THE ECOLOGY OF A HOST-PARASITE RELATIONSHIP:

HAEMOGREGARINES & THE EASTERN WATER SKINK,

EULAMPRUS QUOYII

Thesis submitted by

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Daniel Joseph Salkeld

ABSTRACT

The study of wildlife disease has gained importance in the last two decades as a result of theoretical insights into its possible roles in host evolution, population biology and ecology. However, knowledge of how hosts and parasites interact in natural systems remains limited, and there is a critical need for further research. Therefore, this thesis examines the ecology and interactions between wild populations of hosts, the eastern water skink, *Eulamprus quoyii*, and a parasite, the haemogregarine protist *Hepatozoon hinuliae*. I carried out a two-year mark-recapture study of eastern water skinks at Blackdown Tableland, Queensland, Australia, and analysed blood slides to measure haemogregarine blood parasite infection. Prevalence (the proportion of the host population infected) increased with host age, did not differ between the sexes, and varied little during the two-year study. Parasite load (the intensity of infection within individuals) was significantly higher in males than in females, and is highly correlated in individuals over time. Eastern water skinks are viviparous, and therefore reproductive output can be accurately measured by housing pregnant females in captivity shortly before they gave birth. High haemogregarine loads reduced female water skink fecundity, by approximately one offspring per litter, compared to females with low parasite loads. Body condition and fat reserves were not responsible for this reduced fecundity. There was no effect of maternal haemogregarine parasite load on offspring size/number trade-offs, or on the performance of offspring measured by growth rates, sprint speed or competitive ability. Using microsatellite markers, I carried out a preliminary investigation of the effect of blood parasites on female mating strategy. Fifty percent of analysed litters showed evidence of multiple sires, but the propensity to multiple mating was unaffected by female haemogregarine parasite

load. Because *Eulamprus quoyii* occupies a large geographical range spanning the Australian tropical and temperate zones, I investigated whether patterns of parasite abundance are affected by climate. Parasite load, but not prevalence, is related to temperature, but is independent of rainfall. In conclusion, I argue that haemogregarine blood parasites affect the life-history of their natural host, the eastern water skink, and that continued study of the Blackdown Tableland population should offer further insights into the evolutionary ecology of a wild host-parasite relationship.

ACKNOWLEDGEMENTS

I'm interested in things. I'm not a real doctor but I am a real worm, I am an actual worm.

They Might Be Giants

And so, thanks to…

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the road-trip-high, a zen-like consciousness that can only be achieved by speeding for hundred of kilometres on empty roads in a green Hilux Surf, in a sunburnt country of towering skies, acacias, emus and kangaroo carcasses decomposing on the roadside. And the smell of tropical rain hitting equatorial asphalt.

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STATEMENT ON SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any other university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

All research procedures reported in this thesis received the approval of James Cook University and Department of Agriculture, NSW Ethics Committees. Scientific permits to collect animals were granted by National Parks & Wildlife Service of Queensland & New South Wales.

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Daniel Joseph Salkeld

CHAPTER 1

GENERAL INTRODUCTION & METHODS

> Many years later, as he faced the firing squad, Colonel Aureliano Buendia would remember that distant afternoon when his father took him to discover lice.
>
> Misspelling of the first line of 'One Hundred Years of Solitude'[1]
> Gabriel Garcia Marquez

PERSONAL INTRODUCTION

After a vain effort to conceive a gripping first line for this PhD thesis, I have decided instead to bastardise Garcia Marquez and establish a tenuous link between great literature and parasites. It puts me in good stead, for I have bastardised the work of many other scientists while writing this tome, the theme of which is parasites. During travels in South America, I developed an interest in disease ecology when I played host to several infestations of life-threatening bacteria, harboured the progeny of a vindictive botfly in my shoulder whilst in the steaming depths of the Amazon's Rio Tambopata, and contracted a pathogenic fungus from bats skulking in a Bolivian cave. I somehow managed to avoid leishmaniasis, malaria, yellow fever, cholera, typhoid, schistosomiasis and Chaga's disease. This thesis however, is not about the romantic diseases that debilitate much of mankind,

[1] Of course, the real version refers to that distant afternoon when his father took him to discover ice. The fictional characters of the novel would presumably have been familiar with ectoparasites from an early age, although, disappointingly, Garcia Marquez fails to mention this.

but instead discusses the little-known parasites of some Australian reptiles.

THE ECOLOGY OF WILDLIFE DISEASES

Evolutionary ecologists have traditionally assumed that parasites had little impact on their host's biology, arguing that if virulent parasites reduced host survival then they would ensure their own extinction, and so should therefore evolve towards benign relationships with their hosts (Hudson et al. 2002). However, this viewpoint was challenged when studies of host-parasite systems that had coevolved over long periods revealed that parasites still inflicted fitness costs upon their hosts (Keymer & Read 1991; Schall 1990a; Schall 1990b). Subsequent insights into the interactions between virulence, host density and transmission rates have resulted in the consensus that parasites are in fact important selective agents affecting host population biology, ecology and evolution (Dawkins 1990; Gulland 1995; Hudson et al. 2002; Morand & Poulin 2000). Because parasitic diseases can pose major threats to endangered animal populations, have serious economic impacts in agriculture, and can affect public health, further research into host-parasite ecology is critical (Cleaveland et al. 2002; Dobson & Carper 1996; Dobson & Foufopoulos 2001; Galvani 2003; Gog et al. 2002; Lafferty & Gerber 2002; McCallum & Dobson 2002; McCallum & Dobson 1995; Osterhaus 2001). However, in spite of the need of further research, knowledge of how hosts and parasites interact in natural systems remains limited, and consequently, international bodies like the World Health Organisation have advocated an increased study of host-parasite evolution and ecology in wild host populations (Real 1996; Schall & Pearson 2000).

Therefore, this thesis examines the ecology and interactions between wild populations of hosts, the eastern water skink, *Eulamprus quoyii*, and a parasite, the haemogregarine protist *Hepatozoon hinuliae*.

Lizards and blood parasites

Lizards are ideal for ecological studies of natural host-parasite interactions because they are easy to observe and capture, frequently demonstrate site-fidelity, which facilitates mark-recapture studies, and can occur in dense populations allowing researchers to obtain appropriate sample sizes for statistical inferences (Schall 1990a). Previous studies of lizard-parasite systems have demonstrated that natural infections of malaria parasites, *Plasmodium mexicanum*, affect host physiology and induce behavioural alterations and fitness costs in their host, the western fence lizard (*Sceloporus occidentalis*) (Schall 1990a). Infected fence lizards exhibit reduced blood haemoglobin concentrations and an increased abundance of immature erythrocytes, which affects maximal oxygen consumption (Schall et al. 1982). As a result of the altered physiology, *P. mexicanum* infection affects lizard host behaviour (Schall & Sarni 1987), and reduces running stamina (Schall et al. 1982) and male social status (Schall & Dearing 1987; Schall & Houle 1992). Malarial parasite infection is also associated with decreased lipid storage and clutch size in females (Schall 1983) and smaller testis size in males (Schall 1990a). Although *P. mexicanum* reduces host fitness by reducing host fecundity, there is no evidence that malaria parasites increase mortality in its fence lizard hosts (Eisen 2001).

Other blood parasites also affect their lizard hosts. Common European lizards, *Lacerta vivipara*, infected with haemogregarines had lower mean running speeds, lower circular-track endurance, lower haemoglobin concentrations and reduced oxygen consumption when compared to uninfected lizards (Clobert et al. 2000; Oppliger et al. 1996). Tail regeneration was also slower in parasitised lizards (Oppliger & Clobert 1997). Another haemogregarine, *Haemolivia mariae*, is associated with poor condition in male Australian sleepy lizards (*Tiliqua rugosa*) (Smallridge & Bull 2000). Furthermore, haemogregarine (*Hepatozoon* sp.) infection was associated with higher oxygen consumption at rest in the Aruban whiptail lizard (*Cnemidophorus arubensis*) even though there were no demonstrable effects on the host's haematology, physiology, anatomy (organ mass) or behaviour (Schall 1986).

Hosts may also be largely unaffected by parasitic infections. *Plasmodium floridense* and *Plasmodium azurophilum* had no effects on the body condition (SVL/mass) of the Puerto Rican anole *Anolis gundlachi* (Schall & Pearson 2000). Mosquito-borne filarial parasites *Oswaldofilaria chlamydosauri* had no effect on body condition, oxygen consumption, blood haematocrit or haemoglobin concentration of frillnecked lizards (*Chlamydosaurus kingii*) (Christian & Bedford 1995).

Variations in host susceptibility to disease can influence host community ecology. For example, on the Caribbean island of St. Maarten, the competitively superior lizard *Anolis gingivinus* is more susceptible to infections of malaria parasites (*Plasmodium azurophilum*) than *Anolis wattsi*, and the two lizards only coexist

where malaria is present, presumably because infection compromises the competitive ability of *A. gingivinus* (Schall 1992).

Although parasites may appear avirulent in certain hosts, it is important to be aware of two factors when considering studies on a parasite's virulence. First, the effect of parasites may be subtle and difficult to demonstrate in terms of measurable host fitness (Christian & Bedford 1995), and second, the potential impact of parasites on individual hosts may vary depending on circumstances such as host condition or physiology (Newman et al. 2001; Sanz et al. 2002).

THE HOST-PARASITE MODEL

The host: *Eulamprus quoyii*, the eastern water skink

Eulamprus quoyii is a relatively large (mean snout-to-vent length (SVL) = 107.7 mm, up to 133 mm, mean mass = 26.4 g, up to 47.5 g, n = 305; Fig. 1.1) diurnal, heliothermic skink that lives mainly in habitats close to permanent water (Cogger 2000). It is an opportunistic insectivore that is also known to eat adult frogs, tadpoles, fish and other skinks (Daniels 1987; Salkeld in press; Veron 1969a). Eastern water skinks occur throughout eastern Australia from northern Queensland to southern New South Wales, and along the Murray-Darling drainage system to western Victoria and southeastern South Australia (Cogger 2000).

Eulamprus quoyii belongs to a group of five closely related species comprising *E. heatwolei, tympanum, kosciuskoi and leurensis* (Greer 1989). The *Eulamprus* group has been the focus of much ecological research, including recent work on

reproductive biology and life-history (Blomberg & Shine 2001; Doughty & Shine !997, 1998; Rohr 1997; Schwarzkopf 1992, 1993, 1996; Schwarzkopf & Shine 1991; Wilson & Booth 1998), predation (Blomberg & Shine 2000), mating systems (Morrison et al. 2002), sex allocation (Robert & Thompson 2001; Robert et al. 2003) and physiology (Borges Landaez 1999; Robert & Thompson 2000; Thompson 1981; Thompson et al. 2001). The eastern water skink is particularly appropriate for life history studies because females are viviparous, so reproductive output can be accurately measured by isolating females shortly before they give birth. Furthermore, energy reserves (especially those relevant to reproductive output) are stored almost exclusively in the tail and can be estimated simply by measuring tail-base width (Doughty & Shine 1997; Doughty & Shine 1998). It is also a common, abundant, and philopatric skink, making it a good subject for successful mark-recapture studies.

The parasite: *Hepatozoon hinuliae*

Haemogregarines (Apicomplexa: Adeleina) are parasitic protists of the blood, closely related to coccidia and *Plasmodium* (Perkins & Keller 2001). Members of the genus *Hepatozoon* possess a complex life cycle and are transmitted from vertebrate host blood to invertebrate hosts during blood feeding (Smith 1996). Possible vectors include ticks, mites, reduviid bugs, sandflies, fleas, leeches and mosquitoes (Smallridge & Bull 1999; Smith 1996). Subsequent transmission of *Hepatozoon* to another vertebrate host occurs exclusively by ingestion of the infected invertebrate (Smith 1996), and the diet of *E. quoyii* includes haematophagous vectors such as acarinads (ticks and mites), and tabanids (march

flies) (Veron 1969a). However, during this study I found very few ectoparasites on captured animals (only 2 ticks) in Blackdown Tableland and was unable to pursue an investigation of possible vectors. Theoretically, transmission may also occur in *E. quoyii* when other infected lizards are consumed (Salkeld in press). *Hepatozoon* parasites have a global distribution and infect all major lizard families (Smith 1996). Host-specificity is variously reported as being both high (Telford et al. 2001) and low (Smith et al. 1999).

Only one species of haemogregarine has so far been described from *Eulamprus* lizards: *Hepatozoon hinuliae* (formerly *Haemogregarina hinuliae*, reclassified by Smith 1996) which was originally described by Johnston & Cleland (1910) (see Mackerras 1960), from *Eulamprus quoyii* (formerly *Sphenomorphus quoyii*). Within this study, I have assumed that all observed blood parasites are *Hepatozoon hinuliae*, because this is the only species previously described in *Eulamprus* lizards, it resembles the blood parasites found in my study, and because all the parasites I observed were morphologically similar (Fig. 1.2).

Study site

Lizards were caught from a number of study sites (Chapter 6) but most of the work (Chapters 2-5) focused on a population of *Eulamprus quoyii* in Blackdown Tableland National Park, a large sandstone mesa in central Queensland. The main study area was at South Mimosa Creek (149°04'E, 23°47'S, altitude 760m), a creek running through open, dry sclerophyll forest. Small pools of water remained throughout the study period, although many areas dried up. Additional animals

were also caught at North Mimosa Creek and Rainbow Falls, about 3 and 11 km distant, respectively. Field work took place from December 2000 to December 2002.

The woodland adjacent to the creek was *Eucalypt* woodland with an open understorey (Fig. 1.1). Wildfire burnt the study area and much of Blackdown Tableland during October 2002 and had a visible effect upon the lizard habitat in the creek bed, as areas with good cover, previously consisting of heaped leaf litter, dead branches and large logs, were reduced to ash. These types of habitat had previously provided refuge and basking sites for eastern water skinks.

Other lizard species commonly observed in the creek beds at Blackdown Tableland were nobby dragons, *Amphibolurus nobbi,* two-line dragons, *Diporiphora australis*, eastern water dragons, *Physignathus lesuerii*, goannas, *Varanus varius*, rainbow skinks, *Carlia schmeltzii*, major skinks, *Egernia frerei,* fire-tailed skinks, *Morethia taeniopleura*, and a congener, *Eulamprus sokosoma*.

GENERAL METHODS

Capture in the wild

All animals were caught by hand, with hand-held nooses or by using baited sticky traps (Downes & Borges Landaez 1998). Animals were kept temporarily in cloth bags until they were processed (normally on the same afternoon), and then released at the exact sites of capture (within 24 hours of capture, apart from those lizards

being kept in JCU). Lizards were captured during December (summer), March (autumn) and September/October (spring) from December 2000 to December 2002.

Lizards were sexed by everting hemipenes. Snout-to-vent length (SVL) and tail length were measured to the nearest millimetre using a transparent plastic ruler. Obvious regeneration of the tail was noted. Body mass was recorded to the nearest 0.5 g with a 60 g spring balance. Head width, throat width and tail width were recorded to the nearest 0.1 mm using callipers placed just behind the eyes, behind the skull and approx 1 cm behind the cloacal vent respectively. Minimum head, throat and tail width estimates were obtained by gently compressing the relevant body part with the callipers, and then allowing them to spring back with natural tissue resistance. The number of ectoparasites (mites or ticks) was recorded, with particular attention paid to the lizard's nostrils, ears, limb joints and toes. However, very few ectoparasites were found on *Eulamprus quoyii* in Blackdown Tableland, so they are not discussed further. All lizards were marked with a toe clip combination that allowed subsequent individual identification (no more than four toes were removed from an individual).

Figure 1.1 *Top:* th*ii.* This individual has been released after several months in captivity, hence the large fat tail. *Middle:* heavily pregnant female water skink. *Bottom:* prime *'quoyii'* habitat at Blackdown Tableland, Queensland. Note permanent water, vegetation and rock ledges that provide refuge and basking sites.

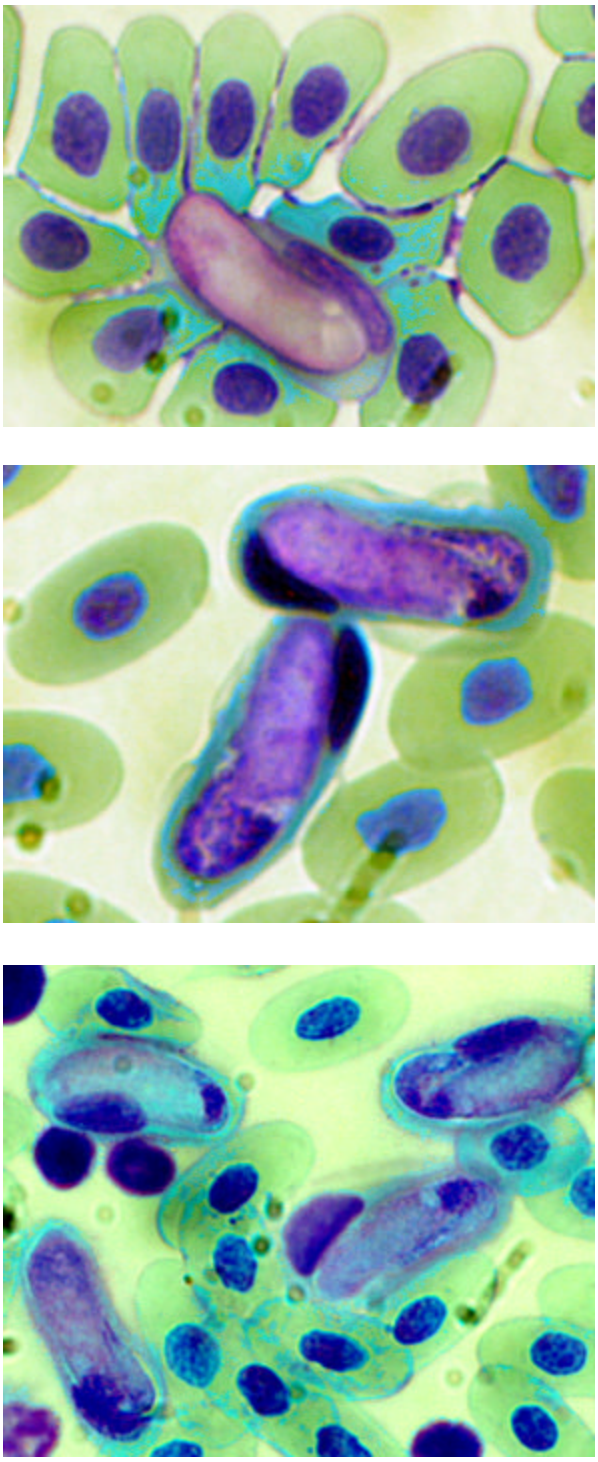

Figure 1.2 The parasite *Hepatozoon hinuliae*. Note the displacement of lizard erythrocyte nuclei in infected cells.

Blood sampling

Lizard blood was sampled from the caudal vein ventral to the tail vertebrae. To prevent blood clotting, porcine heparin solution was taken up in single-use 0.5 ml syringes (TM Terumo, 0.33 x 13 mm) and then expelled before sampling, which left enough heparin to coat the syringe's surfaces. Lizards were held in my left hand, ventral side up, with their tail gently restrained. Needles were inserted, bevel-up, at an acute angle between the scales on the mid-line of the tail. Once the needle was in caudal tissue, the plunger was retracted (to create a vacuum) and the syringe inserted at a near perpendicular angle. The syringe was then pressed slowly into the tail until it pierced the caudal vein and blood entered the vacuum in the syringe barrel. If blood failed to enter the barrel, the needle would contact the vertebrae, at which point slow retraction of the syringe point would generally find the blood vessel. Approximately 0.02 ml of blood was taken and then made into a smear.

Staining & examining slides

Blood slides were air-dried and then fixed in absolute methanol for three minutes. Slides were immediately placed directly (i.e., still wet with methanol) in Giemsa stain (1:10 dilution of Improved R66 GURR solution and phosphate buffer). (The buffer was made with 5.47g of KH_2PO_4 (MW 136.09), 3.8g of Na_2HPO_4 (MW 141.96), 1,000 ml distilled water, and the pH was adjusted to 7.2 by addition of KOH or NaOH in solution). Slides were stained for 45 minutes, before being washed in tap water and then left to dry. Giemsa stain was used only twice (because it oxidises), and methanol only once (because it absorbs moisture). Blood slides were examined under the microscope at 400x magnification. Presence or absence of

haemogregarines was determined by searching transects of the blood smear for up to six minutes. If no haemogregarines were found after six minutes of searching the sample was considered uninfected. Parasite load, was quantified by counting the number of infected cells in a transect consisting of a minimum of 2,000 red blood cells, and then expressed as a percentage. A gridded eyepiece allowed accurate counting of cells. To check that infected cell counts were repeatable, I recorded the number of infected cells observed when counting 2,000 and 10,000 red blood cells on the same slide. Counts of parasitised cells were highly correlated ($n = 58$, $r^2 = 0.91$, $P < 0.000$; Fig. 1.3). Other studies of lizard blood parasites have typically counted parasites present in 1,000 red blood cells (e.g., Eisen 2000), so my study was sufficiently rigorous to detect parasitism.

False positives i.e. scoring animals as infected when they were not, were improbable using this procedure because contamination is very unlikely (separate syringes and slides were used for each animal). False negatives may have been a problem if the number of parasitised cells in an individual declined to undetectable levels. However, PCR studies have shown that visually scanning blood slides results in few false negatives (Perkins et al. 1998).

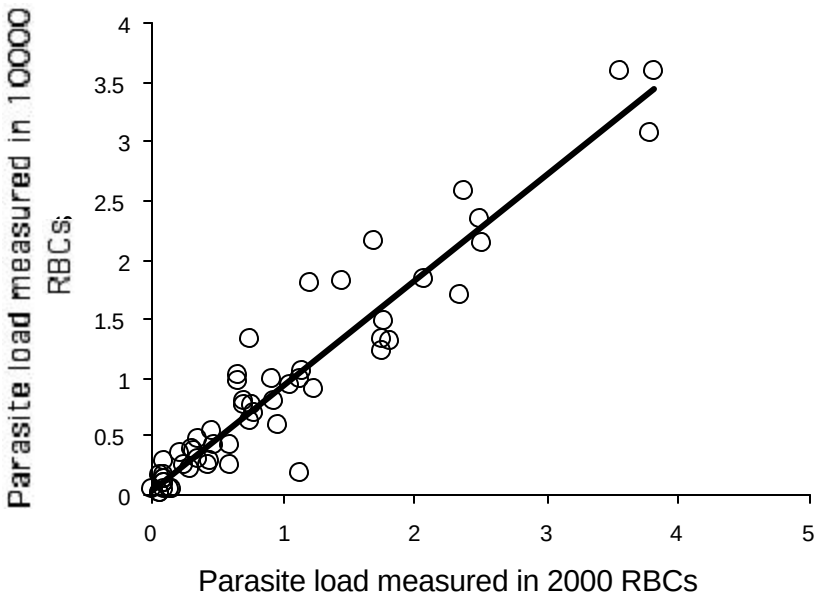

Figure 1.3 The relationship between parasite load (% of infected red blood cells) in counts of 2000 and 10000 red blood cells (RBCs). There was a significant positive correlation between the two count levels.

STUDY AIMS & THESIS STRUCTURE

My study examines a wild host-parasite system, using a population of Australian eastern water skinks, *Eulamprus quoyii*, infected with a haemogregarine, *Hepatozoon hinuliae*. I describe the disease ecology and the parasite's impacts upon host fitness, life-history and behaviour. In addition, I discuss geographical variation in parasite abundance within different host populations.

This thesis is composed of five main chapters, each dealing with a partic ular theme or aspect of host-parasite ecology. Chapter 2 describes the population biology of the blood parasite, and discusses biotic and abiotic factors that determine parasite

abundance within its lizard hosts. Chapter 3 examines the impact of parasite infection on female host reproductive output. I also examine measures of host condition to infer causes of parasite-induced alterations on host fecundity. In Chapter 4, the relationships between maternal parasite load, offspring performance and offspring phenotype are studied. Using microsatellite markers, Chapter 5 investigates whether female parasite load affects the mating strategy of the host. Geographic variation in parasite abundance is discussed in Chapter 6, and I specifically address the possibility that tropical host populations harbour higher parasite loads than non-tropical populations. Finally, Chapter 7 briefly summarises the entire thesis, and presents several possible directions for future research.

CHAPTER 2

EPIZOOTIOLOGY OF *HEPATOZOON HINULIAE* IN THE EASTERN WATER SKINK, *EULAMPRUS QUOYII*: A MARK - RECAPTURE STUDY OF A NATURAL POPULATION

SUMMARY

Knowledge of the population dynamics of parasite populations is important in understanding the impact of parasites on host populations, and in the conservation and evolution of both host and parasite species. Here I present the results of a two-year mark-recapture study of a water skink population infected with haemogregarine blood parasites. Prevalence (the proportion of the host population infected) increased with host age, did not differ between the sexes, and varied little during the two-year study. Parasite load (the intensity of infection within individuals) was significantly higher in males than in females, and is highly correlated in individuals over time. Current understanding of the reptile immune system is reviewed, and it is suggested that the reptile immune system is not primitive, but advanced and competent.

INTRODUCTION

The ecology of hosts and parasites interact simultaneously, such that host population biology affects the ecology and evolution of the parasite, just as parasite dynamics affect host fitness and population growth (Hudson et al. 2002). Therefore, to understand host population ecology it is important to be aware of parasite population biology, and vice versa. Although the importance of parasites in host evolutionary ecology and population biology is well recognised (Dawkins 1990; Hudson et al. 2002), there is still a need for studies of parasite population biology in natural systems (Real 1996; Schall & Pearson 2000), in part because an understanding of parasite population dynamics can provide insights into parasite control and/or eradication, and of conservation strategies for both host and parasite species (Cleaveland et al. 2002; Galvani 2003).

It is well established that parasite abundance can vary in response to numerous factors, both in terms of prevalence, defined as the proportion of the host population that is infected, and parasite load, which is the number of parasites in an individual host. Biotic factors determining parasite abundance include vector biology (Sol et al. 2000), host density (Arneberg et al. 1998), host sex and age (Schall et al. 2000; Smallridge & Bull 2000), host reproductive effort (Nordling et al. 1998; Norris et al. 1994; Salvador et al. 1997; Sanz et al. 2002; Veiga et al. 1998), host condition and physiology (Appleby et al. 1999; Dowell 2001; Nelson & Demas 1996), and host behaviour (Figuerola & Green 2000). Abiotic factors such as temperature and rainfall can either influence parasite abundance directly, or indirectly, by influencing vector abundance and/or host reproductive cycles (Bajer et al. 2001; Bennett & Cameron

1974; Forbes et al. 1994; Fuller 1996; Gregory et al. 1992; Saad & El Ridi 1988; Saad et al. 1990; Veiga et al. 1998). Abiotic factors, and their effects on parasites, may vary geographically (Abu-Madi et al. 1998; Lefcort & Blaustein 1991; Merila et al. 1995; Moller 1998; Tella et al. 1999).

Studies of lizards infected with protist blood parasites have been important in the development of our understanding of host-parasite ecology (Keymer & Read 1991; Schall 1996), but have focused mainly on malaria parasites (*Plasmodium* spp.) in the Americas and West Africa (Schall 1996). Some work has been done on unspecified haemogregarines (protist blood parasites) in common European lizards (*Lacerta vivipara*) (Sorci 1995), and on *Haemolivia mariae* in Australian sleepy lizards (*Tiliqua rugosa*) (Smallridge & Bull 2000; Smallridge & Bull 2001). However, sampling times of these studies were seasonally restricted: Sorci (1995) only reports parasite loads 1 year after initial capture, whereas Smallridge & Bull (2000) only examined lizards during the main activity season.

Here, I examine an additional host-parasite system, *Hepatozoon hinuliae* in *Eulamprus quoyii* from Australia. The study system differs from those of Schall and co-workers mainly because the life span of *Eulamprus quoyii* is long (8 years, Borges Landaez 1999). Host-parasite dynamics in long-lived hosts may differ from those in short-lived hosts. Secondly, unlike malarial systems that involve biting vectors, hosts become infected with *Hepatozoon hinuliae* when they ingest an infected haematophagous vector (Smith 1996). In this chapter, I compare haemogregarine parasite abundance in various sub-populations of hosts, and emphasise parasite dynamics in correlation with host life history using data from a 24-month mark-

recapture survey to examine temporal changes in parasite abundance at the individual and population levels. Trends apparent from the results presented here are discussed in comparison with previous studies, and implications for our understanding of the reptilian immune system are considered.

METHODS

Lizards were caught at Blackdown Tableland, and details of capture methods, blood sampling and study site are included in Chapter 1. Climatic data of the study site during the mark-recapture study are shown in Figure 2.1. Long-term average annual rainfall at Blackdown Tableland is 1150 mm/year but the actual total annual rainfall was 1117 mm, 582 mm, and 567 mm for 2000, 2001 & 2002 respectively.

Eastern water skinks mate during October, and females give birth in December and January. The allocation of host resources to fats and proteins in yolk and testes begins in April/May, occurs throughout the winter period of inactivity (May to September) and peaks by October (Veron 1969b). To study temporal changes in parasite load which might correspond to seasonal changes in host physiology, I caught lizards at four times in the year: (1) September, when lizards began to emerge after the winter period of inactivity; (2) October, when mating was taking place; (3) December, when females are pregnant and close to parturition; and (4) March, after parturition, but before animals begin yolk and testes development. More males than females were captured during September because they emerge from winter inactivity before females. No lizards were caught in October 2002 because fire prevented access to Blackdown Tableland.

Determining age classes

Although snout-to-vent length (SVL) is often indicative of age, lizard growth is continuous and highly variable, so assigning discrete age classes based on size can be difficult (Blomberg & Shine 2001). However, in the Blackdown Tableland population offspring are only born between December and January (austral summer) and this makes it easy to recognise animals born in the previous summer based on both size and appearance. Size-at-recapture data for animals originally caught at known ages (i.e., one- or two-years olds) were used to generate size-at-age classes of lizards for animals up to five years old. Animals older than this were difficult to classify and were all included in the 'Age 6' class. All pregnant animals had SVLs of 90 mm or larger, therefore animals smaller than this size were considered to be juveniles and in their first year of life.

Statistical analysis

I examined both prevalence and parasite load in various host sub-populations. Apart from the analyses of parasite abundance over time in individuals, and because repeated sampling of lizards is not independent, I only used a single measure of parasite load which was determined from the blood sample obtained at first capture. Parasite load was not normally distributed, so I used nonparametric tests to compare the median parasite load among various host sub-populations, and over time.

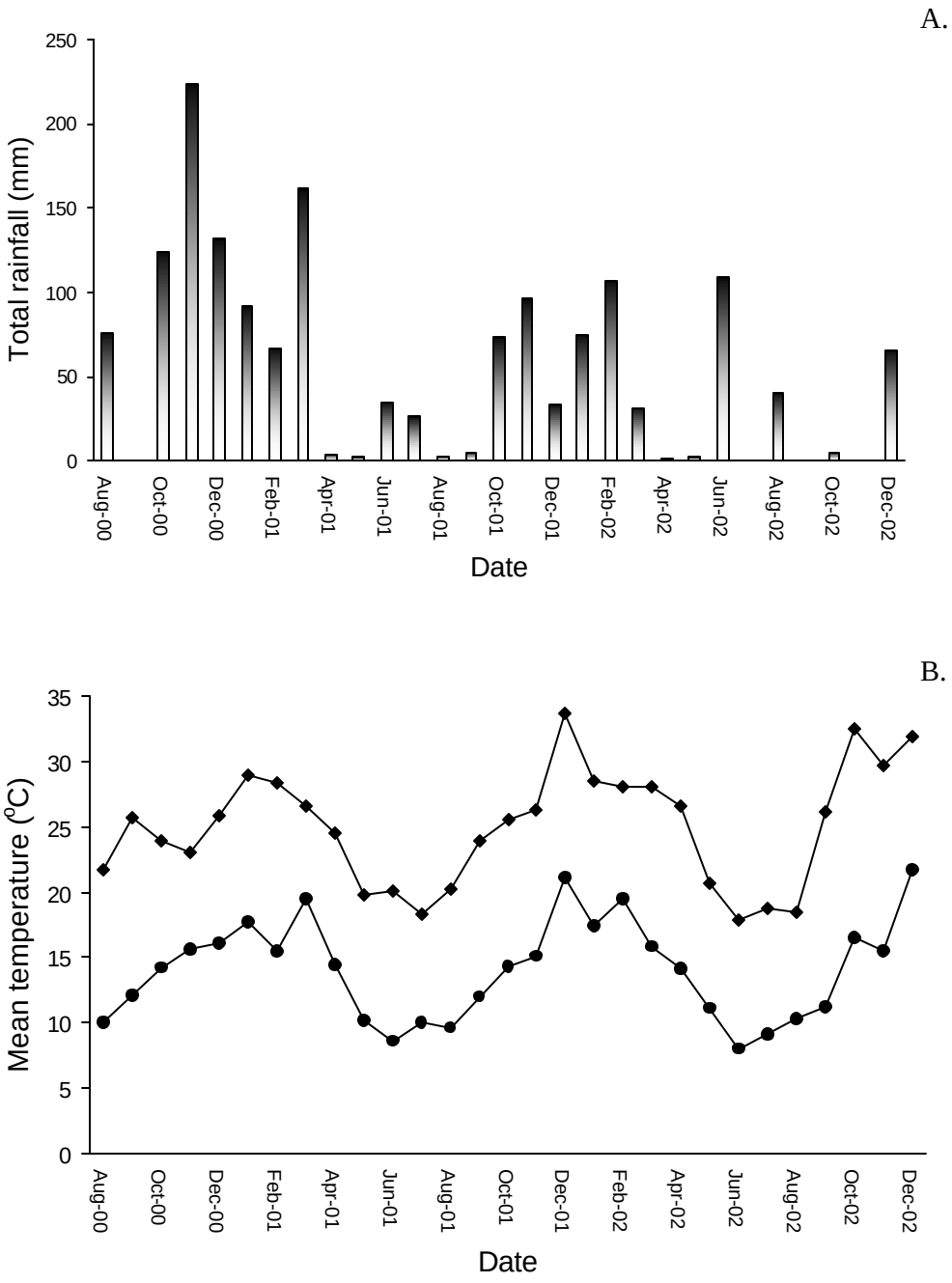

Figure 2.1 Climate data for Blackdown Tableland from August 2000 to December 2002. A. Total rainfall (mm). B. Mean minimum (^{0}C) and maximum (^{0}C) temperatures. Lizard capture occurred between December 2000 and December 2002.

RESULTS

During the two-year mark-recapture study, I made a total of 593 captures (Fig. 2.2), comprising 331 individuals. Overall, there was a high prevalence of blood parasites in water skinks at Blackdown Tableland with 218 / 331 lizards infected (65.9%).

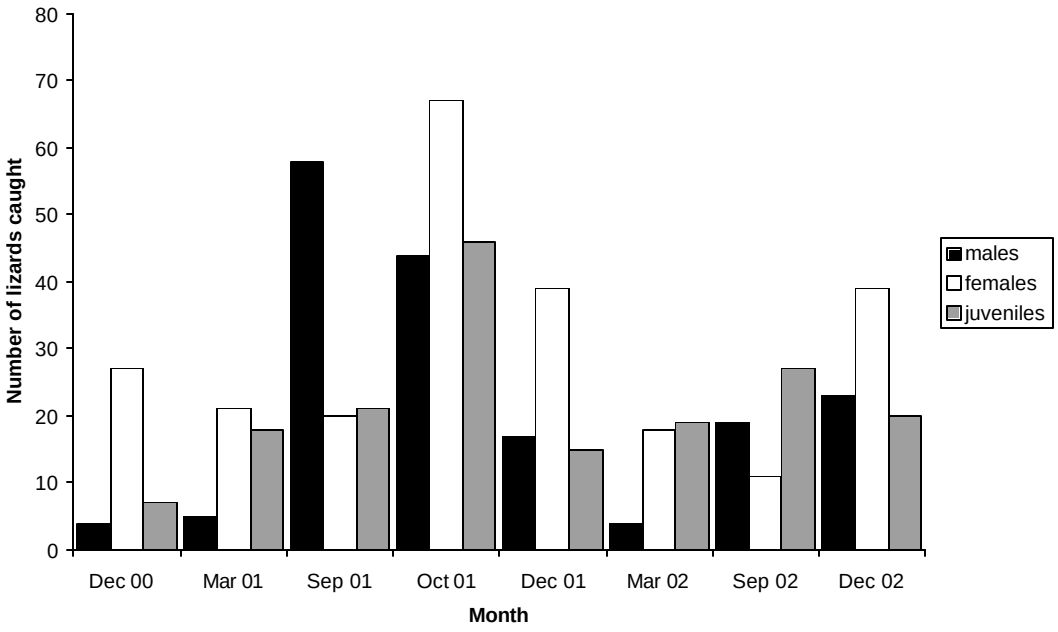

Figure 2.2 The number of male, female and juvenile *Eulamprus quoyii* caught during a 2-year mark-recapture study at Blackdown Tableland, Australia.

Host factors influencing parasite prevalence

Sex

There was no overall difference in prevalence between males and females (no. of males infected = 101/146 = 69.2%; no. of females infected = 117/185 = 63.2%, X^2 = 1.28, P = 0.26, Fig. 2.3). Prevalence was similar in males and females even when considering only adults (no. of adult males infected = 95/107 = 88.8%; no. of females

infected =105/130 = 80.8%, X^2 = 2.86, P = 0.09), or only juveniles (no. of males infected males = 6/39 = 15.4%; no. of females infected =12/55 = 21.8%, X^2 = 0.61, P = 0.44).

Size & Age

Logistic regression revealed that prevalence increased significantly with body size (X^2 = 199.8, P < 0.000; Fig. 2.4). Grouping animals into age classes showed similar results (Fig. 2.3). Juveniles (SVL < 90 mm) were significantly less likely to be infected than adults (SVL = 90 mm) (no. of juveniles infected = 18/94 = 19.1%; prevalence in adults = 200/237 = 84.4%; X^2 = 127.41, P < 0.000).

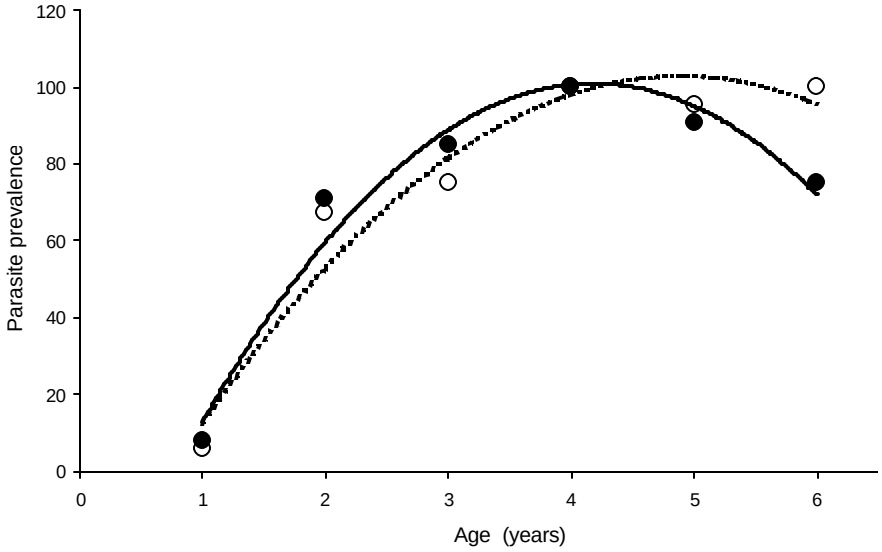

Figure 2.3 Changes in haemogregarine parasite prevalence (proportion of the host population infected) related to age of male (•) and female (o) hosts. Prevalence did not differ significantly between the sexes, but was significantly higher in adult hosts. Polynomial regression curves explain the relationship well (male, r^2 = 0.96, y = -8.7x^2 + 72.8x - 50.9, unbroken line; female, r^2 = 0.94, y = -5.9x^2 + 58.3x - 39.8, broken line).

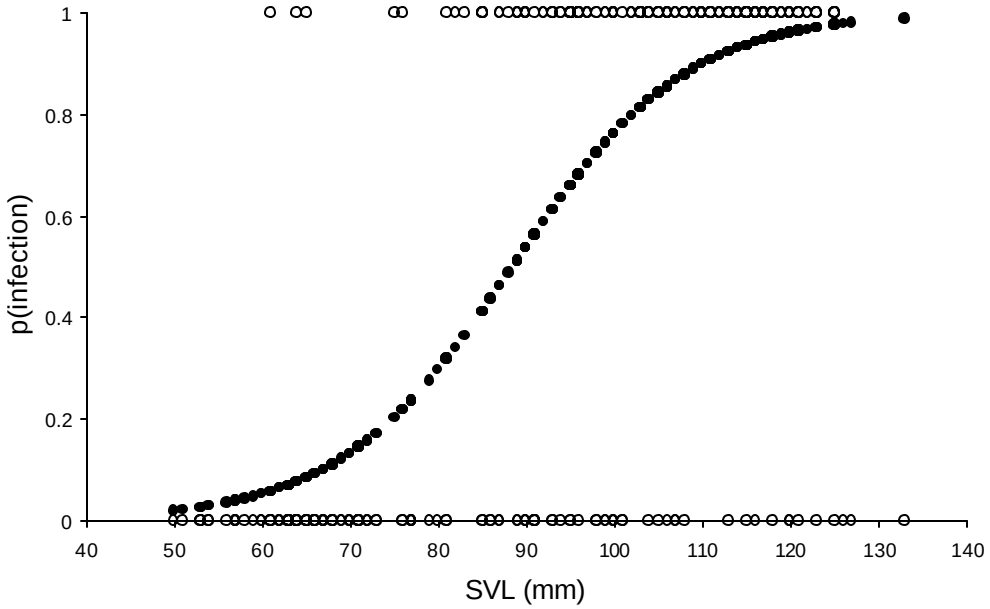

Figure 2.4 Fitted logistic curve showing the relationship between body size (snout-vent length, SVL) and probability of haemogregarine blood parasite infection (prevalence) in eastern water skinks. Open circles are actual data and filled circles are the probabilities of infection.

Patterns of parasite load

Haemogregarine parasite load differed between hosts; many hosts had small parasite loads, and few hosts were heavily infected (Figure 2.5).

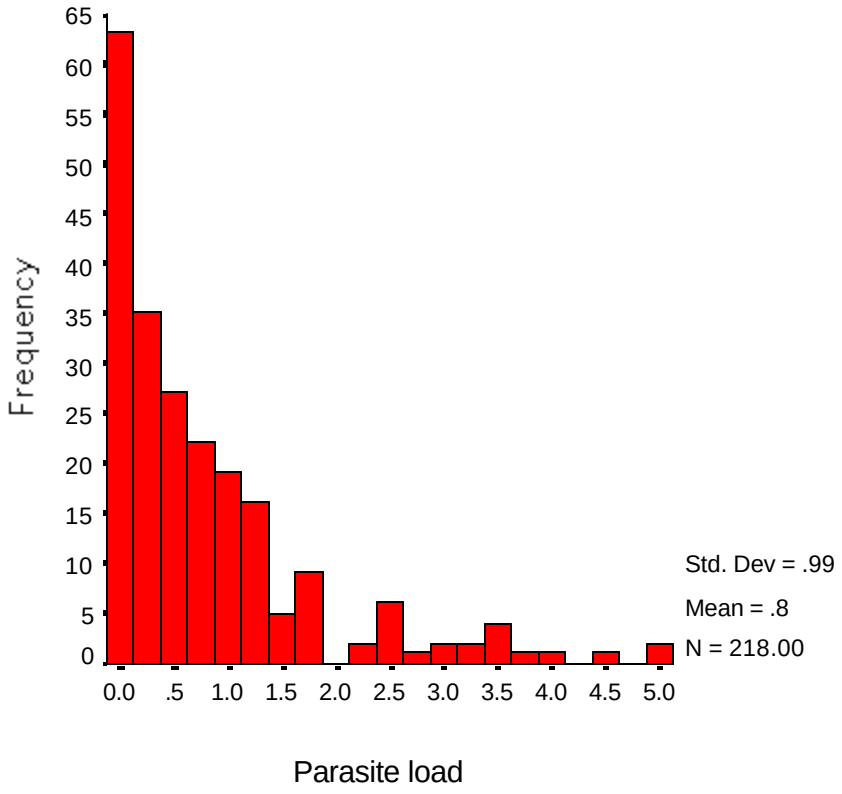

Figure 2.5 Frequency distribution of haemogregarine parasite loads (% infected cells) in infected eastern water skinks, *Eulamprus quoyii*.

Host factors influencing parasite loads

Sex

Haemogregarine parasite load was significantly higher in infected adult males (n = 95) than in infected adult females (n = 105) (Mann Whitney U, z = -3.066, *P* = 0.002; Fig. 2.6). Mean parasite load (+/- SD) in adult males was 1.2% infected cells (+/- 1.0) and reached a maximum of 5.1%. In adult females, mean parasite load (+/- SD) was 0.59% infected cells (+/- 0.74) and reached a maximum of 3.6%. Parasite load was not significantly different in infected juvenile males and females (Mann Whitney, z = -1.41, *P* = 0.16).

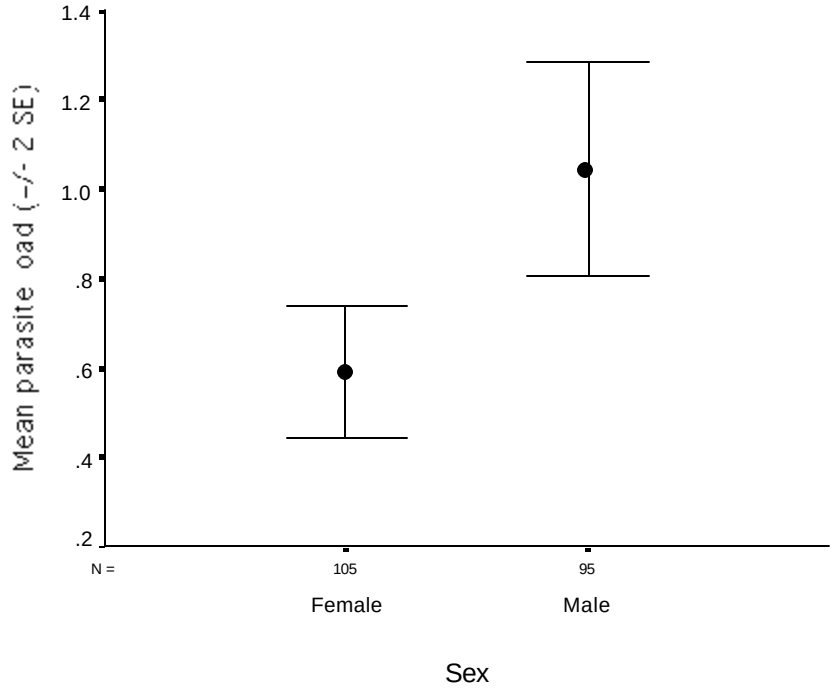

Figure 2.6 Sex-dependent differences in parasite load (mean +/- 2 SE) of *Hepatozoon hinuliae* in adult *Eulamprus quoyii* hosts. Males have significantly higher parasite loads than females.

Age

Age groups (both sexes combined) varied significantly in their haemogregarine parasite load (Kruskal-Wallis test, Pearson's coefficient = 16.01, d.f. = 5, $P = 0.007$; Fig. 2.8), because of significant differences between ages 2 and 3 (Mann-Whitney, Z = -3.01, $P = 0.003$). When host sexes were analysed separately, female parasite load was not related to age (Kruskal-Wallis test, Pearson's coefficient = 8.78, d.f. = 5, $P = 0.12$; Fig. 2.7), but there was a significant difference among males of different ages (Kruskal-Wallis test, Pearson's coefficient = 11.73, d.f. = 5, $P = 0.039$; Fig. 2.7). 3-year old males had significantly higher parasite loads than 2-year olds (Mann-Whitney, Z = -2.57, $P = 0.010$). 3-year old males also had higher parasite loads than 4-year olds, although this difference only approached significance (Mann-Whitney, Z = -1.78, $P = 0.074$).

There was no difference in parasite loads when simply comparing juveniles (SVL < 90 mm) with adults (Mann-Whitney Z = -0.784, $P = 0.433$), even when sexes were separated (female juveniles compared with adult females, Mann-Whitney, Z = -0.75, $P = 0.45$; juvenile males compared to adult males, Mann-Whitney, Z = -0.17, $P = 0.86$).

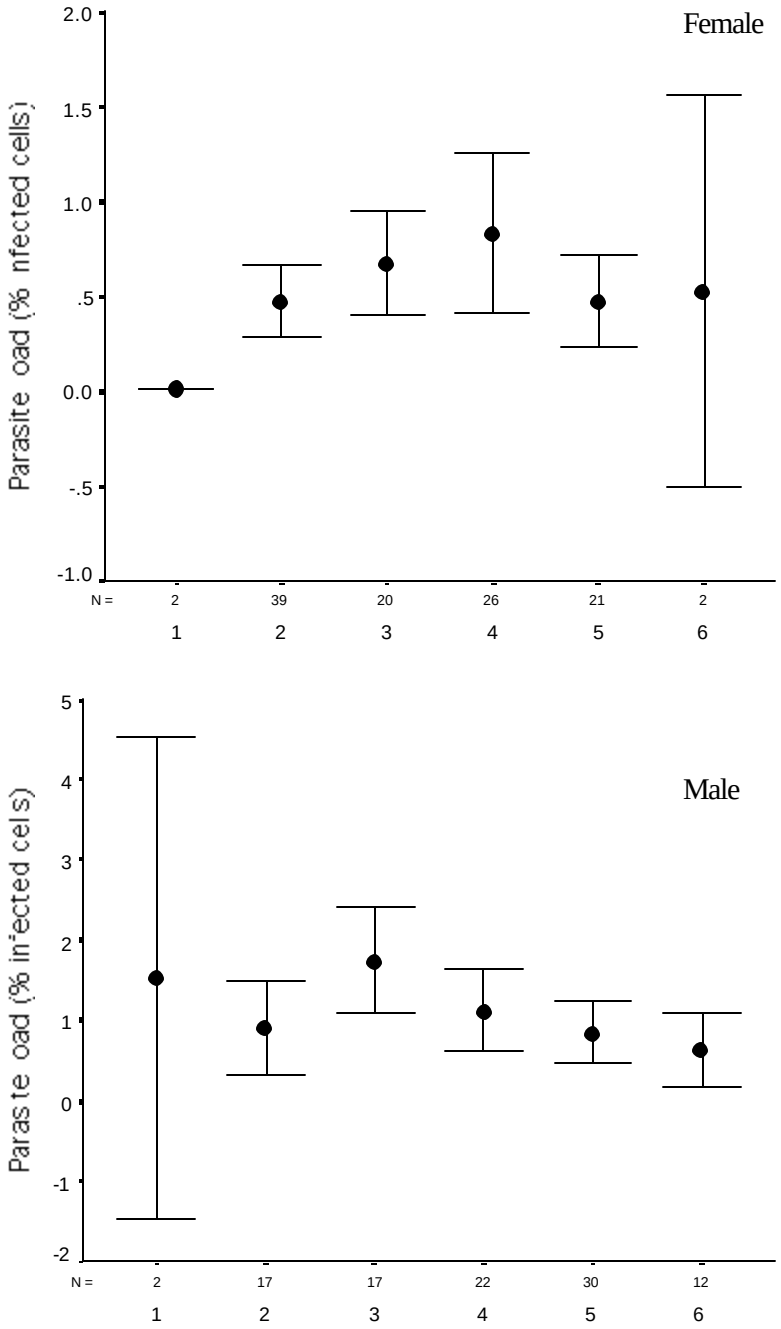

Figure 2.7 Mean haemogregarine parasite load in different age classes of *Eulamprus quoyii*, for females and males (error bars show 2 x SE). Parasite load differed significantly with age in males, but not in females.

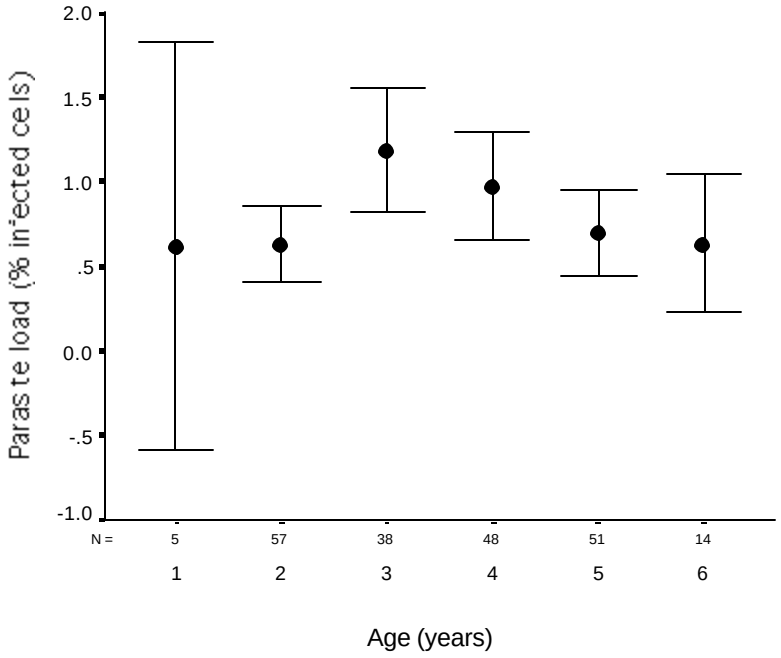

Figure 2.8 Mean haemogregarine parasite load in different age classes of *Eulamprus quoyii*, for both sexes combined (error bars show 2 x SE).

Temporal variations in infection

Prevalence over time

Prevalence changed significantly over time (X^2 = 36.4, d.f. = 7, P < 0.000; Fig. 2.9), and this was due to differences in prevalence in females (females: X^2 = 31.0, d.f. = 7, P < 0.000; males: X^2= 10.8, d.f. = 7, P = 0.15; juveniles: X^2 = 8.32, d.f. = 7, P = 0.31). The variation in parasite prevalence in female hosts was due to low prevalence during March and December 2002.

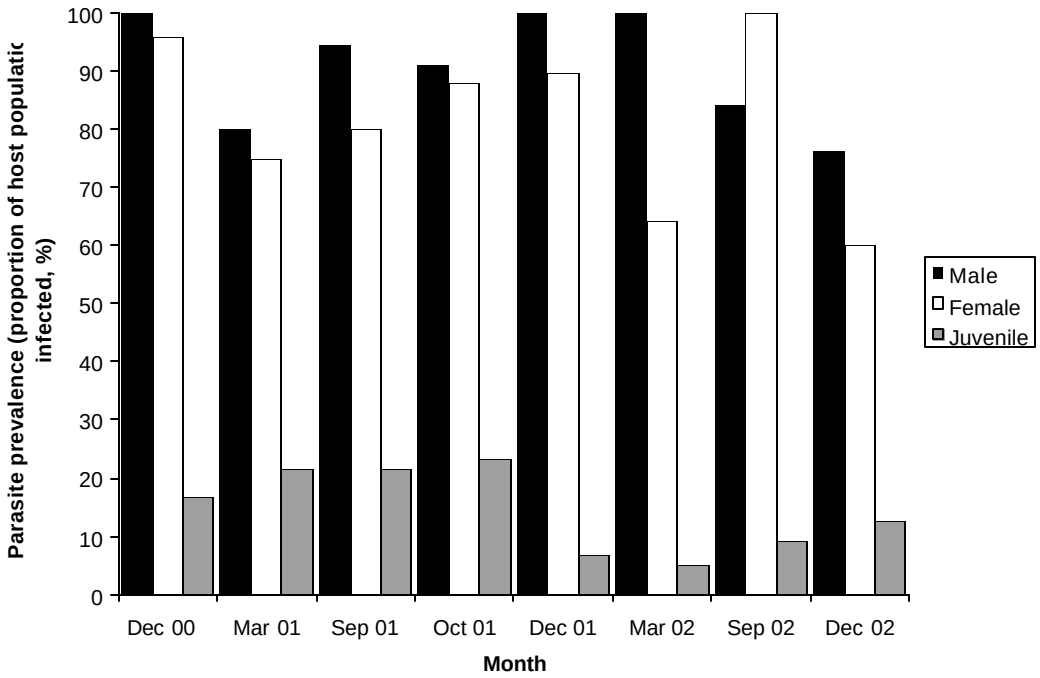

Figure 2.9 Changes in haemogregarine parasite prevalence in the host population over time. Prevalence of infection increases in the juvenile cohort during the year.

Parasite load over time

For both sexes pooled, there was no difference in haemogregarine parasite load during the two-year study, either due to annual changes, or to reproductive cycles (Kruskal-Wallis test Chi = 4.99; d.f. = 7; $P = 0.66$; Fig. 2.10). Parasite load showed no seasonal changes when sexes were separated (adult infected males, Kruskal-Wallis test Chi = 5.50; d.f. = 7; $P = 0.60$; adult infected females Kruskal-Wallis test Chi = 4.46; d.f. = 7; $P = 0.73$).

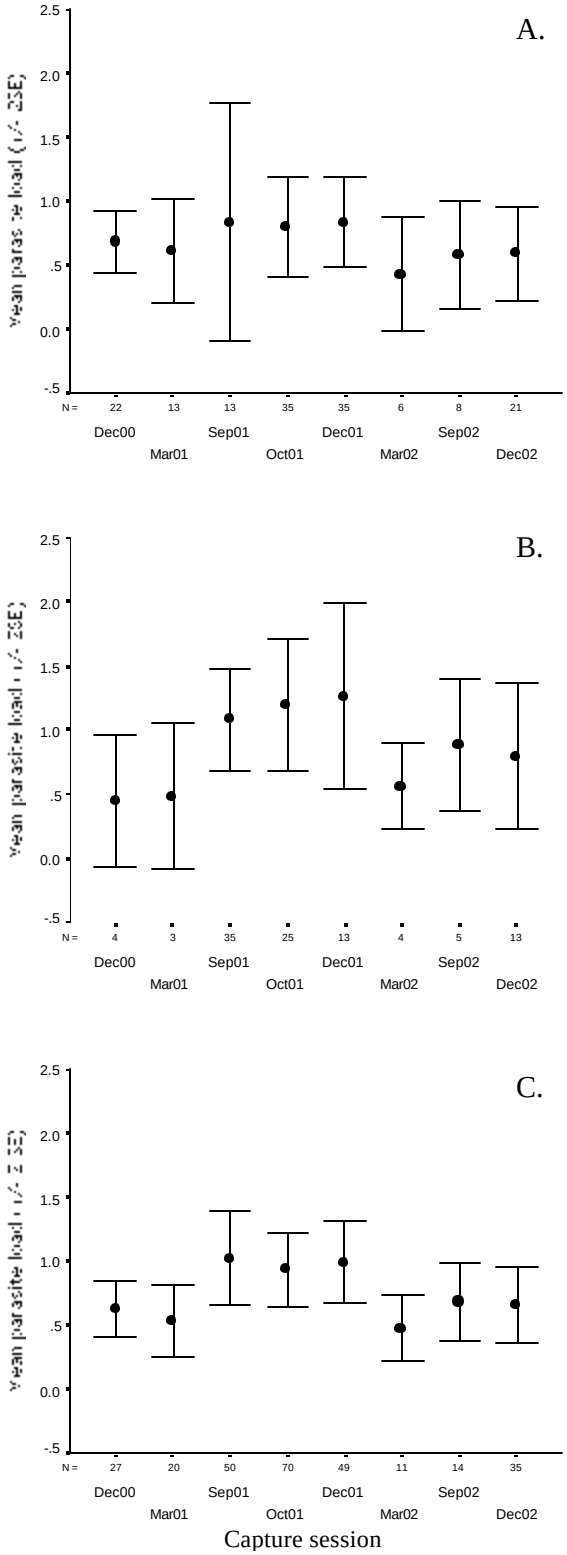

Figure 2.10 Mean parasite load (± 2 SE) for females (A), males (B) and both sexes (C) combined during capture sessions from December 2000 to December 2002.

Temporal variations in infection - within individuals

I compared *Hepatozoon* parasite load at first and second capture of marked individuals (Sorci 1995), and grouped animals from capture intervals that might reflect particular seasonal changes in climate or host reproductive cycle. For example, comparisons were made of animals caught before and during the mating season.

Male reproductive effort is probably greatest during September/October when they compete for mates, although testes development occurs between April/May and September (Veron 1969b). If reproductive effort is correlated with parasite load, changes should be evident between September/October, and the following December. Parasite loads were significantly correlated ($r^2 = 0.82$, $F_{1, 12} = 54.4$, $P < 0.000$; $y = 0.93x - 0.11$) with no uniform trend in rise or fall (Wilcoxon Rank Test, $z = -1.10$, $P = 0.27$). Prevalence was identical in males caught in both September/October and December (100%), in March and September (80%), and in December and September (100%).

Females develop ovaries from April until October when they mate. Pregnancy lasts until December/January. Parasite load was significantly correlated between March (before vitellogenesis begins) and the September/October capture sessions (peak of vitellogenesis) ($r^2 = 0.67$, $F_{1, 6} = 12.27$, $P = 0.013$, $y = 1.72x - 0.27$) and there was no trend in changes in parasite load (Wilcoxon Signed Ranks Test, $Z = -0.28$, $P = 0.78$). Similarly, between September/October and December (i.e., during pregnancy) there was no change in parasite load (Wilcoxon Signed Ranks Test, $Z = -0.98$, $P = 0.33$) and loads were significantly correlated ($r^2 = 0.683$, $F_{1, 17} = 36.7$, $P < 0.000$; $y = 0.71x$

+ 0.17). There were no uniform trend in changes in parasite load between December and March in female water skinks (Wilcoxon Signed Ranks Test, Z = -0.67, P = 0.5) but there was no significant correlation between parasite load at first and second capture (r^2 = 0.04, $F_{1, 3}$ = 0.11, P = 0.76).

Parasite loads of individual males and females were still significantly correlated a year after first capture (r^2 = 0.72, $F_{1, 7}$ = 18.26, P = 0.004, y = 0.86x - 0.06) and were unchanged (Wilcoxon Signed Ranks Test, Z = -0.06, P = 0.95). In fact, when combining data from all recaptures and disregarding the time interval between first and second capture (mean average duration between captures = 138 days, minimum 7, maximum 725), parasite load at each capture was still significantly highly correlated (r^2 = 0.70, $F_{1, 108}$ = 254.4, P < 0.000, y = 0.90x + 0.1; Fig. 2.11) and unchanged (Wilcoxon Signed Ranks Test Z = -0.065, P = 0.95).

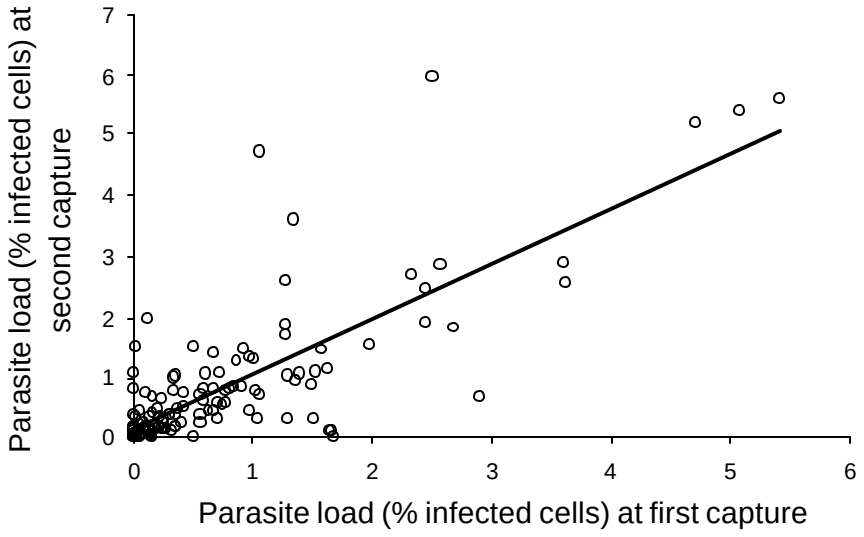

Figure 2.11 Parasite load at first and second capture for all recaptures (capture interval varies).

Evidence of self-curing

Out of 120 individuals that were caught at least twice, six individuals (all female) showed signs of clearing *Hepatozoon* parasite infections, as parasite loads dropped to zero. There was a mean of 223 days (SD = 152) between the previous positive blood slide and the negative one. Mean parasite load, at the sample previous to the negative load, was 0.13% (SD = 0.20). Two lizards recaptured a third time, i.e., once more after being regarded as uninfected, displayed parasitaemia, although with parasite loads of 0.01% or lower. Recrudescence of infection in individuals with low parasite loads may indicate that detecting very low parasite loads was difficult, and subject to error, or it may indicate that these females were reinfected with the disease after having recovered from an infection.

DISCUSSION

Haemogregarine blood parasite infections were common in Blackdown Tableland, with over 65% of animals infected with *Hepatozoon hinuliae*, and prevalence was higher in adults. Malarial parasite systems of American lizards have rates of prevalence approximating 25%, and reaching 40% at tropical sites where lizards are active year-round (Eisen 2000; Staats & Schall 1996). In another Australian study system, 11.5% of a population of sleepy lizards *Tiliqua rugosa* were infected by the haemogregarine blood parasite *Haemolivia mariae* (Smallridge & Bull 2000). In comparison with these other studies, the population of eastern water skinks at Blackdown Tableland appears to have an abnormally high prevalence of blood parasites. It is difficult to know why there is such a high prevalence, but other

populations of *Eulamprus quoyii* infected with *Hepatozoon* exhibit similarly high prevalence (Chapter 6). Factors affecting the population dynamics of *Hepatozoon hinuliae* in *Eulamprus quoyii* are discussed here.

Parasite abundance and host sex

Previous studies have suggested that males of all species may be more prone to parasitic infection than females due to the immunosuppressive action of testosterone (Salvador et al. 1997; Salvador et al. 1996; Schalk & Forbes 1997; Veiga et al. 1998). For example, there is a male-skewed sex-bias in blood parasite prevalence in mammals (Schalk & Forbes 1997). However, there is no overall sex-based bias in blood parasite prevalence in birds, and in cases where a bias exists (e.g., *Haemoproteus* in breeding birds) females are more prone to infections than males (McCurdy et al. 1998).

In my study, prevalence of *Hepatozoon* parasites was not significantly different between males and females of any age class. However, parasite load was significantly higher in adult males than in adult females. Because both males and females are equally susceptible to infections, i.e., prevalence is the same, higher parasite loads in males suggest that the immune response to *Hepatozoon* may be gender dependent. Testosterone may be responsible for this observed difference, although a study of malaria parasites (*Plasmodium mexicanum*) in western fence lizards (*Sceloporus occidentalis*) found little evidence to suggest that testosterone affects the parasite's life-history traits (Eisen & DeNardo 2000). Differences in behaviour may also account for higher parasite loads in males.

There are no reports reviewing sex-biases in blood parasites of lizard or reptile hosts as a group, although reptiles do not exhibit sex-biases in either parasite load or prevalence of helminth infections (Poulin 1996). A review of the literature shows 3/7 studies reveal a higher prevalence of blood parasites in male lizards (Table 2.1), but this phenomenon demands more attention.

Parasite abundance and host age

Adult water skinks are more likely to harbour infections of *Hepatozoon hinuliae* than juveniles. An increased probability of infection in adults may be due either to behavioural changes, changes in diet, the advent of reproductive maturity (immunosuppressive hormones or increased social contact), or simply an increased chance of exposure over time. A study of ontogenetic shifts in habitat of *E. quoyii* suggested that juveniles occupy more marginal habitats than adults, due to intraspecific aggression (Law 1991). If habitat changes do occur, then transmission rates may change, either due to closer association with infected larger animals, or because of changes in vector abundance due to habitat (Eisen & Wright 2001).

In many avian host-blood parasite systems, juveniles harbour higher parasite loads than adults, because of mortality of very heavily infected juvenile hosts, ontogenetic changes in the degree of exposure and risk of transmission, and/or the development of acquired immunity (Smallridge & Bull 2000; Sol *et al.* 2003,). In contrast, my study, and most other studies on lizards, demonstrate higher parasite prevalence in adults (Table 2.2). Lizard neonates often disperse soon after birth and occupy different habitats from their parents, although dispersal may be affected by many factors (Law

1991; Massot et al. 2002; Massot et al. 2003; Meylan et al. 2002). In contrast, the high prevalence of blood parasites in juvenile altricial birds may represent a cost of parental care and sedentary nesting. Further examination of a diversity of host-parasite systems with and without parental care may provide insights into the co-evolution of host life history and the immune system.

Table 2.1 Literature review of sex-bias in prevalence of blood parasites in lizard hosts.

Host	Parasite	Effect	Reference
Aruban whiptail *Cnemidophorus arubensis*	*Hepatozoon* sp.	Male bias in prevalence	Schall 1986
Western fence lizard *Sceloporus occidentalis*	*Plasmodium mexicanum*	Male bias in prevalence No bias in prevalence	Schall & Marghoob 1995 Eisen 2000
Anolis gundlachi	*Plasmodium. azurophilum* & *P. floridense*	Male bias in prevalence	Schall, Pearson & Perkins 2000
Anolis sabanus	*Plasmodium azurophilum*	No bias	Staats & Schall 1996
Agama agama	*Plasmodium giganteum* & *P. agamae*	No bias	Schall & Bromwich 1994
Australian sleepy lizard *Tiliqua rugosa*	*Haemolivia mariae*	No bias in prevalence Parasite load higher in females	Smallridge & Bull 2000
Eastern water skink *Eulamprus quoyii*	*Hepatozoon Hinuliae*	No bias in prevalence Parasite load higher in males	This study

Adults and juveniles did not differ significantly in parasite load, but three-year-old male lizards had higher parasite loads than other age classes. Lower mean parasite loads in older age groups could suggest that highly parasitised animals are suffering parasite-induced mortality, and the lower mean parasite loads of older animals are representative of survivors with smaller parasite loads (Anderson & Gordon 1982). Alternatively, concomitant increase in prevalence in two- and three-year olds suggests that more of these animals may be exhibiting the peak parasite loads typical of newly infected hosts (Smallridge & Bull 2001). The equivalent parasite loads in adults and juveniles suggest that it is variation in the individual host-parasite relationship that determines persistent parasite load, rather than the accumulation of multiple infections over time. The results from recaptured animals further support this.

Table 2.2 Literature review of blood parasite and age-bias in prevalence in lizard hosts.

Host species	Parasite	Effect	Reference
Western fence lizard *Sceloporus occidentalis*	*Plasmodium mexicanum*	Adult bias in prevalence	Schall & Marghoob 1995
		Maximal parasite load higher in juveniles	Bromwich & Schall 1986
Australian sleepy lizard *Tiliqua rugosa*	*Haemolivia mariae*	Adult bias in prevalence	Smallridge & Bull 2000
European common lizard *Lacerta vivipara*	*Haemogregarine*	Adult bias in prevalence	Oppliger & Clobert 1997
Eastern water skink *Eulamprus quoyii*	*Hepatozoon hinuliae*	Adult bias in prevalence	This study

Changes in prevalence

Previous studies suggest that the prevalence of blood parasite infections is often relatively stable for long periods (Bennett & Cameron 1974; Eisen 2000; Schall et al. 2000; Smallridge & Bull 2000), and independent of seasonal climatic changes in temperature (Schall 1986). Prevalence of haemogregarine blood parasites in Australian sleepy lizards (*Tiliqua rugosa*) and the Aruban whiptail (*Cnemidophorus arubensis*) does not fluctuate (Smallridge & Bull 2000, Schall 1986).

In this Blackdown Tableland population, prevalence of blood parasites in males and juveniles did not differ significantly during the two-year study. Although the observed change in prevalence in females is puzzling, (perhaps due to changes in female host biology, or to sampling error among months), the evidence nonetheless suggests that the prevalence of *Hepatozoon hinuliae* in water skinks at Blackdown Tableland remains relatively stable over time. However, short studies can fail to identify temporal trends in prevalence (Schall & Marghoob 1995; Smallridge & Bull 2000), so prolonged study is required to determine whether the parasite population really is unchanging over long periods.

The stable prevalence of blood parasites may be typical of long-lived hosts. Host-parasite systems that experience large fluctuations in prevalence may do so because of large variations in vector abundance, or host immunity, and such fluctuations may be more obvious in host populations with high turnover rates (Bennett and Cameron 1974, Schall and Marghoob 1995, McKenzie *et al.* 2001(McKenzie et al. 2001). For example, western fence lizards are short-lived (two to three years, Schall and

Marghoob 1995, Eisen 2000) and transmission depends on over-wintering lizards acting as reservoirs of infection, followed by vector transmission (Bromwich & Schall 1986). *Eulamprus* lizards are longer lived (8 years for *Eulamprus quoyii*, Borges Landaez 1999) and as parasite infections are also persistent, prevalence in adults will remain fairly stable once infection has occurred.

Parasite load over time

This 2-year study incorporated within-year sampling periods and revealed that parasite loads remain constant over time. A mark-recapture study of the European common lizard, *Lacerta vivipara*, also revealed that haemogregarine (unidentified species) loads one year after first capture are correlated with the original parasite load at first capture (Sorci 1995), and similar relationships have been described in blood parasites of birds (Appleby et al. 1999; Dufva 1996). Other reports of blood parasite infections in lizards also suggest that parasite loads remain reasonably constant after a peak following the initial infection. *Plasmodium mexicanum* infections in western fence lizards, *Sceloporus occidentalis*, commonly rise exponentially before levelling off to a constant load, and rarely disappear from the host (Bromwich & Schall 1986; Eisen 2000). A similar pattern is observed in sleepy lizards, *Tiliqua rugosa*, experimentally infected with *Haemolivia mariae* (Smallridge & Bull 2001). Some authors have suggested that stable parasite loads may be caused by a low-grade or inadequate reptilian immune system (Bromwich & Schall 1986; Sorci 1995). However, other studies have demonstrated that lizard hosts do have the potential to control haemogregarine infections. Experimentally infected lizards (*Sceloporus undulatus*, *Sceloporus poinsetti*, and *Eumeces obsoletus*) react with common and

stage-specific antibodies to *Hepatozoon mocassini*, and experimental haemogregarine infections in unnatural hosts provoke inflammatory immune responses and are rarely successful (Wozniak et al. 1996, and references therein). There is also evidence that lizards and haemogregarines can co-evolve, as local host populations inhibit growth rates of local strains of blood parasites more than they inhibit non-local strains (Oppliger et al. 1999). Furthermore, in experimental infections, mean parasitaemia of *Haemolivia mariae* was significantly lower in sleepy lizards (*Tiliqua rugosa*) previously exposed to the parasite than in naive hosts. Therefore it seems that lizards are capable of mounting immune responses to haemogregarine infections. Competent immune systems combined with individual variation in parasite load that persists over time, suggests that blood parasite load is a function of the individual host-parasite relationship, and this relationship is probably mediated by both genotypic and phenotypic variation.

Long-lived parasite infections may be due to successful evasion of the host immune system. Alternatively, the long life span of reptilian erythrocytes compared to mammals and birds (600-800 days, Campbell 1996) may mean that two-year studies, or even two-year lizard life-spans, are not long enough to observe animals recovering from infection. In other words, if the actual infected red blood cells are not destroyed by the host's immune system, perhaps malaria parasites or *Hepatozoon* can persist in blood cells for longer than the lizards are studied or survive post-infection. Persistent parasite loads may also be a result of re-infection. However, four *Eulamprus quoyii* were held in captivity at the JCU laboratory for periods in excess of 18 months. These animals harboured *Hepatozoon* infections when captured at the study site, and the infections remained in these animals for the entire duration of captivity. Because no

blood-feeding invertebrates were present on the lizards or in the lab, and because captive lizards were fed on commercial cat food and captive-bred crickets, there was no possibility of the long duration of the infections being due to new infections. Thus, it appears that *Hepatozoon* infections can be long-lived.

There was some (weak) evidence in this study of self-curing, although this may be a result of false negatives because infections reappeared subsequently. Continued monitoring of marked individuals within the Blackdown Tableland population should reveal the dynamics of long-lived infections in long-lived hosts. Studies of lizard malaria have argued that parasite genotype accounts for some of the variation expressed in parasite abundance and life history (Eisen & Schall 2000). In the Blackdown Tableland population, there are now two known-age cohorts of parasitically naïve individuals with known matrilineage. Monitoring these cohorts will help to determine the importance of host genotype on parasite load in their lizard hosts.

In conclusion, load of *Hepatozoon* is dependent on host factors such as sex and age. The reason behind individual variation in parasite load is unclear, and maybe due to host behaviour, immune system or genotype. Alternatively, variations in parasite strain or infection stage may be responsible for the observed heterogeneities in parasite load. Prevalence appears reasonably stable in the water skinks of Blackdown Tableland, and suggests that *Hepatozoon hinuliae* is a persistent and endemic parasite of this lizard population.

CHAPTER 3

PARASITE-INDUCED REDUCTIONS IN HOST FECUNDITY: ADAPTIVE TRADE-OFF, DISRUPTED PHYSIOLOGY, OR BOTH?

SUMMARY

Hosts can maximise their fitness after parasitic infection by compromising certa in life history traits such as reproductive output, growth or energy storage. However, measuring the impacts of parasitic infection in wild-living hosts is difficult to achieve. Here, I study the effects of protist blood parasites on the fecundity of free-living Australian eastern water skinks (*Eulamprus quoyii*). During a mark-recapture study, I examined growth rates, energy storage and reproductive output of this viviparous lizard. High parasite loads reduce the reproductive output of female lizards by approximately one offspring per litter. There were no deleterious effects of parasitism on host growth rates or energy storage, although females with high parasite loads had higher lipid reserves at the end of pregnancy. Parasites may reduce reproductive output by disrupting host physiology, but this reduced fecundity may also be an adaptive host trade-off that maximises host fitness.

INTRODUCTION

Parasitic infection can cause reductions in host fecundity (Albon et al. 2002; Grenfell & Gulland 1995; Gulland 1995; Stien et al. 2002; Tompkins & Begon 1999; Tompkins et al. 2002). Hosts may suffer reduced fecundity for two main reasons. First, parasitic infection may reduce the resources available to host reproduction by the direct consumption of the host's nutrients, through damage to host tissues, or by interfering with host physiology (Dawson & Bortolotti 2001; Hurd 2001). Second, reduced reproductive output in response to parasitic infection may be an adaptive response of the host that alleviates the negative effects of infection on survival (Webb & Hurd 1999). It is well established that reproduction, whether as number of reproductive events or current investment, can incur costs in terms of survival (e.g., Schwarzkopf 1993; Schwarzkopf 1994; Shine 1980). By reducing current reproductive output, hosts may mitigate against the potential reduction in survival caused by parasitic infection (Hurd 2001; Sorci et al. 1996). Infected females can then maximise lifetime reproductive success by maintaining the normal number of breeding events, but pay a fitness cost by having a reduced number of young at each breeding event. Alternatively, iteroparous hosts may trade-off other life-history traits with reproduction, such as the immune response or growth, so that they invest maximally in reproduction, but pay costs in terms of survival or lifetime reproductive success (Hurd 2001; Sheldon & Verhulst 1996). For example, parasitised hosts may have lowered growth rates (Soler et al. 2003), and this may reduce lifetime reproductive output if fecundity is related to body size (Schwarzkopf 1992; Schwarzkopf 1993). Consequently, deleterious effects of parasitism may be more obvious in 'subordinate' life-history traits such as growth (Rigby & Moret 2000).

Negative correlations between host fecundity and parasite load suggest that parasites cause a decline in host reproductive output. However, correlations can be misleading because other aspects of the host's biology may be responsible for an observed relationship between reproductive output and infection. For example, both diminished reproductive output and high parasite loads can be a result of poor host condition (Schall 1983; Wiehn & Korpimaki 1998). In contrast, positive correlations between reproductive output and parasite load can occur if the host allocates resources to reproduction at the cost of investment in the immune system (Sorci et al. 1996). Although causal factors are difficult to determine in non-experimental studies, examination of correlations between parasitic infection and other aspects of the host's biology can help to reveal the mechanisms responsible for life-history trade-offs (Doughty & Shine 1997). Consequently, investigations of the influence of parasites on host fecundity should take host quality or condition into account.

Lizard-parasite systems are well suited to research on the effects of parasites on host fecundity because blood parasites are common in lizards (Smith 1996) and can be pathogenic (Schall 1986; Schall 1990a; Schall 1990b). Natural infections of *Plasmodium mexicanum* reduced clutch size in female western fence lizards (*Sceloporus occidentalis*) (Schall 1983). The reduction in clutch size, of approximately 1-2 eggs, was due to reduced fat reserves of infected mothers (Schall 1983). In contrast, haemogregarines (family Haemogregarinidae) have no effect on litter size (number of offspring) in infected European common lizards, *Lacerta vivipara*, although there are weak positive associations between parasite load and offspring size and maternal investment (Sorci et al. 1996).

Here I report on a previously unstudied lizard-parasite system: the Australian eastern water skink, *Eulamprus quoyii*, infected by the haemogregarine *Hepatozoon hinuliae*. *E. quoyii* is a large, heliothermic skink, which gives birth to live young (litter size = 1 – 8 in this study). The genus *Eulamprus* has been studied extensively, and has proved useful for testing theories about life history and reproductive allocation (Doughty & Shine 1997; Doughty & Shine 1998; Robert & Thompson 2001; Robert et al. 2003; Rohr 1997; Schwarzkopf 1993). *Eulamprus* skinks are viviparous, and monitoring reproductive output is simple because they will give birth readily in the laboratory. Furthermore, they rely predominantly on caudal lipid reserves for reproduction (Doughty & Shine 1998; Wilson & Booth 1998) so by measuring these energy stores it is possible to directly examine energy allocation trade-offs between reproduction and other life-history traits.

In this chapter I examine the impacts of parasitism on host reproduction by looking specifically at the effect of blood parasites, *Hepatozoon hinuliae*, on the reproductive output and offspring size of female *E. quoyii*. To understand other potential reasons for changes in fecundity of hosts, I compared growth rates, condition and energy reserves of female water skinks with high and low parasite loads.

METHODS

See Chapter 1 for site description.

Reproductive output

In December 2001, 42 female lizards were captured at Blackdown Tableland. All females were in the very late stages of pregnancy, evident from their swollen abdomens. Collected individuals began to give birth three days after arriving in captivity (18/12/01). All animals were measured in the field (Chapter 1), and then transferred in dampened cloth bags to holding cages at James Cook University (JCU) within 48 hours of capture.

At JCU, females were maintained individually in 10L plastic boxes in constant temperature rooms set at 20^{o}C. Lights operated on a 12:12LD cycle, and heating wires beneath the boxes provided heat for thermoregulation for eight hours a day. Each box contained a water bowl that had sufficient water for the lizards to immerse themselves, paper towel as a substrate, and 'furniture' in the form of rocks or small logs. Females were fed twice a week with commercial cat food. Cages were checked at least twice daily for the presence of newly born offspring (neonates). As soon as umbilical cords had dried or been eaten, I recorded neonate mass (to the nearest 0.001g, using an electronic balance), SVL and tail length (to the nearest mm, using a clear plastic ruler), and sex (by eversion of hemipenes). To prevent neonates eating, and thereby increasing their body weight between birth and detection, food was removed from mother's cage while offspring were being born. Post-litter mass of the female was measured after the entire clutch had been born. All females had given

birth within 30 days of capture. I used this data to determine the following parameters for each mother: litter mass (total mass of all offspring), number of offspring, relative clutch mass (RCM; litter mass divided by female mass post-parturition), and mean offspring mass.

Growth rates

Mark-recapture data from a two-year (December 2000 to December 2002) study at Blackdown Tableland were used to determine growth rates of female skinks in the field. Daily growth rates were calculated by dividing growth increment (SVL at last capture minus SVL from first capture) by the number of days between the two captures, providing the capture interval was greater than 30 days. Because the magnitude of growth rates of juveniles and adults are different, only adult animals (SVL < 90 mm (Caley & Schwarzkopf In press)) were included in the analysis. Adult *Eulamprus quoyii* are typically inactive during the southern hemisphere winter (Veron 1969b) so growth rates calculated for animals recaptured after an intervening winter period are depressed. To compensate for this, I subtracted 123 days (the total number of days in mid-winter: May, June, July & August) from the capture interval of all over-wintering animals. This assumes zero growth for winter, when some growth may occur, so these estimates of growth rate are conservative. Other possible seasonal variations in growth rate were ignored.

In southern populations of other *Eulamprus* species, females do not reproduce every year (*E. tympanum;* (Schwarzkopf 1992); *E. heatwolei;* (Morrison et al. 2002)) and growth rates differ between pregnant females and those females skipping reproduction

(Schwarzkopf 1992). However, in my study population there was little evidence of females skipping reproduction: only 3 of 74 mature females (SVL > 90 mm and captured during the breeding season) were not obviously pregnant in December capture periods (2001 & 2002), and these three females may have given birth before my arrival in the field as neonates were already present at the study site. Because another study of an *E. quoyii* population also found that all females were pregnant in one summer (Veron 1969b), I assumed that all adult female *Eulamprus quoyii* reproduce annually in the Blackdown Tableland population, and therefore that growth rates were similar for all adult females.

Condition and energy stores

Condition was measured using the least-squares regression relationship between body mass (g) and body size (SVL) (Schall & Pearson 2000). Because size/mass indices can sometimes misrepresent condition (Doughty & Shine 1997; Schulte-Hostedde et al. 2001), fat reserves were estimated by measuring the tail width of female *E. quoyii* 1 cm below the cloaca (Doughty & Shine 1997; Doughty & Shine 1998). Lipids are the major source of nutrients for embryo development in *Eulamprus quoyii* and are stored almost exclusively in the mother's tail prior to yolk production, or vitellogenesis (Doughty & Shine 1997; Doughty & Shine 1998; Thompson 1981; Wilson & Booth 1998). As part of a two-year study of the Blackdown Tableland population, skinks were caught and measured on three occasions: (1) March, before lipids are allocated to yolk, so tail width should be at a maximum, (2) October, when fertilisation takes place and follicles will have completed yolking (Veron 1969b), so tail width should be minimal, and (3) December, shortly before females gave birth so

tail width should be intermediate. I compared the tail widths of females with low versus high parasite load in these three periods, including only females large enough to be in breeding condition (SVL > 90 mm in October and December, and SVL > 100 mm in March; smaller animals in March would not have been large enough to have reproduced in the previous spring).

Determining high and low parasite loads

See Chapter 1 for details on calculating parasite load.

Only 3 of the 42 females used for reproductive output analysis were uninfected, and this low sample size of uninfected animals prevented the direct comparison of infected and uninfected animals. Therefore, mothers were separated into groups of low and high parasite load. Low parasite loads were in the lowest quartile of ranked parasite loads, and high parasite load was composed of the remaining 3 quartiles. For the sake of consistency I used the quartile method to determining high versus low parasite loads for all analyses in this chapter, i.e., measures of reproductive output, growth rates, condition etc. although each analysis used different hosts. Adjusting the cut-off point for high and low parasite loads (both above and below the first quartile) did not produce results that differed qualitatively from those presented below. Figure 3.1 shows the parasite load distribution of the mothers used in the analyses of reproductive output.

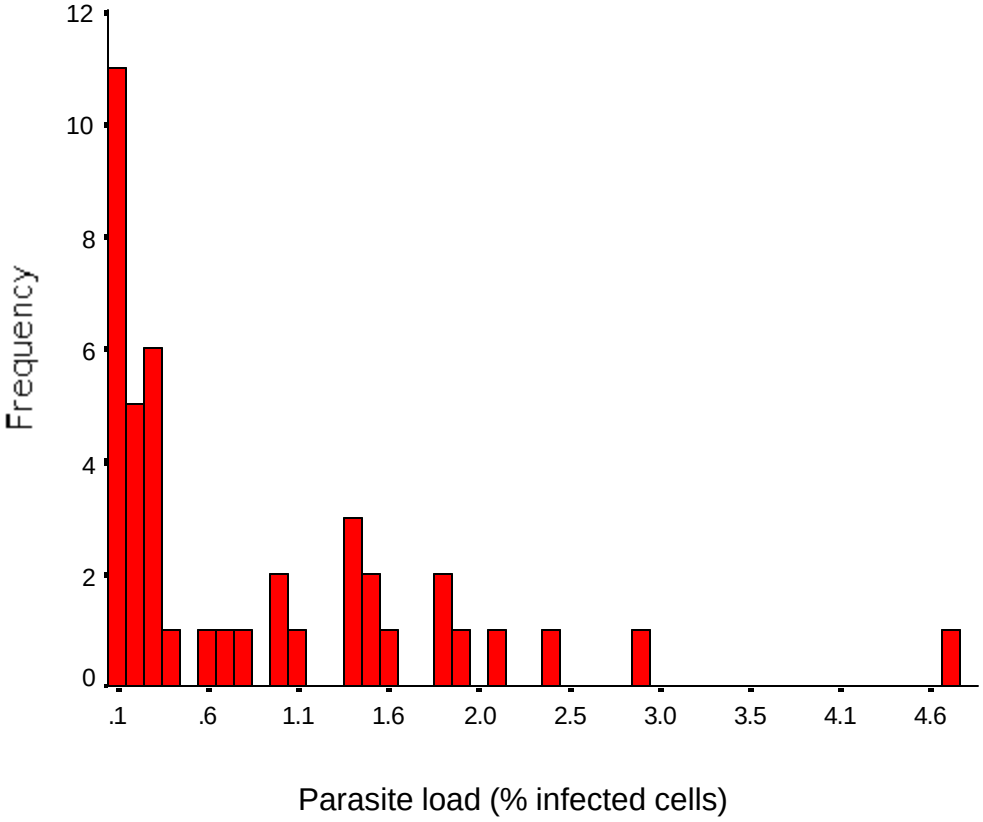

Figure 3.1 The distribution of parasite load in the 42 mothers analysed for reproductive output. Low parasite load was determined using the first quartile of ranked parasite load, in this particular case corresponding to the 11 mothers with parasite loads ranging from 0.00 to 0.09 % infected cells. The remaining females (n = 31) had parasite loads ranging from 0.1 to 4.7 % infected cells.

Statistical analysis

Because reproductive output is related to female size in water skinks (Schwarzkopf 1992), I used ANCOVA to measure the effects of parasites upon reproductive output (Schall 1983) using parasite load (high or low) as a factor, and SVL as covariate. I also used ANCOVA to determine differences between growth rates, condition and tail base width of females with low and high parasite loads, again using SVL as a covariate. Data for condition and tail base width were $\log_{10}$ transformed to meet assumptions of homogeneity of variance. Variances among treatments were not significantly different (Levene's test, $P > 0.05$).

RESULTS

Reproductive output

Maternal SVL, tail length, date of birth, mass pre- and post-parturition, were not significantly different between groups with low and high parasite loads (low parasite load n = 11, high parasite load n = 31, $P > 0.05$).

There was a significant, positive correlation between maternal body size (SVL) and number of offspring (low parasite load: no. of offspring = 0.233 (maternal SVL) - 20.6; $r^2 = 0.85$, $P < 0.000$; high parasite load: no. of offspring = 0.213 (maternal SVL) x - 19.3; $r^2 = 0.56$, $P < 0.000$) and elevation was significantly higher in females with low compared to high parasite loads (ANCOVA, $F_{1, 39} = 5.7$ $P = 0.02$; Fig. 3.2). Slopes were not significantly different (ANCOVA, $F_{1, 38} = 0.16$, $P = 0.70$). In

biological terms, for a given SVL, females with low parasite loads had approximately one additional offspring, compared to females with high parasite loads.

Litter mass was also significantly, positively correlated with maternal SVL (low parasite load: litter mass = 0.241 (maternal SVL) - 21.0; $r^2 = 0.82$, $P < 0.000$; high parasite load: litter mass = 0.227 (maternal SVL) - 20.4; $r^2 = 0.61$, $P < 0.000$), and there was also a difference in elevation between the two parasite load groups, with females with low parasite load having a larger litter mass for their size, than females with high parasite loads (ANCOVA, $F_{1, 39} = 6.58$, $P = 0.01$; Fig. 3.2). The slopes of these relationships were not significantly different (ANCOVA, $F_{1, 38} = 0.07$, $P = 0.79$).

There was also a significant, positive correlation between RCM and SVL (high parasite load: RCM = 0.005 (maternal SVL) - 0.41; $r^2 = 0.30$, $P = 0.002$; low parasite load: RCM = 0.006 (maternal SVL) - 0.42; $r^2 = 0.52$, $P < 0.012$). The elevation, but not the slope, of this relationship was also significantly lower in females with a high parasite load compared to those with a low parasite load (ANCOVA, $F_{1, 38\ slopes} = 0.05$, $P = 0.83$; ANCOVA, $F_{1, 39\ elevation} = 4.57$, $P = 0.039$; Fig. 3.3).

Finally, mean offspring size was not related to maternal body size (low parasite load $r^2 = 0.23$, $P = 0.13$; high parasite load infected $r^2 = 0.05$, $P = 0.26$), and there was no difference in the mean offspring size of mothers with high or low parasite loads ($t = 0.22$, $df = 40$, $P = 0.83$; Fig. 3.3).

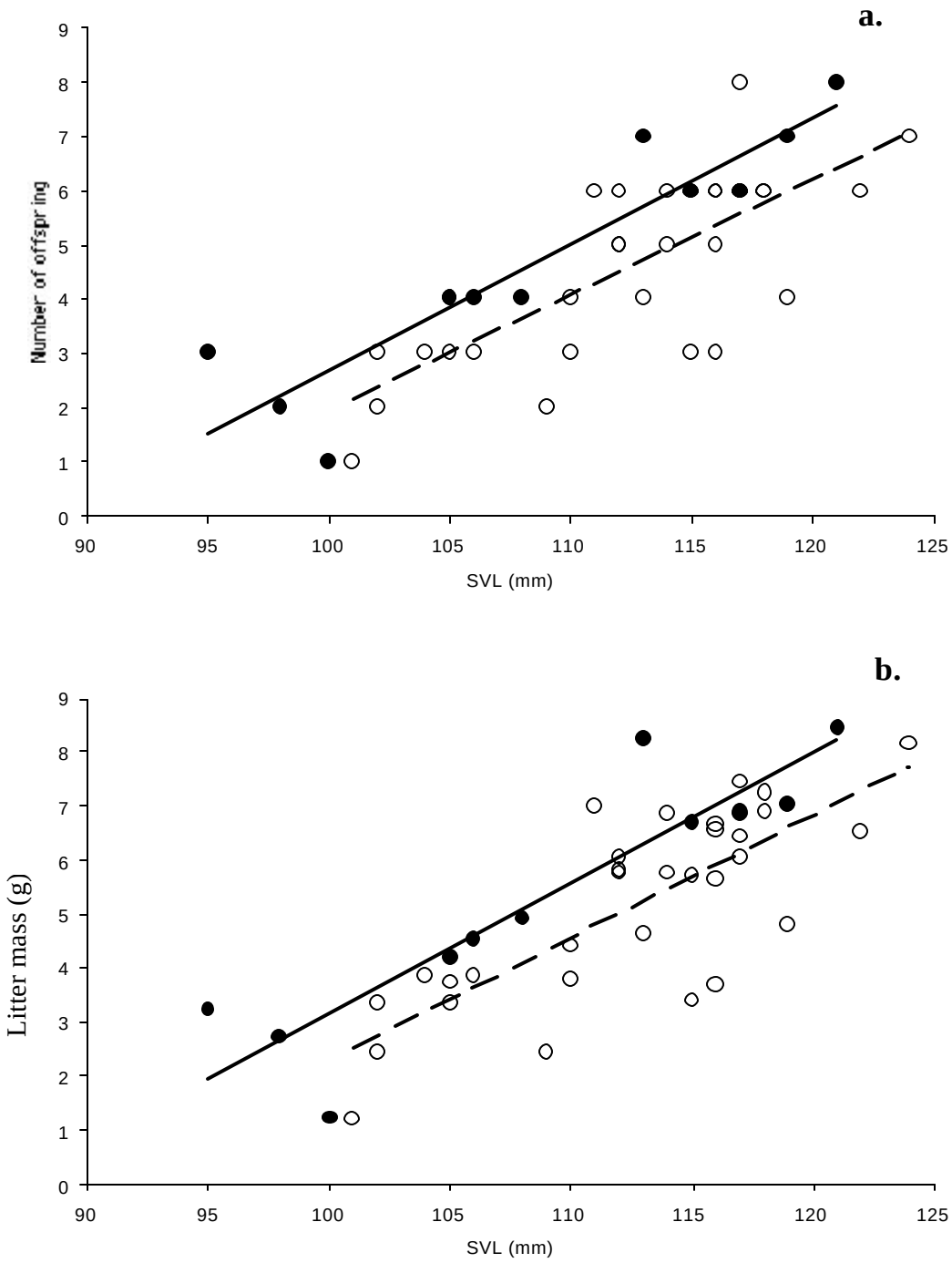

Figure 3.2 The relationship between maternal SVL (mm) and (a.) number of offspring or litter size, and (b.) litter mass. The low (•, unbroken line) and high (o, dashed line) parasite load groups differ significantly in the elevation of the relationship.

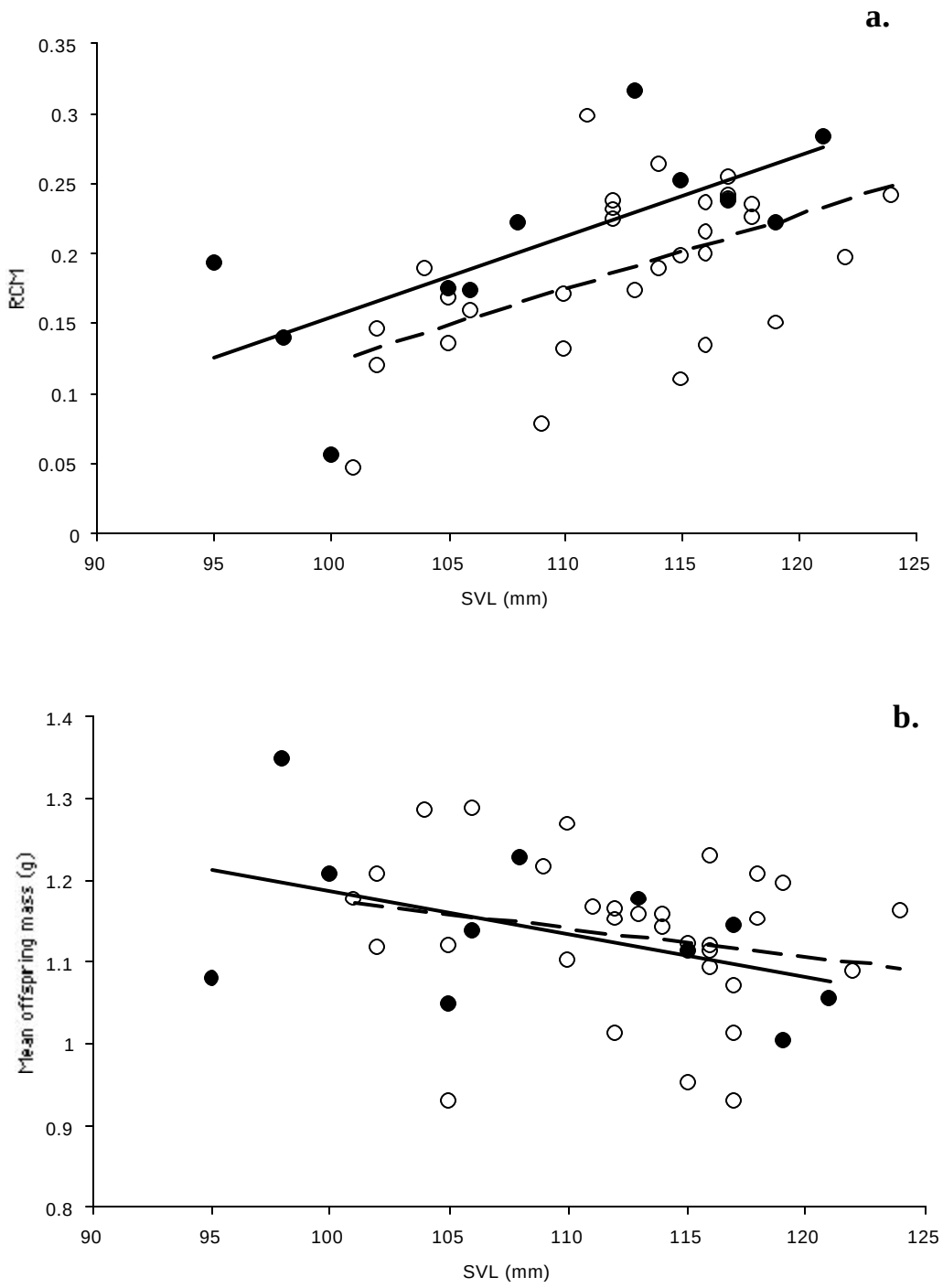

Figure 3.3 The relationship between maternal SVL (mm) and (a.) RCM (relative clutch mass), and (b.) mean offspring mass. Parasite load groups (low •, unbroken line, and high o, broken line) differ significantly in RCM, but there is no significant difference between groups for mean offspring mass.

Growth rates

Growth rates for all recaptured adult females (mean = 0.036 mm/day (SD = 0.03) was significantly, negatively correlated with SVL at first capture ($y = -0.0015x + 0.19$; $r^2 = 0.2$, n = 43, $P = 0.003$). There was no significant difference between growth rates of the low and high parasite group in either slope or elevation (ANCOVA $F_{1, 39\ slope} = 1.19$, $P = 0.28$; ANCOVA $F_{1, 40\ elevation} = 0.03$, $P = 0.87$; Fig. 3.4) even though females with low parasite loads were generally smaller than females with high parasite loads.

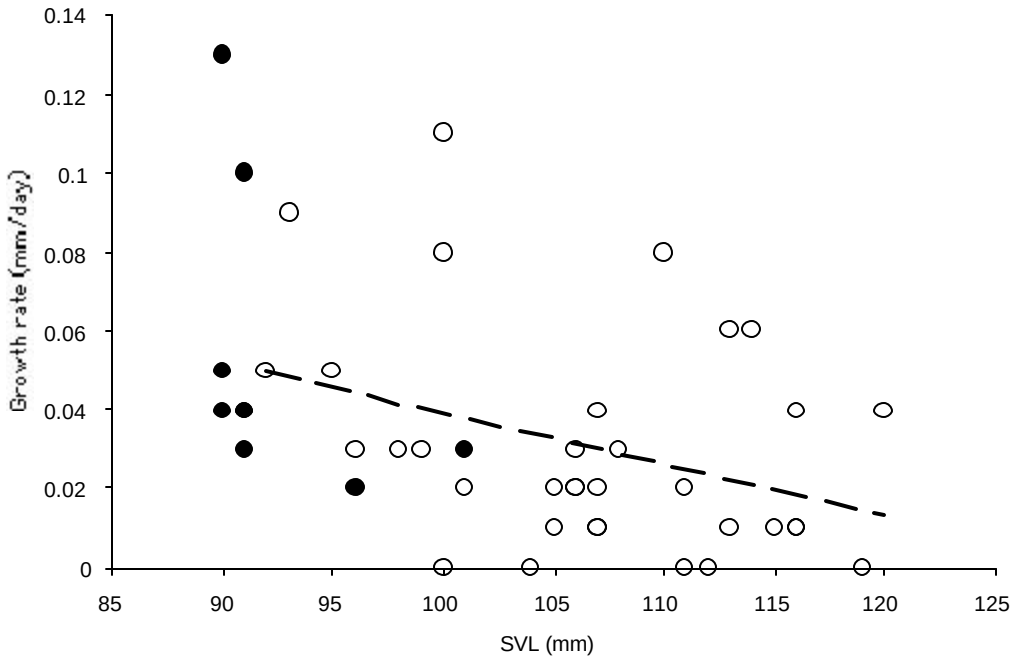

Figure 3.4 The relationship between female SVL (mm) at first capture and growth rate (mm/day) for both low (•, unbroken line) and high (o, dashed line) parasite load groups. There was no significant difference in growth rate between the groups.

Energy stores and condition

During the two-year mark-recapture study, I caught 22 females in March (6 low parasite load, 16 high parasite load), 58 females in October (15 low parasite load, 43 high parasite load), and 63 in December (15 low parasite load, 48 high parasite load).

In all three sampled months, condition was not significantly different between females with high or low parasite loads either in slope, (March ANCOVA $F_{1, 18} = 0.74$, $P = 0.4$; October ANCOVA $F_{1,53} = 0.01$, $P = 0.93$; or December ANCOVA $F_{1, 59} = 2.05$, $P = 0.16$) or elevation (March ANCOVA $F_{1, 19} = 0.50$, $P = 0.49$; October ANCOVA $F_{1, 54} = 0.001$, $P = 0.98$, or December ANCOVA $F_{1, 60} = 0.29$, $P = 0.60$; Fig. 3.5).

During March, there was a significant positive correlation between body size (SVL) and tail base width (TBW) for females with both low and high parasite loads (low parasite load: TBW = 0.11 (SVL) - 4.1; $r^2 = 0.50$, n = 18, $P = 0.001$; high parasite load: TBW = 0.08 (SVL) + 0.3; $r^2 = 0.39$, n = 20, $P = 0.003$; Fig. 3.6). Variances of these two groups were heterogeneous, and transformations did not remedy this, so ANCOVA was not used to determine the relationship between condition and parasite load in March. There was a strong relationship between SVL and tail base width in October (low parasite load: TBW = 0.08 (SVL) - 1.1; $r^2 = 0.81$, n = 10, $P < 0.000$; high parasite load: TBW = 0.08 (SVL) -1.1; $r^2 = 0.65$, n = 48, $P < 0.000$) but there was no significant difference between either slope (ANCOVA $F_{1, 54} = 0.08$, $P = 0.79$) or elevation (ANCOVA $F_{1, 55} = 0.58$, $P = 0.45$) between the parasite load groups (Fig. 4.5). During December, there was a good relationship between SVL and tail base width (low parasite load: TBW = 0.09 (SVL) - 3.2; $r^2 = 0.78$, n = 14, $P < 0.000$;

high parasite load: TBW = 0.06 (SVL) + 0.9; $r^2 = 0.18$, n = 49, $P = 0.003$). Low parasite load animals had a significantly lower regression elevation than did high parasite load animals (ANCOVA, $F_{1, 60} = 7.23$, $P = 0.009$), indicating wider tail bases for their body size in the high parasite load group, but slopes were not significantly different (ANCOVA, $F_{1, 59} = 1.433$, $P = 0.236$).

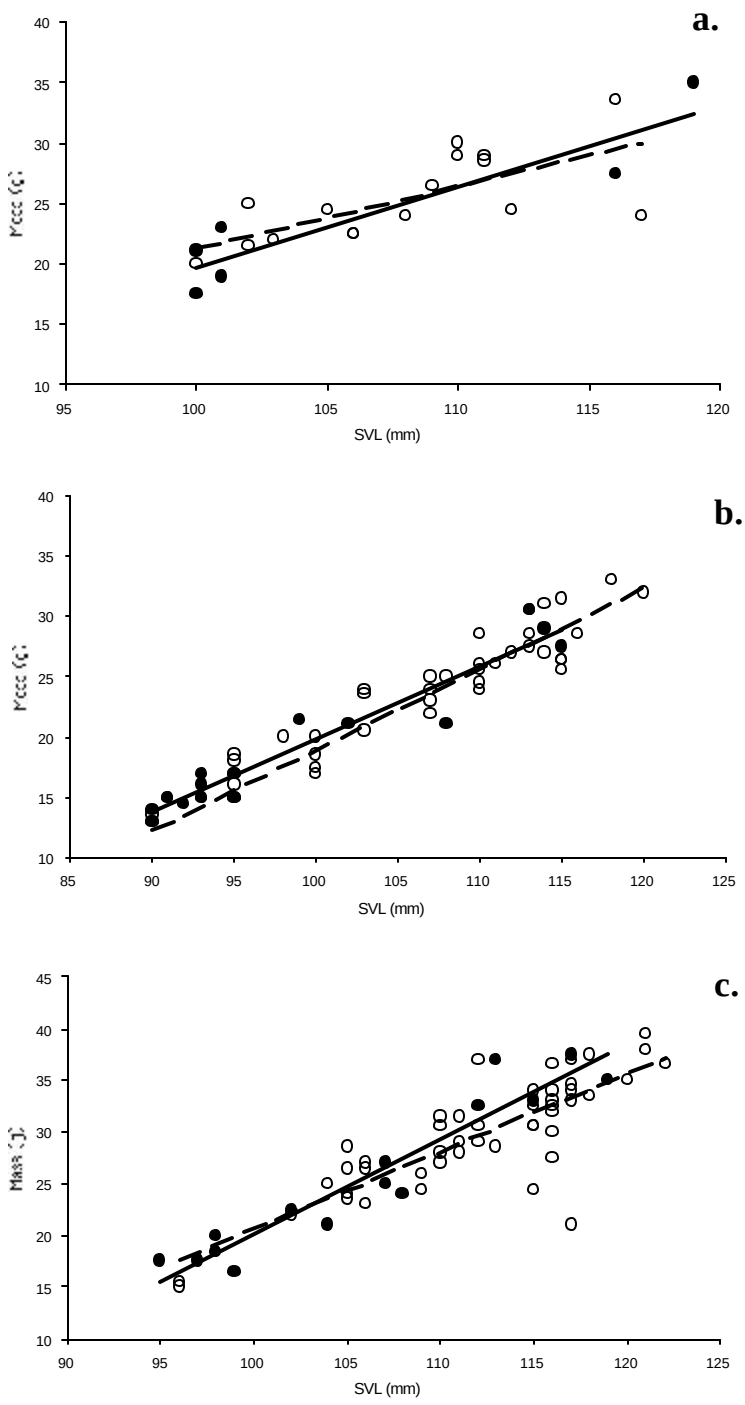

Figure 3.5 Condition, depicted by size-mass (mm-g) relationships, for adult female water skinks caught during (a.) March (post-reproductive females), (b.) October (pre-reproductive females), & (c.) December (pregnant females). Condition was not affected by high (o, broken line) or low (•, unbroken line) parasite load. Overall relationship equations are: March: Mass = 0.6 (SVL) - 39.7, October: Mass = 0.64 (SVL) - 44.5, & December: Mass = 0.79 (SVL) - 58.1

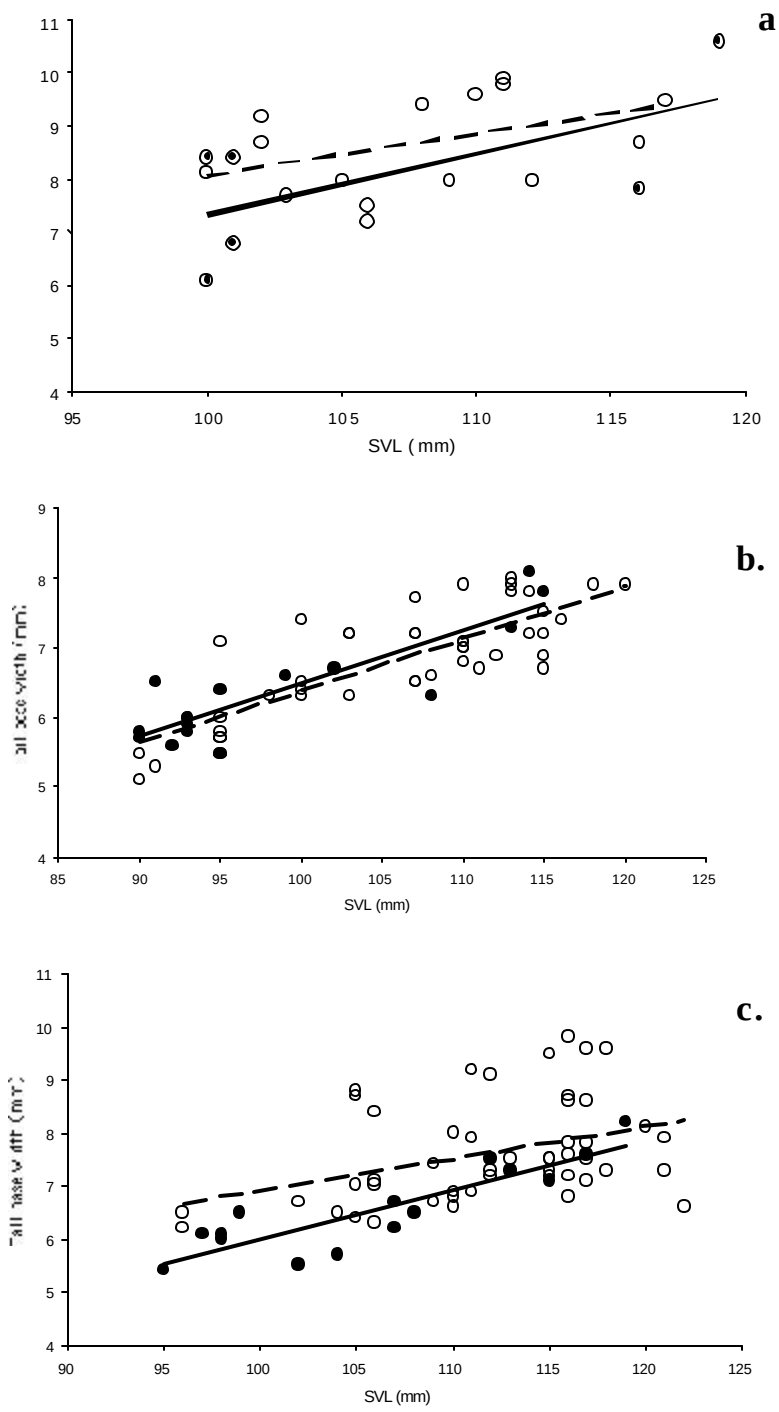

Figure 3.6 The relationships between female size and tail-base width (mm), an indicator of lipid stores. Measurements are from (a.) March (post reproductive females), (b.) October (prereproductive females) & (c.) December (pregnant females). Relationships were only significantly different between high (o, broken line) and low (•, unbroken line) parasite load groups in December.

DISCUSSION

Parasites and host reproductive output

Typically, documented cases of parasite-induced decreases in reproductive effort have involved macroparasites, such as nematodes and arthropods (Tompkins & Begon 1999; Tompkins et al. 2002). In contrast, the influence of protists (microparasites, Hudson et al. 2002) on fecundity in wild hosts remains equivocal, despite the importance of protists in the development of host-parasite evolutionary-ecological theory (Keymer & Read 1991; Read 1991; Thomas et al. 1995a). For example, infections of *Haemoproteus majoris* reduced clutch size of blue tits (*Parus caeruleus*) compared to uninfected animals, irrespective of age, and condition of fledglings was dependent on parental infection status (Merila & Andersson 1999). Furthermore, if infections of *H. majoris* and *Leucocytozoon majoris* were reduced by medication, hosts had greater reproductive success (higher fledging success and lower nestling mortality) than untreated birds (Merino et al. 2000). In contrast, infection with malaria parasites, *Plasmodium* spp., was correlated with increased clutch size in great tits (*Parus major*) (Oppliger et al. 1997). Finally, several studies have failed to establish any correlation between blood parasite infections and reproductive output of hosts such as American kestrels (*Falco sparverius*) (Dawson & Bortolotti 2001), pied flycatchers (*Ficedula hypoleuca*) (Siikamaki et al. 1997), and red winged blackbirds (*Agelaius phoeniceus*) (Weatherhead 1990).

I found that natural infections of *Hepatozoon hinuliae* reduce number of offspring and litter mass, but not mean offspring size, in the eastern water skink, *Eulamprus quoyii*. The extent of reduction in number of offspring of water skinks infected with

haemogregarines is similar to that of fence lizards infected with malaria parasites (Schall 1983). However, the results of my study contrast with the lack of effect of haemogregarines on number of offspring in European common lizards, *Lacerta vivipara* (Sorci et al. 1996). The disparity between the different studies may be due to the nature of the different host-parasite systems. The degree to which parasites deleteriously affect their hosts (virulence) is highly dependent on the host and parasite species involved (Thomas et al. 1995b) and different species and/or populations of haemogregarines may vary in their interactions with their hosts (Martin et al. 2001; Oppliger et al. 1999).

Blood parasites also reduce fecundity of blue tits (Merila and Andersson 1999, Merino, Moreno et al 2000) and western fence lizards (Schall 1983), but both these hosts are relatively short lived (adult survival rate of blue tits is only 20-30%, (Merila & Andersson 1999), and fence lizards normally live for less than two years, (Schall & Marghoob 1995)). In short-lived host species, parasites may have a strong negative impact on their relatively heavy investments in current (as opposed to future) reproductive output (Merila and Andersson 1999). In long-lived hosts, reductions in host fecundity may be more subtle, especially if the costs imposed by parasitism are absorbed by plastic traits such as growth rates or behaviour (Rigby & Moret 2000; Schwarzkopf 1994). *E. quoyii* is relatively long-lived with a life-span up to 8 years (Borges Landaez 1999). This is the first example that I am aware of, in which an endemic blood parasite causes reductions in fecundity of a long-lived wild host. It supports the theory that haemogregarines can be pathogenic to their hosts (Oppliger et al. 1996; Oppliger & Clobert 1997; Schall 1986).

Many studies only compare infected versus uninfected hosts (e.g.,Schall 1983), but my study has demonstrated that actual parasite load may be important in determining the pathogenic effects of parasitic infection, an idea that has previously been neglected in the literature (Sol et al. 2003). High parasite loads may exert higher costs on hosts by intensifying parasite-induced damage to host tissues, or by elevating levels of biochemical disruption (Sol et al. 2003).

Parasites and host condition

Variations in nutritional status were not responsible for the observed reductions in water skink fecundity observed in the high parasite load group. Body condition (measured as the relationship between SVL and mass) was not significantly different in animals with high or low parasite loads. Caudal lipid reserves (as indicated by tail base width) were the same or larger in females with a high parasite load compared to those with low parasite loads. A lack of a reduction in body condition in animals with high parasite loads suggests that the smaller litter masses, number of offspring and RCMs characteristic of this group were not the result of insufficient energy reserves in females with high parasite loads. In contrast, western fence lizards, *Sceloporus occidentalis,* infected with malaria parasites (*Plasmodium mexicanum*) had reduced lipid reserves as well as reduced clutch sizes (Schall 1983). Although malaria parasites may have been responsible for the reduced lipid reserves, it is also possible that inadequate lipid reserves may have reduced the fecundity of female fence lizards and concurrently reduced host defences against *Plasmodium* infection. High loads of blood parasites have also been associated with poor body condition in male sleepy lizards, (*Tiliqua rugosa*) infected with *Haemolivia mariae* (Smallridge & Bull 2000).

Other studies have failed to demonstrate a relationship between blood parasite infections and host body condition (*Plasmodium* spp. in *Anolis gundlachi*) (Schall & Pearson 2000).

A possible mechanism for the effect of parasites upon host fecundity

As suggested in the introduction, reductions in fecundity caused by parasitic infections might be a host response to infection that maximises possible lifetime reproductive output post-infection. This may be the case with *Hepatozoon* infections in reproducing female *Eulamprus quoyii*. Infected lizards invest less in their litters, in terms of number of offspring, and in the ratio of litter mass to female mass post-parturition (RCM), which may reduce the costs of reproduction (Schwarzkopf 1993). Furthermore, females with high parasite loads caught towards the end of pregnancy (December) had wider tail bases, indicating larger energy stores. The larger energy stores may be a result of reduced investment in the litter, and may account for the lack of an observed effect of parasites on growth or condition in the other months. Because water skinks are long-lived, and because size (and therefore growth) is related to fecundity, costs of parasitism may be paid during current reproduction, but minimised in future reproduction.

However, the parasite-induced reduction in host fecundity may also be non-adaptive, and simply a result of disrupted host physiology. An understanding of the reproductive physiology of water skinks suggests a possible mechanism for the *Hepatozoon*-induced reductions in female *Eulamprus quoyii* fecundity. *Eulamprus* lizards are lecithotrophic, i.e., they possess large, yolky eggs that provide most of the

nutrition for developing embryos (Thompson 1981; Thompson et al. 2001). In *Eulamprus quoyii,* allocation of yolk substances (predominantly lipids) to the ovaries begins during the autumn (April), continues during the winter dormancy, and ceases before ovulation and fertilisation in September/October (Thompson 1981; Veron 1969b). Litter mass and number of offspring are determined by available lipid reserves in the tail prior to mating (Doughty and Shine 1997, 1998; Wilson and Booth 1998), although lipid storage in the liver may also play a role (Thompson 1981). Variation in offspring size is probably determined by uptake of water and inorganic ions during pregnancy (Thompson 1981; Doughty and Shine 1998). Because *Hepatozoon* infections had an impact on measures of reproductive output associated with vitellogenesis (fecundity and litter mass), but had no effect on measures of reproductive output associated with water and ion uptake (offspring size), it suggests that *Hepatozoon hinuliae* could disrupt the metabolic processes involved in vitellogenesis.

Previous studies have demonstrated disruption of host vitellogenesis by parasitic infection. For example, rat tapeworm metacestodes (*Hymenolepis diminuta)* affect the fecundity of mealworms, *Tenebrio molitor*, because the presence of the parasite interferes with the synthesis of the yolk protein vitellogenin (Webb & Hurd 1999). Similarly, blood parasite (*Plasmodium yoelii nigeriensis*) infection in mosquitoes (*Anopheles gambiae*) reduces egg production and viability by directly affecting the processes of vitellogenesis, due to a decline in abundance of vitellogenin and impairment of ovary function, rather than through nutrient deprivation (Ahmed et al. 2001). Malaria parasites (*Plasmodium falciparum*) in humans are also known to alter lipid biochemistry (Faucher et al. 2002).

Physiologically based explanations of parasite-induced reductions in host fecundity are not mutually exclusive of host life-history adaptation, as these processes may coevolve together. For example, if parasites interfere with vitellogenesis reducing yolk availability, then perhaps a reduction in fecundity, as occurred in *E. quoyii*, rather than in the size of individual offspring, is the best fitness response to that constraint.

Future study

I found that blood parasites are associated with reductions in host fitness in the *Eulamprus-Hepatozoon* system. Observational studies are of great importance in illustrating trade-offs in the wild (Zera & Harshman 2001), but an experimental approach is required to reveal the mechanisms underlying possible haemogregarine-induced reductions in host fecundity. Whilst techniques exist to artificially inoculate reptiles with haemogregarines (Smallridge & Bull 2001; Smith et al. 2000; Wozniak & Telford 1991), experimentally infecting animals in the wild has important ethical and ecological connotations, and the use of this technique in wild populations should be carefully considered before it is adopted. Unfortunately, although curing parasitised individuals would clarify the role of haemogregarines in host fecundity, lizards are typically unresponsive to antimalarial therapy (Schall 1996) and attempts to cure lizards of *Hepatozoon* infections in the Blackdown Tableland population proved unsuccessful (unpublished data). An understanding of the mechanism by which *Hepatozoon* affects vitellogenesis could be achieved by examining the effects

of haemogregarines on production of vitellogenin (Webb & Hurd 1999), or by the use of palliatives that compensate for disruption of vitellogenesis.

Females with high parasite loads have litter sizes reduced by an average of one offspring per reproductive event. A high proportion of the adult female population are infected by *Hepatozoon hinuliae* (Chapter 2), and using the same rationale that I used to divide high from low parasite loads in this study, 75% of females have a reduced reproductive output as result of blood parasite infection. Furthermore, *Hepatozoon hinuliae* infections are long-lived (Chapter 2) so this reduction of reproductive output may last for several breeding seasons. Finally, the impact of the parasite may vary with other factors: for example harsh environmental conditions, such as drought, may accentuate the impacts of parasites by influencing host condition and health (Newman et al. 2001; Sanz et al. 2002; Schall & Pearson 2000). This study was carried out in a relatively dry year. Examination of the interactions between *Hepatozoon* and female *Eulamprus quoyii* reproductive output in the same population in additional years will enhance our understanding of the parasite's impact on its host's fitness and population biology.

CHAPTER 4

MATERNAL PARASITE LOAD & OFFSPRING TRAITS

They fuck you up, your mum and dad.
They may not mean to, but they do.
They fill you with the faults they had
And add some extra, just for you.

This Be The Verse, Phillip Larkin

SUMMARY

To maximise reproductive success, females may trade-off the number of offspring against individual offspring size. Parasitic infection may affect this trade-off in pregnant females. This chapter examines whether size-number trade-offs occur in the litters of *Eulamprus quoyii*, and whether maternal haemogregarine parasite load affects these trade-offs. In addition, I examine whether maternal parasite load affects neonate performance. Trade-offs did exist as females with larger litter sizes had smaller offspring, but there was no effect of maternal blood parasite load on these trade-offs. Offspring size was related to various performance indicators such as sprint speed, growth rate and competitive ability but maternal parasite load did not affect offspring performance.

INTRODUCTION

Maternal reproductive success is dependent on offspring fitness, and if offspring fitness is related to size at birth, then there is likely to be a trade-off between the number and the size of offspring produced by mothers with limited resources (Caley et al. 2001; Roff 1992; Schwarzkopf et al. 1999; Stearns 1992). This trade-off occurs because larger offspring may have increased chances of survival compared to smaller offspring, but high fecundity may improve the probability that offspring recruit to the adult breeding population. Previous studies have demonstrated that offspring size/number trade-offs can and do occur, but that the relationship is dependent on maternal and environmental conditions and trade-offs are not always observed (Schwarzkopf 1992; Sinervo 1990).

Theoretically, parasites can affect the offspring size/number trade-off because of their impacts on other co-varying life history traits such as survival and host fecundity (Chapter 3). Infection may also affect offspring size (Rolff 1999) by affecting the availability of maternal resources available to offspring growth, or by affecting the physiological processes involved in offspring development. Reduced offspring size may compromise the offspring's fitness in terms of survival, reproductive success and/or life-history traits such as growth rate (Lindstrom 1999; Metcalfe & Monaghan 2001; Roff 1992; Sinervo & Doughty 1996). However, few studies have examined the impact of parasitic infection on host offspring number/size trade-offs. Furthermore, the influence of parasites on offspring fitness may be subtler than simply causing changes in offspring size. For example, maternal infections of ectoparasitic mites (Lealapidae) have no effect on offspring mass or snout-vent length (SVL), but do

affect sprint speeds of neonate European common lizards (*Lacerta vivipara*) (Sorci & Clobert 1995; Sorci et al. 1994). Growth rates of year-old offspring were also related to maternal parasite load, and condition of female offspring after they had had their own offspring was lower in those with heavily infected mothers (Sorci & Clobert 1995). Unfortunately, because the lizards studied by Sorci & Clobert (1995) were held in captivity, ectoparasite loads were significantly higher than those seen on lizards in the wild, and therefore the results of their study may not be indicative of the impacts of parasites in natural systems. Although reduced offspring viability due to maternal parasite loads or immune system function has been observed in other taxa (Kristan 2002a; Kristan 2002b; Lozano & Ydenberg 2002), the phenomenon has not been frequently studied.

In this chapter, I use the *Eulamprus-Hepatozoon* system to determine whether maternal parasite load can affect reproductive trade-offs and the traits of offspring. Eastern water skinks (*Eulamprus quoyii*) with high levels of *Hepatozoon hinuliae* infections have reduced litter size, but offspring size is unaffected (Chapter 3). However, the impact of parasites upon offspring phenotype has not previously been examined. Therefore, in this chapter, I ask two questions: (1) Is there a trade-off between the size and number of offspring in *E. quoyii*, and if so, how is this trade-off influenced by maternal parasite load? (2) How does maternal parasite load affect the quality of offspring as measured by locomotor ability (sprint speed), growth rate, or competitive ability?

METHODS

Forty-two pregnant female lizards were captured at Blackdown Tableland in December 2001, and nineteen additional females were captured in December 2002. Chapter 3 provides details on capture, handling, measuring and housing of mothers, and Chapter 1 provides details on the study site. Data from both years were used to investigate relationships between offspring size and number, parasite load, and offspring growth. Competition and sprint trials were only performed in 2002-2003.

Newly born offspring were removed from the mother's cage as soon as they were detected and their umbilical cord had dried. I recorded mass (+/- 0.001g) using an electronic balance, snout-to-vent length (SVL) and tail length (to nearest mm) using a transparent plastic ruler, and sex (by eversion of hemipenes). Offspring were then individually housed in plastic boxes (30L x 12W x 9H cm) lined with paper towel and containing rocks or wood branches and a water container that allowed total immersion. Lights operated on a 12:12LD cycle, and heating wires beneath the boxes provided heat for thermoregulation for eight hours a day. Offspring were fed commercial cat food and small crickets (*Acheta domesticus*) every three days, and were toe clipped with unique combinations only before release into the wild (except those used in competition experiments, which were not included in the experiments monitoring growth).

Growth

Offspring SVL was measured on the day of birth before they had access to food. Offspring were remeasured, prior to feeding, 9-11 days after birth. Growth rates were

established by dividing the difference in SVL by the number of days between birth and day of measurement. Growth rates and size at birth do not vary between the sexes in this species (Caley & Schwarzkopf In press), so data from males and females were not kept separate for this analysis.

Locomotor performance

I quantified locomotor performance of lizards on the day of birth and before they were fed. This measure of performance therefore measures offspring quality at birth. Neonates were raced along a wooden racetrack that was 120L x 5W x 15H cm. Running speeds were measured in a temperature-controlled room at 28^{o} C, which is within the range of preferred body temperatures of *Eulamprus quoyii* in the wild (Schwarzkopf 1998). Along the racetrack were four pairs of infra-red photocells, spaced at 20-cm intervals, connected to an electronic timer. This timer was automatically activated when the neonate passed through the first pair of photocells, and passage through subsequent photocell pairs stopped it, thus providing three timed trials. Neonates were given 45 minutes to equilibrate to room temperature before the first trial, and had at least 45 minutes of rest between trials. Neonates were induced to run down the racetrack by chasing them with an artist's paintbrush. I disregarded trials in which neonates were performing sub-optimally (e.g., turning round in the racetrack, making frequent stops, and/or obvious non-maximal running) (Losos et al. 2002). Previous studies have shown no difference in running speeds between the sexes of *Eulamprus quoyii* (Borges Landaez 1999), so data from males and females were not separated. The maximum sprint speed of each individual over a 20-cm section of track was used to generate litter averages for maximum speed.

Competition

The importance of size in competitive interactions of newly born lizards was examined by housing two unrelated neonates together in one cage. Cages (30L x 12W x 9H cm) contained two rocks, water for immersion, and a single food dish. Offspring were fed with commercial cat food and small crickets, and the same light and heat regimes as the individually housed neonates. Offspring were two days old when placed together, and of the same sex. Two treatments were used: the two neonates either differed in birth weight by approximately 0.2 g (difference in mass = 0.2 +/- 0.035 g, n = 6, t-test of difference in mass, t = 11.4, df = 9, P < 0.000), or were approximately the same mass (control, difference in mass = 0.01 +/- 0.015 g, n = 6). Six pairs of equal-sized offspring (four male and two female) and six pairs of unequal-sized offspring (three male and three female) were used. Only offspring from mothers caught in December 2002 were used in these experiments.

Offspring infection

Theoretically, offspring may become infected with haemogregarines when they consume parts of the placenta after birth. I analysed blood slides of 43 offspring of heavily infected mothers but found no evidence of infection in these animals.

Statistical Analysis

Reproductive output and trade-offs can vary between years in lizards (Jordan & Snell 2002; Schwarzkopf 1992), and the interactions between parasite load, environment and reproduction are subject to annual variation (Sanz et al. 2002). Therefore the data from the two years were not combined.

Within-litter averages were used for analyses, as data from individual offspring are not independent (Merino et al. 1996; Zar 1999). Low parasite loads were defined as those females in the lower quartile of the range of parasite loads, and high parasite loads consisted of the remainder (Chapter 3 used the same method). ANCOVAs were used to examine the effects of parasite load on the offspring size-number trade-off, using SVL as a covariate, and to measure the effect of maternal parasite load on offspring running speeds, using offspring SVL as a covariate. Because of a smaller sample size, in December 2002 there were only 4 females with low parasite loads by the definition used above. Therefore, instead of separating litters into those with high and low maternal parasite loads, I used bivariate and partial correlations to examine the relationship between maternal parasite load and offspring sprint speed. Finally, sample sizes differ in the analyses because offspring used in the competition experiments were excluded from analyses on growth and running.

RESULTS

Offspring size-number trade-off

In 2001-2002, there was a significant negative relationship between offspring size and number of offspring ($r^2 = 0.29$, $P < 0.000$, Figure 4.1). Maternal parasite load (high or low) did not affect the relationship between size and number of offspring (ANCOVA, $F_{1, 38\ slopes} = 0.12$, $P = 0.74$, $F_{1, 39\ elevation} = 0.14$, $P = 0.71$). In 2002-2003 there was no significant relationship between offspring size and number ($r^2 = 0.03$, $P = 0.54$; Fig. 4.2).

Growth rates

The relationship between growth rate and offspring size at birth was significant and negative for offspring born in December-January 2001-2002 ($r^2 = 0.18$, $P = 0.005$; Fig. 4.3), and was not affected by maternal parasite load (ANCOVA, $F_{1, 37 \text{ slopes}} = 2.58$, $P = 0.12$, $F_{\text{elevation } 1, 38} = 0.01$, $P = 0.92$). There was no relationship between growth rate and size of offspring in 2002-2003 ($r^2 = 0.03$, $P = 0.67$; Fig. 4.4).

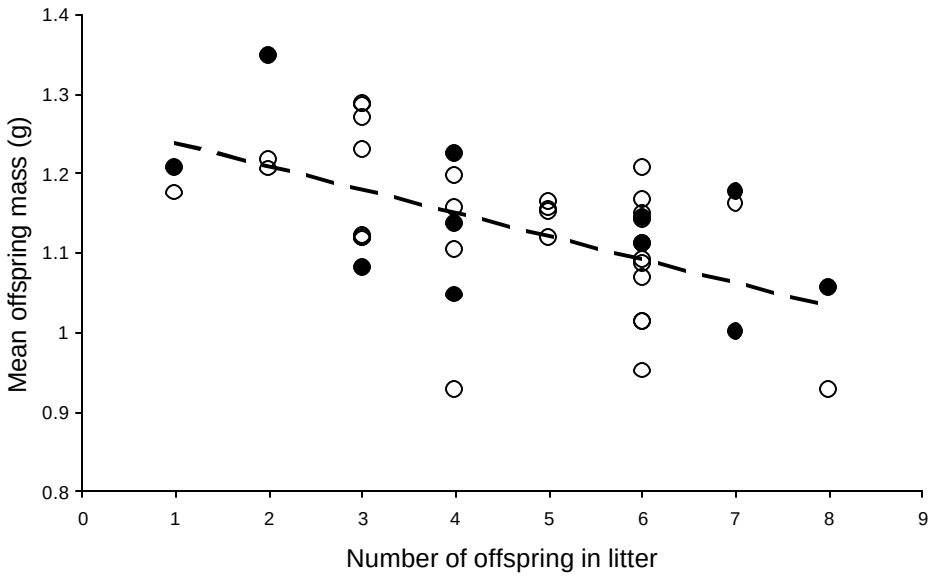

Figure 4.1 The relationship between number of offspring and mean offspring mass in 2001-2002. Maternal parasite load (low (•) and high (o)) had no significant effect on the relationship.

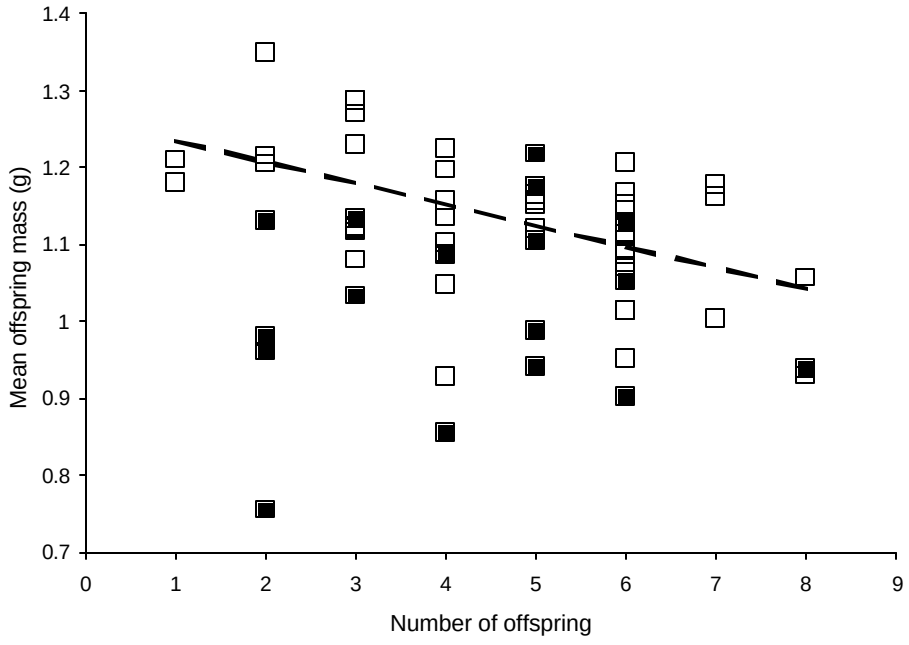

Figure 4.2 Interannual variation in the size/number offspring trade-offs of female eastern water skinks. There was a significant negative relationship in 2001-2002 (broken line,), but no significant relationship in 2002-2003 (■).

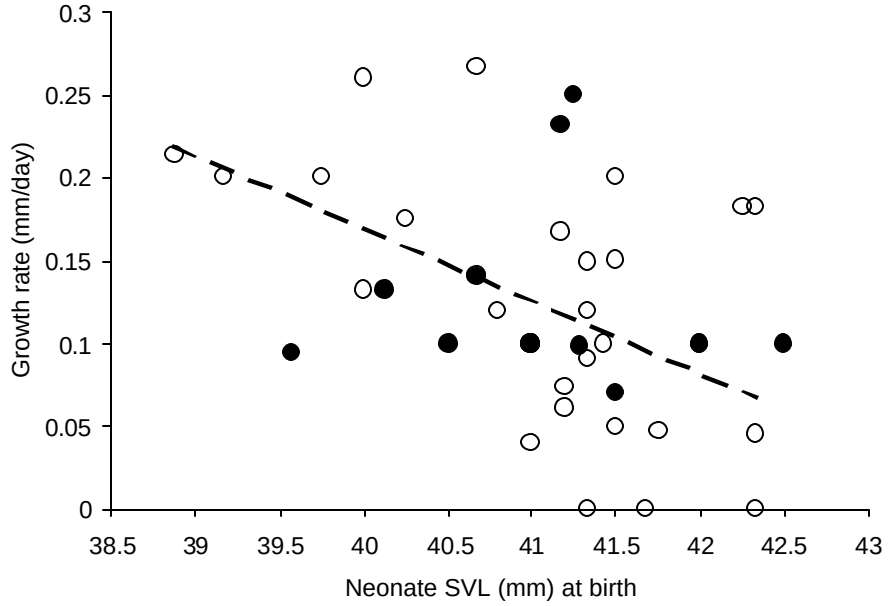

Figure 4.3 Relationship between neonate snout-vent length (SVL) and neonate growth rate during the first 10 days of life (2001-2002). Maternal parasite load (low (•) and high (o)) had no significant effect on the relationship.

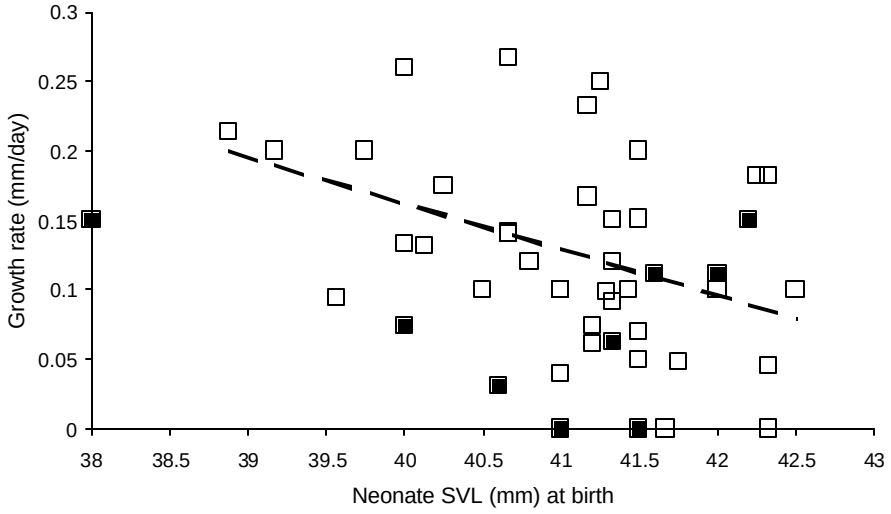

Figure 4.4 Interannnual variation in the relationship between neonate lizard size and growth rate. There was a significant relationship in 2001-2002 (broken line,), but no significant relationship in 2002-2003 (■). The lack of a relationship may be due to the smaller sample size.

Sprint speed

The time taken to cover 20 cm was significantly correlated with neonate size (SVL, mm), ($r^2 = 0.27$, $P = 0.039$; Fig. 4.5); larger animals ran faster. Maternal parasite load had no effect on offspring running speed (r = -0.16, n = 16, $P = 0.58$) even when controlling for the relationship between offspring SVL and running speed (partial correlation r = 0.04, df = 12, $P = 0.9$).

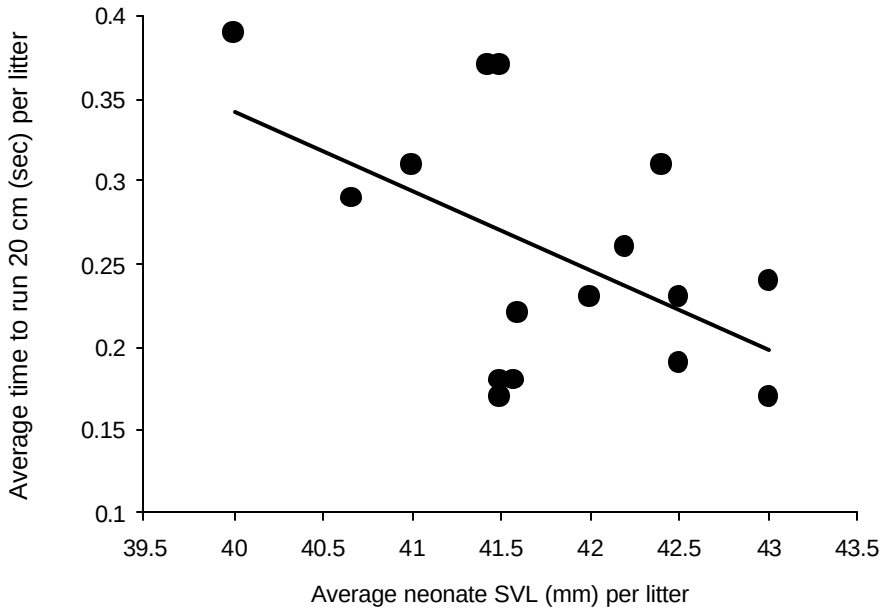

Figure 4.5 The relationship between neonate SVL (mm) of newly born eastern water skinks and fastest recorded times to run a 20 cm distance.

Competition & offspring size

This experiment was terminated after five days due to high levels of mortality caused by competitive interactions. By day 5, five lizards had been killed by their competitors although all lizards were alive on day 3. There was no significant

difference in mortality rates between the equal- and unequal-sized pairings (1 death in 6 trials with equal-sized offspring, 4 deaths in 6 trials of unequal-sized offspring, 2-tailed Fisher's exact test, P = 0.24). The lack of a significant effect may be due to the small number of trials. I had intended to compare growth rates of neonates, but instead adopted a post-hoc method of determining 'winners' and 'losers' in the unequal-sized offspring trials. 'Losers' were either dead, or if alive, had damaged tails (bitten or lost). 'Winners' were either the survivor, or had undamaged tails. The larger individuals in the unequal-sized offspring trials were significantly more likely to be the winners (1-tailed Fisher's exact test, $P = 0.04$). From 6 unequal-sized offspring trials, 3 trials resulted in the larger individual killing the smaller competitor; 2 trials resulted in the smaller individuals having broken tails although the larger individuals' tails were intact; and in the remaining trial, the larger offspring had been killed by the smaller offspring.

DISCUSSION

Offspring size-number trade-off

Offspring size and fecundity (number of offspring) are two life-history traits where trade-offs are possible, phenotypically and genotypically, so that a mother with limited resources can maximise her lifetime reproductive success (Roff 1992; Stearns 1992). The trade-off exists because larger offspring may have increased chances of survival compared to smaller offspring, whereas high fecundity may improve the probability that offspring recruit to the adult population.

Female eastern water skinks exhibited a trade-off between offspring size and number during 2001-2002, although there was no significant relationship in 2002-2003. The trade-off in offspring size and number has been observed in other lizard species, but it is not universally observed, and may vary annually (Schwarzkopf 1992; Sinervo 1990). The difference between years in the trade-off seen in my study may be due to annual variation in maternal condition prior to pregnancy, but does not seem to be due to the smaller sample size in 2002-2003 (Fig. 4.2). In both years, maternal parasite load did not affect the offspring size-number trade-off, even though females with high parasite loads had fewer offspring (Chapter 3).

Growth rates

In 2001-2002, there was a negative relationship between growth rate and body size of neonates. The phenomenon of faster growth in smaller individuals is commonly observed in lizards (Sinervo 1990; Sinervo & Adolph 1994). Another study of growth in eastern water skinks failed to detect a relationship between growth rate and offspring size at birth (Caley & Schwarzkopf In press). However, growth rates were measured over a much longer period in the Caley & Schwarzkopf study so an early relationship between size and initial growth may have gone undetected.

There was no effect of maternal parasite load on offspring growth rate. Females had reduced fecundity as a result of high parasite load (Chapter 3), suggesting that costs of parasitism are manifested in terms of offspring number, but not in terms of the remaining offspring's quality. The lack of a significant relationship between growth

rates and size in 2002-2003 may be due to the small sample size; 2002-2003 data values were distributed within the spread of 2001-2002 data values (Fig. 4.4).

Sprint Speed

In the wild, juvenile water skinks flee predators (e.g., scientific researchers) by sprinting to cover (e.g., vegetation clumps, leaf litter or rocks) (pers. obs.), and therefore measurement of sprint speed is relevant to the animal's natural escape behaviour, and possibly survival (Vanhooydonck & Van Damme 2003). Like most previous studies of squamate reptiles (Elphick & Shine 1998; Shine & Harlow 1993; Sinervo 1990; Sorci et al. 1994), sprint speed was significantly related to body size in newly born *Eulamprus quoyii*. The infection status of the mother had no influence on sprint speed in my study, once again suggesting that reduction in clutch size, rather than in clutch quality, occurs in infected individuals.

Competition & offspring size

A previous report indicated little aggression between communally housed *Eulamprus quoyii* offspring (Borges Landaez 1999). However, in this study, competition between offspring was intense, and often (5/12 trials) caused the death of one of the competitors. Other trials showed evidence of fighting, as tails were either bitten or had been partially lost by one of the competitors. Typically, the larger offspring "won" the encounter, either killing the smaller lizard, or causing extensive physical damage by biting. Therefore size is important in determining the outcome of competitive interactions in this species, although the size of the container used may have exacerbated the severity of aggressive contests.

In summary, this research demonstrates that offspring size does trade-off against offspring number in eastern water skinks, and that the trade-off may occur partly because of the differential fitness of offspring of varying size. *Eulamprus* offspring phenotype can be affected by maternal environment during embryonic development (Caley & Schwarzkopf In press; Robert & Thompson 2001; Robert et al. 2003; Shine & Harlow 1993). However, unlike previous research on the impact of ectoparasites on European common lizards (*Lacerta vivipara*) (Sorci & Clobert 1995; Sorci et al. 1994), I found no effect of maternal blood parasite load on offspring size (Chapter 3) or quality (sprint speed, growth, competitive ability). The lack of effect of maternal parasite load on *Eulamprus* offspring-number trade-offs occurs despite the reduced litter sizes of heavily infected females (Chapter 3). Therefore, it appears that females pay a cost of parasitism by reducing fecundity, but that the offspring are otherwise unaffected (although see Future Research below).

Two explanations account for the contrary effects of maternal parasite loads on offspring fitness in European common lizards and Australian water skinks. The first is the different parasite types: blood parasites may affect vitellogenesis in breeding females, but have little effect upon condition or energy reserves that are important in determining offspring size (Chapter 3). In contrast, ectoparasites remove blood, and therefore nutrients and oxygen from the host and its offspring. The second is that this study examined parasite loads manifested naturally in the wild, whereas a by-product of the work on *L. vivipara* was an artificially elevated ectoparasite load (Sorci & Clobert 1995).

The importance of offspring quality

Neonate traits measured at birth do not necessarily translate to fitness and lifetime reproductive success. Performance abilities at birth may have little relation to survival in the wild due to trade-offs with other traits such as resistance to parasites (Barber et al. 2001), or to variation in environmental conditions such as predation pressure or food availability (Ferguson & Fox 1984). For example, endurance ability of neonate European common lizards (*Lacerta vivipara*) was unrelated to subsequent survival, because lizards with high endurance suffered increased predation pressure, whilst neonates that had exhibited reduced endurance were more prone to blood parasite infection (Clobert et al. 2000). Consequently, it may be short sighted to simply equate higher neonate performance indicators with higher fitness for two reasons. Differences in traits observed shortly after birth may disappear: for example, compensatory growth patterns can allow offspring to mature at similar sizes despite challenges such as maternal parasite loads (Bize et al. 2003; Kristan 2002a). Second, 'good' performance may be inversely related to actual fitness, e.g. higher growth rates of juvenile Tasmanian snow skinks (*Niveoscincus microlepidotus*) were associated with reduced survival (Olsson & Shine 2002).

However, other studies have demonstrated long-term effects of offspring phenotype (Caley & Schwarzkopf In press; Sinervo & Doughty 1996). Differences in running speed of neonate *Eulamprus heatwolei* caused by experimental maternal basking regimes were still apparent two months after birth (Shine & Harlow 1993). In *E. quoyii*, offspring size at birth correlates with survival in predator-free outdoor enclosures (Borges Landaez 1999), and neonate growth rates are indicative of long-

term patterns of growth (Caley & Schwarzkopf In press). Although the long-term fitness implications of neonate performance measured by sprint speed, growth rates, and competitive ability characteristics are uncertain, offspring size was significantly related to these traits, and this may help explain why there is a trade-off between offspring size and number.

Future research

This study raises two further questions. First, how does offspring phenotype affect fitness (survival and reproductive output) under natural conditions? Morphological and behavioural traits such as running speed, growth rate and competitive ability might be expected to influence fitness in natural environments, but it is necessary to relate these traits to offspring survival under field conditions (Borges Landaez 1999; Elphick & Shine 1998; Jordan & Snell 2002). In my study, all offspring born in captivity were individually marked and then released in the field within a month of being born. Monitoring these cohorts of offspring in 2004 and beyond will demonstrate how offspring size affects survival and reproductive biology.

Second, how does maternal parasite load affect offspring fitness in the long term? Like the measurements of fitness related to neonate size, subtle effects of maternal parasite load may only become apparent with time (Sorci & Clobert 1995). In addition, if there is a genetic component to parasite load (as suggested in Chapter 2), examination of parasite loads, and age of first infection of lizards with known matrilineage will help determine the heritable element to the *Eulamprus-Hepatozoon* relationship.

CHAPTER 5

PARASITES AND MULTIPLE MATING IN EASTERN WATER SKINKS

SUMMARY

Multiple mating by females may increase reproductive output, and may also confer fitness benefits on the offspring. For example, multiple mating may be a strategy to increase genetic variation of the offspring, and therefore increase parasite resistance of the offspring which may aid survival. Parasites can also affect host behaviour, although the role of female infection status in mating behaviour has received little attention. Using the *Eulamprus-Hepatozoon* host-parasite system, this chapter describes a preliminary attempt to employ microsatellite markers to determine whether haemogregarine parasite load affects a female's propensity to mate with multiple partners. In addition, I compare growth rates of offspring from mothers with single and multiple mates. Of 14 litters, 7 were sired by 2-3 individuals, confirming that *Eulamprus quoyii* engages in multiple mating. Although sample sizes were small, this investigation indicated that parasite load had no impact on female multiple mating behaviour. There was no significant difference between growth rates of litters with one or multiple fathers.

INTRODUCTION

The theoretical link between parasites and host mate choice has been debated since Hamilton and Zuk (1982) proposed that there should be a relationship between a species' sexual ornamentation and the risk of disease; if parasites have significant effects upon host fitness, and if resistance to disease is at least partially heritable, then individuals that select mates with low parasite loads should have higher reproductive success. The Hamilton & Zuk (1982) hypothesis is difficult to test and is still controversial (Atkinson 1991; Folstad & Karter 1992; Hamilton & Poulin 1997; John 1997; Read & Harvey 1989; Thomas et al. 1995). Nonetheless, work on some animal models has demonstrated the ability of females to discern the parasite loads of prospective mates, and to choose unparasitised males (Milinski & Bakker 1990). In addition, the Hamilton & Zuk (1982) hypothesis has provoked much research on links between mating systems and parasites (Westneat & Birkhead 1998). For example, risk of disease has been correlated with host mating systems in birds (McCurdy et al. 1998; Read 1991) and humans (Low 1990). Experimental work has demonstrated that certain mating strategies can increase the offspring's resistance to disease. For example, higher levels of polyandry in bumble bees (*Bombus terrestris*) decreased parasite load, prevalence and parasite species richness in subsequent colonies (Baer & Schmid-Hempel 1999). Indeed, increased resistance to disease may explain why female multiple mating can increase reproductive success (Stockley 2003).

Although the role of parasites in the evolution of mating strategies and mate choice has received much theoretical attention, few studies have examined the role of the female's parasitic infection status in her choice of mates (Poulin 1994; Poulin &

Vickery 1993). Laboratory studies that have incorporated female parasite load into their experiments have demonstrated that parasites can affect the cost-benefit trade-offs of female mate choice. For example, larval trematodes *Telogaster opisthorchis* affect the mate choice of a New Zealand fish host, the upland bully (*Gobiomorphus breviceps*). Infected females reduced mate inspection behaviours and were more likely to choose smaller males than were uninfected females (Poulin 1994). Female guppies, *Poecilia reticulata*, infected with the monogenean parasite *Gyrodactylus turnbulli* fail to choose between attractive and unattractive males, probably due to reduced energy levels (Lopez 1999). Similarly, female spadefoot toads (*Scaphiopus couchii*) showed no preference for male call traits when parasitised by a blood-feeding monogenean worm (*Pseudodiplorchis americanus*) (Pfennig & Tinsley 2002), while female crickets (*Requena verticalis*) mated more frequently if infected with protozoan gut parasites (Sporozoa: Gregarinida) (Simmons 1994). Changes in female mating behaviour due to parasitic infection may occur because parasites may alter female resource or energy availability. Infected females may be less inclined to make energetically expensive mate choice decisions, or become unable to reject advances or resist male copulation attempts (Lopez 1999; Milinski & Bakker 1992; Real 1990). Physiological mechanisms of mate discrimination may also be disrupted by infection so that females are unable to choose between males (Moore 2002; Poulin & Vickery 1996).

Ideally, three conditions should be met before studies are performed on the importance of parasites in mating behaviour (Poulin & Vickery 1993). There must be (i) knowledge of the natural infection dynamics, (ii) an understanding of the pathogenic effects induced by various parasite intensities, and (iii) the males made

available to females should be representative of male phenotypes in the wild. This study uses a lizard-blood parasite system to fulfil these three requirements. The eastern water skink, *Eulamprus quoyii*, is frequently infected with the protist *Hepatozoon hinuliae*. Chapter 2 examined natural infection dynamics in *E. quoyii* at Blackdown Tableland. In this system, at similar body sizes, females with high parasite loads have a reduced fecundity compared to females with low parasite loads (Chapter 3). Finally, I analyse the mating strategies of females that mated in the wild, and therefore could select from the natural spectrum of males.

Previous studies of lizard mating systems have shown that females commonly engage in multiple mating within clutches (Abell 1997; Gullberg et al. 1997; Laloi et al. 2004; Lebas 2001; Morrison et al. 2002). These studies have determined that spatial proximity is often related to paternity, and that the nutrient content of sperm and seminal fluid is negligible, thereby imparting little useful energy to the females (see references above). Apart from behavioural observations of male fence lizards (*Sceloporus occidentalis*) infected with malaria parasites (*Plasmodium mexicanum*) (Schall & Dearing 1987; Schall & Houle 1992), and a literature review investigating the Hamilton & Zuk (1982) hypothesis (Lefcort & Blaustein 1991), the role of parasites in lizard mating systems has not been studied.

In this chapter, levels of female multiple mating in a wild population of eastern water skinks were estimated using microsatellite markers recently developed for the congener *Eulamprus kosciuskoi* (see Scott et al. 2001). This investigation acts as a pilot study exploring how useful these microsatellite markers might be in investigating mating systems within the Blackdown Tableland *E. quoyii* population,

and at the same time determines whether parasite load affects the mating behaviour of female water skinks in terms of the number of mates they select. In addition, I compare the size and growth rate of offspring born from mothers mated with high and low numbers of mates.

METHODS

Female eastern water skinks were caught towards the end of pregnancy from Blackdown Tableland National Park and housed in individual cages in James Cook University until they gave birth (Chapter 1, Chapter 3). Data from mothers with similar-sized litters were separated into groups of high and low parasite loads. In the Blackdown Tableland water skink population, mean parasite load of adult females (+/- SD) is 0.59% (+/- 0.74) and reaches a maximum of 3.6% (n = 105) (Salkeld 2004). Here, low parasite load females were categorized as those with a mean parasite load (+/- SD), of 0.03% (+/- 0.04) (n = 12), and high parasite load females had a mean parasite load (+/- SD) of 1.93% (+/- 0.99) (n = 12). Growth rates of neonates were calculated as described in Chapter 4.

Microsatellite genotyping

DNA of a subset of captured mothers (n = 24) and their offspring was extracted from toe clip and tail tip samples that had been stored in 100% ethanol. Samples were genotyped using microsatellite markers previously isolated from the congener *Eulamprus kosciuskoi* (see Scott et al. 2001 & unpublished communications from the authors) although loci were redesigned to accommodate a slightly different genotyping method, which favoured smaller products. The markers used were: Ek17

forward primer TGAGTCGCCTTGGCAGAAT & reverse primer CCGAATCTCCGCAACTTT; Ek 37 forward primer GTGCCAATTTACTTGATGCC & reverse primer AAGGTCCCCATTGAAATGAG, Ek 107 forward primer TGACACCATGCAAAACCTTT & reverse primer TGAGATCCATTGGCTTGGAA, Ek122 forward primer AAATGCAGGTCCTTCGAAGA, & reverse primer CATTTGAAACAGGATCCCAGA.

DNA was extracted from the tissue samples using a standard salt precipitation protocol (Miller et al. 1988; Strassmann et al. 1996). DNA was then resuspended in 200 µl of sterile, ultrapure water.

Loci were amplified using the polymerase chain reaction (PCR) in a Perkin Elmer 9700 thermocycler using 10 µl reactions (1 part Promega Buffer with 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 µM of fluorescent forward primer, 0.5 µM of unlabelled reverse primer, and 0.25 units Promega *Taq* Polymerase and diluted genomic DNA). Reactions were denatured at 94°C for 2 min, followed by 35 cycles at 94°C for 30 sec, annealing temperatures appropriate for each locus for 30 seconds, and 72°C for 45 sec. There was a final extension period of 2 min at 72°C. Five per cent polyacrylamide gels were used to reveal loci using a GelScan 2000 electrophoresis system (Corbett Research). A standard ladder sample marked with MapMarker 400 was run with each sample, allowing accurate sizing of alleles and comparison between gels.

Assessing number of mates

Because females had mated in the wild, paternal genotypes were not available. Therefore I estimated the number of males who had sired offspring in each litter by sorting the litters into groups of full siblings using the computer programme *Kinship 1.4* (Goodnight & Queller 1999a). *Kinship 1.4* compares a hypothesized relationship between pairs of individuals to determine whether it is significantly more likely than a user-specified alternative relationship. Because I wanted to sort full- and half-siblings into their sibling groups I tested whether each pair of offspring was significantly more likely to be full-siblings than half-siblings. Based on the results of *Kinship 1.4*, and Mendelian principles of inheritance, the offspring of each litter were assembled into the minimum number of sibling groups that could account for their genotypes, given the genotype of their mother.

For 10 litters, maternal DNA was unavailable (some samples from 2001-2002 had been lost, and accidents during the genotyping protocol compromised 4 others) making it impossible to estimate the number of fathers. Therefore I also calculated the Queller and Goodnight index of relatedness (*R*), a relative measure that is calibrated by the frequency of alleles and number of individuals in the sample (Queller & Goodnight 1989). *R* was calculated using the computer programme *Relatedness 5.0* (Goodnight & Queller 1999b). A high value of *R* implies few fathers for a litter. Confidence intervals were calculated by jackknifing over loci within litters, and over litters for the overall value of *R*.

RESULTS

Parasite load and female mating behaviour

I analysed the genotypes of litters of 24 female water skinks from Blackdown Tableland. Lizards with high (n = 12) and low (n = 12) parasite loads did not differ in body length (SVL), condition (mean tail base width, mean mass post-birth) or reproductive output (mean litter size, mean litter mass or mean date of birth) (t-tests, $P > 0.74$). The ave rage litter size was 5.8 +/- 1.1 (n = 24).

The loci present indicated that female water skinks engage in multiple mating. From fourteen litters with known maternal genotype, seven litters (i.e., 50%) exhibited evidence of multiple mating. Five litters (36%) had a minimum of two fathers, and two litters (14%) had a minimum of three fathers. Mean relatedness (*R*) for all litters was 0.45 +/- 0.18 (n = 24).

The degree of relatedness was not significantly different between mothers with low or high parasite loads (t-test, t = -0.247, n = 24, df = 22, $P = 0.81$). Also, there was no relationship between relatedness and parasite load ($r^2 = 0.0$, n = 24, $F_{1,22} = 0.002$, $P = 0.96$) or parasite load and number of sibling groups ($r^2 = 0.0$, n = 14, $F_{1, 12} = 0.001$, $P = 0.98$).

Female size did not significantly affect relatedness of their offspring ($r^2 = 0.001$, n = 24, $F_{1,22} = 0.023$ P = 0.88). Female investment, measured as Relative Clutch Mass (litter mass / mass of female post-birth), was not affected by relatedness ($r^2 = 0.09$, $P = 0.16$) or number of fathers ($r^2 = 0.09$, $P = 0.29$). Mean offspring size was unrelated

to Relatedness (*R*) ($r^2 = 0.02$, n = 24, *P* = 0.46) and no. of sibling groups ($r^2 = 0.06$, n = 14, *P* = 0.41).

Female mating behaviour & offspring traits

Multiple mating had no effect upon average litter growth rates (SVL) (Relatedness: $r^2 = 0.01$, n = 24, *P* = 0.60; no. of sibling groups: $r^2 = 0.01$, n = 14, *P* = 0.70).

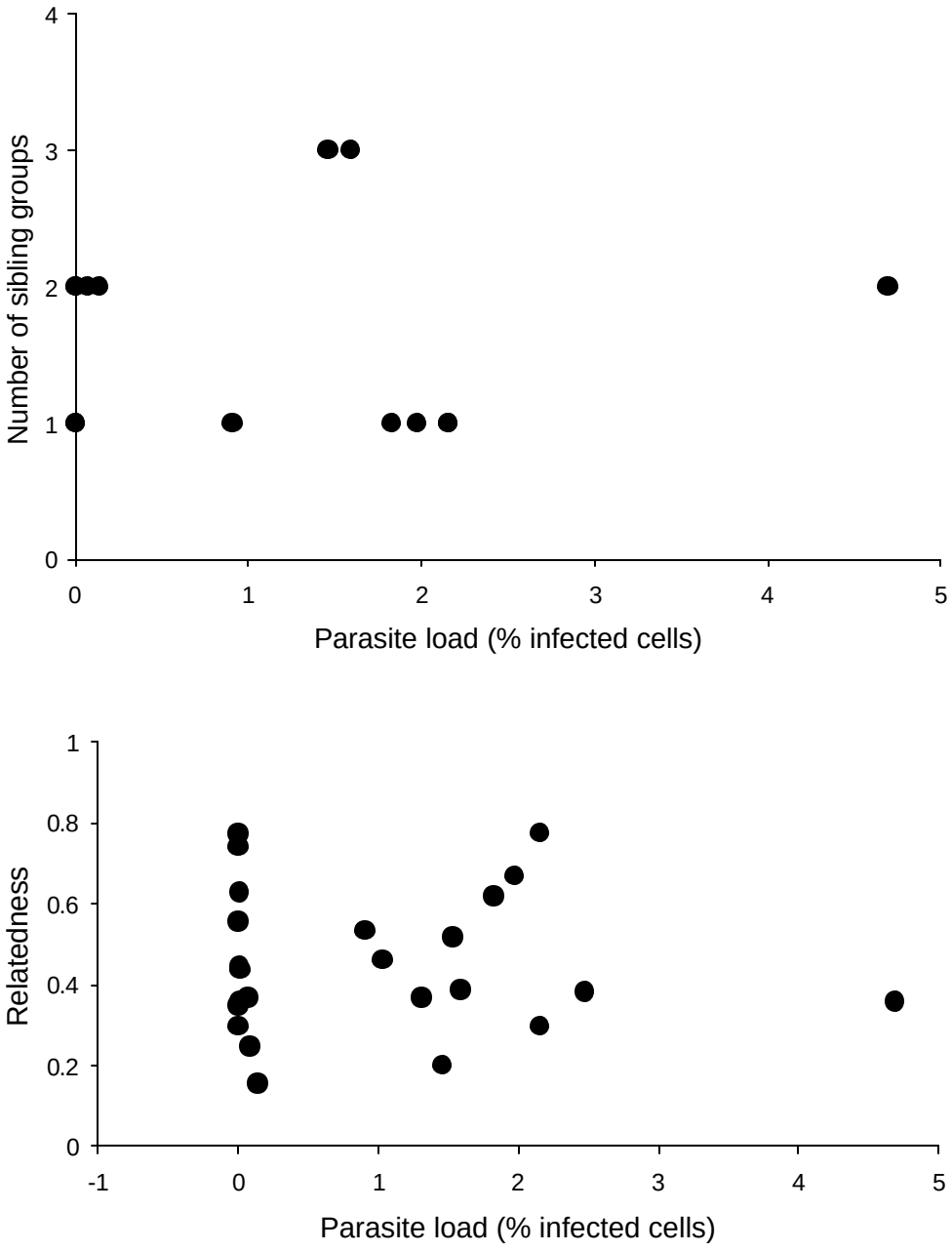

Figure 5.1 The relationship between haemogregarine parasite load (% infected cells) and female mating behaviour in terms of the number of sibling groups (top) and relatedness (bottom). There was no significant relationship in either case.

Table 5.1 Parasite load, litter size, relatedness (*R*), and number of sibling groups for each litter (NM = No Maternal DNA).

Mother ID	Maternal parasite load (% infected cells, High/Low)	Litter size	Relatedness (CI)	No of sibling groups
1	0.00 LOW	6	0.298 (0.535)	2
2	0.00 LOW	7	0.554 (0.228)	1
3	0.00 LOW	5	0.773 (0.508)	1
4	0.00 LOW	5	0.347 (0.813)	2
5	0.00 LOW	3	0.741 (0.211)	NM
6	0.01 LOW	7	0.627 (0.617)	NM
7	0.01 LOW	6	0.357 (0.896)	NM
8	0.01 LOW	5	0.444 (0.483)	NM
9	0.02 LOW	6	0.436 (0.582)	NM
10	0.07 LOW	8	0.368 (0.327)	2
11	0.08 LOW	5	0.246 (0.282)	NM
12	0.14 LOW	6	0.153 (0.414)	2
13	0.91 HIGH	6	0.534 (0.360)	1
14	1.03 HIGH	6	0.461 (1.227)	NM
15	1.31 HIGH	5	0.368 (0.268)	NM
16	1.46 HIGH	5	0.200 (0.523)	3
17	1.54 HIGH	5	0.516 (0.599)	NM
18	1.59 HIGH	8	0.387 (0.297)	3
19	1.83 HIGH	6	0.617 (0.148)	1
20	1.98 HIGH	5	0.669 (0.647)	1
21	2.16 HIGH	5	0.776 (0.398)	1
22	2.16 HIGH	7	0.297 (0.471)	1
23	2.48 HIGH	6	0.381 (0.355)	NM
24	4.70 HIGH	6	0.358 (0.236)	2

DISCUSSION

Parasite load and female mating behaviour

Multiple mating by males is regarded as a strategy to maximise reproductive output, and therefore lifetime reproductive success, because sperm is cheap. In contrast, the reason for multiple mating by females is less clear, particularly in situations when there are few direct benefits to females or their offspring, i.e., if no additional resources or territorial advantages are gained from multiple mating. Various hypotheses have suggested that the benefits of polygamy to females are the avoidance of genetic incompatibility and inbreeding, promotion of sperm competition, and/or maximising offspring fitness (Baer & Schmid-Hempel 1999; Jennions & Petrie 2000; Konior et al. 2001; Stockley 2003; Tregenza & Wedell 2002; Zeh & Zeh 2001).

This study demonstrates that 50% of female eastern water skinks engage in multiple mating. Previous studies have also found multiple mating in wild skinks (Morrison et al. 2002; Stow & Sunnucks 2004), lacertids (Gullberg et al. 1997; Laloi et al. 2004), agamids (Lebas 2001) and phrynosomatids (Abell 1997; Zamudio & Sinervo 2000).

Although parasite load may alter host mating behaviour and mate choice in other taxa (Poulin 1994, Lopez 1999), there was no significant difference in mating behaviour of female *Eulamprus quoyii* with high and low haemogregarine blood parasite loads. Indeed, although parasites can affect lizard fitness and life history (Chapters 1 & 3), there is no explicit evidence to suggest that parasites play a role in lizard mating systems (Lefcort & Blaustein 1991). An explanation for this may be that lizards are unable to determine the infection status of other lizards. However, lizard sensory

systems are capable of detecting kin (Aragon et al. 2001; Bull et al. 2001; Lopez & Martin 2002), and the ability to discern parasite infection has evolved in fish, birds and mammals (Horak et al. 2001; Klein et al. 1999; Merila et al. 1999; Milinski & Bakker 1990), so why not lizards? Interestingly, studies of lizard species with bright ornamentation have also failed to demonstrate that such extravagant characteristics are important to female choice. Instead, ornaments may play more of a role in male-male competition and territoriality (Lebas 2001; Lebas & Marshall 2001; Olsson 2001). Alternatively, because my study was part of a preliminary investigation into the possible use of microsatellite markers in determining mating systems in *Eulamprus quoyii*, the small sample size may be inadequate to reveal the effects of parasites on female mating behaviour. Genotyping of additional litters would remedy this problem.

Perhaps other factors, such as proximity to males or predator avoidance, are more important than parasite load in determining female mating behaviour in the wild. Examination of male genotypes, and possible correlations with phenotype, will help to further explain female mate choice behaviour, if it occurs in this species. However, a study of the congener *Eulamprus heatwolei* revealed that both male body size and home range ownership were unrelated to male reproductive success (Morrison et al. 2002). Conceivably, random mating is the most evolutionarily stable strategy for species where phenotypes do not obviously correlate with mating success, and genotypes that endow disease resistance or other qualities are difficult to recognise (Hosken & Blanckenhorn 1999). Studies of male behaviour and reproductive success may also show impacts of blood parasitism, and these effects may be independent of female preferences for male phenotype, but still could affect the evolution of mating

systems. For example, when compared to uninfected males, male western fence lizards (*Sceloporus occidentalis*) infected with malaria parasites (*Plasmodium mexicanum*) exhibited courtship displays less often, were less dominant in social interactions, and had smaller testes, reduced titres of basal plasma testosterone and higher levels of corticosterone (Dunlap & Schall 1995; Schall 1990a; Schall & Dearing 1987; Schall & Houle 1992), indicating that infected males probably have reduced reproductive success. However, the effect of parasite loads on male lizard reproductive success, measured by number of offspring, has not been explicitly examined.

Female mating behaviour & offspring traits

Multiple mating by females is potentially costly due to increased energy expenditure and predation risk, but these costs may be offset if offspring gain fitness benefits (Baer & Schmid-Hempel 1999; Tregenza & Wedell 1998; Tregenza & Wedell 2002). For example, early offspring mortality is reduced in mammalian species with multiply mating females (Stockley 2003). Also, the number of mates of female sand lizards, *Lacerta agilis*, was significantly correlated with hatching success, offspring mass and offspring survival in the first year (Olsson et al. 1994a; Olsson et al. 1994b). In my study of eastern water skinks, offspring from females with high and low levels of multiple mating did not significantly differ in their early growth rate. This does not mean that multiple mating has no effect on offspring. The benefits of multiple mating may only be observable when considering survival and future mating success, and may even only be apparent in the second generation (Konior et al. 2001). However, in order to investigate the possibility that multiple mating does affect offspring fitness,

individually marked offspring will be monitored in the wild, as described in Chapter 4. An additional explanation is that there are no benefits of multiple mating to the offspring, but instead, benefits accrue to the female (Schmoll et al. 2003).

In conclusion, this chapter has examined the effects of parasite load on female mating strategy, and found that in a small sample, there was no significant difference between the mating behaviour of females with high and low haemogregarine parasite loads. The impact of maternal mating strategy on offspring traits can be determined by further observations of offspring survival and reproductive success in the wild. Further genotyping of DNA samples from water skink litters, as well as males, in the Blackdown Tableland population will help in revealing the mating systems adopted by eastern water skinks, although more markers specific to *Eulamprus quoyii* need to be developed to accurately determine paternity of wild fathers. Such an effort would establish which male characteristics influence reproductive success, and may explain variation in the degree of relatedness of offspring observed in the females studied here. Optimistically, such information may allow us to infer whether female choice or male-male competition is dominant in shaping water skink mating strategies. This study has nonetheless demonstrated that the use of microsatellite markers can provide an understanding of eastern water skink mating behaviour.

CHAPTER 6

GEOGRAPHICAL VARIATION IN PARASITE ABUNDANCE; HIGHER PARASITE LOADS IN THE TROPICS

SUMMARY

Parasites are important selective forces, and some hypotheses have suggested that high species diversity in the tropics may be a result of high parasite abundance. However, few studies have directly compared parasite abundance both in and outside the tropics in a single host species. Eastern water skinks inhabit a large geographical range covering much of the east coast of Australia. I examined *Hepatozoon hinuliae* abundance in seven host populations ranging from southern New South Wales (temperate) to far north Queensland (tropical). Prevalence (the proportion of the host population infected) was unrelated to geography or climate. Parasite load was significantly higher in lizard populations in the tropics, and was significantly related to temperature, but not to rainfall. Possible causes of the observed relationship are variations in host density, host reproductive behaviour and physiology, vector dynamics, host metabolism and parasite growth rates, and I conclude that host life history is an important causal factor dictating the relationship between latitude and parasite load.

INTRODUCTION

Parasites can affect host evolution and community structure (Minchella & Scott 1991; Morand & Poulin 2000; Roberts et al. 2002; Schall 1992), and high parasite abundance or virulence may have contributed to the evolution of high biodiversity in the tropics (Connell 1978; Moller 1998; Rosenzweig 1995). Theory suggests that low seasonal variability in the tropics may allow parasite abundance to remain high throughout the year, resulting in high transmission rates and, consequently, high virulence (virulence is defined here as the negative effects of parasitic infection upon host fitness) (Moller 1998). High virulence imposes a strong selective force, which may stimulate higher rates of speciation, and hence higher biodiversity, in the tropics compared to the temperate zone (Moller 1998). Other theories also suggest that there should be high parasite abundance in the tropics. For example, in areas with high host birth rate, or favourable environments for host metabolism, high levels of parasitism may co-evolve with high levels of host immune investment (Hochberg & van Baalen 1998). However, in spite of the theoretical importance of high parasite abundance as a selective factor producing high diversity in the tropics, few studies have measured and compared differences in parasite abundance between the tropical and temperate zones.

To test the hypothesis that parasites are more abundant in the tropics, Moller (1998) compared measures of immune function (spleen sizes and circulating leucocyte titres) of phylogenetically matched pairs of bird species from the tropical and non-tropical zones. Tropical birds had higher levels of circulating leukocytes, and larger spleen sizes for a given body size than their temperate counterparts, suggesting that tropical parasites may be more virulent, although actual parasite abundance was not measured.

Unfortunately, although differences in the size of immune system organs, e.g., the spleen, can be due to the evolution of high levels of host immunity, variation in organ size may also be a result of recent immune challenges within the animal's lifetime. Therefore, caution should be used in interpreting spleen size as a measure of the evolution of immune investment (Brown & Brown 2002; Owens & Wilson 1999; Smith & Hunt 2004). A more direct approach is to compare actual parasite abundance in the tropics and in temperate zones. One study has demonstrated that blood parasite infections are more common in Nearctic than in Neotropical bird species (Ricklefs 1992). However, the degree to which parasites affect their hosts' evolution and life history is probably determined by variation in parasite load within individuals as well as the prevalence of the parasite within the host population (Poulin & Vickery 1993; Schall 2002). Furthermore, it would be advantageous to measure geographical patterns of parasite abundance within a single host species that inhabits both tropical and temperate zones so that patterns in the host-parasite relationship are not compromised by variation in the host or parasite's behaviour, phylogeny, geographic range or habitat (Smith & Hunt 2004).

In this study, I examined geographical variation in the patterns of infection of a haemogregarine blood parasite in a lizard host species that occupies tropical and temperate zones in eastern Australia. See Chapter 1 for details on the host and parasite. To determine the possible cause of gradients in parasite population structure, patterns of parasite abundance (load, prevalence) were correlated with latitude, rainfall and temperature. Thus, I directly tested the hypothesis that tropical hosts have higher parasite loads and prevalence than temperate hosts.

METHODS

Lizard capture and blood sampling techniques are described in Chapter 1. Prevalence is defined as the proportion of the host population infected with parasites (%), whereas parasite load is the percentage of infected cells within an individual host.

Lizards were caught between December 2000 and February 2003. Parasite load and prevalence can vary as a response to host reproductive effort (Merino et al. 1996; Norris et al. 1994), so populations were sampled when females were in the late stages of pregnancy or when newly born offspring were present in the population (i.e., just after parturition). In any case, levels of blood parasite infections in lizard hosts tend to remain relatively stable (Bromwich & Schall 1986; Sorci 1995, Chapter 2), so observed patterns of parasite abundance were likely to be representative of parasite abundance in the population regardless of when the population was sampled. I only included adult lizards (SVL > 90 mm) in my analysis, because prevalence is significantly lower in juveniles (Chapter 2), and the number of juveniles varied at different sites depending on the sampling dates.

Study sites

I caught lizards from seven populations along a transect spanning tropical and temperate zones (Figure 6.1 & Table 6.1). At three latitudes, I sampled lizards at both low and high altitudes and assumed that high altitude sites would be more seasonal than low altitude sites at the same latitude. I used data on rainfall and temperature from nearby meteorology stations (www.bom.com.au) or from National Park Ranger Stations. I pooled data from Royal National Park (33°53'S 151°13'E) and Heathcote

National Park (34°07'S 150°58'E) because habitat and location were similar, and refer to it as Royal National Park. I also pooled data for the lizards caught in Brisbane Cultural Centre (27°22' S 153°03' E) and the Brisbane Botanic Gardens at Mt Cootha (27°28' S 153°02' E), and refer to it as Brisbane.

Habitats were fairly similar at all the sites, as eastern water skinks normally reside near areas of flowing, permanent water. Most sites were open, rock creek beds.

Statistical analysis

Parasite loads, even after transformation, were not normally distributed, so non-parametric tests were used to compare parasite loads of different populations. For analysis of parasite loads I only used data from infected animals and ignored uninfected animals, i.e., mean parasite load is the average parasite load of infected animals only, and not of the entire sample.

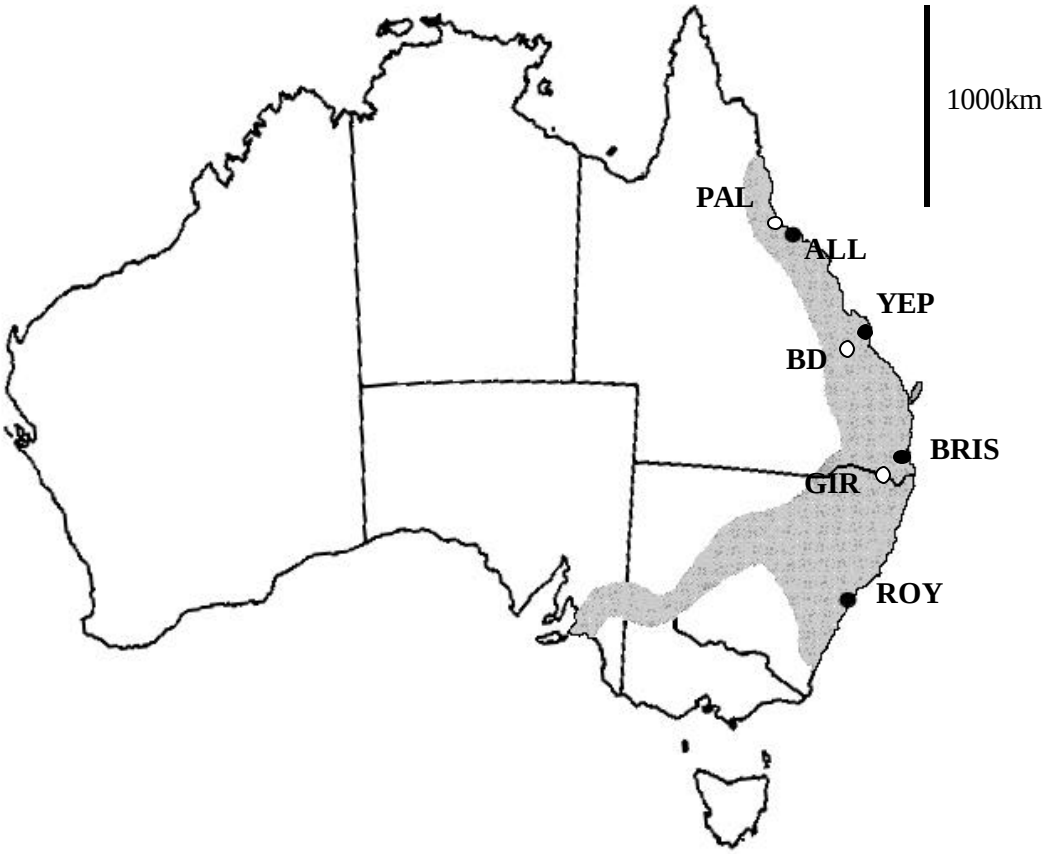

Figure 6.1 Map showing the locations of the seven study populations. PAL, Paluma; ALL, Alligator Creek; YEP, Yeppoon; BD, Blackdown Tableland; BRIS, Brisbane; GIR, Girraween; ROY, Royal National Park. Black circles denote lowland sites and white circles denote high altitude sites. Shaded region depicts the range of the eastern water skink, *Eulamprus quoyii*. The distance between Paluma and Royal is approximately 1600 km.

Table 1. Collecting localities and their latitude and longitude, collecting dates and sample sizes of *Eulamprus quoyii* caught, showing sexes separately. *(AR = annual rainfall)*

Location	Latitude (S), Longitude (E)	Altitude (m)	Dates	No. of males caught	No. of females caught	Total number caught	Mean Annual Rainfall	Mean max. temp.	Mean min. temp.	Prevalence % (no infected / no caught)	Mean parasite load (SD) (% infected cells)
Paluma	19° 00' 146 ° 12'	High (892)	Mar 2002	6	6	12	2618	21.3	13.9	8.3 (1/12)	0.01
Alligator Ck	19° 35' 147 ° 10'	Low	Nov 2001 Jan 2003	6	11	17	1390	28.8	20.4	58.8 (10/17)	2.68 (2.69)
Blackdown Tableland	23 °47' 149° 04'	High (760)	Dec 2000 Mar 2001	9	46	55	1150	23.57	13.5	87.3 (48/55)	0.74 (0.72)
Yeppoon	22° 47' 150° 43'	Low	Jan 2001 Nov 2001 Dec 2002	9	15	24	920	25.7	18.5	95.8 (23/24)	3.31 (2.73)
Girraween	28°46' 151° 54'	High	Feb 2003	8	23	31	770	21.6	8.8	48.4 (15/31)	0.23 (0.26)
Brisbane	27° 28' 153° 02'	Low	Jan 2001 Feb 2003	7	24	31	1146	25.5	15.7	3.2 (1/31)	0.01
Royal Nat. Park	33°53' 153° 13'	Low	Dec 2000 Feb 2001	8	47	55	1380	22.2	10.4	65.5 (36/55)	0.62 (1.02)

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RESULTS

Overall, 235 adult lizards were caught from seven populations (Table 6.1). Sample sizes in all but one population, exceeded 15 (the minimum number required to produce consistent prevalence values, Tella 2002). Only 12 adults were caught in the Paluma population, but the prevalence there was low, and even if three more infected animals had been caught, prevalence would still be low compared to other populations (a potential maximum of 26.7% compared to an average of 71.2% in the other highly infected populations). Previous studies have used smaller sample sizes (n > 9) for similar analysis (McCurdy et al. 1998).

Parasite pre valence

Hepatozoon hinuliae prevalence was significantly different among populations (X^2 = 95.16, d.f. = 6, P < 0.000), ranging from 95.8% to 3.2% (Yeppoon and Brisbane respectively, Table 6.1), but showed no relationship with latitude (r^2 = 0.002, P = 0.91), mean annual rainfall (r^2 = 0.28, P =0.22), mean minimum temperature (r^2 =0.01, P = 0.86) or mean maximum temperature (r^2 = 0.01, P = 0.89).

Parasite load

Two populations, Paluma and Brisbane, had a very low prevalence of parasite infection (number of infected animals = 1) so they were not included in the analyses involving parasite load. Parasite load differed significantly among the remaining populations (Kruskal-Wallis test: X^2 = 51.0, d.f. = 4, P < 0.000; Fig. 6.2). Parasite load in the two tropical populations, Alligator Creek and Yeppoon, was not

significantly different (Mann-Whitney test, U = 82.0, Z = -1.14, $P = 0.26$). However, parasite load at Yeppoon was significantly different from Blackdown Tableland which lies at the same latitude, but at a higher (760m) altitude (Mann-Whitney U = 108.0, Z = -5.31, $P < 0.000$). Similarly, parasite load differed significantly between Blackdown Tableland and Alligator Creek (Mann-Whitney U = 89.0, Z = -3.11, $P = 0.002$). The two highest latitude populations, Girraween and Royal, did not differ in parasite load (Mann-Whitney U = 253.5, Z = - 0.70, $P = 0.49$) but both differed from Blackdown Tableland (Royal & Blackdown Tableland Mann-Whitney U = 623.0, Z = -2.18, $P = 0.029$; Girraween and Blackdown Tableland Mann-Whitney U = 180.0, Z = -3.17, $P = 0.002$).

Parasite load was not significantly different between the sexes (Mann-Whitney, $P > 0.05$) in all populations, apart from Blackdown Tableland (Mann-Whitney, Z = -2.57, $P = 0.01$). Analysing sexes separately did not affect the nature of the reported relationships.

Parasite load was significantly and negatively related to latitude ($r^2 = 0.11$, $F_{1,130} = 15.3$, $P < 0.000$; Fig. 6.3), but was not related to rainfall ($r^2 = 0.01$, $F_{1, 130} = 1.8$, $P = 0.18$; Fig. 6.3). Parasite load was significantly and positively related to mean annual minimum temperature ($r^2 = 0.25$, $F_{1, 130} = 43.2$, $P < 0.000$; Fig. 6.4), and to mean annual maximum temperature ($r^2 = 0.29$, $F_{1, 130} = 53.8$, $P < 0.000$; Fig. 6.4).

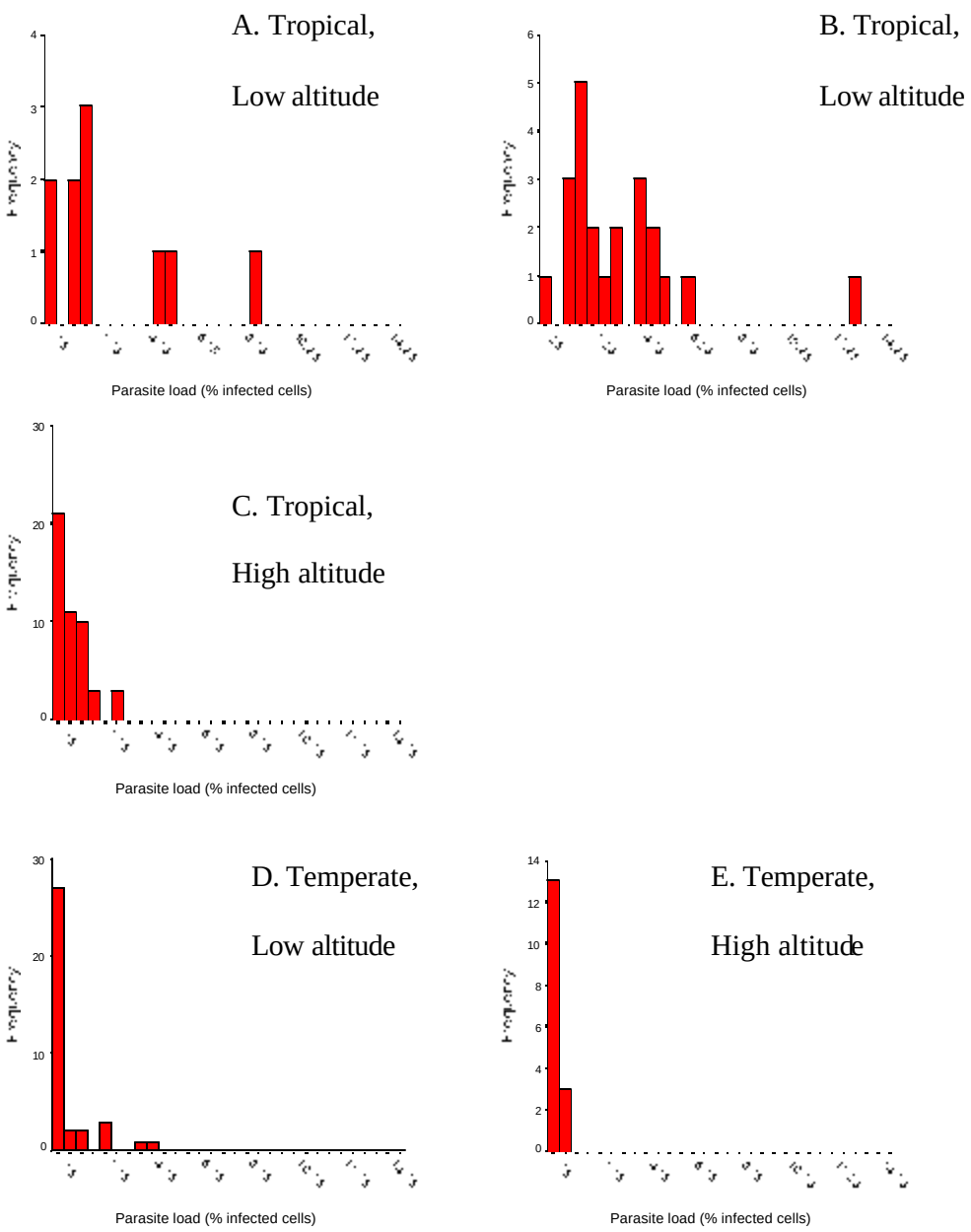

Figure 6.2 *Hepatozoon* parasite loads for populations of lizards sampled over an altitudinal and latitudinal gradient. A. Alligator Creek, B. Yeppoon, C. Blackdown Tableland, D. Royal National Park, and E. Girraween.

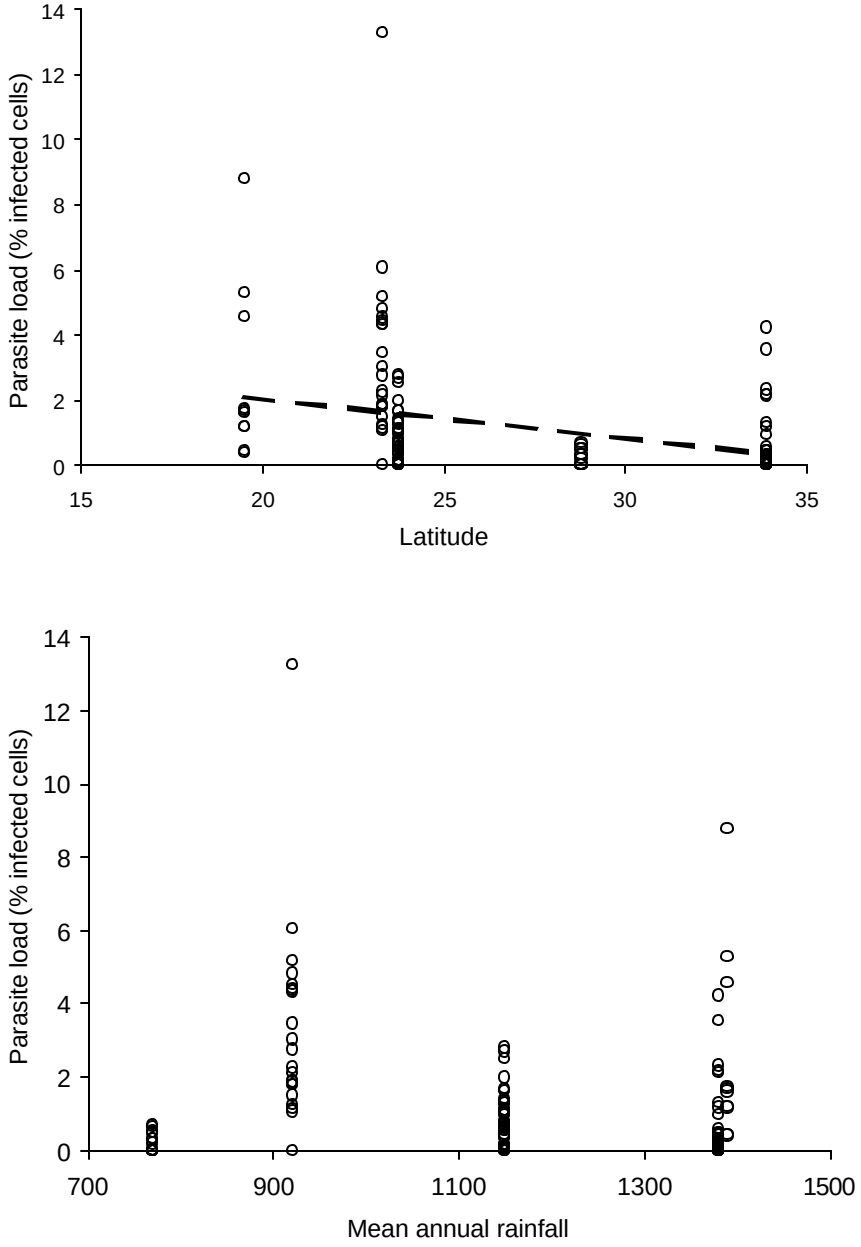

Figure 6.3 Haemogregarine parasite load was significantly related to latitude, and was higher nearer the tropics (top). Parasite load was unrelated to annual rainfall (bottom).

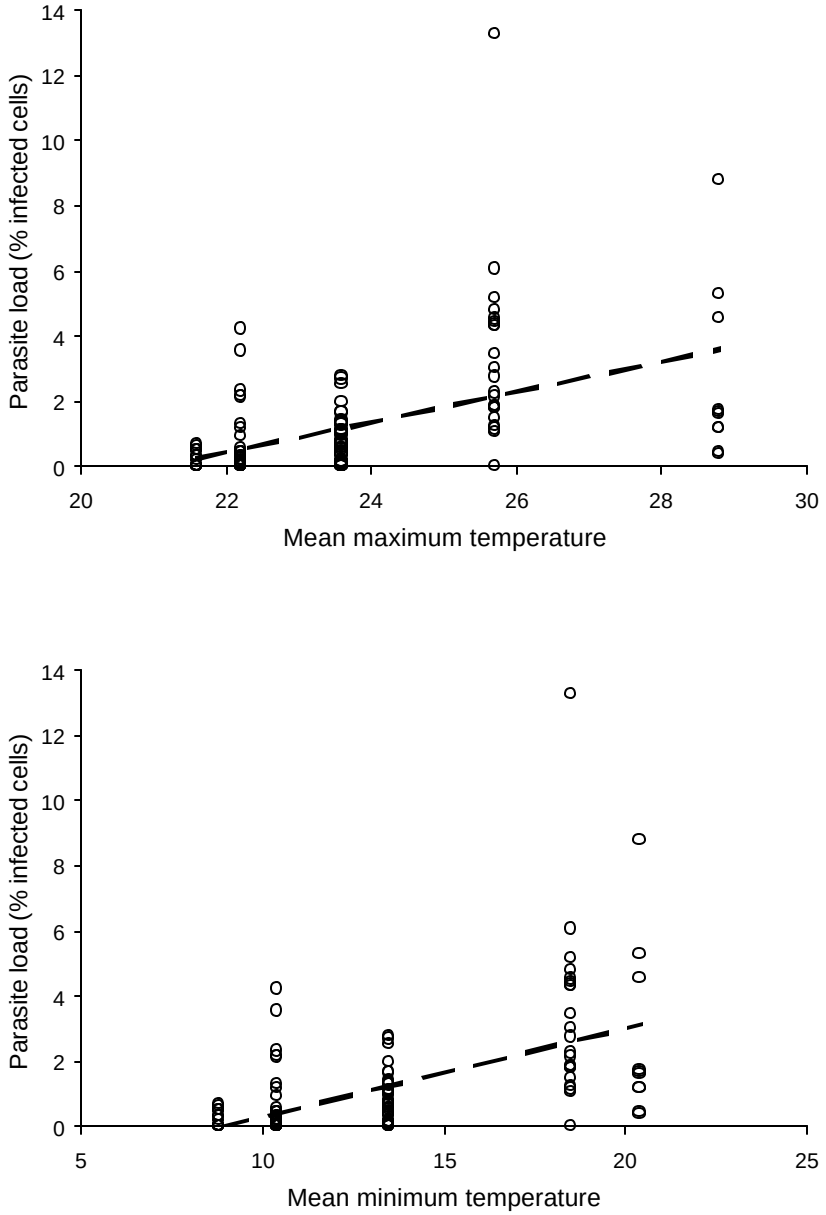

Figure 6.4 *Hepatozoon hinuliae* load was significantly related to both mean annual maximum (top) and mean annual minimum temperatures (bottom).

DISCUSSION

Patterns in parasite prevalence

There have been few macrogeographical studies of parasite-host evolutionary ecology since the 1970s (Freeman-Gallant et al. 2001; Hochberg & van Baalen 1998; Hochberg & Van Baalen 2000; Moller 1998) and consequently, few clear trends arise from the literature. Blood parasites were more prevalent in temperate bird species than in tropical bird species by a factor of 2.6 (Ricklefs 1992). In contrast, there was no relationship between geographic location and prevalence of blood parasites in European greenfinches (*Carduelis chloris*) (Merila et al. 1995). High latitude populations of Savannah sparrows (*Passerculus sandwichensis*) were less likely to have malaria (*Plasmodium* spp.) than lower latitude populations (Freeman-Gallant et al. 2001). Other studies found links between various climatic variables and prevalence (Fuller 1996; Semple et al. 2002). For example, rodent species inhabiting mesic conditions exhibit a higher prevalence of coccidia than rodents in drier habitats (Ford et al. 1990).

In my study, there was no geographical pattern (determined by rainfall, temperature or latitude) in the prevalence of *Hepatozoon hinuliae* infecting *Eulamprus quoyii*. Instead, parasite prevalence was patchy, with two populations of hosts exhibiting very low parasite prevalence (Brisbane [temperate, low altitude] & Paluma [tropical, high altitude]). Of all the sampled populations, Brisbane and Paluma were nearest to human habitation, so perhaps blood parasite prevalence had been reduced by anthropogenic factors, such as insecticide spraying that reduces vector abundance. Alternatively, the low prevalence at these sites may be due to fragmentation effects

upon host species community composition and consequently on parasite transmission dynamics (Allan et al. 2003). The absence of *Hepatozoon hinuliae* at these sites may also be natural, and illustrates the danger of inferring species-wide infection dynamics by sampling from only a few populations (Freeman-Gallant et al. 2001).

Like mine, other studies have found that saurian blood-parasite prevalence is independent of environmental conditions: for example, prevalence of malaria, *Plasmodium mexicanum*, in western fence lizards was unrelated to rainfall or mean low temperature of the current or previous year (Schall & Marghoob 1995). Similarly, in Puerto Rico, prevalence of *Plasmodium azurophilum* and *P. floridense* in *Anolis gundlachi* remained relatively stable throughout a nine-year period (Schall et al. 2000), and prevalence of haemogregarines in Aruban whiptails, *Cnemidophorus arubensis*, did not differ between wet and dry seasons (Schall 1986).

Altitude may have an influence on parasite prevalence (Lefcort & Blaustein 1991). For example, *Plasmodium mexicanum* is not present in hosts living at altitudes above 500m (Schall & Marghoob 1995). However, in my study, prevalence of *Hepatozoon hinuliae* did not differ between high and low altitude sites in a predictable manner. This may simply be due to the patchy distribution of the parasite: the Paluma-Alligator Creek and Girraween-Brisbane paired samples (samples from the same latitude but different altitude) were confounded by the near absence of the parasite in the Paluma and Brisbane. Further investigations may reveal a pattern, although the altitudinal range inhabited by *Eulamprus quoyii* may be insufficient to exhibit differences in prevalence.

Patterns in parasite load

As far as I am aware, this is the first study that directly compares parasite load in both tropical and temperate zones in a single host-parasite system, and it shows that parasite load is indeed higher in the tropics. In this study, parasite load increased towards the equator, and was correlated with mean minimum and mean maximum air temperatures, but was unrelated to annual rainfall. Many factors, biotic and abiotic, contribute to variation in parasite abundance in host populations. For example, latitudinal variations in vector abundance, host density and host susceptibility may result in higher parasite loads in the tropics. Possible causes of high parasite loads in the tropics are discussed below.

Vectors

Vector abundance may be less seasonal in the tropics because there are no harsh winters to reduce invertebrate populations, and so, continual high abundance of vectors in the tropics will result in higher rates of parasite transmission (Moller 1998). However, most tropical regions are not actually aseasonal; instead, much of the tropics show distinct seasonal patterns or unpredictable variations in rainfall (Jones 1987a; Jones 1987b) that drive patterns in insect abundance (i.e., potential vectors) (Wolda 1978a; Wolda 1978b). Temporal variation in insect abundance may also be driven by subtle changes in photoperiod (Dowell 2001; Wolda 1989), and it is therefore not surprising that insect abundance in the tropics (e.g., Panama) fluctuates no less than insect abundance in the temperate zones (Wolda 1978a; Wolda 1978b). It seems unlikely then, that higher parasite loads in the tropics are due simply to a higher constant abundance of invertebrate parasite vectors. Indeed, personal observations of

the abundance of biting insects in all the tropical sites visited during this study suggest that insect abundance is seasonal in these areas, peaking during the rainy season, although there may be occasional instances of high insect abundance at other times.

Alternatively, the life history of the host may be more important to disease dynamics than that of the vectors. Whereas coordinated pulses of high concentrations of vectors are as likely to occur in both temperate and tropical zones (Wolda 1978a, b), host behaviour may be a more important cause of macrogeographical patterns in parasite abundance. Susceptibility to parasites may vary because of geographical differences in activity season, movement, reproductive and hormonal cycles, immune system characteristics and local genetic adaptation (Dowell 2001; Oppliger et al. 1999; Saad & El Ridi 1988; Saad et al. 1990).

Host density

Patterns of parasite abundance can be influenced by host density. In mammals, parasite load can be strongly correlated with host population density, probably because transmission rates are dependent on host population density (Arneberg et al. 1998). However, tropical mammal species often exist at lower densities than temperate species (Johnson 1998) suggesting that parasite abundance should actually be lower in the tropics. If temperate host species usually occur at higher densities than do tropical hosts, this could explain the higher prevalence of parasites observed in temperate bird species (Ricklefs 1992). Unfortunately, comparative densities of lizard host populations in the tropics versus temperate zones have not been explicitly examined and may not follow the trends demonstrated by mammal populations. I did

not measure host density, and it was not possible to make comparable catch per unit effort (measured as time) estimates, because lizards were more wary in the tropical populations. Therefore, it is possible that lizards occur at higher densities in the tropics than in temperate populations and that the higher tropical parasite load was due to higher population density.

Host reproduction

Reproduction influences parasite load due to changes in host physiology and hormone titres that occur during breeding (Merino et al. 1996; Norris et al. 1994; Richner et al. 1995; Salvador et al. 1997; Salvador et al. 1996). If tropical host populations have less seasonal or more prolonged reproductive periods than temperate host populations, then certain sub-populations of reproducing hosts, such as mating males and pregnant females, may be present for longer parts of the year. Parasite abundance may be higher if mating sub-populations are more susceptible to new infection, or if reproductive hormones suppress the immune system, and therefore allow the parasites to reach high levels. Certainly, *Eulamprus quoyii* populations in the tropics have more extended breeding seasons than do those in the temperate zone (temperate breeding season: September to December, tropical breeding season: September to May (Caley & Schwarzkopf In press)). Since *E. quoyii* females do not store sperm (Schwarzkopf, pers. obs.), the longer breeding season of females suggests that males in low latitude and low altitude populations may be sexually active and have high levels of testosterone for longer parts of the year. A longer period of sexual activity in males may be correlated with higher parasite loads (Salvador et al. 1997; Salvador et al. 1996). It is important to note, however, that blood parasite loads in *Eulamprus quoyii*

at Blackdown Tableland do not appear to change in response to reproductive effort (Chapter 2), suggesting that in this species, reproduction may not be correlated with increased parasite load. However, water skinks in Blackdown Tableland breed only once a year and temporal changes in parasite load may be very different in tropical populations that reproduce more than once a year.

Parasite growth rates

If transmission is more seasonal in the temperate zone (for example, due to host or vector reproductive cycles) the higher parasite loads observed in the tropical populations studied here may be caused by aseasonality in initial infections. Measures of parasite load are dependent on the life-cycle stage of the parasite. For example, in sleepy lizards, *Tiliqua rugosa*, experimentally infected with *Haemolivia mariae*, there is a pre-patent period (approximately 4-10 weeks) before parasitaemia peaks (around 11 weeks) and then gradually declines, although infections were still detectable in laboratory animals 17 months after the initial inoculation (Smallridge & Bull 2001). In western fence lizards, *Sceloporus occidentalis*, parasitaemia of malaria, *Plasmodium mexicanum*, commonly rises exponentially before levelling off to a constant load (Bromwich & Schall 1986). If temperate populations have a strictly seasonal pulse of transmission (determined either by vector abundance, birth of a new naïve host cohort, or seasonal variations in behaviour) then subsequent sampling may only detect parasite loads at low or chronic levels because they have missed the annual peak parasitaemias. In contrast, if transmission were less seasonal in the tropics (for whatever reason) then sampling may detect parasite levels at different stages of their life cycles, i.e., some will be peaking whilst others are chronic. The

impression given will be that parasite loads are higher, but this may simply be an artefact of the sampling dates. To mitigate against this possibility, I sampled all populations when females were pregnant or had recently given birth. However, the hypothesis cannot currently be rejected with the data I collected.

Host seasonal activity

Like reproductive cycles, there may be latitudinal trends in host activity cycles, i.e., in reptile hosts, the duration of host inactivity will be longer at latitudes that have longer winters or dry seasons (Adolph & Porter 1993). The duration of host activity season may affect patterns of exposure to parasite infection. Australian lizards are often inactive during the Austral winter/dry season, and the low latitude populations of *Eulamprus quoyii* emerge slightly earlier in the spring and 'disappear' later in the winter than do higher latitude populations (Caley & Schwarzkopf In press). Consequently, in the tropics there may be a longer activity period favourable to parasite transmission in the same manner as described for prolonged reproductive seasons. Higher loads may be a result of longer activity combined with observations of the parasite infection at different life history stages.

Host metabolism

A theoretical model has predicted that in areas of high host birth rate, or favourable environments for host metabolism, higher levels of parasitism may co-evolve simultaneously with high levels of host immune investment (Hochberg & van Baalen 1998). Compared to eastern water skinks in temperate zones, eastern water skinks in the tropics reach sexual maturity at an earlier age, have larger litter sizes (number of

offspring), and the reproductive season persists throughout the summer (Caley & Schwarzkopf In press). In addition, water skinks are active for more of the year in tropical locations compared to temperate locations (Schwarzkopf, pers. obs.), which suggests that the tropical environment is more favourable for host metabolism in these ectothermic animals. In areas where conditions are less favourable, animals may be unable to sustain high parasite loads, and therefore are absent from sampling because they have not survived. Therefore, interactions between the environment, host metabolism, and the relative costs of parasitism may explain why mean minimum and mean maximum temperatures were significantly related to parasite load in this study.

Tropical virulence

Moller (1998) suggested that virulence might be related to parasite abundance. However, several studies conclude that the link between parasite load and virulence is uncertain. Host response to parasite loads may depend on variations in host or environmental conditions (Newman et al. 2001; Sanz et al. 2002). Furthermore, host-parasite coevolution may result in high parasite loads without concurrent costs to the host's fitness (Hochberg & van Baalen 1998; Medley 2002; Roy & Kirchner 2000).

Nonetheless, current understanding of the evolution of virulence suggests that increased opportunities for parasite transmission may allow the development of higher virulence (Bull 1994; Herre 1993; Schall 2002; Tompkins et al. 2002). For example, nematodes had larger impacts on the lifetime reproductive success of fig wasps when host life history increased the opportunities for nematode transmission (Herre 1993). Similarly, rabbit haemorrhagic disease virus appears more pathogenic in host

populations with high birth rates (White et al. 2002; White et al. 2001). Therefore, if virulence is related to the opportunity for parasites to transmit rapidly to naïve hosts, then tropical host life histories, with high birth rates, may be more likely to sustain virulent parasite populations. Tropical populations of eastern water skinks have higher birth rates, lower ages of maturity, and longer reproductive seasons than temperate populations (Caley & Schwarzkopf In press), suggesting that parasites may have the opportunity to become virulent. However, it is difficult to determine from survey studies whether tropical life histories allow the evolution of high parasite virulence, or whether such life histories are an adaptation to mitigate against the costs and risks of higher parasite loads and virulence (Hansen & Koella 2003; Horak et al. 2001; Schall 2002).

Future research

The experimental use of novel parasites from tropical and temperate zones in naïve tropical and temperate hosts may provide some understanding of the evolution of virulence (Oppliger et al. 1999). Furthermore, the use of techniques that measure immune response independent of parasitic infection (Rigby & Moret 2000), such as the phytohaemagglutinin response assay, may reveal whether actual immune responses (as opposed to organ measurements after uncertain infection histories) are related to geography.

Geographical patterns of parasite abundance and virulence, and the evolution of host life history and/or immune system may also be strongly influenced by parasite diversity. Several studies have found higher parasite diversity in the tropics (Gordon

& FitzGibbon 1999; Poulin & Rohde 1997; Rohde 1999), and a relationship between parasite diversity and immune function (Morand & Poulin 2000; Wegner et al. 2003). Increased parasite diversity can theoretically result in increased virulence (van Baalen & Sabelis 1995). Future investigations of geographical aspects of host-parasite evolution should take parasite diversity into account.

Conclusion

Tropical host populations of *E. quoyii* harboured higher *Hepatozoon* parasite loads than did temperate host populations. Although I examined only one parasite species in one host species, this study is the first to reveal such an effect in a single system without the complications inherent in studying multiple host or parasite species. Contrary to previous predictions (Moller 1998), I suggest that these differences are due to latitudinally dependent differences in the host's biology, rather than seasonal fluctuations in vector biology. Current understanding of the evolution of virulence is insufficient to allow me to predict that tropical parasites are necessarily more virulent than temperate parasites. In agreement with Moller (1998), I hope to stimulate further thought and research on the role of parasites in the tropics. In particular, I suggest that studies on the importance of parasites in the evolution of tropical biodiversity and host life history traits must primarily determine whether parasite diversity, prevalence and load differ predictably between the tropics and elsewhere, and whether these patterns actually reflect the evolution of virulence.

CHAPTER 7

CONCLUSIONS AND FUTURE RESEARCH

The study of wildlife disease has gained importance in the last two decades (Hudson et al. 2002) as a result of theoretical insights into its possible roles in host evolution, population biology and ecology (Anderson & May 1978; Anderson & May 1979; Dawkins 1990; Hamilton & Zuk 1982; Minchella & Scott 1991). Recently, wildlife disease has gained additional publicity because of novel pathogens such as the HIV, SARS and West Nile viruses, that have emerged from animal hosts or reservoirs and have deleteriously affected both human and animal populations (Daszak et al. 2000; Dobson & Foufopoulos 2001; Osterhaus 2001). Despite the risks to public health and to wildlife populations, our understanding of wildlife disease is limited, in part because of the lack of knowledge of the ecology of disease in natural hosts.

My work has sought to understand a co-evolved host-parasite relationship: the blood parasite, *Hepatozoon hinuliae*, in the eastern water skink (*Eulamprus quoyii*). My PhD has demonstrated a high prevalence of *Hepatozoon hinuliae* in many water skink populations throughout eastern Australia (Chapters 2 & 6). Parasite loads were stable in the host population during the two-year study, but varied substantially between individuals possibly as a result of host genotype (Chapter 2). Most skinks appear to become infected during their first year of reproduction (Chapter 2) and, although high parasite loads reduce female skink fecundity (Chapter 3), they have no obvious effect on offspring characteristics (Chapter 4) or female mating behaviour (Chapter 5). It is

clear though, that long-term study is required to fully examine the implications of parasite load on host life history. Importantly, the *Eulamprus-Hepatozoon* system offers advantages over other host-parasite study systems for prolonged study: (i) reproductive output of viviparous reptiles can be accurately measured; (ii) the allocation of energy to different life-history traits can be assessed by examining fat reserves in the tail; (iii) *Eulamprus* lizards are easy to catch, abundant, and show high levels of site-fidelity so the system is amenable to mark-recapture studies; (iv) the parasite is reasonably easy to quantify, & (v) infection status can be determined without killing the host. Because the Blackdown Tableland population has already been studied for two years, and because more than 350 individuals of a known age have been released, this population offers a great chance to learn more about the ecology of a long-lived vertebrate and its blood parasite.

For example, although parasite dynamics appeared stable during the two years of this study, the climate was also relatively dry during this time. More rainfall, or more variation in rainfall, may have a large influence on parasite transmission via changes in vector abundance or host condition. Extended study of the Blackdown population will also more fully reveal the impact of parasite load on lizard life-history. A two-year study of an animal that can live up to 8 years is unlikely to accurately measure rates of parasite-induced host mortality. The impacts of parasites upon host survival are more likely to be understood by monitoring cohorts of hosts through time (Anderson & Gordon 1982). Two cohorts of marked neonates (approximately 350 individuals) of known matrilineage have been released during my study, so parasite-induced host mortality can be measured by observing infection rates and survival of these individuals over time. The recapture of marked individuals will also provide

data on the impact of maternal parasite load, maternal condition and offspring phenotype on offspring fitness and behaviour in the wild, as well as heritable aspects of immunity in the host-parasite relationship.

Identifying the vector of *Hepatozoon hinuliae* would be of great benefit to the study of the host-parasite relationship. It would then be possible to answer questions on the relationship between vector abundance and environmental factors such as rainfall, or biotic factors such as host density. Optimistically, PCR analysis of vector stomach contents could determine the lizard species on which vectors first feed, as has been previously achieved with birds and mosquitoes (Lee et al. 2002). This information would determine the host specificity of *Hepatozoon hinuliae*, as well as the community ecology of the host-parasite relationship. Do certain species harbour infections without suffering pathology? Is the community assembly of lizards mediated by varying immunity to blood parasites? Excitingly, ascertaining the identity of the vector would also allow the possibility of experimental infections that could, for example, reveal courses of infection in hosts of known ages, known infection histories, and condition.

The geographic distribution of *Eulamprus quoyii* presents additional research opportunities. Little is known about geographic aspects of host-parasite relationships, but Chapter 6 showed that parasite abundance varies between populations. Although parasite dynamics were examined in the Blackdown Tableland water skink population (Chapter 2), parasite population dynamics and transmission may differ between host populations and environmental conditions. Again, if the vector species were known, it

could help explain why and how parasite load differs between the tropics and elsewhere.

Study of reptile immune systems may also offer interesting insights into life-history theory. Some scientists have suggested that the reptile immune system is low-grade and inefficient (Chapter 2), but in fact, it is probably complex and interacts with endocrine systems (Saad & El Ridi 1988; Saad et al. 1990; Veiga et al. 1998). The immune system operates with costs and benefits: a competent immune system can fight parasitic infection and therefore increase host survival, but it has costs because it may be energetically expensive to mount and can cause within-host pathologies (Owens & Wilson 1999; Sheldon & Verhulst 1996). Therefore, like growth and reproduction, the immune system should be regarded as a life-history trait: it has costs and benefits and relies on variable allocation of an organism's allocation of limited resources. Indeed, it has been suggested that the immune system is the most important determinant of reproductive success and fitness for many species (Lochmiller & Deerenberg 2000). Previous studies, mostly of birds, have demonstrated reductions in reproductive success as a result of experimental challenges to the immune system (Bonneaud et al. 2003; Ilmonen et al. 2000; Raberg et al. 2000). Also, birds investing heavily in reproduction are unable to mount immune responses and suffer higher rates of mortality because of blood parasite infections (Nordling et al. 1998). The immune response, like reproductive effort and the impact of parasites, is also mediated by the relationship between environmental and host conditions (Christe et al. 2001), and consequently, some studies have linked immune performance to life-history evolution (Martin et al. 2001; Moller 1998; Tella et al. 2002). Benefits of directly challenging the immune system include avoiding the risk of introducing pathogens to wild host

populations, and being able to control the type and quantity of materials that the organisms are exposed to (live pathogen strains and quantities are difficult to control). However, other studies have failed to observe costs of immune responses (Williams 1999) and the acceptance of the immune system as an important and previously neglected aspect of life history biology is not universally accepted (Owens & Wilson 1999; Smith & Hunt 2004).

Sheldon & Verhulst (1996) predicted that although most examples of studies on ecological immunology have focused on avian models, an increased range of taxa would be investigated over time. However, although lizards have proved useful models in the development of life-history theory (Zera & Harshman 2001), there have been few studies of the immune system within an ecological framework (for exceptions see Olsson et al. 2000; Veiga et al. 1998). Because the immune system is closely linked to metabolism (Ots et al. 2001; Raberg et al. 2002), and lizards are ectothermic, studies of the interaction between metabolism and immune system may be particularly rewarding in lizards. For example, it would be possible to manipulate the resources available to the immune system and reproduction by experimentally removing the tails of lizards that typically store energy in regenerative tails. Or, one could easily manipulate interactions between the immune system and metabolism by controlling the thermoregulation regimes of captive lizards. Another interesting aspect would be that of the concurrent evolution of the immune system and viviparity, especially in relation to metabolic constraints.

REFERENCES

Abell, A. J. 1997 Estimating paternity with spatial behaviour and DNA fingerprinting in the striped plateau lizard, *Sceloporus virgatus* (Phrynosomatidae). *Behav. Ecol. Sociobiol.* **41**, 217-226.

Abu-Madi, M. A., Behnke, J. M., Lewis, J. W. & Gilbert, F. S. 1998 Descriptive epidemiology of *Heligmosomoides polygyrus* in *Apodemus sylvaticus* from three contrasting habitats in south-east England. *Journal of Helminthology* **72**, 93-100.

Adolph, S. C. & Porter, W. P. 1993 Temperature, activity, and lizard life histories. *American Naturalist* **142**, 273-295.

Ahmed, A. M., Maingon, R., Romans, P. & Hurd, H. 2001 Effects of malaria infection on vitellogenesis in *Anopheles gambiae* during two gonotrophic cycles. *Insect Molecular Biology* **10**, 347-356.

Albon, S. D., Stien, A., Irvine, R. J., Langvatn, R., Ropstad, E. & Halvorsen, O. 2002 The role of parasites in the dynamics of a reindeer population. *Proc. R. Soc. Lond. B* **269**, 1625-1632.

Allan, B. F., Keesing, F. & Ostfeld, R. S. 2003 Effect of forest fragmentation on Lyme disease risk. *Conservation Biology* **17**, 267-272.

Anderson, R. M. & Gordon, D. M. 1982 Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373-398.

Anderson, R. M. & May, R. M. 1978 Regulation and stability of host-parasite population interactions. I. Regulatory processes. *J. Animal Ecology* **47**, 219-247.

Anderson, R. M. & May, R. M. 1979 Population biology of infectious diseases: part I. *Nature* **280**, 361-367.

Appleby, B. M., Anwar, M. A. & Petty, S. J. 1999 Short-term and long-term effects of food supply on parasite burdens in tawny owls, *Strix aluco*. *Functional Ecology* **13**, 315-321.

Aragon, P., Lopez, P. & Martin, J. 2001 Chemosensory discrimination of familiar and unfamiliar conspecifics by lizards: implications of field spatial relationships between males. *Behav. Ecol. Sociobiol.* **50**, 128-133.

Arneberg, P., Skorping, A., Grenfell, B. T. & Read, A. F. 1998 Host densities as determinants of abundance in parasite communities. *Proc. R. Soc. Lond. B* **265**, 1283-1289.

Atkinson, D. 1991 Sexual showiness and parasite load: correlations without parasite coevolutionary cycles. *J. theor. Biol* **150**, 251-260.

Baer, B. & Schmid-Hempel, P. 1999 Experimental variation in polyandry affects parasite load and fitness in a bumble-bee. *Nature* **397**, 151-154.

Bajer, A., Pawelczyk, A., Behnke, J. M., Gilbert, F. S. & Sinski, E. 2001 Factors affecting the component community structure of haemoparasites in bank voles (*Clethrionomys glareolus*) from the Mazury Lake District region of Poland. *Parasitology* **122**, 43-54.

Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J. & Huntingford, F. A. 2001 Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc. R. Soc. Lond. B* **268**, 71-76.

Bennett, G. F. & Cameron, M. 1974 Seasonal prevalence of avian hematozoa in passeriform birds of Atlantic Canada. *Can. J. Zool.* **52**, 1259-1264.

Bize, P., Roulin, A., Bersier, L.-F., Pfluger, D. & Richner, H. 2003 Parasitism and developmental plasticity in Alpine swift nestlings. *J. Animal Ecology* **72**, 633-639.

Blomberg, S. P. & Shine, R. 2000 Size-based predation by kookaburras (*Dacelo novaeguineae*) on lizards (*Eulamprus tympanum*: Scincidae): what determines prey vulnerability? *Behav. Ecol. Sociobiol.* **48**, 484-489.

Blomberg, S. P. & Shine, R. 2001 Modelling life history strategies with capture-recapture data: evolutionary demography of the water skink *Eulamprus tympanum*. *Austral Ecology* **26**, 349-359.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B. & Sorci, G. 2003 Assessing the cost of mounting an immune response. *American Naturalist* **161**, 367-379.

Borges Landaez, P. A. 1999 PhD dissertation: Maternal thermoregulation and its consequences for offspring fitness in the Australian eastern water skink (*Eulamprus quoyii*). Sydney: University of Sydney.

Bromwich, C. R. & Schall, J. J. 1986 Infection dynamics of *Plasmodium mexicanum*, a malarial parasite of lizards. *Ecology* **67**, 1227-1235.

Brown, C. R. & Brown, M. B. 2002 Spleen volume varies with colony size and parasite load in a colonial bird. *Proc. R. Soc. Lond. B* **269**, 1367-1373.

Bull, C. M., Griffin, C. L., Bonnett, M., Gardner, M. C. & Cooper, S. J. B. 2001 Discrimination between related and unrelated individuals in the Australian lizard *Egernia striolata* . *Behav. Ecol. Sociobiol.* **50**, 173-179.

Bull, J. J. 1994 Virulence. *Evolution* **48**, 1423-1437.

Caley, M. J. & Schwarzkopf, L. In press Complex growth rate evolution in a latitudinally widespread species. *Evolution*.

Caley, M. J., Schwarzkopf, L. & Shine, R. 2001 Does total reproductive output evolve independently of offspring size? *Evolution* **55**, 1245-1248.

Campbell, T. W. 1996 Clinical Pathology. In *Reptile Medicine and Surgery* (ed. D. R. Mader), pp. 248-257. Philadelphia: W.B. Saunders Company.

Christe, P., de Lope, F., Gonzalez, G., Saino, N. & Moller, A. P. 2001 The influence of environmental conditions on immune responses, morphology and recapture probability of nestling house martins (*Delichon urbica*). *Oecologia* **126**, 333-338.

Christian, K. A. & Bedford, G. S. 1995 Physiological consequences of filarial parasites in the frillneck lizard, *Chlamydosaurus kingii*, in northern Australia. *Can. J. Zool.* **73**, 2302-2306.

Cleaveland, S., Hess, G. R., Dobson, A. P., Laurenson, K. M., McCallum, H. I., Roberts, M. G. & Woodroffe, R. 2002 The role of pathogens in biological conservation. In *The Ecology of Wildlife Diseases* (ed. A. P. Dobson), pp. 139-150. Oxford: Oxford University Press.

Clobert, J., Oppliger, A., Sorci, G., Ernande, B., Swallow, J. G. & Garland, T. J. 2000 Trade-offs in phenotypic traits: endurance at birth, growth, survival, predation and susceptibility to parasitism in a lizard, *Lacerta vivipara*. *Functional Ecology* **14**, 675-684.

Cogger, H. G. 2000 *Reptiles & amphibians of Australia*. Singapore: Reed New Holland.

Connell, J. H. 1978 Diversity in tropical rain forests and coral reefs. *Science* **199**, 1302-1310.

Daniels, C. B. 1987 Aspects of the aquatic feeding ecology of the riparian skink *Sphenomorphus quoyii*. *Australian Journal of Zoology* **35**, 253-258.

Daszak, P., Cunningham, A. A. & Hyatt, A. D. 2000 Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science* **287**, 443-449.

Dawkins, R. 1990 Parasites, desiderata lists and the paradox of the organism. *Parasitology* **100**, S63-S73.

Dawson, R. D. & Bortolotti, G. R. 2001 Sex-specific associations between reproductive output and haematozoan parasites of American kestrels. *Oecologia* **126**, 193-200.

Dobson, A. P. & Carper, E. R. 1996 Infectious diseases and human population history. *Bioscience* **46**, 115-126.

Dobson, A. P. & Foufopoulos, J. 2001 Emerging infectious pathogens of wildlife. *Phil. Trans. R. Soc. Lond. B* **356**, 1001-1012.

Doughty, P. & Shine, R. 1997 Detecting life history trade-offs: measuring energy stores in 'capital' breeders reveals costs of reproduction. *Oecologia* **110**, 508-513.

Doughty, P. & Shine, R. 1998 Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). *Ecology* **79**, 1073-1083.

Dowell, S. F. 2001 Seasonal variation in host susceptibility and cycles of certain infectious diseases. *Emerging Infectious Diseases* **7**. www.eid.org

Downes, S. & Borges, P. 1998 Sticky traps: an effective way to capture small terrestrial lizards. *Herpetological Review* **29**, 94.

Dufva, R. 1996 Blood parasites, health, reproductive success, and egg volume in female great tits *Parus major. Journal of Avian Biology* **27**, 83-87.

Dunlap, K. D. & Schall, J. J. 1995 Hormonal alterations and reproductive inhibition in male fence lizards (*Sceloporus occidentalis*) infected with the malarial parasite *Plasmodium mexicanum*. *Physiological Zoology* **68**, 608-621.

Eisen, R. J. 2000 Variation in life-history traits of *Plasmodium mexicanum*, a malaria parasite infecting western fence lizards: a longitudinal study. *Can. J. Zool.* **78**, 1230-1237.

Eisen, R. J. 2001 Absence of measurable malaria-induced mortality in western fence lizards (*Sceloporus occidentalis*) in nature: a 4-year study of annual and over-winter mortality. *Oecologia* **127**, 586-589.

Eisen, R. J. & DeNardo, D. F. 2000 Life history of a malaria parasite (*Plasmodium mexicanum*) in its host, the western fence lizard (*Sceloporus occidentalis*): host testosterone as a source of seasonal and among-host variation? *J. Parasitology* **86**, 1041-1045.

Eisen, R. J. & Schall, J. J. 2000 Life history of a malaria parasite (*Plasmodium mexicanum*): independent traits and basis for variation. *Proc. R. Soc. Lond. B* **267**, 793-799.

Eisen, R. J. & Wright, N. M. 2001 Landscape features associated with infection by a malaria parasite (*Plasmodium mexicanum*) and the importance of multiple scale studies. *Parasitology* **122**, 507-513.

Elphick, M. J. & Shine, R. 1998 Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biological Journal of the Linnean Society* **63**, 429-447.

Faucher, J.-F., Ngou-Miama, E., Missinou, M. A., Ngomo, R., Konmbila, M. & Kremsner, P. G. 2002 The impact of malaria on common lipid parameters. *Parasitology Research* **88**, 1040-1043.

Ferguson, G. W. & Fox, S. F. 1984 Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its caused and evolutionary significance. *Evolution* **38**, 342-349.

Figuerola, J. & Green, A. J. 2000 Haematozoan parasites and migratory behaviour in waterfowl. *Evolutionary Ecology* **14**, 143-153.

Folstad, I. & Karter, A. J. 1992 Parasites, bright males, and the immunocompetence handicap. *American Naturalist* **139**, 603-622.

Forbes, M., Weatherhead, P. J. & Bennett, G. F. 1994 Blood parasites of blue grouse: variation in prevalence and patterns of interspecific association. *Oecologia* **97**, 520-525.

Ford, P. L., Duszynski, D. W. & McAllister, C. T. 1990 Coccidia (Apicomplexa) from heteromyid rodents in the southwestern United States, Baja California, and northern Mexico with three species from *Chaetodipus hispidus*. *J. Parasitology* **76**, 325-331.

Freeman-Gallant, C. R., O'Connor, K. & Breur, M. E. 2001 Sexual selection and the geography of *Plasmodium* infection in Savannah sparrows (*Passerculus sandwichensis*). *Oecologia* **127**, 517-521.

Fuller, C. A. 1996 Population dynamics of two species of *Eimeria* (Apicomlexa: Eimeriidae) in deer mice (*Peromyscus maniculatus*): biotic and abiotic factors. *J. Parasitology* **82**, 220-225.

Galvani, A. P. 2003 Epidemiology meets evolutionary ecology. *TREE* **18**, 132-139.

Gog, J., Woodroffe, R. & Swinton, J. 2002 Disease in endangered metapopulations: the importance of alternative hosts. *Proc. R. Soc. Lond. B* **269**, 671-676.

Goodnight, K. F. & Queller, D. C. 1999a Kinship 1.4 [software]. Goodnight Software. Available at: http://gsoft.smu.edu/GSoft.html.

Goodnight, K. F. & Queller, D. C. 1999b Relatedness 5.0 [software]: Goodnight Software.

Gordon, D. M. & FitzGibbon, F. 1999 The distribution of enteric bacteria from Australian mammals: host and geographical effects. *Microbiology* **145**, 2663-2671.

Greer, A. E. 1989 *The Biology and Evolution of Australian Lizards*. Sydney: Surrey Beatty.

Gregory, R. D., Montgomery, S. J. J. & Montgomery, W. I. 1992 Population biology of *Heligmosomoides polygyrus* (Nematoda) in the wood mouse. *J. Animal Ecology* **61**, 749-757.

Grenfell, B. T. & Gulland, F. M. D. 1995 Ecological impact of parasitism on wildlife host populations. *Parasitology* **111**, S3-S14.

Gulland, F. M. D. 1995 Impact of infectious diseases on wild animal populations, a review. In *Ecology of Infectious Diseases in Natural Populations* (ed. A. P. Dobson), pp. 20-51. Cambridge: Cambridge University Press.

Gullberg, A., Olsson, M. & Tegelstrom, H. 1997 Male mating success, reproductive success and multiple paternity in a natural population of sand lizards: behavioural and molecular genetics data. *Molecular Ecology* **6**, 105-112.

Hamilton, W. D. & Zuk, M. 1982 Heritable true fitness and bright birds: a role for parasites? *Science* **218**, 384-387.

Hamilton, W. J. & Poulin, R. 1997 The Hamilton and Zuk hypothesis revisited: a meta-analytical approach. *Behaviour* **134**, 299-320.

Hansen, M. H. H. & Koella, J. C. 2003 Evolution of tolerance: the genetic basis of a host's resistance against genetic manipulation. *Oikos* **102**, 309-317.

Herre, E. A. 1993 Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259**, 1442-1445.

Hochberg, M. E. & van Baalen, M. 1998 Antagonistic coevolution over productivity gradients. *American Naturalist* **152**, 620-634.

Hochberg, M. E. & Van Baalen, M. 2000 A geographical perspective of virulence. In *Evolutionary Biology of Host-Parasite Relationships: Theory Meets Reality* (ed. A. Skorping), pp. 81-96. Amsterdam: Elsevier Science B.V.

Horak, P., Ots, I., Vellau, H., Spottiswoode, C. & Moller, A. P. 2001 Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. *Oecologia* **126**, 166-173.

Hosken, D. J. & Blanckenhorn, W. U. 1999 Female multiple mating, inbreeding avoidance, and fitness: it is not only the magnitude of costs and benefits that counts. *Behavioral Ecology* **10**, 462-464.

Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, J. A. P. & Dobson, A. P. 2002 *Ecology of Wildlife Diseases.* Oxford: Oxford University Press.

Hurd, H. 2001 Host fecundity reduction: a strategy for damage limitation? *Trends in Parasitology* **17**, 363-368.

Ilmonen, P., Taarna, T. & Hasselquist, D. 2000 Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proc. R. Soc. Lond. B* **267**, 665-670.

Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? A review of the genetic benefits. *Biol. Rev. (Cambridge)* **75**, 21-64.

John, J. L. 1997 The Hamilton-Zuk theory and initial test: an examination of some parasitological criticisms. *International Journal for Parasitology* **27**, 1269-1288.

Johnson, C. N. 1998 Rarity in the tropics: latitudinal gradients in distribution and abundance in Australian mammals. *J. Animal Ecology* **67**, 689-698.

Jones, R. E. 1987a Reproductive seasonality in the tropical genus *Eurema* (Lepidoptera: Pieridae). *Biotropica* **19**, 7-16.

Jones, R. E. 1987b Reproductive strategies for the seasonal tropics. *Insect. Sci. Applic.* **8**, 515-521.

Jordan, M. A. & Snell, H. L. 2002 Life history trade-offs and phenotypic plasticity in the reproduction of Galapagos lava lizards (*Microlophus delanonis*). *Oecologia* **130**, 44-52.

Keymer, A. E. & Read, A. F. 1991 Behavioural ecology: the impact of parasitism. In *Parasitism: coexistence or conflict?* (ed. L. Bolis), pp. 37-61. Oxford: Oxford University Press.

Klein, S. L., Gamble, H. R. & Nelson, R. J. 1999 *Trichinella spiralis* infection in voles alters female odor preference but not partner preference. *Behav. Ecol. Sociobiol.* **45**, 323-329.

Konior, M., Radwan, J. & Kolodziejczyk, M. 2001 Polyandry increases offspring fecundity in the bulb mite. *Evolution* **55**, 1893-1896.

Kristan, D. M. 2002a Maternal and direct effects of the intestinal nematode *Heligmosomoides polygyrus*on offspring growth and susceptibility to infection. *J. Exp. Biol.* **205**, 3967-3977.

Kristan, D. M. 2002b Effects of intestinal nematodes during lactation: consequences for host morphology, physiology and offspring mass. *J. Exp. Biol.* **205**, 3955-3965.

Lafferty, K. D. & Gerber, L. R. 2002 Good medicine for conservation biology: the intersection of epidemiology and conservation theory. *Conservation Biology* **16**, 593-604.

Laloi, D., Richard, M., Lecomte, J., Massot, M. & Clobert, J. 2004 Multiple paternity in clutches of common lizard *Lacerta vivipara*: data from microsatellite markers. *Molecular Ecology* **13**, 719-723.

Law, B. S. 1991 Ontogenetic habitat shift in the eastern Australian water skink (*Eulamprus quoyii*)? *Copeia* **1991**, 1117-1120.

Lebas, N. R. 2001 Microsatellite determination of male reproductive success in a natural population of the territorial ornate dragon lizard, *Ctenophorus ornatus*. *Molecular Ecology* **10**, 193-203.

Lebas, N. R. & Marshall, N. J. 2001 No evidence of female choice for a condition-dependent trait in the Agamid lizard, *Ctenophorus ornatus*. *Behaviour* **138**, 965-980.

Lee, J. H., Hassan, H., Hill, G., Cupp, E. W., Higazi, T. B., Mitchell, C. J., Godsey Jr, M. S. & Unnasch, T. R. 2002 Identification of mosquito avian-derived blood meals by polymerase chain reaction-heteroduplex analysis. *Am. J. Trop. Med. Hyg*. **66**, 599-604.

Lefcort, H. & Blaustein, A. R. 1991 Parasite load and brightness in lizards: an interspecific test of the Hamilton and Zuk hypothesis. *J. Zoology, London* **224**, 491-499.

Lindstrom, J. 1999 Early development and fitness in birds and mammals. *TREE* **14**, 343-348.

Lochmiller, R. L. & Deerenberg, C. 2000 Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87-98.

Lopez, P. & Martin, J. 2002 Chemical rival recognition decreases aggression levels in male Iberian wall lizards, *Podarcis hispanica*. *Behav. Ecol. Sociobiol.* **51**, 461-465.

Lopez, S. 1999 Parasitized female guppies do not prefer showy males. *Animal Behaviour* **57**, 1129-1134.

Losos, J. B., Creer, D. A. & Schulte II, J. A. 2002 Cautionary comments on the measurement of maximum locomotor capabilities. *J. Zoology, London* **258**, 57-61.

Low, B. S. 1990 Marriage systems and pathogen stress in human societies. *Amer. Zool.* **30**, 325-339.

Lozano, G. A. & Ydenberg, R. C. 2002 Transgenerational effects of maternal immune challenge in tree swallows (*Tachycineta bicolor*). *Can. J. Zool.* **80**, 918-925.

Mackerras, M. J. 1960 The haematozoa of Australian reptiles. *Australian Journal of Zoology* **9**, 61-134.

Martin, T. E., Moller, A. P., Merino, S. & Clobert, J. 2001 Does clutch size evolve in response to parasites and immunocompetence? *Proc. Nat. Acad. Sci. USA* **98**, 2071-2076.

Massot, M., Clobert, J., Lorenzon, P. & Rossi, J.-M. 2002 Condition-dependent dispersal and ontogeny of the dispersal behaviour: an experimental approach. *J. Animal Ecology* **71**, 253-261.

Massot, M., Huey, R. B., Tsuji, J. & van Berkum, F. H. 2003 Genetic, prenatal, and postnatal correlates of dispersal in hatchling fence lizards (*Sceloporus occidentalis*). *Behavioral Ecology* **14**, 650-655.

McCallum, H. & Dobson, A. 2002 Disease, habitat fragmentation and conservation. *Proc. R. Soc. Lond. B* **269**, 2041-2049.

McCallum, H. & Dobson, A. P. 1995 Detecting disease and parasite threats to endangered species and ecosystems. *TREE* **10**, 1995.

McCurdy, D. G., Shutler, D., Mullie, A. & Forbes, M. R. 1998 Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. *Oikos* **82**, 303-312.

McKenzie, F. E., Killeen, G. F., Beier, J. C. & Bossert, W. H. 2001 Seasonality, parasite diversity, and local extinctions in *Plasmodium falciparum* malaria. *Ecology* **82**, 2673-2681.

Medley, G. F. 2002 The epidemiological consequences of optimisation of the individual host immune response. *Parasitology* **125**, S61-S70.

Merila, J. & Andersson, M. 1999 Reproductive effort and success are related to haematozoan infections in blue tits. *Ecoscience* **6**, 421-428.

Merila, J., Bjorklund, M. & Bennett, G. F. 1995 Geographic and individual variation in haematozoan infections in the greenfinch, *Carduelis chloris*. *Can. J. Zool.* **73**, 1798-1804.

Merila, J., Sheldon, B. C. & Lindstrom, K. 1999 Plumage brightness in relation to haematozoan infections in the greenfinch *Carduelis chloris*: bright males are a good bet. *Ecoscience* **6**, 12-18.

Merino, S., Moreno, J., Sanz, J. J. & Arriero, E. 2000 Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proc. R. Soc. Lond. B* **267**, 2507-2510.

Merino, S., Potti, J. & Moreno, J. 1996 Maternal effort mediates the prevalence of trypanosomes in the offspring of a passerine bird. *Proc. Nat. Acad. Sci. USA* **93**, 5726-5730.

Metcalfe, N. B. & Monaghan, P. 2001 Compensations for a bad start: grow now, pay later? *TREE* **16**, 254-260.

Meylan, S., Belliure, J., Clobert, J. & de Fraipont, M. 2002 Stress and body condition as prenatal and postnatal determinants of dispersal in the common lizard (*Lacerta vivipara*). *Hormones and Behavior* **42**, 319-326.

Milinski, M. & Bakker, T. C. M. 1990 Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**, 330-333.

Milinski, M. & Bakker, T. C. M. 1992 Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proc. R. Soc. Lond. B* **250**, 229-233.

Miller, S.A., Dykes, D.D., & Polesky, H.T. 1988 A simple salting procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**, 1215.

Minchella, D. J. & Scott, M. E. 1991 Parasitism: a cryptic determinant of animal community structure. *TREE* **6**, 250-253.

Moller, A. P. 1998 Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. *Oikos* **82**, 265-270.

Moore, J. 2002 *Parasites and the behavior of animals*. Oxford: Oxford University Press.

Morand, S. & Poulin, R. 2000 Nematode parasite species richness and the evolution of spleen size in birds. *Can. J. Zool.* **78**, 1356-1360.

Morrison, S. F., Keogh, J. S. & Scott, I. A. W. 2002 Molecular determination of paternity in a natural population of the multiply mating polygynous lizard *Eulamprus heatwolei*. *Molecular Ecology* **11**, 535-545.

Nelson, R. J. & Demas, G. E. 1996 Seasonal changes in immune function. *Quart. Rev. Biology* **71**, 511-548.

Newman, C., Macdonald, D. W. & Anwar, M. A. 2001 Coccidiosis in the European badger, *Meles meles* in Wytham Woods: infection and consequences for growth and survival. *Parasitology* **123**, 133-142.

Nordling, D., Andersson, M., Zohari, S. & Gustafsson, L. 1998 Reproductive effort reduces specific immune response and parasite resistance. *Proc. R. Soc. Lond. B* **265**, 1291-1298.

Norris, K., Anwar, M. & Read, A. F. 1994 Reproductive effort influences the prevalence of haematozoan parasites in great tits. *J. Animal Ecology* **63**, 601-610.

Olsson, M. 2001 No female mate choice in Mallee dragon lizards, *Ctenophorus fordi*. *Evolutionary Ecology* **15**, 191-141.

Olsson, M., Gullberg, A., Tegelstrom, H., Madsen, T. & Shine, R. 1994a 'Promiscuous' matings enhance maternal fitness in lizards. *Nature* **369**, 528.

Olsson, M., Madsen, T., Shine, R., Gullberg, A. & Tegelstrom, H. 1994b Rewards of promiscuity. *Nature* **372**, 230.

Olsson, M. & Shine, R. 2002 Growth to death in lizards. *Evolution* **56**, 1867-1870.

Olsson, M., Wapstra, E., Madsen, T. & Silverin, B. 2000 Testosterone, ticks and travels: a test of the immunocompetence-handicap hypothesis in free-ranging male sand lizards. *Proc. R. Soc. Lond. B* **267**, 2339-2343.

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

Oppliger, A., Christe, P. & Richner, H. 1997 Clutch size and malarial parasites in female great tits. *Behavioral Ecology* **8**, 148-152.

Oppliger, A. & Clobert, J. 1997 Reduced tail regeneration in the common lizard, *Lacerta vivipara*, parasitized by blood parasites. *Functional Ecology* **11**, 652-655.

Oppliger, A., Vernet, R. & Baez, M. 1999 Parasite local maladaptation in the Canarian lizard *Gallotia galloti* (Reptilia: Lacertidae) parasitized by haemogregarian blood parasite. *J. Evol. Biol.* **12**, 951-955.

Osterhaus, A. 2001 Catastrophes after crossing species barriers. *Proc. R. Soc. Lond. B* **356**, 791-793.

Ots, I., Kerimov, A. B., Invankina, E. V., Ilyina, T. A. & Horak, P. 2001 Immune challenge affects basal metabolic activity in wintering great tits. *Proc. R. Soc. Lond. B* **268**, 1175-1181.

Owens, I. P. F. & Wilson, K. 1999 Immuncompetence: a neglected life history trait or conspicuous red herring? *TREE* **14**, 170-172.

Perkins, S. L. & Keller, A. K. 2001 Phylogeny of nuclear small subunit rRNA genes of hemogregarines amplified with specific primers. *J. Parasitology* **87**, 870-876.

Perkins, S. L., Osgood, S. M. & Schall, J. J. 1998 Use of PCR for detection of subpatent infections of lizard malaria: implications for epizootiology. *Molecular Ecology* **7**, 1587-1590.

Pfennig, K. S. & Tinsley, R. C. 2002 Different mate preferences by parasitized and unparasitized females potentially reduces sexual selection. *J. Evol. Biol.* **15**, 399-406.

Poulin, R. 1994 Mate choice decisions by parasitized female upland bullies, *Gobiomorphus breviceps*. *Proc. R. Soc. Lond. B* **256**, 183-187.

Poulin, R. 1996 Sexual inequalities in helminth infections: a cost of being a male? *American Naturalist* **147**, 287-295.

Poulin, R. & Rohde, K. 1997 Comparing the richness of metazoan ectoparasite communities of marine fishes: controlling for host phylogeny. *Oecologia* **110**, 278-283.

Poulin, R. & Vickery, W. L. 1993 Parasite distribution and virulence: implications for parasite-mediated sexual selection. *Behav. Ecol. Sociobiol.* **33**, 429-436.

Poulin, R. & Vickery, W. L. 1996 Parasite-mediated sexual selection: just how choosy are parasitized females? *Behav. Ecol. Sociobiol.* **38**, 43-49.

Queller, D. C. & Goodnight, K. F. 1989 Estimating relatedness using genetic markers. *Evolution* **43**, 258-275.

Raberg, L., Nilsson, J.-A., Ilmonen, P., Stjernman, M. & Hasselquist, D. 2000 The cost of an immune response: vaccination reduces parental effort. *Ecology Letters* **3**, 382-386.

Raberg, L., Vestberg, M., Hasselquist, D., Holmdahl, R., Svensson, E. & Nilsson, J.-A. 2002 Basal metabolism rate and the evolution of the adaptive immune system. *Proc. R. Soc. Lond. B* **169**, 817-821.

Read, A. F. 1991 Passerine polygyny: a role for parasites? *American Naturalist* **138**, 434-459.

Read, A. F. & Harvey, P. H. 1989 Reassessment of comparative evidence for Hamilton and Zuk theory on the evolution of secondary sexual characters. *Nature* **339**, 618-620.

Real, L. 1990 Search theory and mate choice. I. Models of single-sex discrimination. *American Naturalist* **136**, 376-404.

Real, L. A. 1996 Sustainability and the ecology of infectious disease. *Bioscience* **46**, 88-97.

Richner, H., Christe, P. & Oppliger, A. 1995 Paternal investment affects prevalence of malaria. *Proc. Nat. Acad. Sci. USA* **92**, 1192-1194.

Ricklefs, R. E. 1992 Embryonic development period and the prevalence of avian blood parasites. *Proc. Nat. Acad. Sci. USA* **89**, 4722-4725.

Rigby, M. C. & Moret, y. 2000 Life-history trade-offs with immune defenses. In *Evolutionary Biology of Host-Parasite Relationships: Theory Meets Reality* (ed. A. Skorping), pp. 129-142. Amsterdam: Elsevier Science B.V.

Robert, K. A. & Thompson, M. B. 2000 Influence of feeding on the metabolic rate of the lizard, *Eulamprus tympanum*. *Copeia* **2000**, 851-855.

Robert, K. A. & Thompson, M. B. 2001 Viviparous lizard selects sex of embryos. *Nature* **412**, 698-699.

Robert, K. A., Thompson, M. B. & Seebacher, F. 2003 Facultative sex allocation in the viviparous lizard *Eulamprus tympanum*, a species with temperature-dependent sex determination. *Australian Journal of Zoology* **51**, 367-370.

Roberts, M. G., Dobson, A. P., Arneberg, P., de Leo, G. A., Krecek, R. C., Manfredi, M. T., Lanfranchi, P. & Zaffaroni, E. 2002 Parasite community ecology and biodiversity. In *The Ecology of Wildlife Diseases* (ed. A. P. Dobson), pp. 63-82. Oxford: Oxford University Press.

Roff, D. A. 1992 *The Evolution of Life Histories*. New York: Chapman & Hall.

Rohde, K. 1999 Latitudinal gradients in species diversity and Rapoport's rule revisited: a review of recent work and what can parasites teach us about the causes of the gradients? *Ecography* **22**, 593-613.

Rohr, D. H. 1997 Demographic and life-history variation in two proximate populations of a viviparous skink separated by a steep altitudinal gradient. *J. Animal Ecology* **66**, 567-578.

Rolff, J. 1999 Parasitism increases offspring size in a damselfly: experimental evidence for parasite-mediated maternal effects. *Animal Behaviour* **58**, 1105-1108.

Rosenzweig, M. L. 1995 *Species diversity in space and time*. Cambridge: Cambridge University Press.

Roy, B. A. & Kirchner, J. W. 2000 Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* **54**, 51-63.

Saad, A. H. & El Ridi, R. 1988 Endogenous corticosteroids mediate seasonal cyclic changes in immunity of lizards. *Immunobiol* **177**, 390-403.

Saad, A. H., Khalek, N. A. & El Ridi, R. 1990 Blood testosterone level: a season-dependent factor regulating immune reactivity in lizards. *Immunobiol* **180**, 184-194.

Salkeld, D. J. in press *Eulamprus quoyii* predation. *Herpetological Review*.

Salvador, A., Veiga, J. P., Martin, J. & Lopez, P. 1997 Testosterone supplementation in subordinate, small male lizards: consequences for aggressiveness, color development, and parasite load. *Behavioral Ecology* **8**, 135-139`.

Salvador, A., Veiga, J. P., Martin, J., Lopez, P., Abelenda, M. & Puerta, M. 1996 The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behavioral Ecology* **7**, 145-150.

Sanz, J. J., Moreno, J., Arriero, E. & Merino, S. 2002 Reproductive effort and blood parasites of breeding pied flycatchers: the need to control for interannual variation and initial health state. *Oikos* **96**, 299-306.

Schalk, G. & Forbes, M. R. 1997 Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon. *Oikos* **78**, 67-74.

Schall, J. J. 1983 Lizard malaria: cost to vertebrate host's reproductive success. *Parasitology* **87**, 1-6.

Schall, J. J. 1986 Prevalence and virulence of a haemogregarine parasite of the Aruban whiptail lizard, *Cnemidophorus arubensis*. *J. Herpetology* **20**, 318-324.

Schall, J. J. 1990 The ecology of lizard malaria. *Parasitology Today* **6**, 264-269.

Schall, J. J. 1990a The ecology of lizard malaria. *Parasitology Today* **6**, 264-269.

Schall, J. J. 1990b Virulence of lizard malaria: the evolutionary ecology of an ancient parasite-host association. *Parasitology* **100**, S35-S52.

Schall, J. J. 1992 Parasite-mediated competition in *Anolis* lizards. *Oecologia* **92**, 58-64.

Schall, J. J. 1996 Malarial parasites of lizards: diversity and ecology. *Advances in Parasitology* **37**, 255-333.

Schall, J. J. 2002 Parasite virulence. In *The Behavioural Ecology of Parasites* (ed. M. V. K. Sukhdeo), pp. 283-313. Wallingford: CABI Publishing.

Schall, J. J., Bennett, A. F. & Putnam, R. W. 1982 Lizards infected with malaria: physiological and behavioural consequences. *Science* **217**, 1057-1059.

Schall, J. J. & Dearing, M. D. 1987 Malarial parasitism and male competition for mates in the western fence lizard, *Sceloporus occidentalis*. *Oecologia* **73**, 389-392.

Schall, J. J. & Houle, P. R. 1992 Malarial parasitism and home range and social status of male western fence lizards, *Sceloporus occidentalis*. *J. Herpetology* **26**, 74-76.

Schall, J. J. & Marghoob, A. B. 1995 Prevalence of a malarial parasite over time and space: *Plasmodium mexicanum* in its vertebrate host, the western fence lizard *Sceloporus occidentalis*. *J. Animal Ecology* **64**, 177-185.

Schall, J. J. & Pearson, A. R. 2000 Body condition of a Puerto Rican anole, *Anolis gundlachi*: effect of a malaria parasite and weather variation. *J. Herpetology* **34**, 489-491.

Schall, J. J., Pearson, A. R. & Perkins, S. L. 2000 Prevalence of malaria parasites (*Plasmodium floridense* and *Plasmodium azurophilum*) infecting a Puerto Rican lizard (*Anolis gundlachi*): a nine-year study. *J. Parasitology* **86**, 511-515.

Schall, J. J. & Sarni, G. A. 1987 Malarial parasitism and the behavior of the lizard, *Sceloporus occidentalis*. *Copeia* **1987**, 84-93.

Schmoll, T., Dietrich, V., Winkel, W., Epplen, J. T. & Lubjuhn, T. 2003 Long-term fitness consequences of female extra-pair matings in a socially monogamous passerine. *Proc. R. Soc. Lond. B* **270**, 259-264.

Schulte-Hostedde, A. I., Millar, J. S. & Hickling, G. J. 2001 Evaluating body condition in small mammals. *Can. J. Zool.* **79**, 1021-1029.

Schwarzkopf, L. 1992 Annual variation of litter size and offspring size in a viviparous skink. *Herpetologica* **48**, 390-395.

Schwarzkopf, L. 1993 Costs of reproduction in water skinks. *Ecology* **74**, 1970-1981.

Schwarzkopf, L. 1994 Measuring trade-offs: a review of studies of costs of reproduction in lizards. In *Lizard Ecology: historical and experimental perspectives* (ed. E. R. Pianka), pp. 7-29. Princeton, NJ: Princeton University Press.

Schwarzkopf, L. 1996 Decreased food intake in reproducing lizards: a fecundity-dependent cost of reproduction? *Australian Journal of Zoology* **21**, 355-362.

Schwarzkopf, L. 1998 Evidence of geographic variation in lethal temperature but not activity temperature of a lizard. *J. Herpetology* **32**, 102-106.

Schwarzkopf, L., Blows, M. W. & Caley, M. J. 1999 Life-history consequences of divergent selection on egg size in *Drosophila melanogaster*. *American Naturalist* **29**, 333-340.

Schwarzkopf, L. & Shine, R. 1991 Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: why do gravid females bask more? *Oecologia* **88**, 562-569.

Scott, I. A. W., Hayes, C. M., Keogh, J. S. & Morrison, S. F. 2001 Isolation and characterization of novel microsatellite markers from the Australian water skink *Eulamprus kosciuskoi* and cross-species amplification in other members of the species-group. *Molecular Ecology Notes* **1**, 31.

Semple, S., Cowlishaw, G. & Bennett, P. M. 2002 Immune system evolution among anthropoid primates: parasites, injuries and predators. *Proc. R. Soc. Lond. B* **269**, 1031-1037.

Sheldon, B. C. & Verhulst, S. 1996 Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *TREE* **11**, 317-321.

Shine, R. 1980 'Costs' of reproduction in reptiles. *Oecologia* **46**, 92-100.

Shine, R. & Harlow, P. 1993 Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**, 122-127.

Siikamaki, P., Ratti, O., Hovi, M. & Bennett, G. F. 1997 Association between haematozoan infections and reproduction in the pied flycatcher. *Functional Ecology* **11**, 176-183.

Simmons, L. W. 1994 Courtship role reversal in bush crickets: another role for parasites? *Behavioral Ecology* **5**, 259-266.

Sinervo, B. 1990 The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* **44**, 279-294.

Sinervo, B. & Adolph, S. C. 1994 Growth plasticity and thermal opportunity in *Sceloporus* lizards. *Ecology* **75**, 776-790.

Sinervo, B. & Doughty, P. 1996 Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* **50**, 1314-1327.

Smallridge, C. & Bull, C. M. 2001 Infection dynamics of *Haemolivia mariae* in the sleepy lizard *Tiliqua rugosa* . *Parasitology Research* **87**, 657-661.

Smallridge, C. J. & Bull, C. M. 1999 Transmission of the blood parasite *Hemolvia mariae* between its lizard and tick hosts. *Parasitology Research* **85**, 858-863.

Smallridge, C. J. & Bull, C. M. 2000 Prevalence and intensity of the blood parasite *Hemolivia mariae* in a field population of the skink, *Tiliqua rugosa*. *Parasitology Research* **86**, 563-569.

Smith, G. C. & Hunt, J. L. 2004 On the use of spleen mass as a measure of avian immune system strength. *Oecologia* **138**, 28-31.

Smith, T. G. 1996 The genus *Hepatozoon* (Apicomplexa: Adeleina). *J. Parasitology* **82**, 565-585.

Smith, T. G., Kim, B. & Desser, S. S. 1999 Phylogenetic relationships among *Hepatozoon* species from snakes, frogs and mosquitoes of Ontario, Canada, determined by ITS-1 nucleotide sequences and life cycle, morphological and developmental characteristics. *International Journal for Parasitology* **29**, 292-304.

Smith, T. G., Kim, B., Hong, H. & Desser, S. S. 2000 Intraerythrocytic development of species of *Hepatozoon* infecting ranid frogs: evidence for convergence of life cycle characteristics among apicomplexans. *J. Parasitology* **86**, 451-458.

Sol, D., Jovani, R. & Torres, J. 2000 Geographical variation in blood parasites in feral pigeons: the role of vectors. *Ecography* **23**, 307-314.

Sol, D., Jovani, R. & Torres, J. 2003 Parasite mediated mortalityand host immune response explain age-related differences in blood parasitism in birds. *Oecologia* **135**, 542-547.

Soler, J. J., de Neve, L., Perez-Contreras, T., Soler, M. & Sorci, G. 2003 Trade-off between immunocompetence and growth in magpies: an experimental study. *Proc. R. Soc. Lond. B* **270**, 241-248.

Sorci, G. 1995 Repeated measurements of blood parasite levels reveal limited ability for host recovery in the common lizard (*Lacerta vivipara*). *J. Parasitology* **81**, 825-827.

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.

Sorci, G., Clobert, J. & Michalakis, Y. 1996 Cost of reproduction and cost of parasitism in the common lizard, *Lacerta vivipara*. *Oikos* **76**, 121-130.

Sorci, G., Massot, M. & Clobert, J. 1994 Maternal parasite load increases sprint speed and philopatry in female offspring of the common lizard. *American Naturalist* **144**, 153-164.

Staats, C. M. & Schall, J. J. 1996 Distribution and abundance of two malarial parasites of the endemic *Anolis* lizard of Saba Island, Netherlands Antilles. *J. Parasitology* **82**, 409-413.

Stearns. 1992 *The Evolution of Life Histories*. Oxford: Oxford University Press.

Stien, A., Irvine, R. J., Ropstad, E., Halvorsen, O., Langvatn, R. & Albon, S. D. 2002 The impact of gastrointestinal nematodes on wild reindeer: experimental and cross-sectional studies. *J. Animal Ecology* **71**, 937-945.

Stockley, P. 2003 Female multiple mating behaviour, early reproductive failure and litter size variation in mammals. *Proc. R. Soc. Lond. B* **270**, 271-278.

Stow, A. J. & Sunnucks, P. 2004 High mate and site fidelity in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology* **13**, 419-430.

Strassmann, J. E., Solis, C. R., Peters, J. M. & Queller, D. C. 1996 Strategies for finding and using highly polymorphic DNA microsatellite loci for studies of genetic relatedness and pedigrees. In *Molecular Zoology: Advances, Strategies and Protocols* (ed. S. R. Pulumbi), pp. 163-180, 528-549. New York: Wiley-Liss.

Telford, S. R. J., Wozniak, E. J. & Butler, J. F. 2001 Haemogregarine specificity in two communities of Florida snakes, with descriptions of six new species of *Hepatozoon* (Apicomplexa: hepatozoidae) and a possible species of *Haemogregarina* (Apicomplexa: haemogregarinidae). *J. Parasitology* **87**, 890-905.

Tella, J. L. 2002 The evolutionary transition to coloniality promotes higher blood parasitism in birds. *J. Evol. Biol.* **15**, 32-41.

Tella, J. L., Blanco, G., Forero, M. G., Gajon, A., Donazar, J. A. & Hiraldo, F. 1999 Habitat, world geographic range, and embryonic development of hosts explain the prevalence of avian hematozoa at small spatial and phylogenetic scales. *Proc. Nat. Acad. Sci. USA* **96**, 1785-1789.

Tella, J. L., Scheuerlein, A. & Ricklefs, R. E. 2002 Is cell-mediated immunity related to the evolution of life-history strategies in birds? *Proc. R. Soc. Lond. B* **269**, 1059-1066.

Thomas, F., Renaud, F., de Meeus, T. & Cezilly, F. 1995a Parasites, age and the Hamilton-Zuk hypothesis: inferential fallacy? *Oikos* **74**, 305-309.

Thomas, F., Renaud, F., Rousset, F., Cezilly, F. & de Meeus, T. 1995b Differential mortality of two closely related host species induced by one parasite. *Proc. R. Soc. Lond. B* **260**, 349-352.

Thompson, J. 1981 A study of the sources of nutrients for embryonic development in a viviparous lizard, *Sphenomorphus quoyii*. *Comparative Biochemistry & Physiology Part A* **70**, 509-518.

Thompson, M. B., Speake, B. K., Russell, K., J. & McCartney, R. J. 2001 Nutrient uptake by embryos of the Australian viviparous lizard *Eulamprus tympanum*. *Physiological & Biochemical Zoology* **74**, 560-567.

Tompkins, D. M. & Begon, M. 1999 Parasites can regulate wildlife populations. *Parasitology Today* **15**, 311-313.

Tompkins, D. M., Dobson, A. P., Arneberg, P., Begon, M., Cattadori, I. M., Greenman, J. V., Heesterbeek, J. A. P., Hudson, P. J., Newborn, D., Pugliese, A., Rizzoli, A. P., Rosa, R., Rosso, F. & Wilson, K. 2002 Parasites and host population dynamics. In *The Ecology of Wildlife Diseases* (ed. A. P. Dobson), pp. 45-62. Oxford: Oxford University Press.

Tregenza, T. & Wedell, N. 1998 Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution* **52**, 1726-1730.

Tregenza, T. & Wedell, N. 2002 Polyandrous females avoid costs of inbreeding. *Nature* **415**, 71-73.

van Baalen, M. & Sabelis, M. W. 1995 The dynamics of multiple infection and the evolution of virulence. *American Naturalist* **146**, 881-910.

Vanhooydonck, B. & Van Damme, R. 2003 Retionships between locomotor performance, microhabitat use and antipredator behaviour in lacertid lizards. *Functional Ecology* **17**, 160-169.

Veiga, J. P., Salvador, A., Merino, S. & Puerta, M. 1998 Reproductive effort affects immune response and parasite infection in a lizard: a phenotypic manipulation using testosterone. *Oikos* **82**.

Veron, J. E. N. 1969 The reproductive cycle of the water skink, *Sphenomorphus quoyii*. *J. Herpetology* **3**, 55-63.

Veron, J. E. N. 1969a An analysis of stomach contents of the water skink, *Sphenomorphus quoyii*. *J. Herpetology* **3**, 187-189.

Veron, J. E. N. 1969b The reproductive cycle of the water skink, *Sphenomorphus quoyii*. *J. Herpetology* **3**, 55-63.

Weatherhead, P. J. 1990 Secondary sexual traits, parasites, and polygyny in red-winged blackbirds, *Agelaius phoeniceus*. *Behavioral Ecology* **1**, 125-130.

Webb, T. J. & Hurd, H. 1999 Direct manipulation of insect reproduction by agents of parasite origin. *Proc. R. Soc. Lond. B* **266**, 1537-1541.

Wegner, K. M., Reusch, T. B. H. & Kalbe, M. 2003 Multiple parasites are driving major histocompatability complex polymorphism in the wild. *J. Evol. Biol.* **16**, 224-232.

Westneat, D. F. & Birkhead, T. R. 1998 Alternative hypotheses linking the immune system and mate choice for good genes. *Proc. R. Soc. Lond. B* **265**, 1065-1073.

White, P. J., Norman, R. A. & Hudson, P. J. 2002 Epidemiological consequences of a pathogen having both virulenct and avirulent modes of transmission: the case of a rabbit haemorrhagic disease virus. *Epidemiol. Infect.* **129**, 665-677.

White, P. J., Norman, R. A., Trout, R. C., Gould, E. A. & Hudson, P. J. 2001 The emergence of rabbit haemorrhagic disease virus: will a non-pathogenic strain protect the UK? *Phil. Trans. R. Soc. Lond. B* **356**, 1087-1095.

Wiehn, J. & Korpimaki, E. 1998 Resource levels, reproduction and resistance to haematozoan infections. *Proc. R. Soc. Lond. B* **265**, 1197-1201.

Williams, T. D. 1999 Enhanced immune function does not depress reproductive output. *Proc. R. Soc. Lond. B* **266**, 753-757.

Wilson, R. S. & Booth, D. T. 1998 Effect of tail loss on reproductive output and its ecological significance in the skink *Eulamprus quoyii. J. Herpetology* **32**, 128-131.

Wolda, H. 1978a Fluctuations in abundance of tropical insects. *American Naturalist* **112**, 1017-1045.

Wolda, H. 1978b Seasonal fluctuations in rainfall, food and abundance of tropical insects. *J. Animal Ecology* **47**, 369-381.

Wolda, H. 1989 Seasonal cues in tropical organisms. Rainfal? Not necearily! *Oecologia* **80**, 437-442.

Wozniak, E. J., Kanitz, C., Homer, B., Kreisle, R., Telford, S. R. J. & McLaughlin, G. 1996 Demonstration of common and stage-specific anti-*Hepatozoon mocassini* antibodies in experimentally infected unnatural lizard hosts. *International Journal for Parasitology* **26**, 131-133.

Wozniak, E. J. & Telford, S. R. J. 1991 The fate of *Hepatozoon* species naturally infecting Florida black racers and watersnakes in potential mosquito and soft tick vectors, and histological evidence of pathogenicity in unnatural host species. *International Journal for Parasitology* **21**, 511-516.

Zamudio, K. R. & Sinervo, B. 2000 Polygyny, mate-guarding, and posthumous fertilisation as alternative male mating strategies. *Proc. Nat. Acad. Sci. USA* **97**, 14427-14432.

Zar, J. H. 1999 *Biostatistical Analysis*. London: Prentice-Hall International.

Zeh, J. A. & Zeh, D. W. 2001 Reproductive mode and the genetic benefits of polyandry. *Animal Behaviour* **61**, 1051-1063.

Zera, A. J. & Harshman, L. G. 2001 The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics* **32**, 95-126.