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Short communication

Tick parasitism in the Mediterranean spur-thighed tortoise in the Maamora forest, Morocco

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ABSTRACT

Macroparasites in general, and ectoparasites in particular, have the potential to regulate host population dynamics. In this context, this study addresses the tick parasitism traits of the Mediterranean spur-thighed tortoise (*Testudo graeca*) in the core area of its distribution range (northwestern Morocco, Maamora). 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, respectively. The observed parasitization rates were among the highest reported worldwide for *T. graeca*, which could relate to density-depended effects driving host-parasite interactions. The main tick species that parasitized the tortoises were *Hyalomma aegyptium* (95.6% of the ticks and in the 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 might favour a higher host-tick effective contact in adults than in juveniles. The fact that males are more active in spring - the breeding season - might explain the observed male-bias in tick abundance and may also be responsible for the negative effect of male body condition on tick infestation rate in contrast to 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 this tortoise species.

1. Introduction

Macroparasites have the potential to regulate host population dynamics (e.g. Fitze et al., 2004; Bull and Burzacott, 2006), and these effects should be taken into account in wildlife management and conservation programmes. Host-parasite relationships are modulated by different factors that act at individual, population and landscape levels (Alzaga et al., 2009). 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 (e.g. Moore and Wilson, 2002) also in reptiles (e.g. Luiselli, 2006; Gharbi et al., 2015). The effect of host population density may also be related to parasite abundance, since parasite transmission rates are usually a positive function of host density, i.e. density-dependence (Krasnov et al., 2002; but see Sorci et al., 1997).

Ticks are common ectoparasites of reptiles. However, although detailed information on the infestation of reptiles by ticks can be found worldwide (e.g. Horag et al., 2006; Siroky et al., 2006), studies analysing the main drivers of parasitism are scarce, with some exceptions such as those carried out with lizard *Tiliqua rugosa* (Belan and Bull, 1995; Bull and Burzacott, 2006) and with the tuatara species *Sphenodon* sp. (Godfrey et al., 2010). Indeed, there is evidence that ectoparasites infest Palearctic tortoises of the *Testudo* genus, which are reported to be the predominant host of some tick species of the *Hyalomma* genus, such as *Hy. aegyptium* (e.g. Schleich et al., 1996; Siroky et al., 2006, 2009), but studies on the drivers of parasitism are not available.

The Mediterranean spur-thighed tortoise *T. graeca* distribution ranges from high-quality areas in northern and southern Morocco, which are considered to be core areas (e.g. Lawton, 1993), to Algeria and Tunisia, which are reported to be medium and suboptimal areas, respectively (Anadón et al., 2012). As host population size has a central influence on host-parasite interactions (Papkou et al., 2016) and more suitable areas are associated with higher densities of hosts than are peripheral ones, hypothetically tick parasitization rates at the core area will be higher (Gharbi et al., 2015; Tiar et al., 2016). We, therefore, hypothesize that tick parasitism on the Mediterranean spur-thighed tortoise will differ throughout its distribution range owing to higher

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population densities at the core area. In this context, the aims of this study were to: i) describe the tick species that feed on a population of the Mediterranean spur-thighed tortoise in Maamora forest and the rate at which parasitize tortoises in spring, and ii) analyse the role of individual and population related factors – e.g. host age, sex, body condition and population density - as drivers of tick parasitism in the species.

2. Material and methods

The study area has a low elevation (72–185 m a.s.l.) and sandy soil and is located in the Maamora forest (northwestern Morocco; 34° 2′ 54.19″ N, 6° 27′ 19.24″ W). 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 *Phyllirea latifolia* and mastic *Pistacia lentiscus* and a sparse understory. 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. The forest has high livestock densities.

The tortoises were captured by hand in the period April-May 2017 (n = 296; 104 males, 168 females and 24 juveniles; see Table S1 in Supplementary Material). Collection and tick extraction was carried out within a private initiative for the conservation of the species in Maamora Forest (Ecological assessment of *Testudo graeca* in Maamora Forest) and following approved ethical protocols for wildlife capture and management. Each individual encountered was sexed, the body mass estimated (± 1 g using precise balance), its body size measured (carapace length ± 1 mm using a calliper; CL), the age estimated by means of scute rings (e.g. El Mouden et al., 2002), marked and released immediately at the place of capture. The adults were sexed using classical criteria for Testudinae (e.g. Slimani et al., 2002).

All adult ticks attached to the tortoise body were sexed and counted in the field, and a representative subsample was collected for species identification. The subsample corresponded to all the ticks found on those tortoises with a small number of ticks (< 10) and 10 ticks from those with a large number (> 10). The ticks were stored in tubes with ethanol (70%) and identified to species level using appropriate morphological keys (Hoogstraal, 1956; Walker et al., 2014). Data on infestation prevalence (%; number of infested tortoises/number of examined tortoises × 100), mean infestation intensity (number of ticks/number of infested tortoises) and tick abundance (number of ticks/number of examined tortoises) were calculated for each sex- and age-class in the population.

The tortoise body condition, i.e. body mass scaled by body size (e.g. Nagy and Medica, 1986; Henen, 1997), was estimated using residual values obtained by means of linear regression (all individuals pooled), with the natural logarithm (ln) of body mass as the dependent variable and ln CL as the independent variable (e.g. Speakman, 2001). The 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). Tortoise population density in the three sampling areas was estimated in a previous study using capture-recapture approaches that assumed 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); densities were: 26.5 indiv/ha in A, 19.6 indiv/ha in B and 5.5 indiv/ha in C. See Appendix 1 in Supplementary Material for details on sampling design and analytical methods.

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 Poisson distribution is used to model count data with no negative values. The sampling area (A, B or C) was included as a fix factor. The most parsimonious model was selected using a forward stepwise procedure based on the Akaike information criterion (AIC; Akaike, 1974). All statistical analyses were performed with R 3.4.3 software (R Development Core Team, 2017).

3. Results and discussion

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: *Hyalomma aegyptium* (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). Inmature stages of *Hyalomma* feed mainly on birds and small mammals such as, the Algerian hedgehog *Atelerix algirus* and the Cape hare *Lepus capensis* whereas the adults positively select ungulates for feeding (Walker et al., 2014). *Testudo graeca* is a relevant host of *Hy. aegyptium* throughout its distribution range (e.g. Gharbi et al., 2015; Tiar et al., 2016). In the Maamora forest, *T. graeca* shares its habitat with wild species and – especially – with high densities of livestock (Fennane and Rejdali, 2015), being the high diversity of potential hosts a plausible cause of the presence of different tick species on the tortoises.

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, respectively. The characteristics of infestation intensity for all the tortoises are summarized in Table 1. The tick prevalence and intensity on the Mediterranean spur-thighed tortoises in the Maamora forest were higher than anywhere previously reported (see Table S2 in Supplementary Material), with the exception of Djelfa in Algeria (Tiar et al., 2016). The difference between the results obtained in our study area and in Djelfa might be explained by the high sample of immature tick stages (75% of the ticks collected) found on the Algerian tortoises. Even the lower sample of Algerian hosts (n = 41) may have played a part in these differences, but it must be admitted that the sampling year and the location generate variations among areas in the peak tick activity. Host-parasite interactions in the core area might explain the high tick parasitism on Mediterranean spur-thighed tortoises in Maamora, probably as a result of density-dependent relationships, as it was also suggested by our statistical model (see below).

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 2). The model showed that the

Table 1
Characteristics of *Hyalomma* spp. infestation intensity in Mediterranean spur-thighed tortoises in Maamora in 2017.

Tortoise age class and sex (n)		Tick	Male			Female			Adults (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 2
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. The explained deviance of the final model was 33%. See Table S3 (Supplementary Material) 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

males had a significantly higher abundance of ticks than 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 decreases in males in relation to their body condition but not in juveniles (see also Fig. S1 in Supplementary Material). Tortoise age-related behavioural differences might favour a higher host-tick effective contact rate in adults than in juveniles. The lack of sexual maturity may lead juveniles to behave less conspicuously than adults, resulting in a lower movement rate and a lower tick encounter chance since they remain sheltered under cover (Lagarde et al., 2002). The tick infestation rate was male-biased, as has been observed in other reptiles and tortoises (e.g. Robbins et al., 1998; Aubret et al., 2005). Despite the fact that male tortoises are smaller and, therefore, have a lower surface for the ticks to feed on when compared to the females, 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). In addition, males share refuges and therefore there are major individual aggregations and therefore higher tick loads (e.g. Leu et al., 2010).

Our results suggest that ticks can compromise tortoises' body condition (e.g. Godfrey et al., 2016), which could be linked to sex. In Maamora male tortoises with a greater number of parasites had a lower body condition. This fact could influence long-term pairing behaviour (females selecting actively males with lower average tick loads), even playing a role in shaping mate selection as it was observed in Australian lizards (Bull and Burzacott, 2006). Although this pattern was also found in females, it was less evident than in males. Conversely, ticks do not seem to compromise the body condition of juveniles. This finding might be explained by the differences related to growth patterns between juvenile and adult tortoises. Indeed, juveniles have 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).

Despite the increasing amount of research carried out on ticks, it is still necessary to improve the knowledge regarding tick–tortoise interactions. In this study we showed that *T. graeca* is a primary target for exophilic ticks, mainly *Hy. aegyptium,* in the Maamora forest and, tick abundance was mainly determined by individual and population factors, namely sex, age, body condition and host population density. At this respect, our results suggest that ticks should be taken into account in the conservation and management programmes of this singular and threatened tortoise species.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2018.11.002.

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