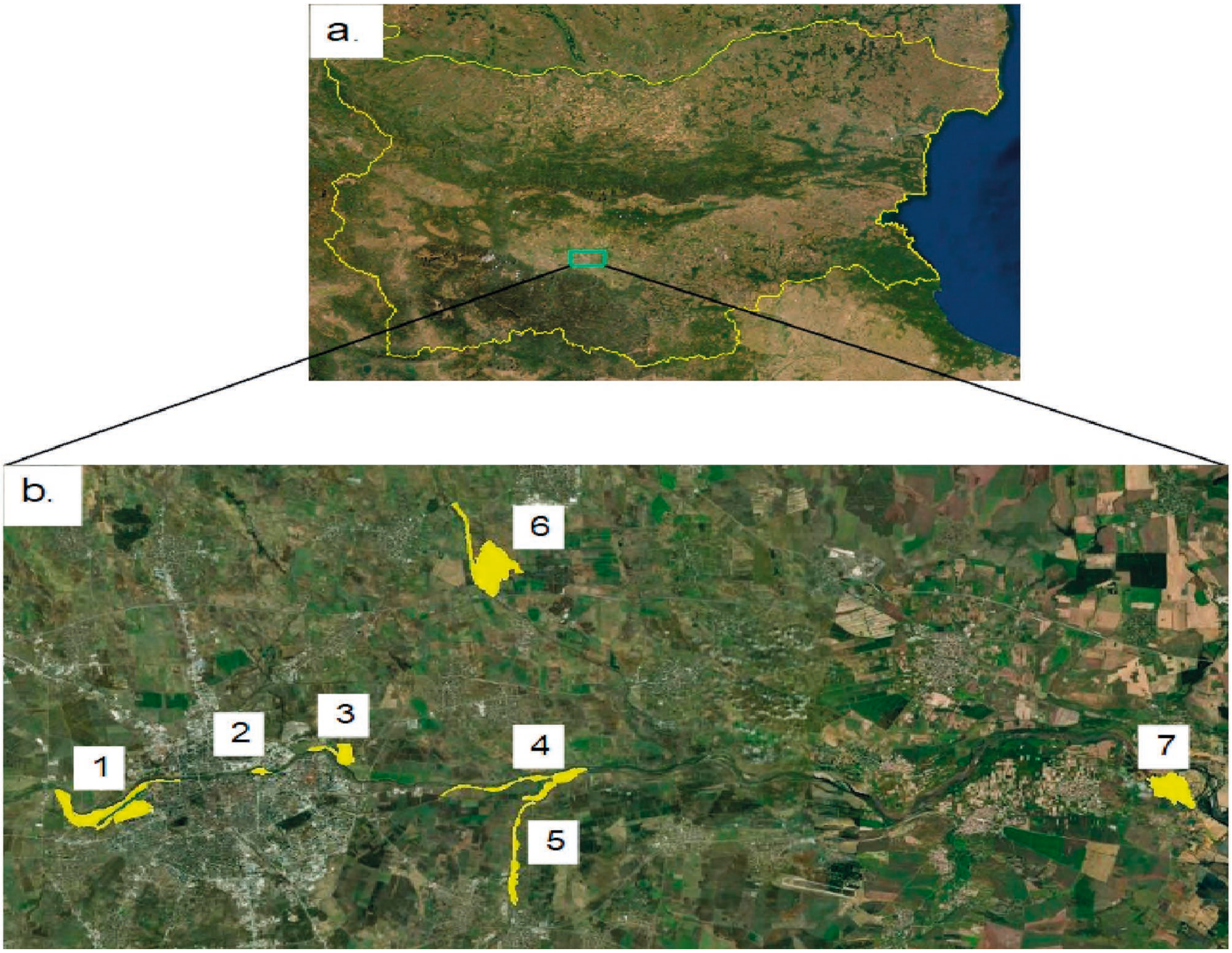

Figure 1. Patches where surveys were carried out in the surroundings of Plovdiv, Bulgaria. Map source: Base map ArcGIS desktop (ESRI 2015).

lected patches should be broadly distributed throughout the landscape in such a manner as to be surrounded by different land cover types. All selected patches were separated from each other by agricultural landscape, urban areas, and/or highways.

Fieldwork was performed from the beginning of April to mid-May in 2015 and from mid-June to the beginning of July in 2017. In this region, males become active after the winter pause in mid-March, with females following suit at the beginning of April. The reproduction season of this species lasts until mid- or late May, and both sexes are active and have a unimodal activity pattern during this time (Grimm et al. 2014). As temperature increases with the advance of spring and the beginning of summer, both sexes remain active, but their daily activity pattern shifts to bimodal with a pause during midday. Thus, survey times were adjusted depending on the season: in the spring of 2015, surveys were performed from 9:00 though 19:00 h, and in the summer of 2017, surveys started very early in the morning, at 7:00 h, when temperatures were already suitable for lizard activity (~27°C) and continued until 20:00 h with a pause of two to three hours during midday, depending on temperature.

Each population was visited on multiple days until 20 individuals had been captured. Populations 1, 2, 3, and 4 were sampled only in 2015, whereas populations 5 and 6 wee sampled in both years, and population 7 was visited only in 2017. For each captured individual, sex, age (adult or subadult), and the number of ticks (*Ixodes ricinus*) it hosted were recorded. Individuals were weighed with a digital scale to the nearest 0.01 g, and snout–vent length (SVL) was measured to the nearest 1 mm. Individuals were classified by sex and age based on visual inspection of size, colour pattern, and reproductive organs in the field. Then, we checked for SVL distribution, and our classification in the field matched the information provided by Sagonas et al. (2018) for Greek populations of *L. viridis* about minimum size of sexually mature individuals (adult males > 80 mm, adult females > 76 mm).

Calculation of body condition index (BCI)

We calculated the scaled mass index (SMI) proposed by Peig & Green (2009) as a measurement of BCI, which is calculated as follows:

$$\text{scaled mass index: } \hat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

where M_i and L_i are the body mass and linear measurement of size of individual 'i'; L_o is any value of L within the range of values measured. Firstly, a standardized major axis regression (SMA) of ln-transformed body mass on ln-transformed SVL was performed to identify outliers and then refitted without outliers to calculate the scale parameter. SMI was calculated for all individuals, using the mean of SVL (87 mm) as L_o and a scale parameter of 3.285. BCI values were log-transformed for further analysis.

Estimation of population density

We applied a distance sampling survey with predetermined sampling effort to collect data and estimate the densities of *L. viridis* populations (Buckland et al. 1993, Andersen & Steidl 2020). In the context of a broader study, populations 1–6 were surveyed to estimate density. In each patch, two 1-hour surveys were carried out, each corresponding to 1200 m of total walking distance at a speed of 20 m/min, which is slow enough to detect lizards. The 1200 m transects were split into several units of variable length. The number and lengths of transects depended on the different habitat types within each patch and the relative area covered by each habitat type. This was estimated using satellite imagery (see Prieto-Ramírez et al. 2020). Each transect was placed within one habitat type only. A strip of 2.5 m wide at each side of the transects was scanned to visually search for lizards, and the perpendicular distance to the transect line was recorded for each detected lizard.

We estimated densities with the aid of the software Distance (Thomas et al. 2010). We applied global detection probability estimation with stratified density estimation. The best-model framework to estimate detection probability was the Conventional simple-distance sampling (CSD) engine with a uniform parametric key function and hermit polynomial adjustment term, and with empirical estimated variance. We selected the model based on the Akaike Information Criterion (AIC), the coefficient of variation (%CV), and the Kolmogorov-Smirnov test (K-S test) of goodness of fit (AIC = 1167.2, %CV = 4.02, K-S test: estimate = 0.1199, p = 0.092). Values of population density used as predictors of tick load are presented in Appendix 2.

Environmental variables

To assess the impacts of habitat loss on body condition and parasite load we evaluated the effects of variables characterizing patches, habitat configuration, and landscape composition. For populations 1–6, values of all variables used herein were extracted from a previous study (Prieto-Ramirez et al. 2020) in which patch digitalization had been performed in ArcMap version 10.3.1 (ESRI 2015) to calculate patch characteristics and habitat configuration variables, and to classify land cover, in order to calculate landscape composition variables. For population 7, digitalization of the patch was performed in this study in the same manner in order to calculate patch characteristics and habitat configuration, but neither habitat quality nor landscape composition variables were available for this patch. Geometric characteristics of each patch include area, perimeter and perimeter/area ratio. Perimeter/area ratio is one of the most common metrics to study edge effects following habitat loss and fragmentation (Fletcher et al. 2007), and hypotheses regarding edge effects were therefore tested by evaluating the effects of this variable.

To evaluate the influence of habitat quality, we tested the effects of vegetation structure. *L. viridis* is a mesothermophilic and mesophilic species, and in the study region it is highly dependent on enough vegetation cover that will offer shade and elevated humidity levels, as well as on refuges and open spots for basking (Mollov & Velcheva 2015, Vacheva et al. 2020). Thus, it uses habitats with comparatively higher vegetation structure than those used by other lizard species in the region (Petrov 2007, Vacheva et al. 2020). Vegetation structure was calculated as a 'foliage height diversity' index (MacArthur & MacArthur 1961) based on information gathered at microhabitat scale in several plots in each patch (Prieto-Ramírez et al. 2018).

Habitat configuration was characterized by patch isolation, calculated as the edge-to-edge Euclidean distance to the nearest patch (isolation). For each patch, the isolation values were calculated with respect to the factual nearest neighbouring patch. Neighbouring patches are not necessarily included in the present study, but were part of an occupancy study performed earlier in the same region (Prieto-Ramirez et al. 2020). Landscape composition variables included the proportion of crop fields and pastures (cropland), the proportion of urbanized areas, and the proportion of habitat surrounding patches. These predictors were calculated at different buffer distances around patches (scales) selected based on dispersal distances reported for *L. viridis* (Saint-Girons & Bradshaw 1989, Mangiacotti et al. 2013, Grimm et al. 2014,). Selected scales were: 50, 150, 250, 500, 750 m, 1, 1.5, 2, 2.5 and 3 km (Prieto-Ramírez et al. 2020). The values of variables corresponding to each patch are summarized in Appendix 3.

Statistical analyses

All statistical analyses were performed with the softweare R (R Core Team 2018), using the relevant functions (see below). In the second year, only very few juveniles that were born in that year were captured, and therefore, for all subsequent analyses, we classified individuals into two age classes: adults and subadults (including juveniles). We applied a Levene's test of homogeneity of variance to test for differences between years. No significant differences were found ($F = 0.013$, $p = 0.908$), and data was therefore combined for all subsequent analyses.

Data from all populations were used to test the effects of geometric patch characteristics and isolation, but only from populations 1–6 to analyze the effects of population density, habitat quality, and landscape composition variables. Mixed models with patch identity as random effect were applied to analyze the data of all traits. Count data of tick load was analyzed with GLMM. Count data is usually analyzed with a Poisson distribution, but when parasite data is over-dispersed, it is recommended that a negative binomial distribution be applied (Wilson et al. 1996). Hence, we tested for over-dispersion to define the distribution to be applied. Our over-dispersion test showed that our data is not over-dispersed (dispersion = 0.027, $p = 0.884$), and therefore, we performed the analysis with a Poisson distribution. We tested the effects of sex and age by applying mixed models. To evaluate the relationships of tick load with BCI and population density, we first applied a Pearson correlation to test correlations between tick load and BCI, and tick load and population density. Then we tested the effect of tick load on BCI by means of LMM. Because the relationship between tick load and BCI can be bidirectional, the response of tick load to BCI was also tested. We also evaluated the response of tick load to host population density. Finally, we tested the additive and interactive effects of BCI and population density on tick load.

To test the effects of habitat loss on tick load, we first identified the scale of effect of each landscape composition variable – cropland, urban and habitat – on tick load by fitting univariate GLMMs with each of these variables at each scale. The scale with the highest R^2 value was selected as the scale of effect (Martin & Fahrig 2012) and used in subsequent analyses. We built single- and multi-scale models. Single-scale models included patch variables, isolation and landscape composition variables at the respective single scale. For multi-scale models, landscape composition predictors were added at their scales of effect. We evaluated separately the individual effects of each patch and landscape parameter on tick load and the effects of the interaction between each parameter and sex and age. To test hypotheses regarding the effects of interactions between parameters, we constructed models with first-order interactions between patch area, perimeter/area, vegetation structure, isolation, proportion of habitat, proportion of cropland, and proportion of urbanized areas. Additionally, the effects of interaction terms were evaluated in combination with sex or age (second-order interaction terms). To avoid collinearity, we tested correlations amongst variables using a Spearman rank test. Only models including non-correlated variables ($r < 0.60$) were tested.

A large number of multiple comparisons were performed to evaluate the effects of habitat loss, which by nature increases the risk of Type I errors. This implies that a family-wise error rate (FWER) correction, like the Bonferroni alpha adjustment, has to be applied (Pedhazur & Schmelkin 1991). However, there are some disadvantages associated with the application of Bonferroni corrections. It increases the Type II error rate and makes the significance of a single test highly dependent on the number of subsequent tests regardless of the effect of the predictor itself (Perneger 1998, White et al. 2019). Therefore, to avoid high Type I error rates while minimizing the shortcomings of the application of Bonferroni corrections, we followed the common null hypothesis principle (Miller 1981, Rice 1989), defining 'family' as the subgroup of models corresponding to a single prediction (Bender & Lange 2001). Thus, a family of models includes the model testing the effect of a single patch or landscape parameter, or of an interaction between parameters, and two models testing the difference of that effect in sex or age classes. Then, the calculated alpha threshold (0.05/3) corresponded to 0.016

and was applied to all models testing effects of landscape and patch parameters. Also, in order to identify the stronger effects, a Cohen's f^2 test was applied to evaluate effect sizes of significant variables or interaction terms (Selya et al. 2012). Results are focused on those variables (or interaction terms) that had medium to high effect sizes (> 0.1) (Cohen 1988).

Results

A total of 158 lizards were captured from the seven populations investigated, being 114 adults and 44 subadults. Sexing was possible in 145 of these lizards, of which 66 were females and 79 were males. Males had a higher tick load compared to females (males: range = 0–35, mean=6.89, females: range = 0–23, mean = 3.94, t = 6.178, p = $6.5e^{-10}$), and adults compared to subadults (adults: range = 0–35, mean = 6.1, subadults: range = 0–27, mean = 2.93, t = -13.784, p < $2e^{-16}$). The total number of individuals excluding population 7 was 141, comprising 110 adults and 31 subadults. Sexing was possible in 130 of these individuals, of which 65 were females and 65 males.

Relationship of tick load to BCI and population density

BCI values ranged from 1.034 to 1.443 with a mean value of 1.215. Correlations of tick load with BCI and population density were not significant (BCI: r = -0.4, p = 0.354, population density: r = -0.55, p = 0.257). BCI was not affected by tick load (ß = -0.0008 ± 0.0005,t = -1.445, p = 0.151), but BCI had a significant negative effect on tick load (ß = -1.969 ± 0.558, z = -3.524, p = 0.0004). Density did not significantly affect tick load (ß = 0.001 ± 0.002, z = 0.44, p = 0.66). We did not find additive (BCI: ß = 0.2478 ± 0.364, z = 0.68, p = 0.497; population density: ß = -3.873 ± 3.006, z = -1.288, p = 0.198) or interactive effects (ß = -6.083 ± 3.634, z = -1.674, p =0.094) of BCI and population density on tick load.

Effects of habitat loss on tick load

The scale of effect of landscape composition variables on tick load of *L. viridis* is shown in Figure 2. The proportions of habitat and urbanized areas had small scales of effect, 150 and 250 m, respectively. In contrast, the strongest effect of cropland was found at the largest scale, 3000 m.

Tick load increased with the proportions of habitat, cropland and patch area (Figs 3a–c, % of habitat: ß = 27.921 ± 5.709, z = 4.891, p ≤ 0.001, Cohen's f^2 = 0.208, % of cropland: ß = 5.419 ± 1.468, z = 3.692, p = 0.0002, Cohen's f^2 = 0.138, patch area: ß = 3.337 ± 1.002, z = 3.328, p = 0.0008, Cohen's f^2 = 0.104). In contrast, tick load decreased with the proportion of urbanized areas (ß = -8.1434 ± 1.904, z = 4.275, p ≤ 0.001, Cohen's f^2 = 0.154), isolation, and the perimeter/area ratio (Figs 3d–f). In the case of isolation, tick load decreased at different rates for each sex (ß = -0.682 ± 0.12, z = -5.681, p ≤ 0.001, Cohen's f^2 = 0.191), with the rate being higher for males than for females. The effect of isolation was also significantly different between age classes (ß = 1.211 ± 0.227, z = 5.313, p ≤ 0.001, Cohen's f^2 = 0.194), with tick loads decreasing with increasing isolation in adults and slightly increasing in subadults. The decreasing rate of tick load in response to the perimeter/area ratio was significantly different between classes, too (ß = 1.758 ± 0.386, z = 4.553, p ≤ 0.001, Cohen's f^2 = 0.164), being higher in adults compared to subadults.

Tick load also increased with vegetation structure at low levels of isolation, but its effect decreased with increasing isolation (Fig. 4A, ß = -89.29 ± 24.30, z = -3.674, p = 0.0002, Cohen's f^2 = 0.159). Similarly, the effect of vegetation structure decreased with increasing perimeter/area ratios (Fig. 4b, ß = -80.54 ± 27.36, z = -2.944, p = 0.003, Cohen's f^2 = 0.117). The effect of urbanized areas was influenced by patch area (ß = 36.575 ± 11.295, z = 3.238, p = 0.001, Cohen's f^2 = 0.143), with the tick load increasing with the patch area at any proportion of urbanized areas, but at a lower rate at high proportions of this land cover for both sexes, with this difference being more pronounced in males (Fig. 4c). The effect of the perimeter/area ratio was influenced by the proportion of urbanized areas (Fig. 4d), with the interaction having a negative effect on tick load of both males and females, and the tick load being much higher in males at low, but not at high proportions of urban areas (ß = -40.256 ± 11.141, z = -3.613, p = 0.0003, Cohen's f^2 = 0.143).

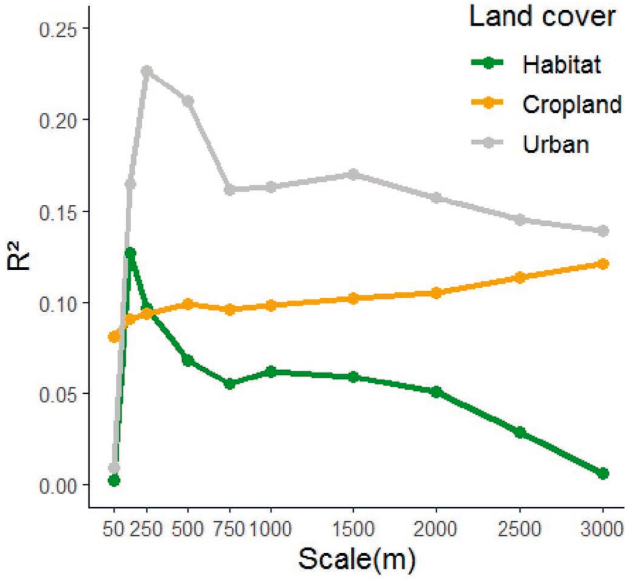

Figure 2. Scale of effect of proportion of habitat (Habitat), proportion of cropland (Cropland), and proportion of urbanized areas (Urban) surrounding habitat patches on the tick load of individuals of *Lacerta viridis*.

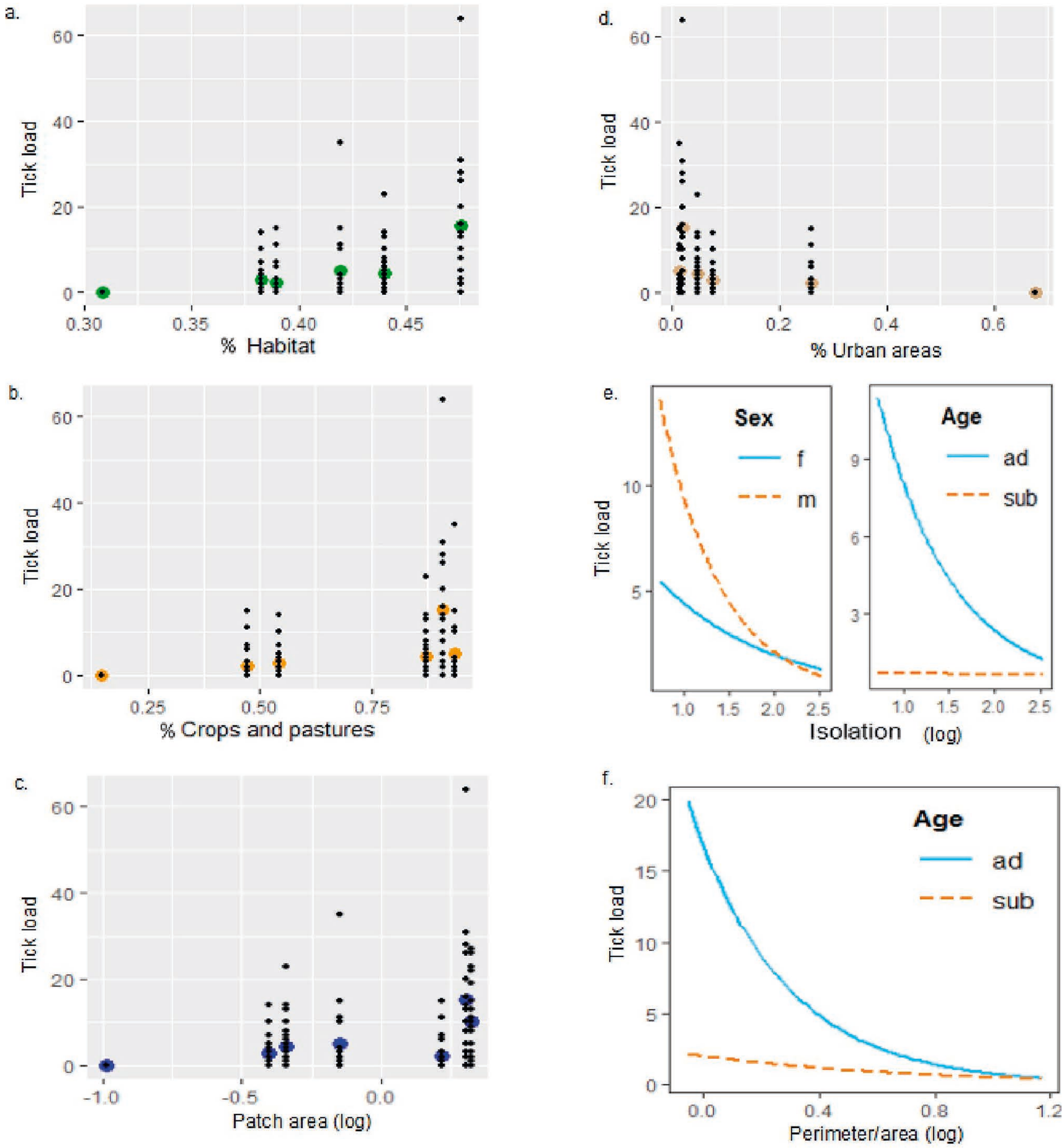

Figure 3. Effects of patch characteristics, isolation and landscape composition variables on the tick load of individuals of *Lacerta viridis*. Coloured dots in a–f represent mean values. Units of untransformed values: patch area in km² and isolation in m.

Discussion

We tested the relationship of tick load in individuals of *L. viridis* with population density and body condition, and evaluated the effects of patch and landscape parameters resulting from habitat loss on tick load. In general, we found that the latter seems to be related to how ticks experience the landscape. We also found a unidirectional relationship between tick load and body condition.

Contrary to our expectation, population density did not affect tick load. This might be related to low density levels of the studied population. In lizards, tick infestation has been found to be related to the number of ticks neighbouring individuals host (Fenner et al. 2011). Therefore, with lower population density, the possibility of having close neighbours that influence tick loads will decrease. The highest population density in our study (1953.5 ind./km²) was already much lower than the density reported for other, less disturbed populations of *L. viridis* in Bulgaria (4535–6000 ind./km², Popgeorgiev & Mollov 2005), and similar to that of populations of the closely related green lizard species *Lacerta bilineata* in modified landscapes (935–1467 ind./km²) in which habitable patches are surrounded by urbanized and agricultural land covers (Maura et al. 2011), which suggests that density has probably already been affected by habitat loss.

Also contrary to our prediction, we did neither find a significant correlation between tick load and body condition nor a negative effect of tick load on BCI. However, we found significant evidence of a negative effect of body condition on the tick load of lizards. Thus, our results suggest that those lizards having already a low body condition index are the ones that are more vulnerable to tick infestations, but not that tick load compromises body condition. A lower immune response has been linked to lower body condition and lower resistance to tick infestations in other lacertid lizards, like *Podarcis muralis* and *Lacerta agilis* (Olsson et al. 2005, Amo et al. 2006). Tick load has been found to have a time-lapsed effect on body condition across years in other lizard species (Godfrey et al. 2010), which is an aspect we could not test in our study.

In our study system, the relationship between body condition and tick load was mediated by characteristics of the modified landscape. Body condition was negatively affected by the proportion of cropland surrounding patches (unpublished data), a parameter that we found to have a positive effect on tick load. Hence, the plausible underpinning mechanism defining the relationship might be a reduction of lizards' physiological status due to negative effects of cropland, which in turn renders them more vulnerable to tick infections. Moreover, a high proportion of cropland around patches can also cause an increment of tick burden in the landscape due to the presence of other host species. For instance, cropland is known to favour the pres-

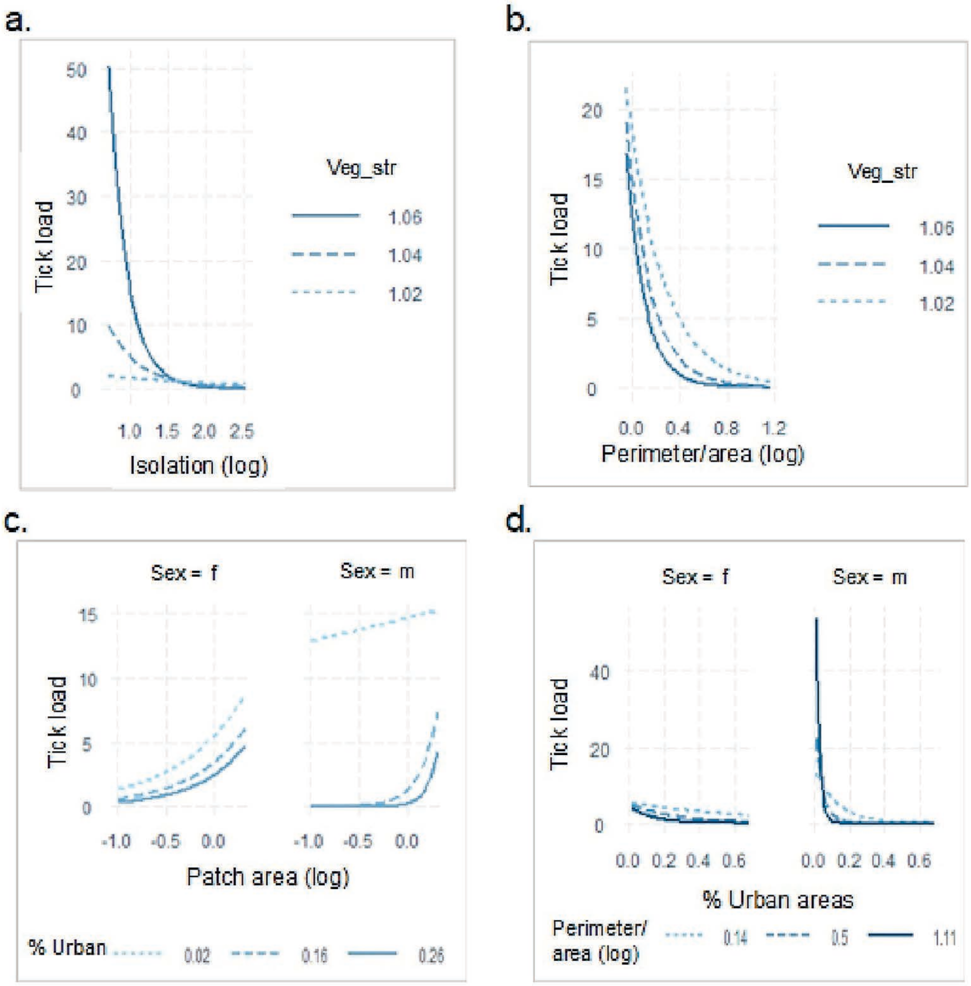

Figure 4. Effects of interaction terms on the tick loads in individuals of *Lacerta viridis*. The values of vegetation structure (veg_str), % urbanized areas in c), and perimeter/area ratio in d), are the first quantile, mean and third quantile of the data. Units of untransformed values: patch area in km² and isolation in m.

ence and abundance of rodents (Fras et al. 2014, Benedek & Sîrbu 2018, Mendoza et al. 2019). Rodents are known to be 'bridge hosts' – host species that cross ecosystem boundaries (Renwick & Lambin 2013, Borremans et al. 2019) and are amongst the most likely reservoirs transferring *Ixodes ricinus* ticks from ecosystems such as pastures and hedgerows around crops to woodlands (Boyard et al. 2008), hence influencing the parasite load in other species (Bolaños-García et al. 2018). Additionally, some of the forested patches visited in this study hold populations of deer, a host of *Ixodes ricinus* ticks whose presence is a key determinant of tick burdens in the habitat (Gilbert et al. 2012, Hofmeester et al. 2017).

Regarding other parameters of habitat loss, we found unexpected positive effects of the proportion of habitat and patch area on tick load. These predictors can also increase the tick burden in the landscape and have been found to have positive effects on the occupancy and abundance of ticks (Allan et al. 2003, Lawrence et al. 2018, Ledger et al. 2019, Simpson et al. 2019). Predictors negatively affecting the tick load of lizards are also related to negative effects on tick abundance and lizard population density. Contrary to our predictions, tick load was reduced by the proportion of urban areas, isolation and perimeter/area ratio. Ticks may be less prevalent in urban wildlife due to a lack of habitat that would be suitable for them (Murray et al. 2019, DeVore et al. 2020) and might be affected by a lack of habitat connectivity (Püttker et al. 2008). The negative effects of perimeter/area ratios on tick loads may correspond to edge effects causing higher turnover and extinction rates in tick populations (Simpson et al. 2019).

Literature about the effects of land conversion and habitat fragmentation on tick loads in individual animals has shown that in the absence of a positive relationship between host density and parasite intensity, tick load increases with increasing isolation and decreasing patch size, habitat quality, and habitat availability at landscape level (Raharivololona & Ganzhorn 2009, van der Mescht 2011, Santos et al. 2018). This is an effect that is expected based on the lack of resistance of individuals to infestations due to the negative effects of habitat loss and fragmentation on their physiological status (Koski & Scott 2001, Sánchez et al. 2018). However, in the case of lizard species, literature to that effect is scarce, and study results are contradictory. For instance, contrary to our results, Pafilis et al. (2013) found a higher prevalence and intensity of tick infestations in habitats with lower vegetation structure. Similarly, Gwiazdowicz et al. (2020) demonstrated that tick load was higher in transformed vs. non-transformed habitats in populations of *Lacerta agilis* and *Zootoca vivipara*, but only in the case of females, whereas it was the opposite for males. Finally, similar to our results, Carabayo et al. (2018) found a positive effect of habitat quality on tick load in both sexes. This reflects the importance of accounting for possible positive effects of patch and landscape characteristics on both lizards and ticks, which makes it less intuitive to expect low tick load levels in less isolated and larger patches, and in landscapes with lower habitat availability.

Finally, regarding the scales of effect of land cover types, that of proportion of urbanized areas and the proportion of suitable habitat on tick load was smaller (150 and 250 m, respectively) than that of the proportion of cropland (3000 m). This might reflect differences on how direct the effects of these land covers are on tick abundance, and in turn, on lizards' tick loads. While habitat and urbanized areas may directly affect tick distribution in the landscape, cropland effects would be mediated by its influence on other host species. Landscape predictors reflecting complex species interactions have scales of effect that reflect the mobility of all the species involved, which is expected to be detected at large rather than localized scales (Martin & Fahrig 2012). In this case, the high mobility of other hosts, like mammals, through cropland areas might be possible to be captured only at broader scales.

Our study focuses on the effects of landscape, patch, population and physiological predictors to understand tick load in lizard populations inhabiting a fragmented landscape. As discussed above, some of the landscape and patch predictors that we analyzed (e.g., habitat availability, vegetation structure) can affect tick populations. However, other parameters, like microclimatic conditions and patch vegetation composition, can also affect tick distribution and abundance (Williams & Ward 2010, Dumas et al. 2022) and, therefore, potentially also the tick load of lizards. Integrating parameters affecting ecological processes and patterns of tick populations with those included in the present study may improve the understanding of host/parasite dynamics in fragmented landscapes, and facilitate a better identification of host species susceptibility (Bower et al. 2019).

Conclusions

The suitability of tick load as an early warning indicator of the negative effects of habitat loss on target reptile species depends on its correlation with physiological parameters like BC, which we encourage be tested in both directions. Based on our results in the studied system, tick load is an indicator of an already compromised body condition. Additionally, because tick load can have time-lapsed negative effects on the body condition of individuals (Godfrey et al. 2010), we recommend this indicator be included in studies evaluating the status of individuals in populations inhabiting modified landscapes across time. Such long-term studies are also necessary to unravel the complex ecological interactions between ticks and host populations in fragmented landscapes. Finally, based on the scales of effect obtained for each landscape composition parameter, we suggest conservation measures for *L. viridis* in the studied system be focused on compensating the negative effects of habitat loss and conversion occurring already at a small scale by protecting and increasing habitat availability at a much broader scale.

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