Consequences of haemogregarine infection on the escape distance in the lacertid lizard, *Podarcis vaucheri*

Isabel Damas-Moreira[1,2,*], D. James Harris[1], Daniela Rosado[1,2], Isabel Tavares[1,2], João P. Maia[1,2,3], Daniele Salvi[1], Ana Perera[1]

[1] *CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, Nº 7, 4485-661 Vairão, Vila do Conde, Portugal. *Corresponding author. E-mail: isabeldamas.m@gmail.com*
[2] *Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre FC4 4169-007 Porto, Portugal*
[3] *Institut de Biologia Evolutiva (CSIC - Universitat Pompeu Fabra). Passeig Marítim de la Barceloneta, 37-49. 08003 Barcelona, Spain*

Abstract. Nowadays it is widely accepted that parasites play a significant role in the community structures in which they occur, and ultimately upon ecosystems. Furthermore, infection by parasites might be associated with considerable deterioration of individual host fitness. While the apicomplexan parasites belonging to the genus *Hepatozoon* can provoke severe deleterious effects in some mammals, impact on other hosts, such as reptiles, is still unclear. We assessed the effect of *Hepatozoon* parasites on *Podarcis vaucheri* flight-initiation distance from a simulated predator, a behaviour that is determinant for a successful escape and is therefore likely to have major implications on a lizard's survival. We found that flight-initiation distance was not dependent on the time of the day or tail condition. Subadults exhibited worse body condition than adults and females had worse body condition than males. Regarding intensity of parasitism, subadults showed higher parasitemia levels. Escape distance was not associated with parasitic load or any of the other studied features, which is indicative of limited impact of the parasite. This negligible effect might explain the remarkably high prevalence (more than 96%) of this parasitic group within this *P. vaucheri* population.

Keywords. Flight-initiation distance, *Hepatozoon*, lacertid.

Parasitism is a major selective force driving organisms' life history traits, and is usually associated with a deterioration of host body condition (Oppliger et al., 1996). The apicomplexan haemogregarines (Apicomplexa: Adeleorina) are the most common blood parasites in reptiles, and among them, the genus *Hepatozoon* Miller, 1908 is one of the most frequent (Telford, 2009). Although the deleterious effects of some *Hepatozoon* species in domestic animals are well-known (Baneth et al., 1998; 2003), the impact for wild reptile hosts is still uncertain and vague. *Hepatozoon* gamonts infect erythrocytes, possibly causing an oxygen deficit as a result of the destruction of red blood cells (Telford, 2009). Therefore, *Hepatozoon* infection consequences might be relevant in situations requiring high oxygen transportation, such

as escaping from predators. Concerning this, haemogregarine load was found to be associated with lower antipredatory performance both in terms of burst speed (Oppliger et al., 1996; Garrido and Pérez Mellado, 2014), and tail regeneration rate (Oppliger and Clobert, 1997).

The distance a lizard allows potential predators to approach before fleeing (the flight-initiation distance), is determinant for a successful escape (Cooper, 2006). The optimal escape theory (Ydenberg and Dill, 1986) is based on the principal that a prey will only escape from its predator when the risk of predation equals the cost of escaping. These costs are not only energetic but also include abdicating foraging (Cooper and Pérez-Mellado, 2004), missing social opportunities (Ydenberg and Dill, 1986) and reducing basking time (Martín and López, 1999).

In this study we analyse the influence of the haemogregarine *Hepatozoon* on the flight-initiation distance in the lizard *Podarcis vaucheri* Boulenger, 1905. Following the optimal escape theory and previous studies, if parasites negatively affect host fitness or locomotor performance, we hypothesise that this can be reflected in lizards' flight-initiation distance. Thus, we expect that lizards with higher parasite load will have a faster response and run to the shelter sooner than non-parasitized lizards, as *Hepatozoon* sp. may decrease their locomotor speed which can be compensated by running away before the predator gets as close.

The tests were performed in May 2013 in Oukaimeden (Morocco, 31o12'N, 7o51'W) on a total of 55 individuals of *Podarcis vaucheri*. Of these, 24 were males (including three subadults), and 31 were females (including seven subadults). Individuals above 45 mm snout-vent length were considered adults, and below, subadults (Schleich et al., 1996). The study area consists mainly of rock outcrops with fissures or large rocks among grass. At this location, *Podarcis vaucheri* presents high densities and *Hepatozoon* parasites have been previously reported and characterized genetically (Maia et al., 2011).

Escape distance trials consisted of standardized approaches to lizards simulating a predator attack. Given that speed and direction of the approach might influence a lizard response (Martín and López, 1999; Cooper, 2006) approaches were always performed in the same way by the same researcher with the same outfit. The researcher simulating the predator (DR) slowly walked towards the lizard, with arms next to the body and making no abrupt movements until the lizard fled. The distance between the researcher and the place where the lizard was at the moment it escaped was recorded using a laser distance measurer (±2 mm over 30 m). The distance from the lizards to the nearest refuge at the time of fleeing was not recorded, as all lizards were no more than 30 centimetres away from a shelter (IDM, pers. obs.). Only one trial was performed per lizard and only individuals that were basking at the initiation of the trial were included.

After each trial, the lizard was captured and tail condition (intact, regenerated or recently lost) and time of capture were recorded. All trials were conducted between 10 and 16 hours. Individuals were measured for snout-vent length (SVL), weighed and a piece of tail tip was taken and stored in 96% ethanol for genetic analyses. Blood resultant from the tail was smeared across a glass slide and air-dried for posterior treatment in the laboratory. All animals were released immediately after at the sample site.

Blood smears were fixed with absolute methanol for 2 min, stained with Giemsa (1:9 distilled water) for 45 min and air-dried. Using an Olympus CX41 microscope, prevalence was estimated as the percentage of infected individuals within the sampled population and the individual parasitemia load was considered as the percentage of infected red blood cells out of 2500 erythrocytes. For these counts, random pictures were taken at x400 using Cell^B 3.4 Olympus® software, and counted manually using the ImageJ 1.46® program.

Molecular methods were used to confirm the identity of the detected parasites. DNA was extracted from the blood of five randomly selected infected lizards using the high salt method (Sambrook et al., 1989). A fragment of the 18S rRNA gene was amplified using the primers HepF300 and HepR900 (Ujvari et al., 2004), as described in Harris et al. (2011). Positive PCR products were purified and sequenced by a commercial facility (Macrogen, The Netherlands). Sequences are available at GenBank with the accession numbers KJ659858 to KJ659862.

All parasite sequences analysed were part of the same lineage of *Hepatozoon* infecting lizard hosts (lineage 2 identified in Maia et al., 2012). Prevalence was 96.4% (53/55) and intensity of infection varied from 0 to 11.7%, with a total mean intensity (± SD) of 1.86% (± 2.44). Six individuals had more than 5% infected erythrocytes and two of these lizards, both females, more than 10% erythrocytes infected.

Prior to statistical analysis all continuous variables were log transformed to meet normality assumptions. In order to infer if the time of capture or tail condition were interfering with the flight-initiation distance, Analysis of Variance (ANOVA, lm function) were performed for each variable independently. In order to assess the need to include SVL and body condition (BC, considered as the ratio of SVL/weight) as covariates in the analysis, Spearman correlations were determined with all the remaining continuous variables (cor function).

Differences between sexes and ages in body size and body condition were assessed using an ANOVA (lm function). Differences in parasite prevalence between sexes and ages were tested using a Generalized Linear Model (GLM) with a logistic regression (MASS package, Venables and Ripley, 2002), while differences in parasite intensity (considering only positive samples) were tested with an Analysis of Covariance (ANCOVAs, with lm function) including BC as covariate.

To evaluate if BC, SVL or intensity of parasitism were related to flight-initiation distance, non-parametric Spearman correlations (cor function) were determined. Differences in flight escape distance were assessed using ANCOVAs, where we tested the effect of host sex, age and presence of the parasite, including BC as covariate. Finally, ANCOVAs were performed only on infected

individuals, to assess for any effect of sex and age, after correcting by BC and intensity as covariates. All statistical tests were conducted using R v. 2.15.2 (R Development Core Team, 2012).

Flight-initiation distance was not dependent on the time of the day (F = 0.973, df = 1, P = 0.328), or tail condition (F = 0.241, df = 2, P = 0.787), and thus these were not considered in further analyses. Males and females did not differ significantly in body size (F = 2.232, df = 1, P = 0.141). Body condition, however, differed between sexes (F = 38.510, df = 1, P < 0.001) and ages (F = 37.918, df = 1, P < 0.001), with females and subadults exhibiting worse BC. All individuals analysed were infected, with the exception of two lizards. Given the low sample size of non-infected individuals, differences in prevalence between sexes or ages could not be tested statistically. Considering only infected individuals and correcting for body condition, intensity of parasitism was not associated with sex (F = 0.346, df = 1, P = 0.56), but it differed between age classes (F = 4.136, df = 1, P = 0.048), with younger lizards having higher parasite load (Table 1).

Flight-initiation distance was not correlated either to body condition (Spearman correlation, R = -0.102, P = 0.458) or to parasite intensity (R = 0.024, P = 0.861). Moreover, escape distance did not differ between infected and uninfected lizards (ANCOVA, F = 0.007, df = 1, P = 0.933), sexes (F = 0.714, df = 1, P = 0.402) or ages (F = 1.175, df = 1, P = 0.284) after correcting for BC. Finally, considering only infected individuals, we did not find differences in escape distance between sexes and ages (ANCOVA, using BC as covariate, in all cases, P > 0.05).

Our results show that flight initiation distance was independent of parasite intensity. One possible explanation for this lack of differences might be that heavily infected individuals suffer higher mortality, and consequently our sampling was performed on individuals that were not exposed to higher infection levels. However, this is unlikely to be the case, since several of the individuals exhibited moderately high parasitemia levels, when compared to other studies with *Podarcis* (0.1% in Amo et al., 2005 b; 0.9% in Garrido and Pérez-Mellado, 2013).

Hepatozoon prevalence in lizards are highly variable: while some studies reported about 20% infected individuals in populations of *Sphenodon, Calotes* and *Hemidactylus* (Herbert et al., 2010; Godfrey et al., 2011; Gupta et al., 2013), others report higher levels in *Podarcis* from the Iberian Peninsula (Amo et al., 2005 b; Martín et al., 2008; Roca and Galdón, 2010). Nevertheless, our prevalence values are extremely high, with only two of the 55 sampled individuals not infected with *Hepatozoon*, which precludes any conclusion regarding the effect of parasite presence on the flight escape distance. In fact, as far as we know, this is the highest prevalence recorded in a continental population of *Podarcis*. This estimate is similar to the values found in the insular *Podarcis lilfordi* showing 95% haemogregarine prevalence of infection, albeit with an average of only 1% parasitemia levels (Garrido and Pérez-Mellado, 2013). These differences may be the result of different availability of competent vectors, or to a seasonal variation of the parasite itself or their vectors, although this still needs to be verified.

Our study revealed that after correcting for body condition, younger individuals were more heavily infected, contradicting the trend found in other studies with reptilian haemogregarines (Amo et al., 2004; Molnár et al., 2013) and also with *Hepatozoon* (Salkeld and Schwar-

Table 1. Number of individuals analyzed (*n*), mean flight-initiation distance (m), mean intensity of infection (%), and prevalence among genders and ages.

	Males		Females	
	Adults	Subadults	Adults	Subadults
	n = 21	*n* = 3	*n* = 24	*n* = 7
Distance				
Mean distance	1.8700	1.4237	1.9588	1.5364
± SD	0.8562	0.2224	0.7736	0.4337
Overall gender mean ± SD	1.814 ± 0.815		1.863 ± 0.727	
Parasites				
Mean intensity	1.72	1.52	1.51	3.11
± SD	1.90	1.61	2.46	3.76
Prevalence (%)	100	66.66	95.83	100
Overall gender mean intensity ± SD	1.77 ± 1.85		1.93 ± 2.84	
Overall gender prevalence (%)	95.83		96.77	

zkopf, 2005). However, this result is also concordant with other works conducted in *Hepatozoon* spp. in reptiles, in which parasite intensity declined with increased host size or age (Madsen et al., 2005; Brown et al., 2006; Godfrey et al., 2011). This may be explained by natural selection removing susceptible reptiles and only those displaying moderate parasite load are able to survive until older ages (Madsen et al., 2005; Brown et al., 2006), or simply occur because younger individuals have had less time to acquire immunity, which might lead to higher infection intensity (Hudson and Dobson, 1997) and plausibly lower body condition, as observed in our study.

These results seem to indicate that *Hepatozoon* infection has no strong impact on the flight-initiation distance of *P. vaucheri* from Oukaimeden. This suggests a solid and stable evolutionary interaction between host and parasite (Combes, 2001; Amo et al., 2005 b), where lizards from this population evolved to tolerate the infection rather than to fight it (Sheldon and Verhulst, 1996). Both intensity and prevalence levels can thus indicate how well established this parasite is in this population, in which the mean intensity (near 2%) might be the result of immune system counterbalancing the high prevalence values (near 96.4%) in the population.

Moreover, flight-initiation distance might not be affected by the virulence of this *Hepatozoon* strain, but can be influenced by other variables, allied or not with the occurrence of parasites. Future research should integrate the study of other endo or ectoparasites, as studies in lizards revealed that their presence can also interfere in host fitness, including nematodes (Amo et al., 2005 a), ticks (Main and Bull, 2000) or mites (Sorci et al., 1995). Despite the lack of evidence of parasite interference in the flight-initiation behaviour obtained in this study, these are complex parasite systems and clearly warrants further investigation.

REFERENCES

Amo, L., Fargallo, J.A., Martínez-Padilla, J., Millán, J., López, P., Martín, J. (2005 a): Prevalence and intensity of blood and intestinal parasites in a field population of a Mediterranean lizard, *Lacerta lepida*. Parasitol. Res. **96:** 413-417.

Amo, L., López, P., Martín, J. (2004): Prevalence and intensity of haemogregarinid blood parasites in a population of the Iberian rock lizard, *Lacerta monticola*. Parasitol. Res. **94:** 290-293.

Amo, L., López, P., Martín, J. (2005 b): Prevalence and intensity of haemogregarine blood parasites and their mite vectors in the common wall lizard, *Podarcis muralis*. Parasitol. Res. **96:** 378-381.

Baneth, G., Aroch, I., Tal, N., Harrus, S. (1998): *Hepatozoon* species infection in domestic cats: a retrospective study. Vet. Parasitol. **79:** 123-133.

Baneth, G., Mathew, J.S., Shkap, V., Macintire, D.K., Barta, J.R., Ewing, S.A. (2003): Canine hepatozoonosis: two disease syndromes caused by separate *Hepatozoon* spp. Trends Parasitol. **19:** 27-31.

Brown, G.P., Shilton, C.M., Shine, R. (2006): Do parasites matter? Assessing the fitness consequences of haemogregarine infection in snakes. Can. J. Zool. **676:** 668-676.

Combes, C. (2001): Parasitism: The Ecology and Evolution of Intimate Interactions. University of Chicago Press, Chicago.

Cooper, W.E. Jr. (2006): Dynamic risk assessment: prey rapidly adjust flight initiation distance to changes in predator approach speed. Ethology **112:** 858-864.

Cooper, W.E. Jr., Pérez-Mellado, V. (2004): Tradeoffs between escape behavior and foraging opportunity by the balearic lizard (*Podarcis lilfordi*). Herpetologica **60:** 321-324.

Garrido, M., Pérez-Mellado, V. (2013): Prevalence and intensity of blood parasites in insular lizards. Zool. Anz. **252:** 588-592.

Garrido, M., Pérez-Mellado, V. (2014): Sprint speed is related with blood parasites, but not with ectoparasites in an insular population of lacertid lizards. Can. J. Zool. **92:** 67-72.

Godfrey, S.S., Nelson, N.J., Bull, C.M. (2011): Ecology and dynamics of the blood parasite, *Hepatozoon tuatarae* (Apicomplexa), in tuatara (*Sphenodon punctatus*) on Stephens Island, New Zealand. J. Wildlife Dis. **47:** 126-139.

Gupta, N., Bhaskar, M., Gupta, D.K. (2013): Ecological attributes of *Hepatozoon lacertilis* susceptibility in Indian lizards, *Hemidactylus flaviviridis* (Gekkonidae) and *Calotes versicolor* (Agamidae). Trop. Biomed. **30:** 97-104.

Harris, D.J., Maia, J.P.M.C., Perera, A. (2011): Molecular characterization of *Hepatozoon* species in reptiles from the Seychelles. J. Parasitol. **97:** 106-110.

Herbert, J.D.K., Godfrey, S.S., Bull, C.M., Menz, R.I. (2010): Developmental stages and molecular phylogeny of *Hepatozoon tuatarae*, a parasite infecting the New Zealand tuatara, *Sphenodon punctatus* and the tick, *Amblyomma sphenodonti*. Int. J. Parasitol. **40:** 1311-1315.

Hudson, P.J., Dobson, A.P. (1997): Host-parasite processes and demographic consequences. In: Host-parasite Evolution - General Principles and Avian Models, pp. 128-154. Clayton, D.H., Moore, J., Eds, Oxford University Press, Oxford.

Madsen, T., Ujvari, B., Olsson, M. (2005): Old pythons stay fit; effects of haematozoan infections on life history traits of a large tropical predator. Oecologia **142:** 407-412.

Maia, J.P.M.C., Harris, D.J., Perera, A. (2011): Molecular survey of *Hepatozoon* species in lizards from North Africa. J. Parasitol. **97:** 513-517.

Maia, J.P.M.C., Perera, A., Harris, D.J. (2012): Molecular survey and microscopic examination of *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina) in lacertid lizards from the western Mediterranean. Folia Parasitol. **59:** 241-248.

Main, A.R., Bull, C.M. (2000): The impact of tick parasites on the behaviour of the lizard *Tiliqua rugosa*. Oecologia **122:** 574-581.

Martín, J., Amo, L., López, P. (2008): Parasites and health affect multiple sexual signals in male common wall lizards, *Podarcis muralis*. Naturwissenschaften **95:** 293-300.

Martín, J., López, P. (1999): When to come out from a refuge: risk-sensitive and state-dependent decisions in an alpine lizard. Behav. Ecol. **10:** 487-492.

Molnár, O., Bajer, K., Mészáros, B., Török, J., Herczeg, G. (2013): Negative correlation between nuptial throat colour and blood parasite load in male European green lizards supports the Hamilton-Zuk hypothesis. Naturwissenschaften **100:** 551-558.

Oppliger, A., Celerier, M.L., Clobert, J. (1996): Physiological and behaviour changes in common lizards parasitized by haemogregarines. Parasitology **113:** 433-438.

Oppliger, A., Clobert, J. (1997): Reduced tail regeneration in the Common Lizard, *Lacerta vivipara*, parasitized by blood parasites. Funct. Ecol. **11:** 652-655.

R Development Core Team (2012): R – a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roca, V., Galdón, M.A. (2010): Haemogregarine blood parasites in the lizards *Podarcis bocagei* (Seoane) and *P. carbonelli* (Pérez-Mellado) (Sauria: Lacertidae) from NW Portugal. Syst. Parasitol. **75:** 75-79.

Salkeld, D.J., Schwarzkopf, L. (2005): Epizootiology of blood parasites in an Australian lizard: a mark-recapture study of a natural population. Int. J. Parasitol. **35:** 11-18.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular Cloning. Vol 2. Cold spring harbor laboratory press, New York.

Schleich, H.H., Kästle, W., Kabisch, K. (1996): Amphibians and reptiles of North Africa. Koeltz Scientific Books, Koenigstein.

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

Sorci, G., Clobert, J., Ecologie, L., Ura, C., Pierre, U. (1995): Effects of maternal parasite load on offspring life-history traits in the common lizard (*Lacerta vivipara*). J. Evol. Biol. **8:** 711-723.

Telford, S.R. (2009): Hemoparasites of the Reptilia – color atlas and text. CRC Press, Boca Raton, Florida.

Ujvari, B., Madsen, M., Olsson, M. (2004): High prevalence of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) infection in Water pythons (*Liasis fuscus*) from Tropical Australia. J. Parasitol. **90:** 670-672.

Venables, W.N., Ripley, B.D. (2002): Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Ydenberg, R.C., Dill, L.M. (1986): The economics of fleeing from predators. Adv. Stud. Behav. **16:** 229-249.