ORIGINAL PAPER

Susceptibility to infection by a haemogregarine parasite and the impact of infection in the Australian sleepy lizard *Tiliqua rugosa*

Menno J. Bouma · Catherine J. Smallridge · C. Michael Bull · Jan Komdeur

Abstract The Hamilton and Zuk hypothesis on haemoparasite-mediated sexual selection and certain studies of fitness are based on the assumption that blood parasite infections are detrimental to their hosts. However, there are few reports that have demonstrated harmful effects of endemic blood parasites on fitness in wild populations, and it has even been suggested that they may be non-pathogenic. In this paper, we show that individuals of the Australian sleepy lizard (*Tiliqua rugosa*) have smaller home ranges when they are infected with the haemogregarine blood parasite *Hemolivia mariae* than when no infection can be detected. An apparently contradictory result was that lizards with larger home ranges were more susceptible to infection under experimental exposure to *Hemolivia*. We propose that lizards sacrifice defence against pathogens by increased activity, perhaps associated with maintaining home ranges and mating opportunities. As a consequence, they gain higher parasite loads, which in turn inhibit their activity. In this case, the parasite–host interaction may act as a buffer of lizard activity.

M. J. Bouma · J. Komdeur
Animal Ecology Group, Centre for Ecological
and Evolutionary Studies, University of Groningen,
P.O. Box 14, 9750 AA Haren, The Netherlands

C. J. Smallridge · C. M. Bull (✉)
School of Biological Sciences,
Flinders University of South Australia,
GPO Box 2100, Adelaide, SA 5001, Australia
e-mail: michael.bull@flinders.edu.au

Present address:
C. J. Smallridge
Entomology Unit, South Australian Research
and Development Institute,
GPO Box 397, Adelaide, SA 5001, Australia

Introduction

Theory suggests that host–parasite interactions are responsible for substantial genetic variation within host populations (Anderson and May 1982), and for the evolution and maintenance of sexual reproduction and certain sexually selected traits (Hamilton and Zuk 1982; Andersson 1994). Although these arguments are based on the premise that parasites reduce the fitness of their hosts (Combes 1997; Goater and Holmes 1997), the effects of parasites on hosts remain controversial. Without knowledge of the effects of a specific parasite on a host species, it is impossible to evaluate the importance of the parasite as an evolutionary force (Price 1980). Parasites can adversely affect host fitness, but the extent of these effects is known to differ depending on the conditions.

This study focuses on the impact of endemic parasites on an Australian lizard. Parasites can induce mortality in lizards in laboratory conditions (Schall 1983, 1986, 1996), but this is rarely reported in studies of natural host–parasite interactions. More commonly, lizards show fitness-related effects of parasites, such as poorer body condition (Sorci and Clobert 1995; Merino et al. 2000) or slower running speed (Oppliger et al. 1996). Parasites can cause lizards to move shorter distances (Main and Bull 2000), occupy smaller home ranges (Main and Bull 2000) or have reduced social status where infected males display fewer courtships and less territorial behaviour than uninfected males (Dunlap and Schall 1995). In these cases, activity and courtship displays could honestly indicate the ability of an individual to cope with parasites. However, in many host–parasite interactions, including those involving lizards, it remains to be established what costs are associated with increased infection. Parasites might evolve to reduce their impact because they rely on host availability. Also, the impact of

parasites may be spatially dependent. Habitats that are favourable for hosts may maintain higher parasite intensities because host availability is higher. In these habitats, the host may be able to meet the cost of being parasitized by gaining access to more favourable resources (Bull and Burzacott 1993; Weatherhead et al. 1995).

Infection dynamics of virulent endoparasite species have been most studied in parasite species that impact human health or have economical consequences (Anderson and May 1991; Scott and Smith 1994). We could gain more understanding about the impact of these parasites on host fitness and their natural dynamics by studying populations that are host to less virulent parasites. In this study, we investigated the effect of the haemogregarine blood parasite *Hemolivia mariae* on the home range and behaviour of the Australian sleepy lizard, *Tiliqua rugosa*. We also examined whether individual traits, including condition and activity of the host lizards, incur secondary costs, such as heightened susceptibility to blood parasite infection.

The sleepy lizard is a large, mainly herbivorous lizard of the family Scincidae. Adults can grow to more than 28 cm snout–vent length and can probably live up to 50 years (Bull 1995). They retain stable home ranges over many years, with male and female lizards occupying similar home range areas (Bull and Freake 1999). Each spring, male and female lizards form monogamous associations for 6–8 weeks before mating in early November, with partners spending much of their time, during spring, within 30 cm of each other (Bull 1988). The pairing intensity, estimated as the proportion of days during spring that the pair is detected together, varies among pairs, with low pairing intensity usually associated with reduced reproductive success in that season (Bull et al. 1998). In the semi-arid region near Mt. Mary, South Australia, these sleepy lizards are naturally infested with two tick species, *Aponomma hydrosauri* and *Amblyomma limbatum* (Bull and Burzacott 1993, 2001). The blood parasite *H. mariae* (Smallridge and Paperna 1997) is a haemogregarine with an indirect life cycle involving either *A. hydrosauri* or *A. limbatum* as invertebrate hosts and the sleepy lizard as the vertebrate host. Lizards become infected by ingestion of infected ticks, and ticks become infected by feeding on infected lizards (Smallridge and Bull 1999).

In this study, we investigated the impact of *H. mariae* infection on fitness of the sleepy lizard under natural environmental conditions. We compared the home range areas of lizards with and without *H. mariae* infection, predicting smaller home ranges in infected lizards if the parasite reduced lizard activity. Also, we experimentally exposed uninfected lizards to *H. mariae* and compared body size, pairing status and home range of lizards with lower and higher susceptibility to *H. mariae* infection. We predicted that both large body size and larger home ranges

may require more energy to sustain and perhaps result in higher susceptibility to infection when exposed to the parasite.

Materials and methods

Study population

The study was conducted over the spring and early summer, the period when sleepy lizards show most of their activity (Bull et al. 1991), of two years (17 August–15 December 1999 and 28 August–25 December 2000). The study site (33°55′S, 139°15′E) was in semi-arid chenopod shrubland near Mt. Mary, South Australia where *H. mariae* is found to infect sleepy lizard red blood cells.

Observations of lizards

We observed 29 adult lizards (15 males and 14 females) in 1999, and another 56 (29 males and 27 females) in 2000. Observations involved different lizards in each year. Small (5 g) Sirtrak radio transmitters were attached to the lizard tails with waterproof surgical tape. Each lizard was located on 4–5 days each week over the study period, and its position was recorded using a global positioning system (GPS).

Each week for each lizard, we measured body mass (±5 g) with a 1-kg spring balance and the number of attached ticks (tick load). We calculated an average tick load for each lizard in each year. We also determined pairing status (paired or unpaired). An adult was considered paired if it was found at least once in the spring period within 30 cm of an adult of the opposite sex (Bull 1988; Bull et al. 1998). A blood sample, taken from a toenail clipping of each individual each week, was smeared onto a microscope slide and air-dried in the field. In the laboratory, the smears were fixed in absolute methanol for 5 min and then stained in Giemsa (diluted 1:7 in phosphate buffer, pH 7) for 15 min. Approximately 10^4 erythrocytes (100 fields of about 100 erythrocytes) were examined at a magnification of ×1,000 to detect the presence of intra-erythrocytic gamonts of *H. mariae*. The intensity of infection was estimated as the number of gamonts per 10^4 erythrocytes, and average values were calculated for each infected lizard each year.

For each lizard in 1999 and 2000, home ranges were calculated from the GPS coordinates of locations as 100% minimum convex polygon area (ha) using Biotas 1.02b (Windows: Ecological Software Solutions). All data were analysed for normality. Data for home range area and tick load were not normally distributed and were square root-transformed. Proportions were arcsine-transformed.

Factors associated with home range area

We used general linear models to test the effects of multiple independent variables on home range area for these 85 lizards. As predictors for home range area, we used *H. mariae* infection (infected vs uninfected), average tick load, pairing status (paired or unpaired), year, sex and average body mass. Potential explanatory terms and two-way interactions were fitted using the stepwise backward removal of non-significant terms. Pairing status and *H. mariae* infection were entered as fixed factors, and other factors were entered as variates. All variable factors and two-way interactions were added to the model, but because no two-way interactions were significant, they are not presented.

We also used correlation analysis to determine the relationship between home range area and infection intensity for the ten naturally infected lizards in 2000. There were too few infected lizards to test this relationship in 1999. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 12.0.1 (SPSS 2001). Means were expressed with standard errors, two-tailed probability tests were used, and null hypotheses were rejected at $P < 0.05$.

Susceptibility to infection

We investigated if home range area or body mass was associated with susceptibility to infection after exposure. We considered absolute home range area to be an indirect measure of activity because more active lizards might have a wider sally zone outside of the core refuge area of their home ranges (Kerr and Bull 2006a). Our prediction, that both large body size and larger home ranges result in higher susceptibility to infection when exposed to the parasite, was tested with a group of 19 different lizards initially free of *H. mariae* (14 males and 5 females) that were experimentally fed with infected ticks. From 20 to 24 September 1999, each of the lizards was fed with the dissected remains of an infected adult *A. limbatum* tick (infected from previous blood meals; Smallridge and Bull 2001). The infection status of each tick was confirmed by examination of a smear from the anterior part of the tick gut. The subsequent infection status of these lizards and their home range areas were determined as described above. Home range areas were estimated only from the first 5 weeks after the experimental feeding, the period before most infections entered the lizard blood cells (Smallridge and Bull 1999). Thus, these results were not directly comparable with the non-manipulated lizards in that season where home ranges estimates were derived over longer periods.

Results

Factors associated with home range area

Table 1 shows the mean home range area for male and female sleepy lizards and the percentage of those lizards infected with *H. mariae* in each year. The overall average home range area was 3.4 ± 0.3 ha, and 16 of the 85 lizards (18.8%) were infected. Home range area was significantly associated with year of study, lizard body mass, pairing status and *H. mariae* infection (Table 2). Lizards that were infected with *H. mariae* had smaller home ranges than uninfected lizards (Fig. 1). Home ranges were smaller in 1999 than in 2000 (Table 1), larger lizards had smaller home ranges, and paired lizards had smaller home ranges than unpaired lizards. Lizard sex and tick load had no influence on home range area. There were no significant interaction effects, suggesting that the impact of parasites was consistent across years, across lizard sexes and sizes and across pairing status classes.

Among the naturally infected lizards from 2000, higher infection intensity was significantly associated with larger home range area ($r^2 = 0.71$, $n = 10$, $P = 0.002$; Fig. 2).

Susceptibility to infection

The uninfected lizards that developed infections after ingesting an infected tick took 4–9 weeks (mean 5.7) from feeding before infections could be detected in blood smears. Lizards were considered to be uninfected if infection could not be detected after 12 weeks. Among the 19 experimental lizards, 9 of 14 males (64.3%) and 1 of 5 females (20.0%) became infected. This apparently higher susceptibility in males was not statistically significant ($\chi^2 = 2.90$, $df = 1$, $P = 0.09$) and would need a larger sample size to confirm the trend.

There was no significant difference in body mass among those that did or did not become infected (Wald = 2.476, $df = 1$, $P = 0.116$). However, those that became infected after ingesting an infected tick had a significantly larger mean home range during the early stages of infection than those that did not become infected (Wald = 11.203, $df = 1$, $P = 0.001$; Fig. 3). Note that this difference would not have resulted

Table 1 Percentage of radio-tracked male and female lizards infected with *H. mariae* and their mean (SE) home range area in 1999 and 2000

Year	Sex	Number	% infected	Home range area (ha)
1999	Male	15	13.3	2.7 (0.4)
	Female	14	28.6	2.5 (0.4)
2000	Male	29	17.2	4.5 (0.5)
	Female	27	18.5	3.2 (0.4)

Table 2 Factors influencing the home range area of lizards ($n=85$; 1999–2000)

Independent variables	Estimate (SE)	F	df	P
Year	**−56.10 (14.40)**	**7.635**	1	0.007
Pair status	**33.57 (14.91)**	**7.040**	1	0.010
Infection	**35.58 (14.30)**	**6.072**	1	0.016
Body mass	**−0.18 (0.07)**	**6.659**	1	0.012
Sex	−6.20 (12.34)	0.260	1	0.612
Tick load	−0.96 (7.12)	0.018	1	0.893

Variables highlighted in bold were left in the minimal adequate model after stepwise removal of non-significant variables.

from the possible sex bias in susceptibility because male and female lizards had similar home range areas.

Discussion

The results of our observations show two apparently contradictory results. First, we showed that lizards that were naturally infected with *H. mariae* had smaller home ranges than lizards without a detectable infection. This result was consistent across two years, and the same trends were apparent in both male and female lizards. It conforms with the prediction that the parasite inhibits lizard activity. Home range area may be reduced because the infected
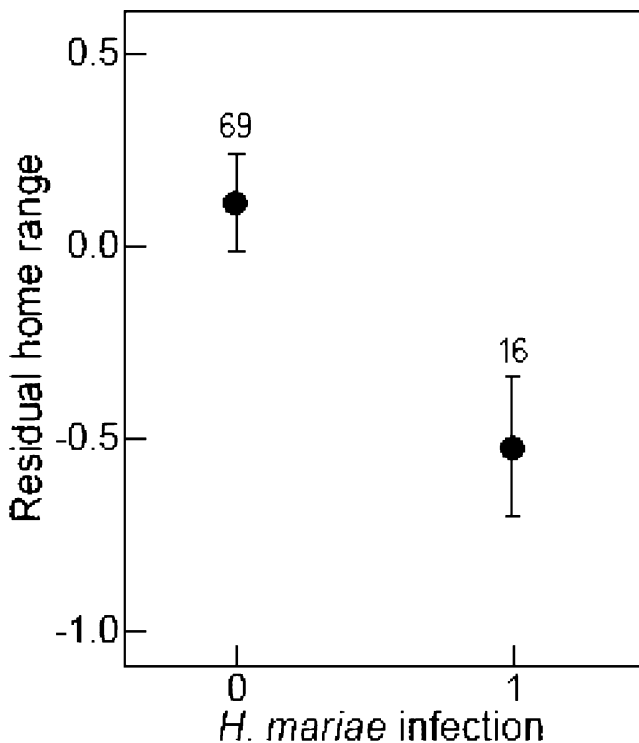

Fig. 1 The influence of *H. mariae* infection on mean (±SE) home range area (ha, controlled for body mass and pairing status). The number of lizards are indicated (1999–2000; see also Table 2)
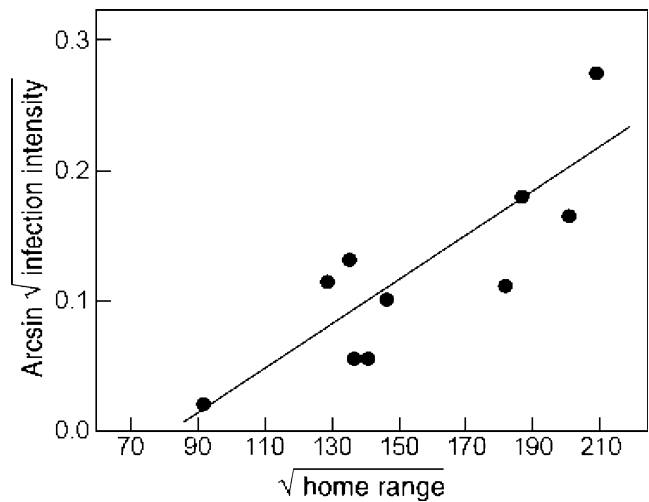

Fig. 2 The influence of home range area (m^2) on *H. mariae* infection intensity (number of gamonts per 10^4 erythrocytes) of infected sleepy lizards in 2000 ($r^2=0.71$, $n=10$, $P=0.002$; $arcsine\sqrt{infection\,intensity} = 0.1384 + 1.7 \times 10^{-3} \times \sqrt{home\,range}$)

lizards move about less or have fewer exploratory ventures beyond their core area of activity. Alternatively, the blood parasite may reduce the vigour of the host lizard, such that it is less able to defend its home range area from neighbours or is inhibited from venturing into overlapping home range areas. There is no regular and overt defence of home ranges in sleepy lizards, and total home ranges overlap extensively (Bull 1994; Bull and Freake 1999). However, they maintain exclusive core areas within their home ranges (Kerr and Bull 2006b), and we have occasional observations of males fighting (Bull and Pamula 1996; Kerr and Bull 2002). Thus, parasites could affect social status and hence home range size.

The second and apparently contradictory result was that, among the home ranges of naturally infected lizards, those
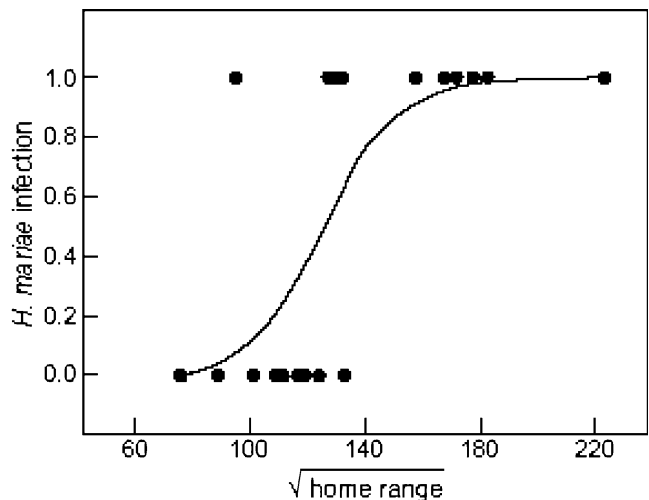

Fig. 3 Home range areas of 19 uninfected sleepy lizards that were fed with an infected *A. limbatum* tick in relation to their chance of becoming infected with *H. mariae* (Wald=11.203, $df=1$, $P=0.001$; fraction infected=$1/(1+e^{-z})$; $z= 0.075(\sqrt{home\,range}) - 9.519$; $n=19$ lizards)

lizards with higher infection intensity had significantly larger home ranges than those with lower intensity infection. In laboratory trials, single infection events of the kind used in our experimental infections generally lead to uniform infection intensity profiles (Smallridge 1998; Smallridge and Bull 1999). The higher infection intensities observed in early stages of infection decline over time because the parasite does not reproduce in lizard red blood cells and because infected red blood cells are gradually replaced by new uninfected cells (Smallridge 1998; Small-ridge and Bull 1999). Alternatively, higher infection intensity might result from multiple infections. We interpret the observed positive relationship between infection intensity and home range size to mean that lizards with larger home ranges are more likely to become infected (and thus might have been infected more often or more recently) rather than that higher infection levels stimulate lizards to occupy larger home ranges.

Our experimental results support this interpretation. Lizards that were initially uninfected were significantly more likely to become infected after exposure to the parasite if they had a larger home range. Why would lizards with larger home ranges become infected more frequently? One obvious explanation is that, by covering a greater area, they are more likely to become exposed to the parasite. The mechanism of transmission is through lizards ingesting ticks that have fed on a previously infected lizard (Smallridge and Bull 1999). Thus, to become newly infected, or for an already infected lizard to become super infected, a lizard must first enter the home range area of another lizard that has been infected, and second, must contact and ingest infected ticks (usually in one of the overnight refuge sites of the infected lizard). The more a lizard moves around, the more likely this is to happen.

However, this cannot be the only explanation for the significant trend for greater infection intensity associated with larger home ranges for two reasons. First, in the observed population, we found no association between home range area and tick load. Second, in the experiment, all lizards had an equal exposure to the parasite independent of their activity and home range sizes; in that, they were each fed a single infected tick. This suggests that lizards with larger home ranges are more susceptible to infection after exposure, and that less active lizards have stronger resistance to infection. The statistically significant trend could be explained if lizards sacrifice some level of parasite resistance for increased activity. In this explanation, ingestion of infected ticks does not always result in infection because lizards can mount a defence against the parasite, but the strength of the defence, and thus the probability of infection, depends on the level of lizard activity.

Studies of birds and mammals have shown that individuals vary in their resistance to infection (e.g. Merino et al. 2000; Ots and Hõrak 1998; Wiehn et al. 1997), and that males that develop secondary sexual characteristics to enhance their mating success may, at the same time, reduce their resistance to infection (e.g. Sheldon and Verhulst 1996; Hillgarth and Wingfield 1997; Wiehn et al. 1997). In sleepy lizards, individuals with higher activity levels and larger home ranges may similarly be compromising their ability to resist infection. Although we did not establish statistical significance, there was a three times higher proportional infection of males than females in our experiment. Bull et al. (1991) and Kerr and Bull (2006a) have shown that male sleepy lizards are active for longer each day and on more days than females in the spring period over which our experiment was conducted, presumably to include mate-searching activity. This is consistent with our explanation of differential levels of susceptibility to infection.

In summary, the interaction between the lizards and their parasites reveals a complex spatial pattern that may result in some buffering of the infection dynamics. First, lizards that are more active and have larger home ranges are more likely both to be exposed to the parasite and to have lower resistance to parasite infection. Thus, these more active lizards are more likely to become infected. Then, however, the longer-term effect of infection is to reduce activity and home range area, thus potentially reducing the probability of exposure and re-infection. The results conform with an emerging trend from other studies for an ecological trade-off between fitness and defence against pathogens (Sheldon and Verhulst 1996).

References

Anderson RM, May RM (1982) Coevolution of hosts and parasites. Parasitology 85:411–426

Anderson RM, May RM (1991) Infectious disease of humans: dynamics and control. Oxford University Press, Oxford

Andersson M (1994) Sexual selection. Princeton University Press, Princeton NJ

Bull CM (1988) Mate fidelity in an Australian lizard *Trachydosaurus rugosus*. Behav Ecol Sociobiol 23:45–49

Bull CM (1994) Population dynamics and pair fidelity in sleepy lizards. In: Vitt LJ, Pianka ER (eds) Lizard ecology: historical

and experimental perspectives. Princeton University Press, Princeton, NJ, pp 159–174

Bull CM (1995) Population ecology of the sleepy lizard, *Tiliqua rugosa*, at Mt Mary, South Australia. Aust J Ecol 20:393–402

Bull CM, Burzacott D (1993) The impact of tick load on the fitness of their lizard hosts. Oecologia 96:415–419

Bull CM, Burzacott DA (2001) Temporal and spatial dynamics of a parapatric boundary between two Australian reptile ticks. Mol Ecol 10:639–648

Bull CM, Freake MJ (1999) Home range fidelity in the Australian sleepy lizard, *Tiliqua rugosa*. Aust J Zool 47:125–132

Bull CM, Pamula Y (1996) Sexually dimorphic head sizes and reproductive success in the sleepy lizard *Tiliqua rugosa*. J Zool 240:511–521

Bull CM, Cooper SJB, Baghurst BC (1998) Social monogamy and extra-pair fertilization in an Australian lizard, *Tiliqua rugosa*. Behav Ecol Sociobiol 44:63–72

Bull CM, McNally A, Dubas G (1991) Asynchronous seasonal activity of male and female sleepy lizards, *Tiliqua rugosa*. J Herpetol 25:426–441

Combes C (1997) Fitness of parasites: pathology and selection. Int J Parasitol 27:1–10

Dunlap KD, Schall JJ (1995) Hormonal alterations and reproductive inhibition in male fence lizards (*Sceloporus occidentalis*) infected with the malarial parasite *Plasmodium mexicanum*. Physiol Zool 68:608–621

Goater CP, Holmes JC (1997) Parasite-mediated natural selection. In: Clayton D, Moore J (eds) Host–parasite evolution: general principles and avian models. Oxford University Press, Oxford, pp 9–29

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218:384–387

Hillgarth N, Wingfield JC (1997) Parasite-mediated sexual selection: endocrine aspects. In: Clayton DH, Moore J (eds) Host–parasite evolution. Oxford University Press, Oxford, pp 78–104

Kerr GD, Bull CM (2002) Field observations of aggressive encounters between male sleepy lizards *Tiliqua rugosa*. Herpetol Rev 33:24–26

Kerr GD, Bull CM (2006a) Movement patterns in the monogamous sleepy lizard (*Tiliqua rugosa*): effects of gender, drought, time of year and time of day. J Zool 269:137–147

Kerr GD, Bull CM (2006b) Exclusive core areas in overlapping ranges of the sleepy lizard, *Tiliqua rugosa*. Behav Ecol 17:380–391

Main AR, Bull CM (2000) The impact of tick parasites on the behaviour of the lizard *Tiliqua rugosa*. Oecologia 122:574–581

Merino S, Moreno J, Sanz JJ, Arriero E (2000) Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). Proc R Soc Lond B Biol Sci 267:2507–9510

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

Ots I, Hõrak P (1998) Health impact of blood parasites in breeding great tits. Oecologia 116:441–448

Price PW (1980) Evolutionary biology of parasites. Princeton University Press, Princeton, NJ

Schall JJ (1983) Lizard malaria: parasite–host ecology. In: Huey RB, Pianka ER, Schoener TW (eds) Lizard ecology: studies of a model organism. Harvard University Press, Cambridge, MA, pp 84–100

Schall JJ (1986) Prevalence and virulence of a haemogregarine parasite of the Aruban whiptail lizard, *Cnemodophorous arubensis*. J Herpetol 20:318–324

Schall JJ (1996) Malarial parasites of lizards: diversity and ecology. Adv Parasitol 37:255–333

Scott ME, Smith G (1994) Parasitic and infectious diseases: epidemiology and ecology. Academic, San Diego

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

Smallridge CJ (1998) Studies on a haemogregarine parasite of the Australian skink, *Tiliqua rugosa*. Ph.D. thesis, Flinders University of South Australia, Adelaide

Smallridge CJ, Bull CM (1999) Transmission of the blood parasite *Hemolivia mariae* between its lizard and tick hosts. Parasitol Res 85:858–863

Smallridge CJ, Bull CM (2001) Prevalence of infection by the protozoan, *Hemolivia mariae*, in ticks. Parasitol Res 87:169–172

Smallridge CJ, Paperna I (1997) The tick transmitted haemogregarinid of the Australian sleepy lizard, *Tiliqua rugosa* belongs to the genus *Hemolivia*. Parasite 4:359–363

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

SPSS (2001) SPSS 12.0.1. SPSS, Chicago, IL

Weatherhead PJ, Barry FE, Brown GP, Forbes MR (1995) Sex ratios, mating behavior and sexual size dimorphism of the northern water snake, *Nerodia sipedon*. Behav Ecol Sociobiol 36:301–311

Wiehn J, Korpimaki E, Bildstein KL, Sorjonen J (1997) Mate choice and reproductive success in the American kestrel: a role for blood parasites. Ethology 103:304–317