Impact of habitat loss on the diversity and structure of ecological networks between oxyurid nematodes and spur-thighed tortoises (*Testudo graeca* L.)

Julieta Benítez-Malvido Corresp., Andrés Giménez, Eva Graciá, Roberto Carlos Rodríguez-Caro, Rocío Ruiz de Ybáñez, Héctor Hugo Siliceo-Cantero, Anna Traveset

Habitat loss and fragmentation are recognized as affecting the nature of biotic interactions, although we still know little about such changes for reptilian herbivores and their hindgut nematodes, in which endosymbiont interactions could range from mutualistic to commensal and parasitic. We investigated the potential cost and benefit of endosymbiont interactions between the spur-thighed tortoise (*Testudo graeca* L.) and adult oxyurid nematodes (Pharyngodonidae order Oxyurida) in scrublands of southern Spain. For this, we assessed the association between richness and abundance of oxyurid species with tortoises’ growth rates and body traits (weight and carapace length) across levels of habitat loss (low, intermediate, and high). Furthermore, by using an intrapopulation ecological network approach, we evaluated the structure and diversity of tortoise-oxyurid interactions by focusing on oxyurid species infesting individual tortoises with different body traits and growth rates across habitats. Overall, tortoise body traits were not related to oxyurid infestation across habitats. Oxyurid richness and abundance however, showed contrasting relationships with growth rates across levels of habitat loss. At low habitat loss, oxyurid infestation was positively associated with growth rates (suggesting a mutualistic oxyurid-tortoise relationship), but the association became negative at high habitat loss (suggesting a parasitic relationship). Furthermore, no relationship was observed when habitat loss was intermediate (suggesting a commensal relationship). The network analysis showed that the oxyurid community was not randomly assembled but significantly nested, revealing a structured pattern for all levels of habitat loss. The diversity of interactions was lowest at low habitat loss. The intermediate level, however, showed the greatest specialization, which indicates that individuals were
infested by fewer oxyurids in this landscape, whereas at high habitat loss individuals were the most generalized hosts. Related to the latter, connectance was greatest at high habitat loss, reflecting a more uniform spread of interactions among oxyurid species. At an individual level, heavier and larger tortoises tended to show a greater number of oxyurid species interactions. We conclude that there is an association between habitat loss and the tortoise-oxyurid interaction. Although we cannot infer causality in their association, we hypothesize that such oxyurids could have negative, neutral and positive consequences for tortoise growth rates. Ecological network analysis can help in the understanding of the nature of such changes in tortoise-oxyurid interactions by showing how generalized or specialized such interactions are under different environmental conditions and how vulnerable endosymbiont interactions might be to further habitat loss.
Impact of habitat loss on the diversity and structure of ecological networks between oxyurid nematodes and spur-thighed tortoises (*Testudo graeca* L.)

Julieta Benítez-Malvido¹, Andrés Giménez², Eva Graciá², Roberto Carlos Rodríguez-Caro², Rocío Ruiz de Ybáñez³, Héctor Hugo Siliceo-Cantero¹ and Anna Traveset⁴

¹Laboratorio de Ecología del Hábitat Alterado, Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México (UNAM), Morelia, Michoacán, México.

²Departamento de Biología Aplicada, Facultad de Ciencias Experimentales, Universidad Miguel Hernández, Elche, Spain.

³Departamento de Sanidad Animal, Facultad de Veterinaria, Campus de Excelencia Internacional Regional “Campus Mare Nostrum”. Universidad de Murcia, Spain.

⁴Global Change Research Group, Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Esportes, Mallorca, Balearic Islands, Spain.

Corresponding author: jbenitez@cieco.unam.mx
Habitat loss and fragmentation are recognized as affecting the nature of biotic interactions, although we still know little about such changes for reptilian herbivores and their hindgut nematodes, in which endosymbiont interactions could range from mutualistic to commensal and parasitic. We investigated the potential cost and benefit of endosymbiont interactions between the spur-thighed tortoise (*Testudo graeca* L.) and adult oxyurid nematodes (Pharyngodonidae order Oxyurida) in scrublands of southern Spain. For this, we assessed the association between richness and abundance of oxyurid species with tortoises’ growth rates and body traits (weight and carapace length) across levels of habitat loss (low, intermediate, and high). Furthermore, by using an intrapopulation ecological network approach, we evaluated the structure and diversity of tortoise-oxyurid interactions by focusing on oxyurid species infesting individual tortoises with different body traits and growth rates across habitats. Overall, tortoise body traits were not related to oxyurid infestation across habitats. Oxyurid richness and abundance however, showed contrasting relationships with growth rates across levels of habitat loss. At low habitat loss, oxyurid infestation was positively associated with growth rates (suggesting a mutualistic oxyurid-tortoise relationship), but the association became negative at high habitat loss (suggesting a parasitic relationship). Furthermore, no relationship was observed when habitat loss was intermediate (suggesting a commensal relationship). The network analysis showed that the oxyurid community was not randomly assembled but significantly nested, revealing a structured pattern for all levels of habitat loss. The diversity of interactions was lowest at low habitat loss. The intermediate level, however, showed the greatest specialization, which indicates that individuals were infested by fewer oxyurids in this landscape, whereas at high habitat loss individuals were the most generalized hosts. Related to the latter, connectance was greatest at
high habitat loss, reflecting a more uniform spread of interactions among oxyurid species. At an individual level, heavier and larger tortoises tended to show a greater number of oxyurid species interactions. We conclude that there is an association between habitat loss and the tortoise-oxyurid interaction. Although we cannot infer causality in their association, we hypothesize that such oxyurids could have negative, neutral and positive consequences for tortoise growth rates.

Ecological network analysis can help in the understanding of the nature of such changes in tortoise-oxyurid interactions by showing how generalized or specialized such interactions are under different environmental conditions and how vulnerable endosymbiont interactions might be to further habitat loss.

Introduction

As a consequence of diverse human activities, habitat loss and fragmentation in terrestrial ecosystems have been generally considered as the main threats to the maintenance of biodiversity worldwide (Brooks et al., 2002; Fahrig, 2003; but see Fahrig, 2019). This is because native species are susceptible to habitat degradation through local extinctions and the reduction in the size of their populations. One aspect of land-use change that could have an important impact on wild populations and biodiversity is the local extinction of native species caused by changes in the nature of biotic interactions, including both mutualistic (e.g., pollination and seed dispersal) and antagonistic (e.g., pests and pathogens) interactions (Harvell et al., 2002; Patz et al., 2004; Aguilar et al., 2006; Aguirre & Tabor, 2008; Tompkins et al., 2011; Trumbore, Brando & Hartmann, 2015; Pringle, 2016). The ecological costs of these changes constitute the loss of species and populations and ecosystem degradation (Harvell et al., 2002; Rizzo et al., 2002; Patz et al., 2004; Aguilar et al., 2006; Aguirre & Tabor, 2008; Vurro, Bonciani & Vannacci, 2010;
There are other biotic interactions, however, that have been little explored in the context of habitat loss and fragmentation, such as commensal interactions in which one species benefits whilst the other receives neither benefit nor damage. In some reptiles, for instance, the symbiotic relationships with hindgut oxyurid nematodes (endosymbionts) have been categorized as commensal, mutualistic and/or parasitic (O’Grady et al., 2005; O’Grady & Dearing, 2006; Jacobson, 2007). In fact, for herbivorous reptiles, hindgut oxyurids and their associated gut microbial community (e.g., bacteria) might be parasitic while also playing a positive role in the digestion and assimilation of plant matter, which shows host-parasite-nutrition relationships (Roca 1999; O’Grady et al., 2005; O’Grady & Dearing, 2006; Donaldson, Lee & Mazmanian, 2016; Midha, Schlosser & Hartmann, 2017). Some studies have suggested a relationship between the kind of oxyurids and the feeding habits of the reptile host. For instance, the Pharyngodonidae (order Oxyurida) nematodes are endosymbionts typical of reptiles with herbivorous diets (Roca 1999; O’Grady et al., 2005; O’Grady & Dearing, 2006). Hindgut oxyurids are frequently found in chelonians and they are generally considered to have a commensal or even a mutualistic interaction with their hosts and are rarely pathogenic (Roca 1999; Mitchell & Figueroa, 2005; Jacobson, 2007).

Habitat loss and fragmentation influence local climate, resource availability and also the distribution, abundance and behaviour of wildlife species and could therefore change the ecology of parasite and/or endosymbiont transmission (Kareiva, 1987; Patz et al., 2004; Aguirre & Tabor, 2008; Fuentes-Montemayor et al., 2009; Benavides et al., 2012; Suzán et al., 2012; Benítez-Malvido et al., 2016; Pringle, 2016). Furthermore, there is evidence that habitat loss and fragmentation reduce individual fitness for several animal species (e.g., bats, primates, rodents, toads, lizards and tortoises) through changes in individual body size, as well as decreased growth...
and reproduction (Patz et al., 2004; Amo, López & Martín, 2007; Fuentes-Montemayor et al., 2009; Janin, Léna & Joly et al., 2011; Benavides et al., 2012; Rodríguez-Caro et al., 2013). In disturbed habitats, less vigorous and stressed individuals are more vulnerable to parasite infection, whereas an infected individual is more prone to be infected by secondary pathogen species concurrently (Patz et al. 2004; Beldomenico & Begon, 2009; Benavides et al., 2012).

The southeastern Iberian Peninsula, as well as other Mediterranean areas, has been an important speciation hot spot and refuge for the survival of several plant and animal species (Hewitt, 2011). Nevertheless, this region has historically been subjected to different human pressures for centuries so that several native species are currently threatened at present because of the increased conversion of natural habitats to agricultural and other land uses (Sánchez-Zapata & Calvo, 1999; Anadón et al., 2006, Anadón, Wiegand & Giménez, 2012; Anadón et al., 2012; Hewitt, 2011; Rodríguez-Caro et al., 2017). In this study, we exclusively used the oxyurids (Pharyngodonidae) present in the hindguts of spur-thighed tortoises, Testudo graeca L. (F. Testudinidae) in scrublands of southeastern Spain as a research model to determine whether the level of habitat loss (low, intermediate and high) is associated with the incidence of oxyurids and individual body traits (i.e., carapace length and tortoise weight) and growth rates. The available information has shown that the abundance of T. graeca within its original distribution range is strongly associated to environmental factors, such as rainfall and temperature, because as ectotherms, terrestrial tortoises tend to react strongly to variations in their environment (Kaspari & Valone, 2002; Anadón et al., 2006, Anadón, Wiegand & Giménez, 2012; Anadón et al., 2012; Rodríguez-Caro et al., 2016). Furthermore, such factors have shown strong shifts in fragmented landscapes when compared to continuous natural habitats in different ecosystems.
elsewhere (Fuentes-Montemayor et al., 2009; Janin, Léna & Joly et al., 2011; Anadón, Wiegand & Giménez, 2012; Anadón et al., 2012; Laurance et al., 2017).

Nematodes are the main helminths infecting terrestrial chelonians, and most of them belong to the orders Ascaridida and Oxyurida, which are transmitted by the orofaecal route and sexual contact (Chávarri et al., 2012). Ascarid infections are associated with carapace deformities and symptoms of upper respiratory tract disease for captive T. graeca individuals (Rideout et al., 1987; Jacobson 2007); whereas no ascarid infections have been reported for wild tortoise populations in the study region (Chávarri et al., 2012). In contrast, the role of oxyurids as a limiting factor of tortoise populations is not well understood. In fact, oxyurids can be highly prevalent and are considered to have an almost commensal relationship with their hosts (Roca, 1999; Jacobson 2007). In addition, oxyurid infections and prevalence increase with age and/or the tortoise’s size, as has been found for other vertebrates (Morand & Poulin 1998; Arneberg 2002; Jacobson 2007; Benavides et al., 2012). For the study region, reductions in growth rates have been reported for T. graeca in disturbed habitats as well as differences in nematode communities between wild and captive tortoises (Chávarri et al., 2012; Rodríguez-Caro et al., 2013). Thus, we expect that tortoises’ body traits, growth rates, oxyurid infestation and their interaction are sensitive to habitat loss and fragmentation.

Ecological networks are increasingly being used as a valuable tool for studying the complexity of biotic interactions at a community level, including both mutualistic and antagonistic interactions (Bascompte et al., 2003; Bascompte & Jordano, 2007; Benítez-Malvido & Dátilo, 2015; Zarazúa-Carbajal et al., 2016; Hernández-Martínez et al., 2018). Within a given community, different organisms and animal species can interact with each other, which generates complex ecological networks (Bascompte & Jordano, 2007). A large number of studies have
found no random structures in ecological networks, including nestedness (Bascompte et al., 2003; Bascompte & Jordano, 2007; Almeida–Neto et al., 2008). A network is considered nested if species with fewer interactions (specialists) are connected with species with the most interactions (generalists) in cohesive subgroups (i.e., more generalized networks) (Guimarães et al., 2006; Bascompte & Stouffer, 2009). Despite the importance and increased knowledge of ecological networks in the literature, most studies have focused on mutualistic interactions (e.g., pollination and seed dispersal; Bascompte & Jordano, 2013), whereas antagonistic and commensal interactions involving animals and their pathogens and endosymbionts have received less attention (however, see Kareiva, 1987; Benítez-Malvido & Dáttilo, 2015; Zarazúa et al., 2016; Hernández-Martínez et al., 2018).

The general aims of this study were the following: (1) to assess if the level of habitat loss alters the local abundance, species composition, richness, and the diversity and structure of ecological networks of oxyurids associated with *T. graeca*, as well as (2) to describe the interaction type between tortoises and oxyurids (i.e., positive, neutral and/or negative) as indicated by the tortoises’ body traits and growth rates. We expected that tortoise’s body traits, growth rates, oxyurid communities and host-oxyurid interactions to vary with the level of habitat loss, with landscapes of low habitat loss differing from the other landscapes considered (Patz et al., 2004; Qian & Ricklefs, 2006; Fuentes-Montemayor et al., 2009; Suzán et al., 2012). It is clear that habitat degradation affects the nature of biotic interactions and ecosystems’ function; for herbivorous reptiles, however, there is a limited understanding of the role that hindgut nematodes have on them. Furthermore, conventional community descriptors have not always been successful in distinguishing among habitats with different levels of degradation, which indicates that alterations of the structure and function of ecological communities might be unnoticed in
conservation research that does not document and quantify species interactions through an ecological network approach (Kareiva, 1987; Tylianakis et al., 2007; Kaiser-Bunbury & Blüthgen, 2015).

Material & Methods

Study area

The study region covering the southeastern *T. graeca* Spanish population comprises approximately 2600 km² of semiarid coastal mountains between the Almeria and Murcia provinces (longitude N38° 23 to N36° 20´ and latitude W0° 30 to W2° 20´; Fig. 1). The climate of the region is semiarid with mild winters, hot summers and low rainfall (200 to 350 mm/year) during the cooler months of the year. This region has been subjected to human intervention for centuries (Hewitt, 2011). Land-use practices are among the most important factors, together with climate, relief and lithology, for determining *T. graeca* presence within its distribution range in southeastern Spain (Anadón et al., 2006). Such practices include traditional agricultural crops (dry crops known as secanos), heavily intensified irrigated agricultural lands, and highways (Anadón et al., 2006). Intensified agricultural practices and fires, as well as habitat reforestation with pines, have been shown to negatively affect *T. graeca* growth, survival and population viability (Anadón et al., 2006; Sanz-Aguilar et al., 2011; Rodríguez-Caro et al., 2013, 2016, 2017; Graciá et al., in press). Furthermore, in this mosaic of land-use practices, tortoise populations require native scrubland patches larger than $\geq 1 \text{ km}^2$ or 75 % scrubland cover at the landscape level to remain viable and prevent local extinctions (Anadón et al., 2006). The study sites were located in landscapes with different levels of land use intensification, habitat loss and fragmentation. Nevertheless, traditional agricultural crops (secanos), when not extensive, are
recognized as suitable habitats for \textit{T. graeca} populations, and therefore the level of habitat loss was established according to the proportion of suitable habitat for tortoises in the landscape. Each site and/or tortoise’s population represents one square kilometre, which correspond to a UTM (Universal Transverse Mercator) cell of 1 × 1 km. In this sense, the landscapes with low habitat loss (sampling sites in Villaltas, Galera and Marinica) comprised on average 95 % of suitable habitat, including 84.59 % scrubland and 10.75 % of traditional agriculture. Landscapes with intermediate habitat loss (Bas Norte, Sierrecica and Chinas) comprised 81.60 % of suitable habitat, including 62.21 % scrubland and 19.39 % traditional agriculture. Finally, landscapes with high habitat loss (Palas, Bas Sur and Misiripalme) encompassed 76.26 % of suitable habitat, including 40.71 % scrubland and 35.54 % of traditional agriculture (Fig. 1). The loss of native scrubland habitat, scrubland fragmentation and land use intensification are related between each other. Native scrubland has been reduced by more than 50 % in landscapes with high habitat loss, whereas traditional agricultural fields (secanos) presented a threefold increase therein, when compared to landscapes with low habitat loss. These changes in landscape configuration and composition have been shown to affect resource distribution and availability, challenging the maintenance of tortoises and other animal populations elsewhere (Anadón et al., 2006; Janin, Léna & Joly et al., 2011; Benítez-Malvido et al., 2016; Pringle 2016; Rodríguez-Caro et al., 2017). The probability of \textit{T. graeca} encounter with less than 40 % scrubland cover is very low (Anadón et al., 2006).

\textbf{The spur-thighed tortoises and the prevalence of oxyurid nematodes}

The Dirección General de Gestión del Medio Natural de la Junta de Andalucía (SGB/FOA/AFR), the Delegación General de Medio Natural de la Comunidad Autónoma de la Región de Murcia
(AUT/ET/UND/48/2010) granted the permissions to sample the tortoises and oxyurids. The spur-thighed tortoise (*T. graeca*) is a midsize (up to 46 cm in length, Werner et al., 2016) terrestrial tortoise and one of the most widely distributed species of terrestrial tortoises. Although mostly distributed throughout North Africa, the Middle East and part of Eastern Europe, there are some small isolated populations in Western Europe (Graciá et al., 2017; Fig. 1). This tortoise species is typically present in arid and semiarid scrublands. The diet of *T. graeca* is based on several parts (i.e., leaves, flowers, fruits and twigs) of wild plants such as alfalfa (*Medicago sativa*), thistles (e.g., *Carthamus* and *Carlina*), dandelion (*Taraxacum*), rosemary (*Rosmarinus officinalis*), etc. (Andreu, Díaz-Paniagua & Keller, 2000; El Mouden et al., 2006). Furthermore, tortoises may disperse the seeds of the consumed plant species (Cobo & Andreu, 1988; Andreu, Díaz-Paniagua & Keller, 2000). The tortoises’ diet also includes animal carcasses, slugs and insects, mainly at early life stages. Currently, wild populations of *T. graeca* are severely threatened throughout their whole distribution range because of habitat loss and fragmentation, as well as over-collection, principally in southeastern Spain (Anadón et al., 2006, 2012; Anadón, Wiegand & Giménez, 2012; Pérez et al., 2012).

From mid March to mid June of 2010, at nine different localities (three for each level of habitat loss), the wild tortoises sighted were captured within standardized transects. Each of the nine sites was visited five times; the sampling time lasted for approximately 2 hours 30 minutes along ca. 1750 m individual transects. After sampling, specimens were marked, and the following individual traits were recorded without harming the tortoises: sex, age estimated from the carapace growth ring (Rodríguez-Caro et al., 2013, 2015), body weight (g) and carapace length (mm). Thereafter, a growth rate score per individual (mm year\(^{-1}\), \(k\)) was calculated using the von Bertalanffy model, the most used to describe chelonians’ growth (Zivkov et al., 2007;
Macale, Scalici & Venchi, 2009). This model assumes that the rate of growth declines with age (Fabens, 1965) and allows estimating the growth of each individual from its birth to the current measurement time. For further details, see Appendix 1.

For the study of oxyurid communities, individual freshly voided faecal samples were obtained immediately following the handling of the animal or from the cloth bag where animals were placed until they defecated. Thereafter, individuals were released in their capture sites, whereas tortoise faecal samples were maintained at 4 °C until processed within 48 h of collection. Firstly, a saline solution was added to the faeces, and under a microscope the adult oxyurids were collected and counted (Mac Arthur et al. 2004; Traversa et al. 2005; Jacobson 2007; Origgi and Paré 2007). Secondly, all collected adult oxyurids were washed in distilled water and subsequently stored in 70 % ethanol. Thirdly, oxyurids were cleared in Amann lactophenol solution and subsequently identified to the species level following several keys (Petter 1966; Bouamer & Morand, 2000, 2002, 2003 a, b, 2005, 2006; Bouamer, Morand & Bourgat, 2001 a, b, 2003; Bouamer, Morand & Kara, 2003). The number of Oxyuridae eggs and larvae present in the faeces were not considered in this study because it was not possible to identify these immature stages to the species level (Traversa et al., 2005). Species identification was necessary for the construction of the tortoise-oxyurid ecological networks. Nevertheless, the prevalence of Oxyuridae eggs was the most abundant life stage since 97 % of the sampled individuals contained eggs in their faeces; whereas oxyurid larvae and adults were present in 36 % and 72 % of the individuals, respectively. Faecal samples containing adult oxyurids always had oxyurid eggs as well (Chávarri et al., 2012). The number of oxyurid eggs, larvae and adults per captured tortoise ranged from 0 to 28 319, from 0 to 1 183, and from 0 to 5 245, respectively. Overall, 14 adult oxyurid species (all within the Oxyurida Family Pharyngodonidae) were
isolated in wild populations of *T. graeca*, including the following: *Tachygonetria dentata*, *T. longicollis*, *T. macrolaimus*, *T. conica*, *T. pusilla*, *T. numidica*, *T. robusta*, *T. setosa*, *T. palearticus*, *T. seurati*, *Alaeuris numidica*, *Mehdiella stylosa*, *M. uncinata* and *M. microstoma*.

We sampled a total of 66 wild tortoises from areas with low (26 individuals), intermediate (20 individuals), and high (20 individuals) habitat loss. In landscapes with low habitat loss, we recorded the following: 14 adult females, seven adult males, four subadults and two juveniles. In landscapes with intermediate habitat loss, we recorded three adult females, six adult males, seven subadults and four juveniles. Finally, for the landscapes with high habitat loss, we recorded nine adult females, four adult males, five subadults and two juveniles. Adults were considered as those individuals > 8 years old, and subadults were those individuals 5-8 years old. Juveniles were all those tortoises 1-4 years old (Sanz-Aguilar et al., 2011).

**Statistical analysis**

Because the levels of oxyurid infection and prevalence increase with age (Chávarri et al., 2012), we performed the statistical tests, firstly, by pooling all sampled individuals (adults, subadults and juveniles), and secondly, on adult tortoises exclusively. For tortoise density (individuals/ha) we analysed the entire sampled population. The data were analysed through generalized linear models (GLMs) by means of the program Statistica 7, and significance was set at the $\alpha = 0.05$ level. Firstly, to test if the level of habitat loss affected tortoise body traits, we used a one-way ANOVA. Body traits values among individual tortoises varied greatly within and across habitat types (e.g., body weight varied between 13 -1 220 g). Therefore except for growth rates, values were log (x+1) transformed to improve normality. Because data on oxyurid species richness and abundance were counts (Poisson distribution), we compared the levels of habitat loss by means
of log-linear models with a chi-square measure of deviance. When overdispersion was found in
the error term of the model, the data were rescaled to make the tests more conservative (Crawley
1993, 2007). We also tested if oxyurid richness and abundance were related to tortoise density
across habitats using log-linear models.

Secondly, to test if tortoise body traits and growth rates were related to oxyurid species
richness and abundance across habitat loss categories (the categorical predictor variable with
three fixed factors), we compared the linear regression slopes among habitats by using one-way
ANCOVAs, with tortoise body traits and growth rates as the dependent variables and oxyurid
species richness and abundance as the independent ones (covariates). In total, we performed 12
one-way ANCOVAs, one for each of the two covariates (oxyurid species richness and
abundance), by again initially pooling all life stages and then for adults only.

Oxyurid nematode species dominance

To test whether the level of habitat loss affected oxyurid species relative abundance and/or
dominance, we constructed rank/abundance plots for the oxyurid communities present in
tortoises at each of the scrubland landscapes (low, intermediate and high habitat loss). For each
level of habitat loss, we plotted the relative abundance of each oxyurid species on a logarithmic
scale against the species’ rank, ordered from the most to the least abundant species (Magurran
2004). To assess whether oxyurid species were evenly distributed (ecological evenness) across
the levels of habitat loss, we compared the slopes of the rank/abundance plots by means of an
ANCOVA. The species evenness of a given community is reflected in the slope of the line that
fits the rank/abundance plot (i.e., logarithmic series relationship). A steep slope indicates low
evenness since the high-ranking species are more abundant than the low-ranking species. A
gentle slope indicates higher evenness because species abundances are more similar (Magurran 2004).

**Patterns of species interactions by using a network approach**

We used a network approach to investigate the structure of individual-based tortoise-oxyurid networks and their underlying mechanisms. Only individuals with adult oxyurids were considered in the interaction network analysis, mainly adult and subadult tortoises. The number of infected tortoises in the three landscapes were 20, 13 and 15, for low, intermediate and high habitat loss, respectively.

Firstly, we included the presence of different oxyurid species infecting *T. graeca* individuals in each of the landscapes (low, intermediate and high habitat loss) as independent interaction networks. We thus constructed three weighted interaction networks, one for each of the habitat loss conditions. Each individual-based tortoise-oxyurid network was built by an adjacency matrix $A$, where $a_{ij}$ = the number of interactions from an individual tortoise $j$ by the oxyurid species $i$, or otherwise 0 (i.e., the absence of interaction). Thus, in these networks, oxyurid species and tortoises are depicted as nodes, and their interactions are represented by links describing the use of tortoise individuals by oxyurid species. Thereafter, we tested if such networks were nested, a pattern in which oxyurid species with fewer interactions (specialists) are connected with tortoise individuals with the most interactions (generalists) in cohesive subgroups. We used the *WNODF* metric (weighted nestedness metric based on overlap and decreasing fill; Almeida–Neto et al., 2008; Almeida-Neto & Ulrich, 2011) to estimate the nestedness value of the networks by using ANINHADO software (Guimarães & Guimarães, 2006). The *WNODF* metric evaluates whether or not the oxyurids of less selective tortoises...
represent subsets of the tortoises that are colonised by a broader number of oxyurids (i.e., species and individuals) for each habitat type. This metric ranges from 0 (no nestedness) to 100 (perfect nestedness). It reduces the chance of overestimating the degrees of nestedness in ecological networks and is less prone to Type I statistical error (Bascompte & Jordano, 2013). We estimated the significance of nestedness with a Monte Carlo procedure, generating 1000 random matrices from the original matrix by using the Null Model II (Bascompte et al., 2003). In this null model, the probability of occurrence of an interaction is proportional to the number of interactions of both oxyurid species and tortoise individuals (Bascompte et al., 2003). Secondly, we considered other network parameters in the analysis for each habitat type as follows (Kaiser-Bunbury & Blüthgen 2015): (i) connectance (the proportion of realized links from the pool of all possible links between the species of a network). Higher connectance indicates greater community stability, reflecting a more uniform spread of interactions among the species in the community; and (ii) interaction diversity ($\text{ID}$). This metric is derived from the Shannon diversity index and ranges from 0 (no diversity) to infinity (Blüthgen, Menzel & Blüthgen, 2006). Interaction diversity is the quantitative analogue to the total number of links. Higher $\text{ID}$ implies higher community stability. Finally, we considered (iii) the specialization index or resource selectivity at the network level ($H_2^\prime$). This selectivity index ranges from 0 (extreme generalization) to 1 (extreme specialization) and is highly robust regarding changes in sampling intensity and the number of interacting species (Blüthgen, Menzel & Blüthgen, 2006). High specialization indicates the high dependency of each oxyurid species on a few exclusive tortoise partners. Low specialization indicates higher functional redundancy (Kaiser-Bunbury & Blüthgen, 2015).

In addition, by using GLMs, we tested if tortoise body traits and growth rates explained
network parameters at the individual level for each habitat type (Kaiser-Bunbury & Blüthgen 2015). We used species-degree (the number of oxyurid species infecting each tortoise) and species-specialization (i.e., Blüthgen’s $d'$, a measure of the exclusiveness of a species’ partner spectrum compared with other species in the network, sensu Kaiser-Bunbury & Blüthgen 2015) as response variables. Species with low species-level specialization indicate opportunistic partner selection compared with other species in the network. For the parameter species-degree, a Poisson type error was used with a log-link function, while for species-specialization, simple linear regression models with Gaussian type errors were used. Network features and plots were obtained by using the Bipartite package in ‘R’ (Dormann, Gruber & Group, 2011; R Development Core Team, 2014).

Results

Overall, we found that the level of habitat loss affected tortoise-oxyurid interactions with consequences for tortoise growth rates, though not for tortoise weight and carapace length (Table 1). Moreover, tortoise density was not related to the richness and abundance of oxyurids. Not all sampled tortoises were infested by adult oxyurids across landscapes, with 27 %, 35 %, and 25 % of individuals being free from oxyurids, in low, intermediate and high habitat loss, respectively. The number of oxyurid species varied between 0 to10 species across tortoises.

Habitat loss, body traits and prevalence of oxyurid nematodes

On the one hand, the one-way ANOVAs and the analysis of deviance showed, through pooling all life stages and by considering only adults, that the level of habitat loss had no significant effect on tortoise body traits and density, nor on oxyurid richness and abundance (Table 1). On
the other hand, species richness and the abundance of oxyurids were significantly associated with growth rates depending on the level of habitat loss, which was evaluated both by pooling all life stages and then focusing on adults. Considering all life stages, the ANCOVA tests showed a significant, positive association between oxyurid species richness with tortoise growth rates, but only in landscapes with low habitat loss, as shown by the habitat loss level x oxyurid species richness interaction term (richness, $F_{2,58} = 3.46, P = 0.038$) (Fig. 2). Considering adult tortoises only, oxyurid infestation and growth rates were not consistently associated across habitat types (Fig. 2). In landscapes with high habitat loss, tortoises sustained greater growth rates than those with low and intermediate habitat loss (richness, $F_{1,2} = 6.39, P = 0.0042$; abundance, $F_{1,2} = 4.42, P = 0.019$), whereas the habitat loss level x oxyurid infestation interaction term was significant for both species richness ($F_{2,35} = 6.17, P = 0.0051$) and oxyurid abundance ($F_{2,35} = 4.24, P = 0.022$) (Fig. 2). While growth rates were positively associated to oxyurid infestation in landscapes with low habitat loss, the opposite was found in landscapes with high habitat loss; at intermediate habitat loss, no association existed between the two variables (Fig. 2).

Habitat loss and oxyurid nematode species dominance

The oxyurid community for the three levels of habitat loss followed a log series model of a small number of abundant species and a large proportion of rare species. The adult oxyurids most commonly found in the faeces were of the species $T. dentata$ and $T. longicollis$ (Fig. 3). The following patterns emerged in the rank-abundance plots: (1) all levels of habitat loss showed the same dominant oxyurid species; (2) although rare species changed ranks across the three levels of habitat loss, they were consistently the same species; and (3) the landscapes with high habitat loss presented one exclusive species, $T. seurati$, whereas $T. palearticus$ was absent from this
landscape type (Fig. 4). The slope analysis in the ANCOVA showed that oxyurid relative abundance differed significantly across habitats with more rare species at low habitat loss ($F_{2, 33} = 28.28, P < 0.001$). Furthermore, the slope at low habitat loss is significantly steeper than the slopes at intermediate and high habitat loss, as shown by the habitat loss level x oxyurid species rank interaction term ($F_{2, 33} = 13.93, P < 0.001$), which indicates that oxyurid species are more evenly distributed in the landscapes with intermediate and high habitat loss than in the landscapes at low habitat loss (Fig. 3).

**Network structure**

The size of our networks (hereafter referred to as tortoise-oxyurid networks) was similar in the number of oxyurid species among landscapes, suggesting that *T. graeca* maintains a stable community of these oxyurids at the three levels of habitat loss (Table 2 and Fig. 4). The lowest diversity of interactions ($ID$), indicating lower community stability, was at low habitat loss. In contrast, the intermediate level of habitat loss showed the greatest specialization level ($H_2'$), implying that individuals were infested by fewer oxyurids in these landscapes, whereas individuals were the most generalized (i.e., showing higher functional redundancy) at high habitat loss. This agrees with the greater connectance in such habitats, reflecting a more uniform spread of interactions among oxyurid species in the community at high habitat loss (Table 2 and Fig. 4). The three levels of habitat loss showed a significant nested pattern of interactions, though the landscapes with intermediate habitat loss exhibited the greatest nestedness values (Table 2). Furthermore, at the individual network level, body traits and growth rates explained to some extent network parameters (Table 2). Consistently in all habitats, heavier and larger tortoises showed a significantly positive relationship with species-degree, i.e., with species richness of
oxyurids in an individual, but not with species-specialization ($d'$). Moreover, species-degree tended to increase with both tortoise weight and carapace length in low (weight, $\chi^2 = 5.76$, df = 1, $P = 0.016$; length, $\chi^2 = 6.01$, df = 1, $P = 0.014$), intermediate (weight, $\chi^2 = 6.88$, df = 1, $P = 0.0087$; length, $\chi^2 = 13.42$, df = 1, $P = 0.0002$) and high habitat loss (weight, $\chi^2 = 4.33$, df = 1, $P = 0.037$). In this case, carapace length was positively related to species degree, though not significantly. In contrast, species-degree was related to individual growth rates only in landscapes with low levels of habitat loss ($\chi^2 = 6.65$, df = 1, $P = 0.01$), indicating that tortoises that grow faster interact with more oxyurid species in this habitat.

**Discussion**

Overall, the level of habitat loss and the conveyed environmental and land use changes were found to affect some aspects involved in the ecological interactions (e.g., at the population and individual level) between the spur-thighed tortoises and their adult hindgut oxyurids. Oxyurid infestation showed differential association only with hosts’ growth rates, across levels of habitat loss (Amo, López & Martín, 2007; Janin, Léna & Joly et al., 2011; Benavides et al., 2012). A possible explanation is that body weight and carapace length are strongly correlated to tortoise’s age (Rodríguez-Caro et al. 2013), and age classes were not homogeneously distributed across landscapes, which lead to great variability among sites. Conversely, individual growth rate depends on the relation between age and size and therefore is not affected by age distribution among populations (Appendix 1). Furthermore, larger and heavier tortoises were infected by more oxyurid species. These changes were evident in spite of the similar oxyurid communities in terms of species richness and composition across landscapes. In general, the association of oxyurid infestation with growth rates was related to the level of habitat loss, ranging from
positive, to neutral, to negative, for landscapes encompassing low, intermediate and high habitat
loss, respectively (Amo, López & Martín, 2007; Janin, Léna & Joly et al., 2011).

**Habitat loss and the costs and benefits of oxyurid nematode infestation**

The modification of the original scrubland vegetation by anthropogenic activities produces
strong shifts in the spatial configuration of the natural landscapes, as well as on their physical
(e.g., rainfall and temperature) and biological environments (e.g., vegetation structure, species
diversity and resources availability) (Patz et al., 2004; Qian & Ricklefs, 2006; Amo, López &
Martín, 2007; Janin, Léna & Joly et al., 2011; Suzán et al., 2012; Pringle 2016). These
environmental shifts may occur at multiple spatial and temporal scales, which affect complex
species interactions, including host-oxyurid interactions of any kind (e.g., positive, negative
and/or neutral) in the case of herbivorous reptiles (Harvell et al., 2002; Amo, López & Martin,
2007; Tylianakis et al., 2007; Janin, Léna & Joly et al., 2011; Benavides et al., 2012; Benítez-
Malvido et al., 2016; Benítez-Malvido, Lázaro & Ferraz, 2018; Hernández-Martínez et al.,
2018). Although our findings represent the standing incidence of adult oxyurids in *T. graeca*
tortoises (i.e., a single observation in time), we detected that changes in landscape configuration,
exemplified by the amount of suitable habitat, influenced some aspects of host-oxyurid
interactions as revealed by differences in oxyurid infestation levels, oxyurid species ecological
evenness, tortoises’ growth rates and by differences in the networks’ structure. There is evidence
showing that the most disturbed and homogenized landscapes (e.g., highly degraded landscapes)
are more susceptible to pest and pathogen invasions because human activities facilitate the
movement of exotic/invasive species to new areas and provide the conditions for their
establishment. An additional reason is that hosts might change their behavioural and
physiological ecology (Patz et al., 2004; Qian & Ricklefs, 2006; Amo, López & Martin, 2007; Janin, Léna & Joly et al., 2011; Tompkins et al., 2011; Suzán et al., 2012). Another factor influencing pathogen transmission and infestation, not detected in the present study, is host population density (Arneberg, 2002). Therefore, the type of endosymbiont interaction (positive, negative and/or neutral) between tortoises and hindgut oxyurids seems to depend on the environmental context, in which differences in resource availability (e.g., host food type and habitat structure) across habitats could be driving the observed variations among them (Tompkins et al., 2011; Pringle, 2016). Large oxyurid infestation was negatively associated with growth rate for adult tortoises in landscapes at high habitat loss, suggesting a parasitic interaction (Fig. 3). Only under unusual circumstances, such as reptiles kept in captivity, are oxyurids pathogenic (Jacobson 2007; Chávarri, et al. 2012). High habitat loss and fragmentation (extreme conditions) might have lowered the capacity of tortoises to cope with a parasite infection in more challenging conditions; alternatively, those individuals with low growth rates might have poorer acquired immunity to oxyurids (Amo, López & Martin, 2007; Janin, Léna & Joly et al., 2011).

Recording oxyurids at all life stages in feces is indicative that oxyurids have been inhabiting tortoise’s hindguts long enough to complete their life cycles, and therefore, probably affecting some aspects of tortoise’s fitness (Jacobson 2007). Our results also showed, however, that high oxyurid species richness and abundance in tortoises are positively related with growth rates at low habitat loss, which suggests that oxyurid infestation could play a positive role in the digestion and thus assimilation of plant matter, possibly indicating a mutualistic interaction (Roca, 1999; Mitchell & Figueroa, 2005; O’Grady et al., 2005; O’Grady & Dearing, 2006; Jacobson, 2007). Furthermore, at low habitat loss, tortoises could have a greater foraging range, i.e., could show higher resource seeking activity enhancing a faster growth though
simultaneously being exposed to greater oxyurid infection, regardless of their parasitic/commensal/mutualistic nature (Amo, López & Martín, 2007; Janin, Léna & Joly et al., 2011). Finally, tortoises within landscapes at intermediate habitat loss showed no significant relationship between oxyurid infestation and growth rates, suggesting a commensal interaction in which oxyurids had no apparent influence on tortoises’ growth. Alternatively, tortoise hosts in these landscapes might be able to sufficiently allocate resources for growth and parasite resistance so that parasite acquisition and resistance balanced out.

In contrast to what might be expected, the ANCOVA showed that overall growth rates were greater at high habitat loss for adult tortoises than at low habitat loss. In this context, some native animal species are able to persist in degraded lands through morphological, physiological and behavioural adjustments (Isabirye-Basuta & Lwanga, 2008; Tompkins et al., 2011). Terrestrial tortoises are ectotherms and therefore may strongly react to environmental changes (Kaspari & Valone, 2002; Anadón et al., 2006). It is possible that reduced scrubland vegetation cover (greater canopy openness) in landscapes with high habitat loss aid tortoises in more effectively regulating their body temperatures (Row & Blouin-Demers, 2006; Poulin & George-Nascimento, 2007; Sato et al., 2014). In addition, in some reptile species, an adequate thermoregulatory behaviour has been found to optimize the digestive performance, which in turn can affect growth rates (Avery et al., 1993; Anguilletta, Hill & Robson, 2002). Further studies should consider other indicators of habitat loss on the resource availability for tortoises and the impact on their populations, such as survival and reproductive success (Rodríguez-Caro et al., 2013).

For the present study region, habitat loss and fragmentation have been found to negatively affect the persistence of tortoise populations, but also the presence of native scrubland
habitat in the vegetation matrix has been reported to enable *T. graeca* to persist in anthropogenic landscapes (Anadón et al., 2006). There are no baseline studies, however, that help us to precisely determine the overall impact of land-use change in *T. graeca* populations because this region has been modified for many centuries by anthropogenic activities of distinct magnitude and intensity (Hewitt, 2011). Therefore, long-term studies on *T. graeca* population dynamics and oxyurid infestation dynamics and their consequences (in survival, growth rates and reproduction) are crucial for understanding habitat loss processes in population’s viability, as well as the wide-ranging role of oxyurid endosymbiotic interactions in tortoises.

Furthermore, it has been indicated that long-term studies are crucial for understanding the impact of sporadic natural disturbances and/or catastrophic events (e.g., flooding, fire and drought) on disease emergence, virulence and transmission (e.g., infection cycles) as well as on overall host health (Baldemonico & Begon, 2009; Tompkins et al., 2011; Zarazúa-Carbajal et al., 2016; Hernández-Martínez et al., 2018). The latter might apply as well for nematode endosymbionts. This information is relevant for the management of wild *T. graeca* populations and cannot be obtained in short-term studies, such as the present one. For instance, the information gathered in the present study was obtained during the spring months when resource availability is high (i.e., with several plant species blooming and fruiting). There is no information, however, on the prevalence and intensity of oxyurid infestation during seasons when food sources are scarce, nor between years, nor on the consequences for tortoise populations (Tompkins et al., 2011; Benavides et al., 2012).

**Ecological networks in the management of spur-thighed tortoise populations**

The oxyurid community infesting *T. graeca* populations was not randomly assembled but nested,
revealing a structured pattern for all levels of habitat loss. Tortoise-oxyurid network properties appear to be maintained in landscapes with contrasting levels of habitat loss, despite differences in species dominance, as well as host number and quality (tortoises’ sex, age and size). Our study contributes to the understanding of the structure of ecological interactions in contrasting landscapes. Within the same host population, we can often find both more selective (those individuals colonized by a few oxyurid species) or more opportunistic (those individuals colonized by many oxyurid species) individuals. Some network distribution metrics, such as $ID$ and $H_2$, may be suitable indicators for detecting human-induced changes in some endosymbiotic interactions (e.g., mutualistic, commensal and parasitic), including tortoise-oxyurid interactions (Kareiva, 1987; Tylianakis, et al., 2007; Kaiser-Bunbury & Blüthgen, 2015). For instance, theory predicts positive correlations between host population density and/or body mass, as well as parasite species richness (Morand & Poulin, 1998; Arneberg, 2002). This relationship could only be detected at the individual network level where tortoise body mass (i.e., weight) was associated with the number of oxyurid species interactions.

We found that as habitat loss increases host specificity decreases. In other words, hosts are infested by a wider array of oxyurids, making tortoises more vulnerable in an already resource-limited environment in case the oxyurid infestation turns out to be parasitic. The greater nested pattern in landscapes with intermediate and high habitat loss may also imply that oxyurids can be more easily transmitted through the entire network, something that would be less likely if the network had a more modular structure (i.e., consisting of very specialized host-oxyurid interactions).

It is worth noting, however, that the lack of identification of oxyurid eggs and larvae could have affected our results in several ways. For instance, oxyurid species richness might be
underestimated, and, thus, some network parameters might have been misinterpreted. The use of
adult oxyurids, however, made our study more conservative, as it was also inconvenient
considering all oxyurid eggs and larvae as “single species” in our ecological networks.

Furthermore, the positive host-parasite-nutrition relationships might be stronger in adult oxyurids
given their more developed gut microbial community; whereas adult oxyurids might, as well, be
more pathogenic than eggs and larvae (Jacobson, 2007).

Conclusions

We aimed to understand the ways habitat loss affected spur-thighed tortoise populations
and to link habitat loss and tortoise-oxyurid interactions by using a network approach. Our
findings indicate that the level of habitat loss is associated with growth rates and host-
endosymbiont interactions; in this sense, this study is the first to provide the structure of such
networks. Animals, at any stage of their life cycles, are subject to infestation by many different
organisms that may modify, improve or interrupt the vital functions that affect their fitness (Patz
et al., 2004; Aguirre & Tabor, 2008; Suzán, et al., 2012; Pringle, 2016). Moreover, molecular
studies -based on barcoding- would be very useful for identifying eggs and larvae and therefore,
to completely describe the oxyurid species conforming the nematode community in the spur-
thighed tortoise hindgut (Jacobson 2007 a, b; Origgi & Paré 2007). On the one hand, the rapid
degradation of natural habitats might cause a large proportion of species and populations to be
vulnerable to disease, and native and exotic pathogens could become a threat for both plant and
animal communities (Anderson et al., 2004; Patz et al., 2004; Vurro, Bonciani & Vannacci,
2010; Tompkins et al., 2011; Benítez-Malvido, Lázaro & Ferraz, 2018). On the other hand,
habitat degradation might also cause the loss of mutualistic and commensal interactions.
Therefore, it is essential to understand the nature and dynamics of symbiotic interactions, the life histories of the animal hosts and the effects of the local environmental conditions on all of them.

Acknowledgements

We warmly thank J. M. Lobato for his careful technical support and to P. M. Mojica and M. Fernández for their help with sampling tortoises. We are also very grateful to M. Chávarri for the isolation and identification of oxyurid species. An anonymous reviewer made very valuable suggestions to previous versions of the manuscript.

References

Aguilar R, Ashworth L, Galetto L, Aizen MA 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968-980 doi:10.1111/j.1461-0248.2006.00927.x.

Aguirre AA, Tabor GM. 2008. Global Factors Driving Emerging Infectious Diseases Impact on Wildlife Populations. Annals of the New York Academy of Sciences. 1149:1-3 doi:10.1196/annals.1428.052.

Almeida–Neto M, Guimaraes P, Guimaraes PR, Loyola RD, Ulrich W. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. Oikos 117:1227-1239 doi: 10.1111/j.0030-1299.2008.16644.x.

Almeida-Neto M, Ulrich W. 2011. A straightforward computational approach for measuring nestedness using quantitative matrices. Environmental Modelling & Software 26: 173-178. doi:10.1016/j.envsoft.2010.08.003.
Amo, IL, López P & Martín, J. 2007. Habitat deterioration affects antipredatory behavior, body condition, and parasite load of female Psammodromus algirus lizards. Canadian Journal of Zoology 85:743–751. DOI: 10.1139/Z07-052.

Anadón JD, Giménez A, Martínez M, Martínez J, Pérez I, Esteve MA. 2006. Factors determining the distribution of the spur-thighed tortoise Testudo graeca in South-East Spain: a hierarchical approach. Ecography 29:339-346 doi.org/10.1111/j.2006.0906-7590.04486.x.

Anadón, JD, Wiegand T, Giménez A. 2012. Individual-based movement models reveals sex-biased effects of landscape fragmentation on animal movement. Ecosphere 3:64 doi:10.1890/ES11-00237.1.

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology and Evolution 19:535-544 doi.org/10.1016/j.tree.2004.07.021.

Andreu AC, Díaz-Paniagua C, Keller C. 2000. La tortuga mora (Testudo graeca L.) en Doñana. Asociación Herpetológica Española, Barcelona.

Anguilletta Jr, MJ, Hill T, Robson MA. 2002. Is physiological performance optimized by thermoregulatory behavior?: a case study of the eastern fence lizard, Sceloporus undulatus. Journal of Thermal Biology 27: 199-204 doi.org/10.1016/S0306-4565(01)00084-5.

Arneberg P. 2002. Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of
mammals. *Ecography* **25**: 88-94. doi.org/10.1034/j.1600-0587.2002.250110.x

Avery, HW, Spotila JR, Congdon JD, Fischer Jr. RU, Standora EA, Avery SB. 1993. Roles of diet protein and temperature in the growth and nutritional energetics of juvenile slider turtles, *Trachemys scripta*. *Physiological Zoology* **66**:902-925 doi.org/10.1086/physzool.66.6.30163746.

Bascompte J, Jordano P, Melian CJ, Olesen J. 2003. The nested assembly of plant-animal mutualistic networks. *Proceedings of Natural Academy of Science of USA* **100**:9383-9387 doi.org/10.1073/pnas.1633576100.

Bascompte J, Jordano P. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology Evolution and Systematics* **38**:567-593 doi.org/10.1146/annurev.ecolsys.38.091206.095818.

Bascompte J, Stouffer DF. 2009. The assembly and disassembly of ecological networks. Philosophical Transactions of the *Royal Society of Biology* **364**:1781-1787 DOI: 10.1098/rstb.2008.0226

Bascompte J, Jordano P. 2013. Mutualistic Networks. Princeton University Press. ISBN9781400848720.

Beldomenico PM, Begon M. 2009. Disease spread, susceptibility and infection intensity: vivious circles? *Trends in Ecology and Evolution* **25**:22-27 doi.org/10.1016/j.tree.2009.06.015.

Benavides JA, Huchard E, Pettorelli N, King AJ, Brown ME, Archer CE, Appleton CHC, Raymond M, Cowishaw G. 2012. From parasite encounter to infection: multiple-scale drivers of parasite richness in a wild social primate population. *American Journal of Physical Anthropology* **147**:52–63. doi.org/10.1002/ajpa.21627
Benítez-Malvido J, Dáttilo W. 2015. Interaction intimacy of pathogens and herbivores with their plant hosts determines the topological structure of ecological networks in different ways. *American Journal of Botany* **102**:1-8 doi.org/10.3732/ajb.1400303.

Benítez-Malvido J, Martínez-Falcón AP, Dáttilo W, González-DiPierro AM, Lombera-Estrada R, Traveset A. 2016. The role of sex and age in the architecture of intrapopulation howler monkey-plant networks in continuous and fragmented rainforests. *Peer J* doi:10.7717/peerj.1809.

Benítez-Malvido J, Lázaro A, Ferraz IDK. 2018. Effect of distance to edge and edge interaction on seedling regeneration and biotic damage in tropical rainforest fragments: A long-term experiment. *Journal of Ecology* doi:10.1111/1365-2745.13003.

Blüthgen N, Menzel F, Blüthgen N. 2006. Measuring specialization in species interaction networks. *BMC Ecology* **6**:12-18 doi:10.1186/1472-6785-6-9.

Bouamer S, Morand S. 2000. Oxyuroids of Palearctic Testudines: new definition of the genus *Thaparia* Ortlepp 1933 (Nematoda: Pharyngodonidae), redescriptions of *T. thapari thapari*, and descriptions of two species. *Comparative Parasitology* **67**:169-180 https://www.cabdirect.org/cabdirect/abstract/20000809684.

Bouamer S, Morand S. 2002. Descriptions of two new species of the genus *Tachygonetria* Wedl, 1862 (Nematoda-Pharyngodonidae) and discussion of the relationships of *Tachygonetria combesin* sp and redescriptions of four species of *Tachygonetria* Wedl, 1862 (Nematoda: Pharyngodonidae), with a new diagnosis of the genus. *Systematic Parasitology* **53**:121-139 doi.org/10.1023/A:102044390

Bouamer S, Morand S. 2003a. Descriptions of two new species of the genus *Tachygonetria* Wedl, 1862 (Nematoda-Pharyngodonidae) and discussion of the relationships
among the species of the genus. *Parasitology Research* 91:68-73 doi.org/10.1007/s00436-003-0932-4.

Bouamer S, Morand S, 2003 b. Phylogeny of Palaearctic Pharyngodonidae parasite species of Testudinidae: a morphological approach. *Canadian Journal of Zoology* 81:1885-1893 doi.org/10.1139/z03-166.

Bouamer S, Morand S, 2005. Descriptions of two new species of the genus *Tachygonetria* Wedl, 1862 (Nematoda, Pharyngodonidae) and redescriptions of five species parasites of Palaearctic Testudinidae. *Zoosystema* 27:193-209 doi.org/10.1007/s00436-003-0932-4.

Bouamer S, Morand S. 2006. Nematodes parasites of Testudinidae (Chelonia): list of species and biogeographical distribution. *Annales Zoologici, Warszawa* 56: 225-240 doi.org/10.3161/000345406778700775.

Bouamer S, Morand S, Bourgat R. 2001a. Oxyuroids of Palaearctic Testudinidae-new definition for *Alaeuris* Seurat, 1918 (Nematoda: Pharyngodonidae) and redescription of *Alaeuris numidica* (Seurat 1918). *Journal of Parasitology* 87:128-133 doi.org/10.1645/0022-3395(2001)087[0128:OOPTND]2.0.CO;2.

Bouamer S, Morand S, Bourgat R. 2001b. Redescription of *Mehdiella microstoma* and description of *Mehdiella petterae* sp. n., with a new definition of the genus *Mehdiella* Seurat, 1918 (Nematoda: Pharyngodonidae). *Folia Parasitologica* 48:132-138.

Bouamer S, Morand S, Kara M. 2003. Redescription of four species of *Mehdiella* from Testudinidae, with a key to the species and discussion on the relationships among the species of this genus. *Parasite* 10:333-342 doi.org/10.1051/parasite/2003104333.
Brooks TM, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Rylands AB, Konstant WR, Flick P, Pilgrim J, Oldfield S, Magin G, Hilton-Taylor C. 2002. Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* **16**:909-923 doi.org/10.1046/j.1523-1739.2002.00530.x.

Chávarri M, Berriatua E, Giménez A, Gracia E, Martinez-Carrasco C, Ortiz JM, de Ybanez RR. 2012. Differences in helminth infections between captive and wild spur-thighed tortoises *Testudo graeca* in southern Spain: A potential risk of reintroductions of this species. *Veterinary Parasitology* **187**:491-497 doi.org/10.1016/j.vetpar.2012.02.007.

Cobo M, Andreu AC. 1988. Seed consumption and dispersal by the spur-thighed tortoise *Testudo graeca*. *Oikos* **51**:267-273 DOI: 10.2307/3565307.

Crawley MJ. 1993. GLIM for ecologists. Blackwell Scientific, Cambridge, United Kingdom.

Crawley MJ. 2007. The R book. West Sussex: John Wiley and Sons.

Donaldson GP, Lee SM, Mazmanian SK. 2016. Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology* **14**: 20–32 doi.org/10.1038/nrmicro3552.

Dormann CF, Gruber B, Group R, 2011. Package Bipartite: visualizing bipartite networks and calculating some ecological indices. R Statistical Software. Available at http://www.r-project.org.

El Mouden EH, Slimania T, Kaddour KB, Lagarde F, Ouhammou A, Bonnet X. 2006. *Testudo graeca graeca* feeding ecology in an arid and overgrazed zone in Morocco. *Journal of Arid Environments* **64**:422-435 doi.org/10.1016/j.jaridenv.2005.06.010.

Fabens AJ. 1965. Properties and fitting of the von Bertalanffy growth curve. *Growth* **29**:265-289 https://www.cabdirect.org/cabdirect/abstract/19661402866.
Fahrig L. 2003. Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution and Systematics* **34**:487-515. doi.org/10.1146/annurev.ecolsys.34.011802.132419.

Fahrig L, Arroyo-Rodríguez V, Bennetta JR, Boucher-Lalonde V, Cazetta E, J. Currie D, Eigenbrod F, Ford AT, Harrison SP, Jaeger JAG, Koper N, Martin AE, Martin JL, Metzger JP, Morrison P, Rhodes JR, Saunders DA, Simberloff D, Smith AC, Tischendorf L, Vellend M, Watling JI. 2019. Is habitat fragmentation bad for biodiversity? *Biological Conservation* **230**:179–186 doi.org/10.1016/j.biocon.2018.12.026.

Fuentes-Montemayor E, Cuarón AD, Vázquez-Domínguez E, Benítez-Malvido J, Valenzuela D, Andresen E. 2009. Living on the edge: roads and edge effects on animal populations. *Journal of Animal Ecology* **78**:857-865 http://hdl.handle.net/1893/16991.

Graciá E, Vargas-Ramírez M, Delfino M, Anadón JD, Giménez A, Fahd S, Corti C, Jdeidi TB, Fritz U. 2017. Expansion after expansion: dissecting the phylogeography of the widely distributed spur-thighed tortoise, *Testudo graeca* (Testudines: Testudinidae) *Biological Journal of the Linnean Society* **121**:641-654 doi.org/10.1093/biolinnean/blx007.

Graciá E, Rodríguez-Caro RC, Sanz-Aguilar A, Anadón JD, Botella F, García-García AL, Wiegand T, Giménez A. (In press). Assessment of the key evolutionary traits that prevent extinctions in human-altered habitats using a spatially explicit individual-based model. *Ecological modelling*.

Guimarães PR Jr, Guimarães PR. 2006. Improving the analyses of nestedness for large sets of matrices. *Environmental Modelling Software* **21**:1512-1513 doi.org/10.1016/j.envsoft.2006.04.002.
Guimarães PR, Rico-Gray V, Dos Reis SF, Thompson JN. 2006. Asymmetries in specialization in ant-plant mutualistic networks. *Proceedings of the Real Society of Biological Science* 273:2041-2047 doi:10.1098/rspb.2006.3548.

Harvell C, Mitchell CE, Ward JR, Atizer S, Dobson AP, Ostfeld RS, Samuel MD. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296:2158-2162. doi.org/10.1126/science.1063699.

Hernández-Martínez J, Morales-Malacara JB, Alvarez-Añorve MY, Amador-Hernández S, Oyama K, Avila-Cabadilla LD. 2018. Drivers potentially influencing host–bat fly interactions in anthropogenic neotropical landscapes at different spatial scales. *Parasitology* 1-15. doi.org/10.1017/S0031182018000732.

Hewitt GM. 2011. Mediterranean Peninsulas: The evolution of hotspots. In: Zachos FE, Habel JC, eds. Biodiversity Hotspots. Distribution and Protection of Conservation Priority Areas. Springer, Berlin. 123-147. ISBN 978-3-642-20992-5.

Isabirye-Basuta GM, Lwanga JS. 2008. Primate populations and their interactions with changing habitats. *International Journal of Primatology* 29:35-48 doi.org/10.1007/s10764-008-9239-8.

Jacobson ER. 2007. Parasites and parasitic diseases of reptiles. In: Infectious diseases and pathology of reptiles. Jacobson ER. Ed. CRC Press. Florida USA. 571-666. ISBN 0-8493-2321-5.

Janin A, Léna JP, Joly P. 2011. Beyond occurrence: body condition and stress hormone as integrative indicators of habitat availability and fragmentation in the common toad. *Biological Conservation* 144:1008-1016. doi.org/10.1016/j.biocon.2010.12.009.

Kaiser-Bunbury CN, Blüthgen N. 2015. Integrating network ecology with applied
conservation: a synthesis and guide to implementation. *AoB PLANTS* 7: plv076;
doi:10.1093/aobpla/plv076.

Kareiva P. 1987. Habitat fragmentation and the stability of predator–prey interactions. *Nature* 326:388-390. doi.org/10.1038/326388a0.

Kaspari M., Valone TJ. 2002. On ectotherm abundance in a seasonal environment-studies of a desert ant assemblage. *Ecology* 83:2991-2996 doi.org/10.1890/0012-9658(2002)083[2991:OEAIAS]2.0.CO;2.

Laurance WF, Camargo JL, Fearnside PM, Lovejoy TE, Williamson GB, Mesquita RCG, Laurance SGW. 2017. An Amazonian rainforest and its fragments as a laboratory of climate change. *Biological Reviews* 93:223-247 doi.org/10.1111/brv.12343.

Macale D, Scalici M, Venchi A. 2009. Growth, mortality, and longevity of the Egyptian tortoise *Testudo kleinmanni* Lortet, 1883. *Israeli Journal of Ecology and Evolution* 55: 133-147. doi.org/10.1560/IJEE.55.2.133.

McArthur S, Meyer J, Innis C. 2004. Anatomy and physiology. In: Medicine and surgery of tortoises and turtles. McArthur S, Wilkinson R, Meyer J. Eds.,Blackwell Publishing Ltd., Oxford, UK, 35–72. SBN: 978-1-405-10889-8.

Magurran AE. 2004. Measuring biological diversity. Oxford: Blackwell. ISBN 0-632-05633-9.

Midha A, Schlosser J, Hartmann S. 2017. Reciprocal interactions between nematodes and their microbial environments. *Frontiers in Cellular and Infection Microbiology* 7:144. doi: 10.3389/fcimb.2017.00144.

Mitchell MA, Figueroa O. 2005. Clinical reptile gastroenterology. *Veterinary Clinics: Exotic Animal Practice* 8: 277-298 doi.org/10.1016/j.cvex.2005.01.008.
Morand S, Poulin R. 1998. Density, body mass and parasite species richness of terrestrial mammals. *Evolutionary Ecology* **12**:717-727 doi.org/10.1023/A:100653760

O’Grady SP, Morando M, Avila L, Dearing D. 2005. Correlating diet and digestive tract specialization: Examples from the lizard family Liolaemidae. *Zoology* **108**:201-210 doi.org/10.1016/j.zool.2005.06.002.

O’Grady SP, Dearing D. 2006. Isotopic insight into host–endosymbiont relationships in Liolaemid lizards. *Oecologia* **150**:355-361 doi.org/10.1007/s00442-006-0487-z.

Origgi, F.C. and Paré, J.A. 2007. Isolation of pathogens. In: Infectious diseases and pathology of reptiles. Jacobson ER. Ed. CRC Press. Florida USA. 667-680. ISBN 0-8493-2321-5.

Jacobson ER. 2007. Parasites and parasitic diseases of reptiles. In: Infectious diseases and pathology of reptiles. Jacobson ER. Ed. CRC Press. Florida USA. 571-666. ISBN 0-8493-2321-5.

Patz JA, Daszak P, Tabor GM, Aguirre AA, Pearl M, Epstein J, Wolfe NA, Kilpatrick AM, Foufopoulos J, Molynex D, Bradley DJ, David J, Butler C. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. Environmental health perspectives **112**:1092-1098 doi.org/10.1289/ehp.6877.

Pérez I, Tenza, A, Martínez J, Anadón JD, Graciá E, Giménez A. 2012. Exurban sprawl increases the extinction probability of an endangered tortoise due to well-intentioned collections. *Ecological Modelling* **245**:19-30 doi.org/10.1016/j.ecolmodel.2012.03.016.

Petter AJ. 1966. Équilibre des espèces dans les populations de nématodes parasites du colon des tortues terrestres. *Mémoires du Muséum National d’Histoire Naturelle. Série A, Zoologie, Paris* **39**:252.
Poulin R, George-Nascimento M. 2007. The scaling of total parasite biomass with host body mass. *International journal for parasitology* **37**:359-364
doi.org/10.1016/j.ijpara.2006.11.009.

Pringle E. 2016. Orienting the Interaction Compass: Resource Availability as a Major Driver of Context Dependence. *Plos One* DOI:10.1371/journal.pbio.2000891.

Qian H, Ricklefs RE. 2006. The role of exotic species in homogenizing the North American flora. *Ecology Letters* **9**:1293-1298 doi.org/10.1111/j.1461-0248.2006.00982.x.

R DEVELOPMENT CORE TEAM. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rideout BA, Montali RJ, Phillips LG, Gardiner CH. 1987. Mortality of captive tortoises due to viviparous nematodes of de genus *Proatractis* (family Atractidae). *Journal of Wildlife Diseases* **23**:103-108 doi.org/10.7589/0090-3558-23.1.103.

Rizzo DM, Garbelotto M, Davidson JM, Salaughter GW, Koike ST. 2002. *Phytophtora ramorum* as the cause of extensive mortality of *Querqus* ssp. and *Lothocarpus densiflorus* in California. *Plant Disease* **86**:205-214 doi.org/10.1094/PDIS.2002.86.3.205.

Roca V.1999. Relación entre las faunas endoparásitas de reptiles y sus tipos de alimentación. *Revista Española de Herpetología* **13**:101-121.

Rodríguez-Caro RC, Gracía E, Anadón JD, Giménez A. 2013. Maintained effects of fire on individual growth and survival rates in a spur-thighed tortoise population. *European Journal of Wildlife Research* **59**:911-913 doi.org/10.1007/s10344-013-0764-1.

Rodríguez-Caro R, Graciá E, Dos Santos RM, Anadón JD, Gimenez A. 2015. One scute ring per year in *Testudo graeca*? A novel method to identify ring deposition patterns in tortoises. *Acta Herpetologica* **10**:77-84 doi.org/10.13128/Acta_Herpetol-14979.
Rodríguez-Caro, R. C., Lima, M., Anadón, J. D., Graciá, E., & Giménez, A. 2016. Density dependence, climate and fires determine population fluctuations of the spur-thighed tortoise *Testudo graeca*. *Journal of Zoology* **300**: 265-273 doi.org/10.1111/jzo.12379.

Rodríguez-Caro RC, Oedekoven CS, Graciá E, Anadón JD, Buckland ST, Esteve-Selma MA, Martínez J, Giménez A. 2017. Low tortoise abundances in pine forest plantations in forest-shrubland transition areas. *Plos One* doi.org/10.1371/journal.pone.0173485.

Row JR, Blouin-Demers G. 2006. Thermal quality influences effectiveness of thermoregulation, habitat use, and behaviour in milk snakes. *Oecologia* **148**:1-11 doi.org/10.1007/s00442-005-0350-7.

Sánchez-Zapata JA, Calvo JF. 1999. Raptor distribution in relation to landscape composition in semi-arid Mediterranean habitats. *Journal Applied Ecology* **36**:254-262 doi.org/10.1046/j.1365-2664.1999.00396.x.

Sanz-Aguilar A, Anadón JD, Giménez A, Ballestar R, Graciá,E, Oro D. 2011. Coexisting with fire: the case of the terrestrial tortoise *Testudo graeca* in Mediterranean shrublands. *Biological Conservation* **144**:1040-1049 doi.org/10.1016/j.biocon.2010.12.023.

Sato CF, Wood JT, Schroder M, Michael DR, Osborne WS, Green K, Lindenmayer DB. 2014. Designing for conservation outcomes: the value of remnant habitat for reptiles on ski runs in subalpine landscapes. *Landscape Ecology* **29**: 1225-1236 doi.org/10.1007/s10980-014-0058-3.

Suzán G, Esponda F, Carrasco-Hernández R, Aguirre AA. 2012 Habitat fragmentation and infectious disease ecology. In: Aguirre AP, Daszak, Ostfeld RS. ed. *Conservation Medicine: Applied cases of Ecological Health*. Oxford University Press. ISBN 978-0-19-973147-3.

Tompkins DM, Dunn AM, Smith MJ, Telfer S. 2011.Wildlife diseases:
from individuals to ecosystems. *Journal of Animal Ecology* **80**:19-38 doi 10.1111/j.1365-2656.2010.01742.x.

Traversa, D., Capelli, G., Iorio, R., Bouamer, S., Cameli, A., Giangaspero, A., 2005. Epidemiology and biology of nematodofauna affecting Testudo hermanni, Testudo graeca and Testudo marginata in Italy. *Parasitological Research*. **98**: 14–20.

Trumbore S, Brando P, Hartmann H. 2015. Forest health and global change. *Science* **349**:814-818 doi.org/10.1126/science. Aac 6759.

Tylianakis JM, Tscharntke T, Lewis OT. 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature* **445**:202-205 doi.org/10.1038/nature05429.

Vurro M, Bonciani B, Vannacci G. 2010. Emerging infectious diseases of crop plants in developing countries: impact on agriculture and socio-economic consequences. *Food Security* **2**:113-132 doi.org/10.1007/s12571-010-0062-7.

Werner YL, Korolker N, Sion G & Göçmen B. 2016. Bergmann's and Rensch's rules and the spur-thighed tortoise (*Testudo graeca*). *Biological Journal of the Linnean Society* **117**: 796-811. doi.org/10.1111/bij.12717.

Zarazúa-Carbajal M, Saldaña-Vázquez RA, Sandoval-Ruiz CA, Stoner KE, Benitez-Malvido J. 2016. The specificity of host-bat fly interaction networks across vegetation and seasonal variation. *Parasitology Research* doi: 10.1007/s00436-016-5176-1.

Zivkov M, Ivanchev I, Raikova-Petrova G, Trichkova T. 2007. First data on the population structure, growth rate and ontogenetic allometry of the tortoise *Testudo hermanni* in eastern Stara Planina (Bulgaria) - *Comptes Rendus de L’Academie Bulgare des Sciences* **60**:1015-1022.
Figure legends

Figure 1. The map shows the distribution of the spur-thighed tortoise (*Testudo graeca*) in the Mediterranean basin. The wild populations sampled for the present study and their localities are indicated with dots: Villaltas, Galera and Marinica, as landscapes with low habitat loss for tortoises; Bas Norte, Sierrecica and Chinas, as landscapes with intermediate habitat loss; and Palas, Bas Sur and Misiripalme, as landscapes with high habitat loss. These wild Spanish populations of *T. graeca* cover approximately 2600 km$^2$ of semiarid coastal mountains between the Almeria and Murcia provinces.

Figure 2. Best-fit linear regressions, with 95% confidence intervals, between oxyurid nematode infestation (i.e., species richness and abundance) and tortoises’ (*Testudo graeca*) growth rates in scrubland landscapes in southern Spain. Tortoises were present in landscapes sustaining different levels of habitat loss (low, intermediate and high). (A and B) Tortoise populations at all life stages. (C and D) Adult tortoise populations only. Significant relationships are indicated by *$P < 0.01$ and/or **$P < 0.001$. The values for the linear regression equation ($y = a + bx$) and the resulting $r$-values are indicated for each case.

Figure 3. Rank/abundance plots for the oxyurid nematode community infesting tortoises (*Testudo graeca*) in scrubland landscapes with different levels of habitat loss (low, intermediate and high) in southern Spain. For each landscape, the relative abundance of each nematode species is plotted on a logarithmic scale against the species’ rank, from the most to the least abundant species. Nematodes included the following species: *Alaeuris numidica* (*A. num*), *Mehdiella stylosa* (*M. styl*), *M. uncinata* (*M. unci*), *M. microstoma* (*M. micro*), *Tachygonetria*...
longicollis (T. long), T. dentata (T. dent), T. conica (T. coni), T. robusta (T. rob), T. macrolaimus (T. mac), T. numidica (T. numi), T. setosa (T. set), T. palearticus (T. pale), T. pusilla (T. pusi), and T. seurati (T. seur). Nematode species not found under all habitat loss conditions are in **bold** red.

Figure 4. Quantitative bipartite plots of the tortoise-oxyurid species ecological networks for each landscape encompassing different levels of habitat loss (low, intermediate and high) in southern Spain. (A) Tortoise-oxyurid ecological network at low habitat loss. (B) Tortoise-oxyurid ecological network at intermediate habitat loss. (C) Tortoise-oxyurid ecological network at high habitat loss. The bar size represents the number of interactions each oxyurid species (black) had with each individual tortoise (*Testudo graeca*) in the population (grey), and the linkage width represents the proportion of oxyurid individuals involved in such interactions.
Table 1 (on next page)

Tortoise traits, growth rates and oxyurid nematode infestation

Body traits (mean ± SE), density and oxyurid nematode infestation (i.e., species richness and abundance) of spur-thighed tortoise populations (*Testudo graeca*) at scrubland landscapes with different levels of habitat loss in southern Spain. The values in **bold** are those exclusively from adult tortoises. There was no significant difference among levels of habitat loss on any variable.
Table 1. Body traits (mean ± SE), density and oxyurid nematode infestation (i.e., species richness and abundance) of spur-thighed tortoise populations (*Testudo graeca*) at scrubland landscapes with different levels of habitat loss in southern Spain. The values in **bold** are those exclusively from adult tortoises. There was no significant difference among levels of habitat loss on any variable.

| Body traits and nematode infestation | Low (N = 26) | Intermediate (N = 20) | High (N = 20) |
|-------------------------------------|--------------|-----------------------|--------------|
| Overall tortoise density (individuals/ha) | 2.57 ± 1.19 | 2.26 ± 0.66 | 0.83 ± 0.07 |
| Weight (g) | 431.79 ± 83.04 | 267.36 ± 59.41 | 410.10 ± 91.13 |
| | **527.00 ± 47.90** | **486.00 ± 75.78** | **534.91 ± 59.95** |
| Carapace length (mm) | 129.95 ± 6.86 | 123.44 ± 7.45 | 132.00 ± 7.84 |
| | **131.12 ± 4.01** | **127.10 ± 6.14** | **128.45 ± 5.45** |
| Growth rate (mm year⁻¹) | 0.115 ± 0.016 | 0.085 ± 0.019 | 0.124 ± 0.018 |
| | **0.131 ± 0.0149** | **0.123 ± 0.019** | **0.157 ± 0.025** |
| Adult oxyurid species richness | 2.59 ± 0.567 | 2.60 ± 0.66 | 3.55 ± 0.66 |
| | **3.82 ± 0.54** | **5.57 ± 1.31** | **5.27 ± 0.90** |
| Abundance of adult oxyurids | 303.63 ± 184.20 | 200.05 ± 214.01 | 360.30 ± 214.01 |
| | **480.82 ± 308.32** | **561.00 ± 311.05** | **614.27 ± 468.36** |
Table 2 (on next page)

Tortoise-oxyurid network parameters

Tortoise-oxyurid ecological network attributes at two hierarchical levels in landscapes that differ in their levels of habitat loss in scrublands of southern Spain.
Table 2. Tortoise-oxyurid ecological network attributes at two hierarchical levels in landscapes that differ in their levels of habitat loss in scrublands of southern Spain.

| Hierarchical level | Metrics               | Low  | Intermediate | High  |
|--------------------|-----------------------|------|--------------|-------|
| **Network level**  | No. of tortoises      | 20   | 13           | 15    |
|                    | Oxyurid species       | 13   | 13           | 13    |
|                    | Nestedness           | 37.23| 45.90        | 43.696|
|                    | (WNODF)              |      |              |       |
|                    | Connectance (C)       | 0.269| 0.308        | 0.364 |
|                    | Interaction diversity | 2.034| 2.371        | 2.397 |
|                    | (ID)                 |      |              |       |
|                    | Specialization index  | 0.125| 0.335        | 0.141 |
|                    | (H₂')                |      |              |       |
| **Individual level** | (mean ± SE)          |      |              |       |
|                    | Specialization index  | 0.228±0.050| 0.187±0.031 | 0.188±0.027|
|                    | (d')                 |      |              |       |
|                    | Number of interactions (degree) | 3.50±0.494| 4.00±0.828 | 4.73±0.777|
Figure 1

Map of *Testudo graeca* tortoises sampling sites

The map shows the distribution of the spur-thighed tortoise (*Testudo graeca*) in the Mediterranean basin. The wild populations sampled for the present study and their localities are indicated with dots: Villaltas, Galera and Marinica, as landscapes with low habitat loss for tortoises; Bas Norte, Sierrecica and Chinas, as landscapes with intermediate habitat loss; and Palas, Bas Sur and Misiripalme, as landscapes with high habitat loss. These wild Spanish populations of *T. graeca* cover approximately 2600 km² of semiarid coastal mountains between the Almeria and Murcia provinces.
Figure 2

Relationship between oxyurid nematode species richness and abundance and tortoises’ growth rates

Best-fit linear regressions, with 95 % confidence intervals, between oxyurid nematode infestation (i.e., species richness and abundance) and tortoises’ (Testudo graeca) growth rates in scrubland landscapes in southern Spain. Tortoises were present in landscapes sustaining different levels of habitat loss (low, intermediate and high). (A and B) Tortoise populations at all life stages. (C and D) Adult tortoise populations only. Significant relationships are indicated by *P < 0.01 and/or **P < 0.001. The values for the linear regression equation \( y = a + bx \) and the resulting r-values are indicated for each case.
(a) All tortoises

(b) Adult tortoises

Growth rate (mm/year) vs. Number of nematode species (log x+1)

Growth rate (mm/year) vs. Abundance of nematodes (log x+1)
Figure 3

Rank/abundance oxyurid nematode species plots

Rank/abundance plots for the oxyurid nematode community infesting tortoises (*Testudo graeca*) in scrubland landscapes with different levels of habitat loss (low, intermediate and high) in southern Spain. For each landscape, the relative abundance of each nematode species is plotted on a logarithmic scale against the species’ rank, from the most to the least abundant species. Nematodes included the following species: *Alaeuris numidica* (*A. num*), *Mehdiella stylosa* (*M. styl*), *M. uncinata* (*M. unci*), *M. microstoma* (*M. micro*), *Tachygonetria longicollis* (*T. long*), *T. dentata* (*T. dent*), *T. conica* (*T. coni*), *T. robusta* (*T. rob*), *T. macrolaimus* (*T. mac*), *T. numidica* (*T. numi*), *T. setosa* (*T. set*), *T. palearticus* (*T. pale*), *T. pusilla* (*T. pusi*), and *T. seurati* (*T. seur*). Nematode species not found under all habitat loss conditions are in **bold red**.
Figure 4

Quantitative bipartite plots of the tortoise-oxyurid species ecological networks encompassing different levels of habitat loss

Quantitative bipartite plots of the tortoise-oxyurid species ecological networks for each landscape encompassing different levels of habitat loss (low, intermediate and high) in southern Spain. (A) Tortoise-oxyurid ecological network at low habitat loss. (B) Tortoise-oxyurid ecological network at intermediate habitat loss. (C) Tortoise-oxyurid ecological network at high habitat loss. The bar size represents the number of interactions each oxyurid species (black) had with each individual tortoise (*Testudo graeca*) in the population (grey), and the linkage width represents the proportion of oxyurid individuals involved in such interactions.
