1 **Tick parasitism in Mediterranean spur-thighed tortoise: first data from the core**
2 **distribution range**

3 Amalia Segura*, Oscar Rodríguez, Francisco Ruiz-Fons, Pelayo Acevedo

4 Instituto de Investigación en Recursos Cinegéticos, IREC (UCLM-CSIC-JCCM)

5 Ronda de Toledo, 12, 13071 Ciudad Real, Spain

6

7 E-mail address:

8 amaliasegura@gmail.com

9

10 Phone: +34 926295450

11

ABSTRACT

Macroparasites in general and ectoparasites in particular have the potential to regulate host population dynamics. Host-parasite relationships are modulated by different factors that act at individual, population and landscape levels, and the relevance of each level depends on the local specific ecological conditions. In this context, this study addresses the tick parasitism traits of the Mediterranean spur-thighed tortoise (*Testudo graeca*) in its core distribution range (North western Morocco, Maamora) during the season in which ticks are principally active. It was discovered that 92.5% of the tortoises were parasitized by ticks in spring, with an infestation intensity and an abundance of 6.7 and 6.2 tick-tortoise⁻¹, respectively. The observed prevalence and infestation rates were among the highest reported throughout the world, which could be explained by host-parasite interactions mediated by a dens-dependent transmission processes. The main tick species that parasitized the tortoises were *Hyalommaegyptium* (95.6% of the identified ticks and 100% of the parasitized tortoises), *Hy. marginatum*, *Hy. excavatum* and *Hy. scupense*. Individual predictors for the tortoises, such as age, sex and the interaction between body condition and sex, were significantly related to tick abundance. Age-related behavioural differences in habitat use might favour a higher host-tick effective contact in adults than in juveniles. The fact that males are more active in spring, which is the breeding season, might explain the male-biased tick abundance observed and may be responsible for the negative relationship between male body condition and tick infestation rate when compared to those of the females. Given the potential role played by parasites as regards modulating population dynamics, our results suggest that ticks should be taken into account in the conservation and management programmes of these singular populations at the core of the species distribution range.

37 **Keywords:** tick, *Testudograeca*, host-population traits, host-individual traits, core
38 distribution

1. Introduction

Macroparasites can reduce the fitness of individual hosts (Vuren, 1996; Neuhaus, 2003; Ujvari and Madsen, 2006; Godfrey et al., 2010; Perez-Orella and Schulte-Hostedde, 2017; but see Raveh et al., 2015), as energy is allocated to the immune system rather than to growth, maintenance and reproduction (Schmid-Hempel, 2003). Macroparasites may, therefore, be able to depress their hosts' immune functioning (Hewitson et al., 2009) and may inflate the risk of background mortality. Indeed, immune depressed hosts are more vulnerable to predation and also more susceptible to opportunistic pathogens (Cornet and Sorci, 2010). Macroparasites in general and ectoparasites in particular have, therefore, the potential to regulate host population dynamics (Khokhlova et al., 2002; Fitze et al., 2004), and these effects should be taken into account in wildlife population management and conservation programmes.

Host-parasite relationships are modulated by different factors that act at individual, population and landscape levels, with non-individual factors being especially relevant for ectoparasites (Alzaga et al., 2009). Parasitism drivers are linked to the ecological traits of both the ectoparasite and the host and also to the ecology of their interaction (Cardonet et al., 2011). The host's age and sex are key individual factors in host-parasite relationships. The host's age affects infestation levels, parasite-induced mortality and parasite distribution among host individuals (Hawlena et al., 2006; Webber et al., 2015). Differences between the impact of ectoparasites according to the specie's sex - there is often a male-biased parasitism - have also been reported (Moore and Wilson, 2002; Gorrell and Schulte-Hostedde, 2008; Ferrari et al., 2004; Perez-Orella and Schulte-Hostedde, 2017) in reptiles (Aubret et al., 2005; Luiselli, 2006; Gharbi et al., 2015; Tiar et al., 2016). Resource partitioning as regards self-maintenance, reproduction and defence against parasites is the result of a basic trade-off undergone by animals (Ruiz-Fons et al.,

64 2013). The breeding system in long-living spam species may lead to drastic resource-
65 allocation changes in individuals and especially in males, whose priorities as regards
66 breeding might be more important than those related to immune competence or growth.
67 The effect of host population factors, such as host population density, may also be related
68 to parasite abundance, since parasite transmission rates are usually a positive function of
69 host population density, i.e. dens-dependence (Krasnov et al., 2002; but see Sorci et al.,
70 1997).

71 Reptiles are commonly infested by ectoparasites. However, although detailed information
72 on the infestation of reptiles by ticks can be found worldwide in, for example, South
73 Africa (Horag et al., 2006), Balkans (Siroky et al., 2006), Brazil (Dantas Torres et al.,
74 2010), Namibia (Tuner et al., 2017), studies analysing the main drivers of parasitism are
75 scarce, with some exceptions such as those carried out for the tuatara species *Sphenodon*
76 sp. (Godfrey et al., 2010). Indeed, there is evidence that ectoparasites infest Palearctic
77 tortoises of the *Testudo* genus, which are reported to be the predominant host of some
78 tick species of the *Hyalomma* genera, such as *Hy. aegyptium* (Schleich et al., 1996; Siroky
79 et al., 2006, 2009; Brianti et al., 2010; Harris et al., 2013), but studies on the drivers of
80 parasitism are not available.

81 The Mediterranean spur-thighed tortoise *T. graeca*'s distribution ranges from high quality
82 areas in northern and southern Morocco, which are considered to be core areas (e.g.
83 Lawton, 1993; Brown et al., 1995), to Algeria and Tunisia, which are reported to be
84 medium and suboptimal areas, respectively (Anadón et al., 2012). As population size has
85 a central influence on host-parasite interactions (Papkou et al., 2016) and core areas are
86 associated with higher densities than are peripheral ones, it is supposed that tick
87 parasitization rates at the core will be higher and that the main drivers may be different
88 to those reported for peripheral *T. graeca* populations (Gharbi et al., 2015; Tiar et al.,

2016). However, no information is currently available for the core populations of the species. We, therefore, hypothesize that tick parasitism on the Mediterranean spur-thighed tortoise will differ throughout its distribution range owing to higher population densities at the core and probably some co-evolutionary adaptations. In this context, the aims of this study were to: i) describe the tick species that feed on a core population of Mediterranean spur-thighed tortoise and parasitism rates in spring, and ii) analyse the role of individual and population related factors as drivers of tick parasitism in the species. Finally, our results are discussed in the light of the differences found between core (optimal areas) and peripheral populations (suboptimal areas).

2. Material and methods

2.1. Study area

The study was conducted in areas with a low elevation (72-185 m a.s.l.) and sandy soil in the Maamora forest (northwest Morocco; 34° 2' 54.19'' N, 6° 27' 19.24'' W; Figure 1). The climate is Mediterranean, with hot and dry summers, and the annual range of average rainfall is between 300 and 500 mm. The Maamora forest is dominated by cork oak trees, *Quercus suber*, with scattered endemic wild pear, *Pyrus mamorensis*, wild olive *Olea europaea*, green olive *Phyllirealatifolia* and mastic *Pistacia lentiscus* and a sparse understory characterised by bush (Mediterranean broom *Genista tinifolia*) and shrub stand (dwarf palm *Chamaerops humilis*, sage-leaved rockrose *Cistus salviifolius*, French lavender *Lavandula stoechas* and *Thymelea lathyroides*). The study took place in three specific areas (A, B, and C), which were close together (separated by 5 km) and covered a total surface of 60 ha.

2.2. Individual and population traits of the Mediterranean spur-thighed tortoise

114 The tortoises were captured by hand in the period April-May 2017 (n=296; 104 males,
115 168 females and 24 juveniles; see Table 1). Each individual encountered was sexed, the
116 body mass (± 1 g using precise balance) and body size measured (carapace length ± 1 mm
117 using calliper; CL), age determined by means of scute rings (Castanet and Cheylan, 1979;
118 Díaz-Paniagua et al., 2001; El Mouden et al., 2002), marked and finally released
119 immediately in the place of capture. The adults were sexed using classical criteria for
120 Testudinae (Andreu et al., 2000; Slimani et al., 2002), assuming a minimum size of
121 maturity of 100 mm for both sexes (El Mouden et al., 2002; Slimani et al., 2002).
122 Individuals below this threshold were considered to be juveniles and longer readily sexed.
123 All adult ticks attached to the tortoise body were sexed and counted in the field, and a
124 representative subsample was collected for the purpose of species identification. The
125 subsample corresponded to all the ticks found on those tortoises with a small number of
126 parasites (<10) and 10 ticks from those with a large number (>10). The ticks were stored
127 in tubes with ethanol (70%) and identified to species level using appropriate
128 morphological keys (Hoogstraal, 1956; Walker et al., 2003). Data on infestation
129 prevalence (%; #infested tortoises/ #examined tortoises* 100), mean intensity infestation
130 (#ticks/ #infested tortoises; SD and range) and tick abundance (#ticks/#examined
131 tortoises) were calculated for the males, females and juveniles at a population level.
132 The condition of a tortoise's body, i.e. body mass scaled by body size (Nagy and Medica,
133 1986; Henen, 1991,1997; Lagarde et al., 2002; Lecq et al., 2014), provides information
134 regarding its nutritional and physiological status, as occurs with all vertebrates
135 (Speakman, 2001). This was estimated using residual values obtained by means of linear
136 regression (all individuals pooled), with the natural logarithm (ln) of body mass as the
137 dependent variable and ln CL as the independent variable (Lagarde et al., 2001;
138 Speakman, 2001; Hayley, 2002; Willemsen and Hayley, 2002; Lecq et al., 2014). The

Comentado [P1]: Lo siento – no entiendo que queréis decir 😊.

individual body-condition index expresses the variation in mass in relation to the values expected according to the size of the animal. Host population density may greatly affect tick burden rates (e.g. Ruiz-Fons et al., 2013), and tortoise population density in the three sampling areas was, therefore, estimated in a previous study using capture-recapture approaches that showed both a closed population and the fact that the adult tortoises have a high philopatry and remain localised during breeding (Ben Kaddour et al., 2006). See Appendix 1 for details on sampling design and analytical methods.

2.3. Statistical analyses

The relationship among the tick infestation rate (tick abundance) and individual (sex, age and body condition) and population factors (density) was tested using a generalized linear model (GLM) with a Poisson distribution and logarithmic link function. The sampling area (A, B or C) was included as a fixed factor. The most parsimonious model was selected using a forward stepwise procedure based on the Akaike information criterion (AIC; Akaike, 1974). Those models separated by less than 2 points of AIC were selected, as they were considered to have similar evidence of strength (Burham and Anderson, 2002). All statistical analyses were performed with R 12.1 software (R DevelopmentCore Team, 2006).

3. Results

We counted a total of 1829 ticks on the 296 tortoises captured. A subsample of 961 ticks, i.e. 52% of the ticks counted on the tortoises, was identified to the species level: *Hyalommaegyptium* (95.6% of the ticks identified and 100% of the parasitized tortoises), *Hy. marginatum* (1.8% of the ticks and 6% of the tortoises), *Hy. excavatum* (1.3% of the

ticks and 4% of the tortoises), and *Hy. scupense* (1.2% of the ticks and 4% of the tortoises). The adult tick sex ratio was biased in favour of males with a mean ratio of 5.4. Overall, 92.5% of the tortoises were parasitized by ticks, ranging from 95% in adult tortoises to 62% in juveniles. Tick infestation intensity and abundance were 6.7 and 6.2 tick·tortoise⁻¹, respectively. The characteristics of infestation intensity for all the tortoises are summarized in Table 2.

The best-fit model showed that tick abundance increased significantly with the tortoise's age and population density and it also differed among study areas (see Table 3). The model showed that the males had a significantly higher abundance of ticks than did the females, and the lowest abundance was observed in juveniles. Finally, a significant interaction between body condition and sex was included in the most parsimonious model, showing that parasitization increases with body condition, but only in juveniles (see also Figure 2). The explained deviance of the final model was 33%.

4. Discussion

Several descriptive studies on the tick parasitism of *T. graeca* have, to date, been carried out in populations from suboptimal habitats and far away from the core distribution areas of the species (Siroky et al., 2006; Gharbi et al., 2015; Tiar et al., 2016). Few studies have, however, paid attention to the factors that affect tick parasitism in reptiles (Godfrey et al., 2010). In this study we show that *T. graeca* is a primary target for exophytic ticks, mainly *Hy.aegyptium*, in the core distribution range and, while it must be admitted that the study of environmental factors is limited, the tick abundance obtained in this study was mainly determined by individual and population factors, namely sex, age, body condition and population density.

4.1. Mediterranean spur-thighed tortoise-tick interaction in the core distribution range

Most of the Mediterranean spur-thighed tortoises in Maamora were parasitized by *Hy.aegyptium* (95.6%) but also by *Hy. marginatum*, *Hy. excavatum* and *Hy. Scupense*, whose common hosts are migrant birds, the Algerian hedgehog *Atelerixalgirus*, the Cape hare *Lepuscapensis* and ungulates (Walker et al., 2003). *Testudograeca* is the principal host of *Hy. Aegyptium* throughout its distribution range (Siroky et al., 2006; Gharbi et al., 2015; Tiar et al., 2016). In the Maamora forest, *T.graeca* shared its habitat with wild species such as the mammals mentioned above, but especially with high densities of livestock (Fennane and Rejdali, 2015), and this may explain the presence of different tick species on the tortoises.

The tick prevalence and intensity on the Mediterranean spur-thighed tortoises in the Maamora forest were higher than anywhere previously reported (see Table 4), with the exception of Djelfa in Algeria (Tiar et al., 2016). The difference between the results obtained in our study area and this Algerian locality might be explained by the high sample of immature tick stages (75% of the ticks collected) found on the tortoises and the possibility of a lower parasitism by adult stages. But even the sampling date, which was in peak tick activity (in May-June) or the lower sample of hosts (n=41) may have played a part in these differences. Host-parasite interactions in core areas might explain the high tick parasitism on Mediterranean spur-thighed tortoises in Maamora, probably as a result of density-dependent relationships, as suggested by the statistical model. In fact, the environment in the core area tends to be less spatially diverse and temporally stable than that at the edge and tortoise densities in the sampling areas were higher (mean average $17.2 \text{ tortoise} \cdot \text{ha}^{-1}$) than others reported in suboptimal habitats, such as Algeria (mean average $5.5 \text{ tortoise} \cdot \text{ha}^{-1}$; Tiar et al., 2016), where the habitat might be characterised by relatively depressed niche diversity owing to the distance to the core range distribution

(e.g. Scudder, 1989). However, dens-dependence does not seem to be a general pattern in all reptiles, since work with common lizards *Lacerta vivipara*, (Sorci et al., 1997) has obtained no clear evidence of it. The aforementioned discrepancy could be explained by different behavioural characteristics between species, such as the overlapping generations of common lizards that shared small and nonexclusive home ranges (Massot et al., 1992). Whether or not ectoparasites can be considered as a cost for reptiles living in high densities merits further studies.

4.2. Host individual factors driving tick parasitism in tortoises

Individual predictors, such as age and the interaction between body condition and sex, were significantly related to tick abundance on Maamora tortoises. Tortoises' age-related behavioural differences as regards habitat use might favour a higher host-tick effective contact in adults than in juveniles. The lack of sexual maturity in juveniles signifies that they behave in a less conspicuous manner than adults, covering smaller areas and remaining sheltered under cover (Lagarde et al., 2002).

The tick infestation rate was male-biased, as has been observed previously in other reptiles and mammals (e. g. Aubret et al., 2005; Luiselli, 2006; Ruiz-Fons et al., 2013; Perez-Orella and Schulte-Hostedde, 2017). Sex differences in the ectoparasite load may be caused by the parasite itself, rather than the host (Perez-Orella and Schulte-Hostedde, 2017). Despite the fact that male tortoises are smaller and, therefore, have a lower surface of tick-contact than females (sexual dimorphism), they move greater distances in spring owing to their attempts to cover a greater number of territories during breeding time (Díaz-Paniagua et al., 2001). The consequences for male tortoises might be the partition energy for growth at the expense of energy for the immune system (Sheldon and Verhulst, 1996) or a dual effect associated with testosterone (immune competence handicap)

enhancing the expression of secondary sexual traits and depressing the immune system (Folstad and Karter, 1992). This sexual hormone may even make male tortoises more susceptible to ticks, thus resulting in the loss of nutrients and in an increase in secondary infections caused by lacerations to the skin (e.g. González Trapaga et al., 2000; but see Sereau et al., 2010). In fact, ticks can compromise tortoises' body condition (e.g. Godfrey et al., 2016), which could be linked to sex, as was found in Maamora male tortoises, where those individuals with a greater number of parasites had a lower body condition. Although this pattern was also found in females, it was less serious than in males. Conversely, ticks do not seem to compromise the body condition of juveniles. This finding might be explained by the differences between juvenile and adult tortoises that are related to growth patterns, cryptic behaviour and habitat use, or even by the lower amount of sampling data obtained for juveniles. Indeed, juveniles had a higher body mass, in accordance with the fact that they have to grow fast in order to survive at this stage of their life (e.g. Nagy, 2000), and they moved shorter distances and remained hidden under vegetation cover, unlike the adults that were easier to spot, especially during the breeding season (Lagarde et al., 2002).

4.3. Final remarks

Despite the increasing amount of research carried out, including that concerning environmental factors and interannual changes, it is still necessary to improve the knowledge regarding tick–tortoise interactions. The present study has shown that those Mediterranean spur-thighed tortoises at the core of their distribution range have a higher prevalence of ticks and infestation rates than most of those reported elsewhere, which could be driven by denso-dependent transmission processes. Given the potential role played by parasites as regards driving population dynamics, our results suggest that ticks

should be taken into account in the conservation and management programmes of these singular populations throughout their distribution range. Our results particularly support the theory that ticks should be considered in studies aimed at understanding the spatio-temporal variation in indicators of both individual and population performance.

Acknowledgements

We are very grateful to Hassan Belhajjamia, Abdessalam Belhajjamia, Hammou El Hazgour and Bouhali Kaddouri for their field assistance when collecting the tortoises. We would also like to thank Greg Trollip and Jacob Mwanzia for their support and interest in wild species conservation. PA and FRF are supported by MINECO-UCLM through a “Ramón y Cajal” contract (RYC-2012-11970 and RYC-2013-12528, respectively).

References

- Akaike, H., 1974. A new look at the statistical model identification. IEEE. Trans. Autom. Control 19: 716-723.
- Alzaga, V., Tizzani, P., Acevedo, P., Ruiz-Fons, F., Vicente, J., Gortazar, C., 2009. Deviance partitioning of host factors affecting parasitization in the European brown hare (*Lepus europaeus*). Naturwissenschaften 96: 1157-68.
- Anadón, J.D., Gimenez, A., Gracia, E., Pérez, I., Fernandez, M., Fahd, S., El Mouden, E.H., Kalboussi, M., Jdeidi, T., Larbes, S., Rohuag, M., Slimani, T., Znari, M., Fritz, U., 2012. Distribution of *Testudo graeca* in the western Mediterranean according to climatic factors. Amphib. Reptil. 33: 285-296.
- Andreu, A.C., Díaz-Paniagua, C., Keller, C., 2000. La tortuga mora en Doñana. Asociación herpetológica española, Barcelona. Monogr. Herpetol., vol 5: 70 pp.

289 Aubret,F., Bonnet, X., Harris, M., Maumelat, S.,2005. Sex differences in body size and
 290 ectoparasite load in the ball python, *Phytonregius*. J.Herpetol.39: 312-315.
 291 Ben Kaddour, K., Slimani, T., El Mouden, E. H., Lagarde, F., Bonnet, X., 2006. Population
 292 structure, population density and individual catchability of *Testudograeca* in the central
 293 Jbilets (Morocco). Vie Milieu 56: 49-54.
 294 Brianti, E., Dantas-Torres, F., Giannetto, S., Risitano, A., Brucato, G., Gaglio, G., Otranto,
 295 D., 2010. Risk for the introduction of exotic ticks and pathogens into Italy through the
 296 illegal importation of tortoises *Testudograeca*. Med. Vet.Ent. 24:336-339.
 297 Brown, J.H., 1984. On the relationship between abundance and distribution of species.
 298 Am. Nat .124: 255-279.
 299 Cardon, M., Loot, G., Grenouillet, G., Blanchet, S., 2011. Host characteristics and
 300 environmental factors differentially drive the burden and pathogenicity of an
 301 ectoparasite: a multilevel causal analysis. J. Animal. Ecol. 80: 657-667.
 302 Castanet, J., Cheylan, M., 1979. Les marques de croissance des os et des
 303 écailles comme indicateur de l'âge chez *Testudohermanni* et *T. graeca*. Can. J. Zool.
 304 57:1649-1665.
 305 Cornet, S., Sorci, G., 2010. Parasite virulence when the infection reduces the host immune
 306 response. Proc. Biol. Sci. 277: 1929-1935
 307 Dantas-Torres, F., Ferreira, D.R., de Melo, L.M., Lima, P.A., Siqueira, D.B., Rameh-de-
 308 Albuquerque, L.C., de Melo, A.V., Ramos, A.J., 2010. Ticks on captive and free-living
 309 wild animals in northeastern Brazil. Exp. Appl.Acarol. 50: 181-189
 310 Díaz-Paniagua, C., Keller, C., Andreu, A.C., 2001. Long-term demographic fluctuations
 311 of the spur-thighed tortoise, *Testudograeca* in SW Spain.Ecography, 24: 707-721.
 312 El Mouden, E.H., Slimani, T., Ben Kaddour, K., 2002. Croissance et dimorphisme sexual
 313 chez la tortue marisque (*Testudo graecagraeca* L. 1758). Chelonii, 3: 325-330.

314 Fennane, M.,Rejdali, M., 2015. The world largest cork oak Maamora forest: challenges
 315 and the way ahead. Fl. Medit. 25: 277-285.

316 Ferrari, N., Cattadori, I.M., Nespereira, J., Rizzoli, A., Hudson, P.J., 2004. The role of host
 317 sex in parasite dynamics: field experiments on the yellow-necked mouse
 318 *Apodemusflavicollis*. Ecol.Lett. 7: 88-94.

319 Fitze, P.S., Tschirren, B., Richner, H., 2004.Life history and fitness consequences of
 320 ectoparasites. J. Anim. Ecol. 73: 216-226.

321 Folstad,I., Karter,A.J.,1992. Parasites, bright males and the immunocompetence handicap.
 322 Am. Nat. 139: 603-622.

323 González Trápaga, R., Aguirre, G., Adest, GA., 2000. Sex-steroids associated with the
 324 reproductive cycle in male and female bolson tortoise *Gopherusflavomarginatus*. Acta
 325 Zool. Mex. 80.

326 Gorrell,J.C., Schulte-Hostedde, A.I., 2008. Patterns of parasitism and body size in red
 327 squirrels (*Tamiasciurushudsonicus*). Can. J. Zool. 89: 99-107.

328 Gharbi, M.,Rjeibi, M.R., Rouatbi, M., Mabrouk, M., Mhadhbi, M., Amairia, S., Amdouni,
 329 Y., Boussaadoun, M.A., 2015.Infestation of the spurtighed tortoise (*Testudograeca*) by
 330 *Hyalommaegyptium* in Tunisia. Ticks Tick Borne Dis. 6: 352-355.

331 Godfrey, S.S., Moore, J.A., Nelson, N.J., Bull, C.M., 2010. Unravelling causality from
 332 correlations:revealing the impacts of endemic ectoparasites on a protected species
 333 (tuatara). Parasitology 137: 275-286.

334 Harris, D.J., Gracia, E., Jorge, F.,, Maia J.P., Perrera, A., Carretero, M.A., 2013. Molecular
 335 detection of *Hemolivia*(Apicomplexa: haemogregarinidae) from ticks of North African
 336 *Testudograeca* (Testudines: testudinae) and an estimation of their phylogenetic
 337 realtionships using 18S rRNA sequences. Comp. Parasitol.80: 292-296.

338 Hawlena, H., Abramsky, Z., Krasnov, B.R., 2006. Ectoparasites and age-dependent
 339 survival in a desert rodent. *Oecologia* 148: 30-9.
 340 Henen, B.T., 1991. Measuring the lipid content of live animals using cyclopropane gas.
 341 *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 261: R752-R759.
 342 Henen, B.T., 1997. Seasonal and annual energy budgets of female desert tortoises
 343 (*Gopherus agassizii*). *Ecology* 78: 283-296
 344 Hewitson, J.P., Grainger, J.R., Maizels, R.M., 2009. Helminth immunoregulation: the role
 345 of parasite-secreted proteins in modulating host immunity. *Mol. Biochem. Parasitol.* 167:
 346 1-11.
 347 Hoogstraal, H., 1956. African Ixodoidea. Volume I. Ticks of the Sudan. (With special
 348 reference to Equatorial province and with preliminary reviews of the genera *Boophilus*,
 349 *Margaropus* and *Hyalomma*). Cairo, Egypt (US NAMRU-3, Research Report NM 005
 350 050.29.07), 1101 pp.
 351 Horag, I.G., McKay, I.J., Henen, B.T., Heyne, H., Hofmeyr, M.D., De Villiers, A.L., 2006.
 352 Parasites of domestic and wild animals in South Africa. XLVII. Ticks of tortoises and
 353 other reptiles. *Onderstepoort J. Vet. Res.* 73: 215-27.
 354 Khokhlova, I.S., Krasnov, B.R., Kam, M., Burdelova, N.I., Degen, A.A., 2002. Energy
 355 costs of ectoparasitism: the flea *Xenopsylla barmesi* on the desert gerbil
 356 *Gerbillus dasyurus*. *J. Zool. (Lond)* 256: 349-354.
 357 Krasnov, B., Khokhlova, I., Shenbrot, G., 2002. The effect of host density on ectoparasite
 358 distribution: an example of a rodent parasitized by fleas. *Ecology*, 83: 164-175.
 359 Lagarde, F., Bonnet, X., Henen, B.T., Corbin, J., Nagy, K.A., Naulleau, G., 2001. Sexual
 360 size dimorphism in steppe tortoises (*Testudo horsfieldi*): growth, maturity and individual
 361 variation. *Can. J. Zool.* 79: 1433-1441.

362 Lagarde, F., Bonnet, X., Corbin, J., Henen, B., Nagy, K., 2002. A short spring before a long
363 jump: the ecological challenge to the steppe tortoise (*Testudo horsfieldi*). Can. J. Zool.
364 80: 493-502.

365 Latif, A.A., Walker, A.R., 2004. An introduction to the biology and control of ticks in
366 Africa. International Consortium on ticks and tick borne diseases.

367 Lawton, J.H., 1993. Range, population abundance and conservation. Trends Ecol. Evol. 8:
368 409-413.

369 Lecq, S., Ballouard, J.M., Caron, S., Livoreil, B., Seynaeve, V., Matthieu, L.A., Bonnet,
370 X., 2014. Body condition and habitat use by Hermann's tortoises in burnt and intact
371 habitats. Conserv. Physiol. 2: cou019.

372 Luiselli, L., 2006. Why do males and females of *Python regius* differ in ectoparasite load?
373 Amphib. Reptil. 27: 469-471.

374 Massot, M., Clobert, J., Pilorge, T., Lecomte, J., Barbault, R., 1992. Density dependence
375 in the common lizard: demographic consequences of a density manipulation. Ecology
376 73: 1742-1756.

377 Moore, S.L., Wilson, K., 2002. Parasites as a viability cost of sexual selection in natural
378 populations of mammals. Science 297: 2015-2018.

379 Nagy, K.A., Medica, P.A., 1986. Physiological ecology of desert tortoises in southern
380 Nevada. Herpetologica 42: 73-92.

381 Nagy, K. A., 2000. Energy costs of growth in neonate reptiles. Herpetol. Monogr. 14: 378-
382 387.

383 Neuhaus, P., 2003. Parasite removal and its impact on litter size and body condition in
384 Columbian ground squirrels (*Spermophilus columbianus*). Biol. Lett. 270: 213-215.

385 Papkou, A., Gokhale, C.S., Traulsen, A., Schulenburg, H., 2016. Host-parasite
386 coevolution: why changing population size matters. Zool. 119: 330-338.

387 Perez-Orella, C., Schulte-Hostedde, A.I., 2017. Effects of sex and body size on ectoparasite
 388 loads in the northern flying squirrel (*Glaucomys sabrinus*). Canad. J. Zool. 10: 1381-
 389 1385.

390 Raveh, S., Neuhaus, P., Dobson, F.S., 2015. Ectoparasites and fitness of female Columbian
 391 ground squirrels. Philos. Trans. R. Soc. Lond. Biol. Sci. 370:20140113.

392 Ruiz-Fons, F., Acevedo, P., Fernandez-de-Mera, I., 2013. Sex-biased differences in the
 393 effects of host individual, host population and environmental traits driving tick
 394 parasitism in red deer. Front. Cell. Infect. Microbiol. 3: 23.

395 Schmid-Hempel, P., 2003. Variation in immune defence as a question of evolutionary
 396 ecology. Proc. R. Soc. Lond. B. 270: 357-366.

397 Scudder, G.G.E., 1989. The adaptive significance of marginal populations: a general
 398 perspective. Can. Spec. Pub. Fish. Aquat. Sci. 105: 180-185.

399 Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and
 400 trade-offs in evolutionary ecology. Trends Ecol. Evol. 11: 317-321.

401 Sereau, M., Lagarde, F., Bonnet, X., El Mouden, H., Slimani, T., Dubroca, L., Trouvé, C.,
 402 Dano, S., Lacroix, A., 2010. Does testosterone influence activity budget in the male
 403 Greek tortoise (*Testudo graeca*)? Gen. Comp. Endocrinol. 167: 181-9.

404 Siroky, P., Petzelkova, K.J., Kamler, M., Mihalca, A.D., Modry, D.,
 405 2006. *Hyalomma aegyptium* as dominant tick in tortoises of the genus Testudo in Balkan
 406 countries, with notes on its host preferences. Exp. Appl. Acarol. 40: 279-290.

407 Siroky, P., Mikulicek, P., Jandzik, D., Kami, H., Mihalca, D., Rouag, R., Kamler, M.,
 408 Schneider, C., Zaruba, M., Modry, D., 2009. Co-distribution of
 409 haemogregarine *Hemolivia mauretanica* (Apicomplexa: Haemogregarinidae) and its
 410 vector *Hyalomma aegyptium* (Metastigmata: Ixodidae). J. Parasitol. 95: 728-733.

Con formato: Inglés (Estados Unidos)

Con formato: Portugués (Brasil)

411 Sorci, G., de Fraipont, M., Clobert, J., 1997. Host density and ectoparasite avoidance in the
412 common lizard (*Lacerta vivipara*). *Oecologia* 111:183-188

413 Speakman, J.R., editor. (Ed). 2001. Body composition Analysis of Animals: A Handbook
414 of Non-destructive Methods. Cambridge University Press, Cambridge, UK.

415 Tiar, G., Tiar-Saadi, M., Benyacoub, S., Rouag, R., Siroky, P., 2016. The dependence of
416 *Hyalommaegyptium* on its tortoise host *Testudo graeca* in Algeria. *Med. Vet. Entomol.*
417 30: 351-9.

418 Tuner, W.C., Kusters, M., Versfeld, W., Horak, I.G., 1996. Ixodid tick diversity on wild
419 mammals, birds and reptiles in and around Etosha National Park, Namibia. *African J.*
420 *Ecol.* 55: 714-721.

421 Ujvari, B., Madsen, T., 2006. Age, parasites and condition affect humoral immune
422 response in tropical phytons. *Behav. Ecol.* 17: 20-24.

423 Vuren, D.V., 1996. Ectoparasites, fitness and social behaviour of yellow-bellied marmots.
424 *Ethology* 102: 686-694.

425 Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A.,
426 Pegram, R.G., Preston, P.M., 2003. Ticks of domestic animals in Africa: a guide to
427 identification species. *BioScience Reports*, Edinburgh, Scotland, U. K.

428 Webber, Q.M.R., McGuire, L.P., Smith, S.B., Willis, C.K.R., 2015. Host behaviour, age
429 and sex correlate with ectoparasite prevalence and intensity in a colonial mammal, the
430 little brown bat. *Behaviour* 152: 83-105.

431 Willemsen, R.E., Hayley, A., 2002. Body mass condition in Greek tortoises: regional and
432 interspecific variation. *Herpetol. J.* 12: 105-114.

433

Con formato: Inglés (Estados Unidos)

Tables

Table 1. Samples (number of males, females and juveniles, including total number) and host population density (tortoise·ha⁻¹) per study area.

Area	Males	Females	Juveniles	Total	Density
A	40	52	6	98	26.4
B	37	53	4	94	19.6
C	27	63	14	104	5.5

Table 2. Characteristics of *Hyalomasp.* infestation intensity in Mediterranean spur-thighed tortoises in Maamora in 2017.

Tortoise age		Adults									
class and sex (n)		Tick	Male			Female			(male+female)		
		Total	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Males	(99)	839	7.18	5.01	1-23	1.29	1.23	0-6	8.47	5.57	1-28
Females	(160)	956	4.95	3.52	1-17	1.02	0.87	0-5	5.97	3.76	1-18
Juveniles	(15)	34	1.66	1.11	1-5	0.6	0.91	0-2	2.26	1.22	1-5
Total	(274)	1829	5.58	4.3	1-23	1.09	1.03	0-6	6.67	4.68	1-28

Table 3. Statistical parameters of the generalized linear model carried out to predict tick burden in tortoises. Only the most parsimonious model (i.e. that with the lowest AIC) is shown. See appendix for the full list of models tested.

Model predictors	Estimate	Std error	Z-value	P-value
Body condition	-0.42	-0.41	-1.02	0.306
Sex: Juvenile	-0.90	0.20	-4.45	< 0.001
Sex: Male	0.28	0.06	5.67	< 0.001

Age	0.0551	0.006	9.11	< 0.001
Density	7.17	0.91	7.87	< 0.001
Area B	51.07	6.46	7.89	< 0.001
Area C	129.40	16.43	7.87	< 0.001
Body condition*Juvenile	1.59	1.35	1.17	0.239
Body condition *Male	-1.56	0.56	-2.77	< 0.001

444

445 Table 4. Tick prevalence (%) and infestation intensity (tick·tortoise⁻¹) and host density

446 (tortoise·ha⁻¹) in different populations in suboptimal habitats for *Testudograeca*.

Country	Region	Host density	Sampling size	Tick prevalence	Tick intensity	Reference
Morocco	Souss Valley			26.9	?	Siroky et al., 2009
Algeria	El Kala	4.4	65	85	4.6	Tiar et al., 2016
Algeria	Aflou	5.8	60	90	5.9	Tiar et al., 2016
Algeria	Djelfa	11.2	41	100	9.4	Tiar et al., 2016
Algeria	Laghouat	1.6	35	9	1.7	Tiar et al., 2016
Tunisia		?	210	66.2	4.3	Gharbi et al., 2015
Africa	Tradetortoise		585	37.8	3.9	Brianti et al., 2010

447

448 **Figures**

449 Figure 1. Climatic suitability for the Mediterranean spur-thighed tortoise
450 (*Testudo graeca*): light grey = suboptimal, darker grey = medium and black = optimal.

451 Adapted from Anadón et al (2012). Location of the study area is shown.

454 Figure 2. Tick abundance variation in relation to the interaction between body condition
455 and age class and sex (see Table 3). Tick abundance decreases with body condition in
456 adults (males and females), whereas this relation is positive in juveniles.
457

458
459

461 **Appendix1. Capture-recapture tortoise sampling and population density**

462 With regard the capture-recapture procedures employed, the tortoises' carapaces were
463 numbered with non-toxic paint. The capture-recapture procedure assumes a closed
464 population; adult tortoises have a high philopatry and remain localized during breeding
465 (Ben Kaddour et al 2006). Population density estimates of the populations studied were
466 determined by employing the Lincoln-Petersen formula with a Seber (1982) correction
467 ($R > 7$):

468
$$N = ((M+1) * (C+1) / (R+1)) - 1,$$

469 where M is the sample of individuals captured and marked during the first survey, C is
470 the number of individuals captured in the second survey and R is the number of
471 individuals captured during the second survey and already marked.

472 The estimated population density in A was 26.4 indiv·ha⁻¹ (302 individuals) with a 95%
473 confidence interval (CI) ranging from 11.8 to 40.2 indiv·ha⁻¹. In B it was 19.6 indiv·ha⁻¹
474 (240 individuals) ranging from 9.6 to 30.3 indiv·ha⁻¹ and in C was 5.5 indiv·ha⁻¹ (95
475 individuals) ranging from 2.6 to 8.4 indiv·ha⁻¹.

476

477 Ben Kaddour, K., Slimani, T., El Mouden, E. H., Lagarde, F., Bonnet, X., 2006. Population
478 structure, population density and individual catchability of *Testudo graeca* in the central
479 Jbilet (Morocco). Vie et Milieu 56: 49-54.

480

481 **Appendix 2. List of models tested**

All	AIC
Tortoise sex*Tortoise body condition+Tortoiseage+Tortoisedensity+Area	1735.8
Tortoise age+Tortoise body condition+Tortoise sex	1811
Tortoise sex*Tortoise body condition	1893.5
Tortoise sex	1911.3
Tortoise age	1935.9
Area	2019.5
Tortoise body condition	2064.7
Tortoise density	2094.2

482