Oecologia (2001) 127:586–589
DOI 10.1007/s004420000626

**Rebecca J. Eisen**

# Absence of measurable malaria-induced mortality in western fence lizards (*Sceloporus occidentalis*) in nature: a 4-year study of annual and over-winter mortality

Received: 5 June 2000 / Accepted: 20 November 2000 / Published online: 21 February 2001


**Abstract** Theoretical models of parasite virulence often quantify virulence by mortality. However, there is a lack of empirical studies of parasite-induced host mortality because it is often difficult to quantify in natural populations. I have estimated annual and over-winter mortality in a population of fence lizards (*Sceloporus occidentalis*) infected with a malaria parasite, *Plasmodium mexicanum*, in northern California. The duration of time a lizard was observed (an estimate of life-span) throughout the 4-year observation period, or following winter, was not related to either infection status or maximum parasitemia. In contrast to previous laboratory studies of this parasite-host system, I found no evidence of parasite-induced host mortality in nature.

**Keywords** *Plasmodium* · Malaria · Parasite-induced host mortality · *Sceloporus occidentalis*

## Introduction

Deleterious effects of parasitism, virulence, can significantly affect the ecology and evolution of parasite hosts. Parasites affect host population growth and regulation (Scott 1987; Holmes 1995; Krebs 1995; Hudson et al. 1998), spatial distribution (Price 1980; van Riper et al. 1986), reproductive success (Schall 1996; Pacejka et al. 1998), and sexual selection (Hamilton and Zuk 1982; Møller et al. 1999). In turn, changes in host behavior, physiology, and survival can affect parasite abundance, reproductive success (transmission) and survival (Holmes and Bethel 1972; Ewald 1983, 1995; Fialho and Schall 1995). Several models of parasite virulence, focusing mainly on parasite-induced host mortality, have been proposed (Bull 1994; Frank 1996). However, there is little empirical evidence of parasite-induced host mortality in natural populations, in large part because these data are difficult to obtain. Methods have been based on mark-recapture techniques, single captures, or laboratory studies (Grenfell and Dobson 1995; Gulland 1995), but the latter cast little light on natural processes. More appropriately, a cohort of the host population should be followed through time in the field and survivorship data recorded based on infection status (infection-status-dependent mortality) and/or parasitemia (parasitemia-dependent mortality) (Anderson and Gordon 1982; Gulland 1995; Hofmeister et al. 1999; Jokela et al. 1999).

A malaria-causing parasite, *Plasmodium mexicanum*, commonly found in western fence lizards (*Sceloporus occidentalis*) has been used as a model organism for studying virulence (Schall 1983, 1990). The parasite is known to have negative effects on host reproduction, hematological profile, social behavior and physiology (Schall 1990). Infected lizards store less fat than uninfected ones and this may affect over-winter mortality (Schall 1996). In addition, a laboratory study showed that infected lizards suffer greater mortality than uninfected ones (Schall 1983). However, studies of *P. mexicanum* -induced lizard mortality have not been conducted under natural conditions. The goal of this 4-year mark-recapture study was to determine if *P. mexicanum* causes mortality in infected lizards either during periods of inactivity in the winter, or during the active period.

R.J. Eisen (✉)
Department of Biology, University of Vermont,
Burlington, VT 05405, USA

*Present address*:
R.J. Eisen, University of California,
ESPM Division of Insect Biology, 201 Wellman Hall,
Berkeley, CA 94720, USA
e-mail: reisen@nature.berkeley.edu
Tel.: +1-510-6438060, Fax: +1-707-7441040

## Materials and methods

### Lizard collection and detection of parasites

Fence lizards were collected from June to August 1996, April to September 1997, March to September 1998 and April to August 1999, within a 4.5-ha oak woodland site at the University of California Hopland Research and Extension Center (HREC) located in southeastern Mendocino County, California. The length of this collection period covered the average life-span of the lizard; only

approximately 3% of the lizards were observed over 3 warm seasons when they are active at the HREC, and none were captured over 4 years (Bromwich and Schall 1986; Eisen 2000).

Lizards were captured by hand or slip noose between 0900 and 1400 hours. Upon first collection, each lizard was marked on its dorsal surface with a number using non-toxic liquid paper, and permanently marked by a unique toe-clip combination. The exact sites of capture were recorded and flagged with surveyor's tape to assure release at the point of capture. All animals were released within 24 h of capture. Recaptures of the same individual were spaced at least 3 days apart.

All animals were transported in cloth bags to a field laboratory where sex and snout-vent length (SVL) were recorded. A drop of blood was drawn from a toe clip of each lizard to prepare thin blood smears which were later stained with Giemsa's stain (pH 7.0, 50 min).

Each slide was examined at 1,000× for 6 min or until a malaria parasite was detected. A 6-min scan allows examination of approximately 10,000 erythrocytes (RBCs) (Bromwich and Schall 1986). Infections with parasitemia <1/10,000 RBC, and thus not detected by this protocol, are rare at HREC (Perkins et al. 1998). When an infection was detected, 1,000 RBCs were counted and the numbers of *Plasmodium* -infected cells (of all stages of infection) were noted.

### Mortality estimates

To detect parasitemia-dependent host mortality, the maximum parasitemia (maximum number of parasites detected per 1,000 RBC counted for each blood smear collected per individual lizard) was calculated for infected lizards captured at least four times throughout the 4-year observation period. Regression analysis was used to determine if the duration of time a lizard was observed (an estimate of life-span) was related to maximum parasitemia.

Because mortality is suspected to be higher during the winter (Schall 1996), a separate analysis was run for winter mortality. Maximum parasitemia was estimated based on the last capture of the year prior to winter. The mean maximum parasitemias between infected lizards that were and were not recaptured the following warm season were compared using a *t*-test.

An analysis of infection-status- dependent parasite-induced host mortality, using longitudinal data collected over 4 years, compared the duration of observation among infected and uninfected lizards. Using only lizards whose infection status did not change throughout the observation period, the duration of observation per individual between infected and uninfected hosts were compared with a *t*-test.

Again, a separate analysis was run to detect infection status-induced winter mortality. Lizards captured for the first time in 1999 were excluded from this analysis because survival during the 1999–2000 winter was unknown. Infection status was based on the latest capture in the season prior to winter per individual and recapture the following warm season was compared by infection status using likelihood ratio statistics (Sokal and Rohlf 1995). All analyses were run using JMP statistical software (SAS Institute, Cary, N.C., USA).

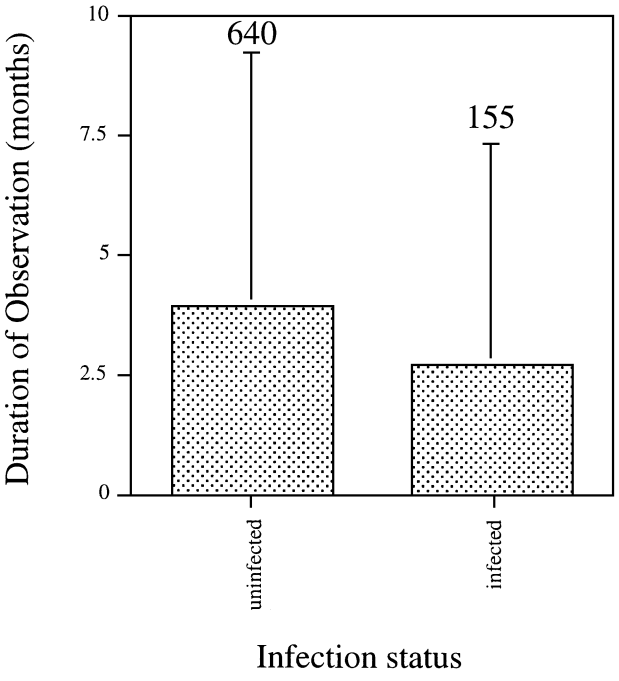


**Fig. 1** Duration of observation by infection status

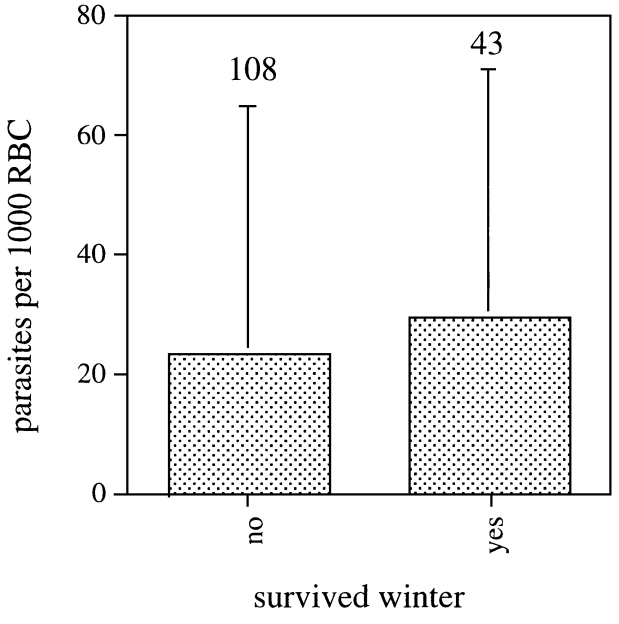


**Fig. 2** Comparison of parasitemia between infected lizards that were captured after the winter and those that were not. Parasitemia is based on the last capture prior to winter (*RBC* red blood cell)

## Results

Throughout the 4- year observation period, the duration of observation per individual was similar for infected and uninfected lizards ($t$=0.21, $P$=0.65, $n$=795) (Fig. 1). In addition, maximum parasitemia was not associated with the duration of observation per individual infected lizard (regression analysis; $r^2$=0.007, $P$=0.93, $n$=67). Power analyses indicated that to detect a significant difference in observation period between infected and uninfected lizards and between maximum parasitemias, at least 15,449 and 36,810 lizards, respectively, would need to be sampled.

*P. mexicanum* had no apparent effect on the host's ability to survive the winter. Infected lizards were as likely as uninfected ones to be recaptured following the winter ($\chi^2$=2.64, $P$=0.10, $n$=722). Furthermore, based on the last parasitemia level measured in the first year of

capture, there was no significant difference in parasitemia between infected animals captured in a single year versus multiple years (*t*-test; $t=0.64$, $P=0.43$; $n=155$) (Fig. 2). Power calculations indicate that at least 913 lizards would need to be sampled to see a significant difference.

## Discussion

I found no evidence of *P. mexicanum*-induced mortality in fence lizards under natural conditions. No infection-status- or parasitemia-dependent mortality were detected for lizards observed over 4 years, or those studied following the winter. Previous field and laboratory studies clearly demonstrate that *P. mexicanum* infections contribute to lizard morbidity. For example, infected lizards have increased numbers of immature RBCs, and decreased hemoglobin, maximum oxygen consumption, running stamina, and fat storage, and are submissive socially to uninfected lizards (Schall 1990, 1996). However, any evidence of parasite-induced host mortality is restricted to lizards maintained in the laboratory (Schall 1983, 1996). The incongruous results obtained from the laboratory study that suggest mortality is significant and the present field study, suggesting the contrary, could have several explanations. First, mortality levels may have been increased by stressful laboratory conditions (Schall 1996). Holding lizards in captivity is known to increase plasma levels of corticosterone, which may reduce immune function and thus exacerbate the negative physiological effects of parasitism (Stimson 1987; Dunlap and Schall 1995). Alternatively, field data may not detect low levels of parasite-induced host mortality detectable under constant environmental conditions in the laboratory. For example, parasite-induced host mortality may be lower than other sources of natural mortality, such as predation or adverse weather conditions (Gulland 1995). However, I know of no studies that compare lizard mortality rates caused by these sources relative to parasitism. Finally, it is possible that natural conditions simply do not exacerbate the negative effects of parasitism (mortality), or may do so only in rare years with extremely cold or wet conditions.

The mortality data presented in this natural study, and in laboratory studies (Schall 1983, 1996) suggest that different methods of evaluating parasite-induced host mortality may yield conflicting results. The main problem encountered in this natural study was that mortality estimates had to be based on recapture success rather than physical evidence of mortality (e.g., if a lizard was not recaptured it was assumed, but could not be confirmed, dead). Further, variation in the quality of lizard territories could have resulted in differences in exposure to environmental triggers exacerbating the effects of parasitism. The advantage of a field study is that it provides insight into the effects of parasitism under natural conditions (e.g., with infections initiated by their natural vectors, *Lutzomyia vexator* or *L. stewartii*, in free-ranging lizards). In contrast, laboratory studies can be used to determine if parasites are capable of inducing host mortality. However, as demonstrated here, such studies need to be validated under natural conditions. An alternative approach would mimic the present study, but would follow a randomly chosen group of lizards that were infected and a matched set left uninfected. The advantage of this method, in addition to those mentioned for the field study, is that spatial variation in lizard habitats could be controlled. However, such an approach was not used in this study for two main reasons. First, in order to prevent uninfected lizards from being exposed to infection, they would have to be kept in vector-proof enclosures. Second, natural vectors are difficult to rear in the laboratory and, thus, artificial methods of inoculation would have been necessary to infect lizards. Previous studies of *P. mexicanum* have demonstrated that the range in parasitemia is similar in naturally infected and needle-inoculated infections, but the central tendencies are often higher for the latter (Eisen 2000; Eisen and Schall 2000; Eisen and DeNardo, 2000). If, as assumed by several theoretical models of virulence (Ewald 1983; Bull 1994; Read 1994), parasitemia is related to mortality, needle-inoculation could have artificially increased estimates of parasite-induced host mortality. Finally, Anderson and Gordon (1982) proposed a model for using point capture data comparing the patterns of parasite load (parasitemia), aggregation and prevalence among host age classes to measure parasite-induced host mortality. This model has been validated in several empirical longitudinal studies of macro-parasites (Gordon and Rau 1982; Thomas et al. 1995a,1995b; Wetzel and Esch 1995; Pampoulie et al. 1999). Such an approach would be less labor-intensive and less obtrusive than the mark-recapture technique. Although this model has been used to measure parasite-induced host mortality of another blood parasite (Sorci 1996), it cannot be used for *Plasmodium* spp. because it assumes that the parasite does not reproduce within its host.

Although *P. mexicanum*-induced lizard mortality was not detected in this study, or in a previous short-term mark-recapture study (Bromwich and Schall 1986), parasite virulence may affect population size and genetic structure. For example, reproductive output of *P. mexicanum*-infected female lizards is reduced by approximately 20% (Schall 1996), which may result in reduced densities of lizards in areas where the parasite is present, as compared to absent. Further, malaria infection appears to reduce the fitness of infected lizards; infected males had reduced testis size and were less capable of defending their territories and competing for mates (Schall 1996). Thus, genetic diversity may be higher in populations lacking the parasite, relative to populations where the parasite is common. Future long-term studies of host population size and genetic structure are required to determine if the proposed effects of *P. mexicanum* infections exist in natural populations.

**Acknowledgements** I thank A. Chatfield, K. Gurski, and S. Osgood for assistance in the field. J. Schall and L. Tälleklint-Eisen offered comments on the manuscript. C. Vaughn, R. Timm, and R. Kieffer, and the staff of the Hopland Research and Extension Center were crucial for the success of the project. Funding was provided by the US-NSF, the Vermont-NSF EPSCoR program to J. J. Schall, and the American Museum of Natural History, and a Graduate Training Grant from NSF to R.J.E.

## References

Anderson RM, Gordon DM (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. Parasitology 85:373–398

Bromwich CR, Schall JJ (1986) Infection dynamics of *Plasmodium mexicanum*. A malaria parasite of lizards. Ecology 67: 1227–1235

Bull JJ (1994) Virulence. Evolution 48:1423–1437

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

Eisen RJ (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 RJ, DeNardo DF (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 Parasitol 86:1041–1045

Eisen RJ, Schall JJ (2000) Life history of a malaria parasite (*Plasmodium mexicanum*): Independent traits and basis for variation. Proc R Soc Lond B267:793–800

Ewald PW (1983) Host-parasite relations, vectors, and the evolution of disease. Ann Rev Ecol Syst 14:465–485

Ewald PW (1995) The evolution of virulence: a unifying link between parasitology and ecology. J Parasitol 81:659–669

Fialho RF, Schall JJ (1995) Thermal ecology of a malarial parasite and its insect vector: consequences for the parasite's transmission success. J Anim Ecol 64:553–562

Frank SA (1996) Models of parasite virulence. Q Rev Biol 71:37–78

Gordon DM, Rau ME (1982) Possible evidence for mortality induced by the parasite *Apatemon gracilis* in a population of brook sticklebacks (*Culea inconstans*). Parasitology 84:41–47

Grenfell BT, Dobson AP (1995) Ecology of infectious diseases in natural populations. Cambridge University Press, Cambridge

Gulland FMD (1995) The impact of infectious diseases on wild animal populations – a review. In: Grenfell BT, Dobson AP (eds) Ecology of infectious diseases in natural populations. Cambridge University Press, Cambridge, pp 20–51

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

Hofmeister EK, Ellis BA, Glass GE, Childs JE (1999) Longitudinal study of infection with *Borrelia burgdorferi* in a population of *Peromyscus leucopus* at a Lyme disease-enzootic site in Maryland. Am J Trop Med Hyg 60:598–609

Holmes JC (1995) Population regulation: a dynamic complex of interactions. Wildl Res 22:11–19

Holmes JC, Bethel WM (1972) Modification of intermediate host behavior by parasites. In: Canning EU, Wright CA (eds) Behavioral aspects of parasite transmission. Academic Press, New York, pp 128–149

Hudson PJ, Dobson AP, Newborn D (1998) Prevention of population cycles by parasite removal. Science 282:2256–2258

Jokela J, Lively CM, Taskinen J, Peters AD (1999) Effect of starvation on parasite-induced mortality in a freshwater snail (*Potamopyrgus antipodarum*). Oecologia 119:320–325

Krebs CJ (1995) Two paradigms of population regulation. Wildl Res 22:1–10

Møller AP, Christe P, Lux E (1999) Parasitism, host immune function and sexual selection. Q Rev Biol 74:3–20

Pacejka AJ, Gratton CM, Thompson CF (1998) Potentially virulent mites affect house wren (*Troglodytes aedon*) reproductive success? Ecology 79:1797–1806

Pampoulie C, Morand S, Lambert A, Rosecchi E, Bouchereau JL, Crivelli AJ (1999) Influence of the treamtode *Aphalloides coelomicola* Dollfus, Chabaud and Golvan, 1957 on the fecundity and survival of *Pomatoschistus microps* (Kroyer, 1838) (Teleostei: Gobiidae). Parasitology 119:61–67

Perkins SL, Osgood SM, Schall JJ (1998) Use of PCR for detection of subpatent infections of lizard malaria: implications for epizootiology. Mol Ecol 7:1587–1590

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

Read AF (1994) The evolution of virulence. Trends Microbiol 2:73–76

Riper C III van, Riper SG van, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol Monog 56:327–344

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

Schall JJ (1990) Virulence of lizard malaria: the evolutionary ecology of an ancient parasite-host association. Parasitology 100:S35-S52

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

Scott ME (1987) Regulation of mouse colony abundance by *Heligmosomoisodes polygyrus*. Parasitology 95:111–124

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. Freeman, New York

Sorci G (1996) Patterns of haemogregarine load, aggregation and prevalence as a function of host age in the lizard *Lacerta vivipara*. J Parasitol 82:676–678

Stimson WH (1987) Sex steroids, steroid receptors and immunity. In: Berczi I, Kovacs K (eds) Hormones and immunity. MTP Press, Norwell, pp 43–53

Thomas F, Lambert A, De Meeüs T, Cézilly F, Renaud F (1995a) Influence of *Microphallus hoffmanni* (Trematoda, Microphallidae) on the survival, sexual selection, and fecundity of *Gammarus aequicauda* (Amphipoda). Can J Zool 73:1634–1639

Thomas F, Renaud F, Rousset F, Cézilly F, De Meeûs T (1995b) Differential mortality of two closely related species induced by one parasite. Proc R Soc Lond B 260:349–352

Wetzel EJ, Esch GW (1995) Effect of age on infectivity of cercariae of *Halipegus occidualis* (Digenea: Hemiuridae) to their second intermediate host. Invert Biol 114:205–210