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REPORT

# Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*

**Anne Oppliger,[1,4] Jean Clobert,[1] Jane Lecomte,[2] Pauline Lorenzon,[1] Katia Boudjemadi,[1] and Henry B. John-Alder[3]**

[1]*Laboratoire d'Ecologie, CNRS-URA 258, Université Pierre et Marie Curie, Bât A, 7ème étage, case 237, 7, quai St-Bernard, 75252 Paris Cedex 05, France.*

[2]*Laboratoire Ecologie, Systématique et Evolution, CNRS-2154, Université Paris-Sud, Bât 362, 91405 Orsay Cedex, France.*

[3]*Rutgers University, Department of Animal Sciences, 84 Lipman Drive, New Brunswick, NJ 08901 U.S.A.*

[4]*Present address: Institut de Zoologie et Ecologie Animale, Bât de Biologie, Université de Lausanne, 1015 Lausanne, Switzerland.*

*E-mail: aopplige@zoolmus.unizh.ch*

## Abstract

Parasites affect the life-histories and fitness of their hosts. It has been demonstrated that the ability of the immune system to cope with parasites partly depends on environmental conditions. In particular, stressful conditions have an immunosuppressive effect and may affect disease resistance. The relationship between environmental stress and parasitism was investigated using a blood parasite of the common lizard *Lacerta vivipara*. In laboratory cages, density and additional stressors had a significant effect on the intensity of both natural parasitaemia and parasitaemia induced by experimental infection. Four weeks after infection, crowded lizards had three times more parasites than noncrowded lizards. After 1 month of stress treatment, naturally infected lizards had a significantly higher level of plasma corticosterone and a higher parasite load than nonstressed individuals. In seminatural enclosures, stress induced by the habitat quality affected both the natural blood parasite prevalence and the intensity of parasitaemia of the host.

## Keywords

Blood parasite, corticosterone, crowding, density, host–parasite interaction, immune system, lizard, stress, susceptibility.

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## INTRODUCTION

The ecological and evolutionary importance of parasites on animals and plants is now widely recognized by biologists (Combes 1995; Begon *et al.* 1996). Parasites are known to strongly affect the life-histories (reviewed by Møller 1997) and fitness (review by Møller *et al.* 1990) of their hosts. As a result, strong selection for antiparasite defence via the immune system is expected. In vertebrates, immunity is partly genetically based (cf. major histocompatibility complex), although environmental effects exist (Chandra 1975; Gershwin *et al.* 1985; Lochmiller *et al.* 1993).

Surprisingly, among the large number of studies concerning host–parasite interactions, very few have examined factors that could affect immune responses (Barnard *et al.* 1994, 1996; Smith *et al.* 1996). The immune response is under the control of several factors, including hormones; e.g. corticosterone acts as an immunosuppressor (Grossman 1988; Finch & Rose 1995; Zuk 1996). This adrenocortical steroid is secreted in response to numerous psychological or somatic stressors and is an integral component of the response to stress (Axelrod & Reisine 1984; Moberg 1985; Guillette *et al.* 1995). Therefore, stressful situations would be predicted to increase the level of corticosterone and decrease immunocompetence. Recently, Lochmiller (1996) suggested that density-dependent interactions influenced survival rates at the population level through their effect on host immunity. In crowded populations, different immunocompetent genotypes may result in different phenotypes depending on nutritional or social stressors.

In free-living populations, the intensity of stress may vary greatly from one population to another depending on population density and habitat quality. The common lizard (*Lacerta vivipara*, Jacquin) is found in many different

habitats, and its population density varies greatly. Moreover, this lizard is the host of a common blood parasite (*Haemogregarina* sp.), which affects both life-history traits (Sorci *et al.* 1996) and physiological parameters of its host (Oppliger *et al.* 1996a; Oppliger & Clobert 1997). If stress decreases immunocompetence, a positive correlation between stress intensity and blood parasitism is predicted. Environmental stress may influence both the prevalence of the parasite in the population (i.e. the proportion of parasitized individuals of the population, equal to the frequency of infection) and the intensity of parasitaemia (number of parasites per infected host). The aim of this study was to examine the relationship between stress, stress hormone levels, and parasitism in a free-living reptile. We investigated: (i) the influence of environmental stress on plasma corticosterone levels, the development of parasitaemia in naturally infected adult *L. vivipara*, and the development of experimentally induced primary infection in yearling *L. vivipara* under laboratory conditions; (ii) the effect of habitat quality on parasite prevalence and the intensity of parasitaemia under seminatural conditions; and (iii) the association between lizard population density and both the prevalence and the intensity of blood parasites in wild lizard populations.

## MATERIALS AND METHODS

### Laboratory experiments, species, and parasitologic status

*Lacerta vivipara* is a widely distributed, small, live-bearing lacertid inhabiting peatbogs and heathland. Snout-vent length ranges from 50 to 70 mm for adults and from 30 to 50 mm for yearlings. A more complete description of the species biology can be found in Pilorge (1987). In June 1996, we captured by hand (visual and auditory detection) 70 yearlings of both sexes and 50 adult males in southern France (Mont Lozère, 44°30′N, 3°45′E, altitude 1450 m). Yearlings were chosen for the experimental infection to insure all individuals were of the same age and that none suffered the debilitating effects of chronic, long-term parasitism, as might occur in older individuals (Sorci *et al.* 1996). Adult males were chosen for the assessment of the effect of stress on plasma hormone levels (testosterone and corticosterone) and on the natural infection dynamics. At the time of capture, we toe-clipped each individual and collected a drop of blood to make a smear for analysing parasite load. After drying, each smear was fixed in methanol and stained with May-Grünwald Giemsa (Colorap de Bioréac, Lausanne). Stained slides were examined by oil immersion microscopy (500 ×) to enumerate blood parasites. Haemogregarine intraerythrocytic parasites are naturally widespread in *L. vivipara*. This protozoan (phylum Sporozoa) has a complex life cycle that involves a blood-feeding mite vector (Manwell 1977) belonging to the family Laelapidae (Mesostigmata). The mites are temporary ectoparasites, living in the soil and climbing on the host to take blood meals. Once in the circulating blood of the host, the protozoan parasites multiply by sporogony (Manwell 1977). We do not know whether, once infected, the lizard is capable of clearing an infection itself. On the other hand, infected lizards can show parasite multiplication without new infection. Parasitaemia of adult males was estimated in a semiquantitative categorical procedure. Regression analysis indicated high repeatability ($n = 24$, $F = 75.055$, $P < 0.001$, $r^2 = 0.773$). Parasitaemia was not affected by the time of day when the host's blood was sampled. Each parasitized individual was assigned to one of the following categories: (i) very rarely parasitized, i.e. one cell parasitized for 10,000–120,000 cells; (ii) rarely parasitized, i.e. 1/400–10,000 cells; (iii) moderately parasitized, i.e. 1/200–400 cells; (iv) strongly parasitized, i.e. 1/80–200; and (v) very strongly parasitized, i.e. 1/10–80 cells. Parasitaemia of experimentally infected yearlings was estimated more precisely, because we made our estimate in the early stages of infection when the intensity of parasitaemia was very low. A quantitative estimation of parasite load was obtained by counting the number of parasites observed per 10,000 red blood cells. A slide was considered as negative when after 5 min (about 300 fields of 400 cells per field) of examination, no parasite was observed. At the beginning of the experiment, body mass and snout-vent length (SVL) were measured for each lizard. To prevent horizontal blood parasite transmission (Sorci *et al.* 1997), all mites (the vector of the blood parasite) were removed from the lizard's skin, by scrubbing with ether soaked cotton. At the end of the experiment, lizards harboured no new mites.

*Development of natural infection under stress treatment*

Forty-four naturally infected adult males were randomly assigned to one of two experimental groups (two replicates per group): "moderately stressed" (MS) and "highly stressed" (HS).

MS lizards were grouped (11 individuals) in one large plastic terrarium (80 × 40 × 40 cm) with damp soil, many shelters, and water dishes. They were fed *ad libitum* with small crickets (*Acheta domesticus*). A heat lamp provided warmth 6 hours per day, allowing lizards to thermoregulate. Stress was induced via social interactions, which were far more frequent than in natural conditions due to crowding in the cages.

HS lizards were also grouped (11 individuals) as above, but to induce additional stress (i.e. other than crowding), only a few shelters were provided. Further stress was induced by feeding the lizard piece by piece during the

day, although the quantity of food provided per individual was the same in both groups. Therefore, each lizard had on average the same quantity of food regardless of treatment group, and crickets were typically still available in the terraria at the end of the day. We simulated predation five times per day by waving one hand in the terraria. A heat lamp was employed as above. After 1 month we again collected a blood sample to estimate the parasite load. One individual died during the experiment and was omitted from analyses.

*Development of experimental infection under stress treatment*

To assess the effect of stress on the development of a primary parasitic infection, we carried out an additional experiment in which we experimentally infected yearling lizards. Each parasite-free yearling was infected in the following manner: we collected 120 μl of parasitized blood by postorbital puncture of three heavily parasitized adult males; this mixed blood was then diluted in 5 ml of physiological saline; each experimental lizard received 50 μl of this dilution by intraperitoneal injection and 50 μl orally. This procedure was repeated three times over three consecutive days. Subsequently, lizards were randomly assigned to experimental groups, each containing 15 individuals. The groups were MS and HS with exactly the same experimental design as above. Unfortunately, for technical reasons we were unable to replicate these groups; however, the two experiments predict the same treatment effect (increase of parasite load), we considered the result of these groups relevant because the results are in the same direction as the other replicates. Moreover, we added a third group called "non-stressed" (NS). NS lizards were individually housed in plastic terraria (20 × 30 × 20 cm) with damp soil, a shelter, and a water dish. They were fed *ad libitum* and warmed as above. The only difference between the MS and the NS group is that NS lizards have no social interactions.

After 30 days, we again collected a blood sample to estimate the number of infected red blood cells. One stressed lizard died at the beginning of the experiment and was omitted from the analyses.

*Hormone level measurement*

Plasma hormone levels were assessed before and after the stress treatment in naturally infected adult male lizards. Blood samples were collected in heparinized microcapillary tubes from the infraorbital sinus behind the eye. Samples were immediatly centrifuged to separate the plasma. Once separated, the samples were frozen until they were analysed. Plasma levels of testosterone (T) and corticosterone (B) were measured by radioimmunoassay (RIA) following published procedures (Wingfield & Farner 1975; Moore 1986; Ball & Wingfield 1987). Briefly, samples of ≈30 μl were extracted twice with diethyl ether and dried under a stream of ultrafiltered air. Steroids were separated via Celite (Sigma, St. Louis, MO) chromatography after samples had been reconstituted in 10% ethyl acetate:90% isooctane (vol/vol). Recoveries of T and B were measured for each sample. After chromatographic separation, samples were dried, reconstituted in assay buffer, and allowed to equilibrate overnight prior to assay. For RIA, tritiated T and B were obtained from DuPont-NEN (Wilmington, DE), T antiserum from Wien Laboratories (Succasunna, NJ), and B antiserum from Endocrine Sciences (Calabasas, CA). Inter-assay variation was no greater than 10.2% (B) and 8% (T), and intra-assay variation was no greater than 8% (B) and 7% (T). Six lizards were omitted from hormone assays because insufficient blood was obtained from them.

## Seminatural experiment

In early summer 1995, 320 *L. vivipara* (64 adult males, 96 adult females, and 160 yearslings of both sexes) were collected at Mont Lozère and were brought to the biological station of Foljuif near Paris, France. After removing all external parasites as above, lizards were randomly distributed into 16 seminatural enclosures (10 m × 10 m), which were covered with nets to exclude avian predators. Each enclosure contained a population of six postgravid females and their offspring, four adults males, and 10 yearlings. Supplementary food (crickets, *Acheta domesticus*) was delivered every 2 days (for details on enclosures, see Lecomte & Clobert 1996). Eight of the enclosures were situated in an unfavourable habitat (wooded environment, dry sand-clay soil, sparse plant cover (10%), 5 h of sunshine a day), which we referred to as "poor habitat". The other enclosures were situated in favourable habitat (open field, wet clay-sand soil, dense and diversified vegetation cover (60%), 9 h of sunshine a day), which we referred to as "rich habitat" (Boudjemadi *et al.* submitted). Our choice of habitat was based on our *a priori* (and measured) knowledge of what is a good and what is a bad habitat for this species (Clobert *et al.* 1994). The differences between poor and rich habitats led to different survival rates, which resulted in a decrease of density in poor habitat enclosures 1 year later (density being decreased by 60% in poor habitat and being increased by 23% in rich habitat) (Boudjemadi *et al.* submitted).

Effects of habitat quality on blood parasite prevalence and parasitaemia were investigated 9 months after the populations had been established in the enclosures. Parasitaemia was estimated in a semiquantitative way as above. Parasite prevalence was computed as the proportion of hosts infected per enclosure. Densities in enclosures were determined by capturing all individuals.

### Field observation: population density and parasitism

We estimated the densities of yearlings in different populations during the summers of 1995–96. We estimated only the yearling densities, because it was the age class for which we had most data. Moreover, the yearling/adult ratio and population density tends to be constant (Pilorge 1987; Massot *et al.* 1992).

All populations studied were situated on Mont Lozère (France) at an altitude of 1410–1465 m. Populations were situated in a 10 $km^2$ area, and each population lived on a distinct area of 2000–3525 $m^2$. The seven populations resided in habitats that differed in the composition and structure of the vegetation. Four populations were situated in relatively dry habitats [vegetation height, 15–20 cm, mainly low shrubs (*Calluna vulgaris*), few small trees (*Pinus uncinata*), and broom plants (*Sarothamus scoparius*), very dry soil and exposed to wind]. The other three populations were situated in relatively wet habitats [vegetation height, 30–50 cm, mainly herbaceous plants (*Nardus strica*, *Molinia coerulea*), few small trees (*Pinus uncinata*), and broom plants (*Sarothamus scoparius*), very wet soil and wind-protected]. Lizards were hand-captured, and individually marked by toe-clipping. Lizards of one population were captured during two consecutive days, and 1 week later, a second 2 days session of capture-recapture occurred and provided data for density estimation. Densities were estimated using the Lincoln Petersen index corrected for small samples (Le Cren 1965; Chapman 1951). An approximately unbiased estimate of the variance of density was calculated according to Seber (1970). In this species, no heterogeneity was apparent in the capture probability (Lebreton *et al.* 1992; Massot *et al.* 1992).

Blood parasite prevalence and parasitaemia were examined as above in gravid females that were collected from the field in early July and subsequently transported to the laboratory for other experiments. The prevalence of blood parasites in gravid females was not different from that in males and was highly correlated to that in yearlings (A.O. unpublished data). Parasite prevalence in a population is constant between years (Sorci 1995).

### Statistics

*Laboratory experiments*

The effect of laboratory treatment on parasitaemia was analysed in a nested analysis of variance in which stress treatment was included as a main effect, while the effect of cage was nested in the main effect. The initial level of parasitaemia was included as a covariate (ANOVA, GLM procedure, SAS Institute 1990). Assumptions underlying the models were checked beforehand. Parasitaemia data of experimental infected lizards were log-transformed and hormone levels were transformed with Blom's correction (Blom 1958) prior to analysis.

*Seminatural experiments*

In the seminatural experiment, we analysed the effect of the habitat (poor or rich) on parasite prevalence and parasitaemia with a logistic regression model (SAS Institute 1990). We used the LOGISTIC procedure for the ordinal response of parasitaemia (i.e. six levels of parasite load) and the GENMOD procedure for the binary response of prevalence (i.e. presence or absence of blood parasites). Because the number of individuals per seminatural enclosure varied with time, we included "final density of enclosure" as a factor in the model.

*Natural populations*

In natural populations, we analysed the relationship between parasitaemia and host density with the LOGISTIC procedure, and we used the GENMOD procedure to estimate the relationship between prevalence and host density. Because the seven studied areas were situated in two different habitats ("wet" or "dry"), we included "habitat" as a factor in the model. The significance levels were determined from the change in deviance of the null model, after the addition of independent variables. The change in deviance is approximated by a $\chi^2$ distribution with corresponding degrees of freedom.

## RESULTS

### Laboratory experiments

*Effect of stress on natural parasite infections*

Before the experiment, there was no significant differences in the mean parasite load among the four cages (initial parasite load: mean $\pm$ SE = 2.45 $\pm$ 0.34; ANOVA, cage effect: $F_{3,39} = 1.21$, $P = 0.318$). After 1 month, the change in parasite load (final–initial parasite load) was significantly higher for the HS group than for the MS group (Fig. 1; ANCOVA using cages nested within stress treatment and stress treatment as factors and initial parasite load as covariate: treatment, $F_{1,38} = 6.35$, $P = 0.016$; cage, $F_{2,38} = 0.33$, $P = 0.719$; initial parasite load, $F_{1,38} = 2.76$, $P = 0.105$).

*Effect of stress on corticosterone and testosterone levels*

Before the experiment, there was no significant difference in the mean testosterone concentration among the cages (ANOVA, cage effect: $F_{3,33} = 1.1$, $P = 0.364$). However, in spite of the fact that animals were chosen randomly for each group, the HS group had a significantly higher level of corticosterone (ANOVA, stress treatment:

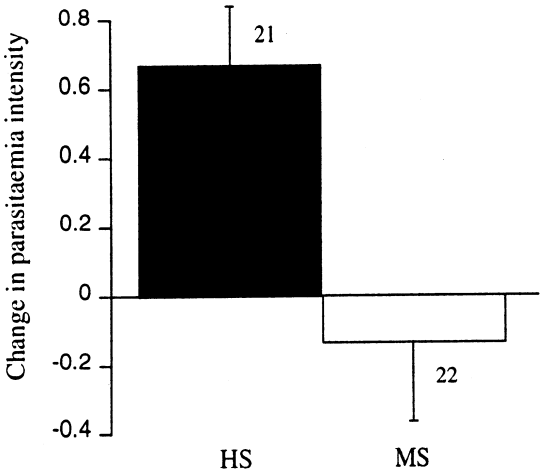


**Figure 1** Mean ± SE of the change in intensity of haemogregarine parasitaemia in naturally infected *L. vivipara* between 17 July and 21 August in response to different stress treatments. HS is the highly stressed group and MS is the moderately stressed group. Numbers beside bars indicate sample sizes.

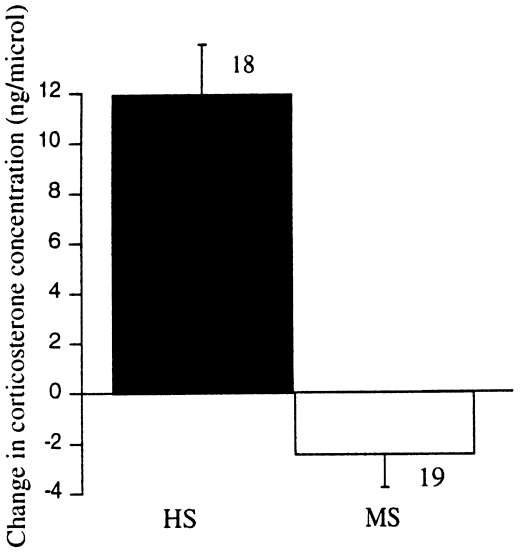


**Figure 2** Mean ± SE of the change in plasma corticosterone concentration between the 17 July and 21 August in naturally infected *L. vivipara* in response to different stress treatments. HS is the highly stressed group and MS is the moderately stressed group. Numbers beside bars indicate sample sizes.

$F_{1,35} = 12.42$, $P = 0.001$). After 1 month of stress treatment, testosterone level was not different between treatment groups, whereas final testosterone level depended significantly on the initial testosterone level (ANCOVA using cage nested within treatment and stress treatment as factors and initial testosterone level as covariate: treatment, $F_{1,32} = 0.28$, $P = 0.59$; cage, $F_{2,32} = 0.265$, $P = 0.086$; initial testosterone level, $F_{1,32} = 16.85$, $P = 0.0003$). On the other hand, after 1 month of stress treatment, the change in corticosterone level (final level of corticosterone–initial level of corticosterone) was significantly higher in HS than in MS groups (Fig. 2), and this was not due to an effect of initial corticosterone level (ANCOVA using cage nested within treatment and stress treatment as factors and initial corticosterone level as covariate: treatment, $F_{1,32} = 13.22$, $P = 0.001$; cage, $F_{2,32} = 0.85$, $P = 0.437$; initial corticosterone level, $F_{1,32} = 0.11$, $P = 0.744$).

*Effect of stress on experimental parasite infections*

Thirty days after the beginning of the experiment, only two injected lizards (from the NS group) did not develop a parasitaemia. NS individuals had on average fewer infected red blood cells than HS and MS individuals (Fig. 3). Two individuals that we could not sex were omitted from the ANOVA.

The mean percentage of infected red blood cells strongly differed among treatment groups and it was not dependent on SVL, sex, or interactions among these factors (ANCOVA with SVL as covariate, sex and treatment group as factors: treatment group, $F_{2,35} = 9.684$, $P < 0.001$; sex, $F_{1,35} = 0.422$, $P = 0.520$; SVL, $F_{1,35} = 0.750$, $P = 0.392$; "sex" × "treatment group", $F_{2,35} = 0.320$, $P = 0.729$). An *a posteriori* comparison showed that the HS

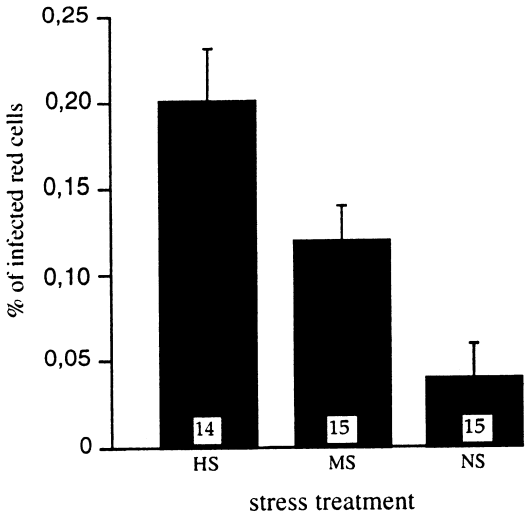


**Figure 3** Mean ± SE of the percentage of parasitized red blood cells observed 30 days after experimental infection of captive *L. vivipara* with haemogregarines in response to different stress treatments. HS is the highly stressed group, MS is the moderately stressed group, and NS is the nonstressed group. Numbers in bars indicate sample sizes.

and MS groups had significantly more infected red blood cells than the NS group, but there was no significant difference in the percentage of parasitized cells between the HS and MS groups (post hoc Fisher's LSD test; HS/NS, $P < 0.001$; MS/NS, $P = 0.003$; HS/MS, $P = 0.132$).

### Experiment in seminatural conditions

Habitat quality affected parasite prevalence. In poor enclosures 55.8% (24 of 43) of lizards were parasitized, whereas in rich enclosures only 36.4% (59 of 162) of individuals were parasitized. Moreover, the infected lizards in poor enclosures were significantly more intensively infected than the infected lizards in rich enclosures (Fig. 4; $\chi^2 = 3.96$, d.f. = 1, $P = 0.046$). Analysis of the relationship between prevalence and type of enclosure (poor and rich) indicated that parasite prevalence depended on the type of enclosure (logistic regression model, $\chi^2 = 5.219$, d.f. = 1, $P = 0.022$). Final density (= number of individuals per enclosure remaining alive 9 months after the beginning of experiment) and the interaction between enclosure type and final density were nonsignificant in their effect on prevalence (final density, $\chi^2 = 0.772$, d.f. = 1, $P = 0.379$; enclosure type × final density, $\chi^2 = 0.735$, d.f. = 1, $P = 0.390$). Final density had no detectable effect on parasitaemia ($\chi^2 = 1.27$, d.f. = 1, $P = 0.259$).

Nine months after the introduction of 10 adults (plus their offspring) and 10 yearslings into each enclosure, final density significantly decreased in poor enclosures ($7.5 \pm 2.6$ SE; paired $t$ test, $N = 8$, $t = 12.500$, $P < 0.001$). Final density in rich enclosures did not differ from the initial density ($24.750 \pm 2.67$ SE; paired $t$ test, $N = 8$, $t = -1.304$, $P = 0.234$).

### Natural populations

Parasite prevalence increased with increased population density (Fig. 5). Analysis of the relationship between parasite prevalence (318 individuals examined) and host density indicated density was significantly positively correlated with parasite prevalence ($\chi^2 = 25.92$, d.f. = 1, $P < 0.001$). Habitat and the interaction between habitat and density had no detectable effect on prevalence (habitat, $\chi^2 = 0.50$, d.f. = 1, $P = 0.49$; habitat × density, $\chi^2 = 0.01$, d.f. = 1, $P = 0.90$), indicating that the effect of density was consistent across the habitats. Parasitaemia also significantly increased with increased population density (Fig. 6; $\chi^2 = 22.66$, d.f. = 1, $P < 0.001$). Habitat had no detectable influence on parasitaemia ($\chi^2 = 2.53$, d.f. = 1, $P = 0.11$).

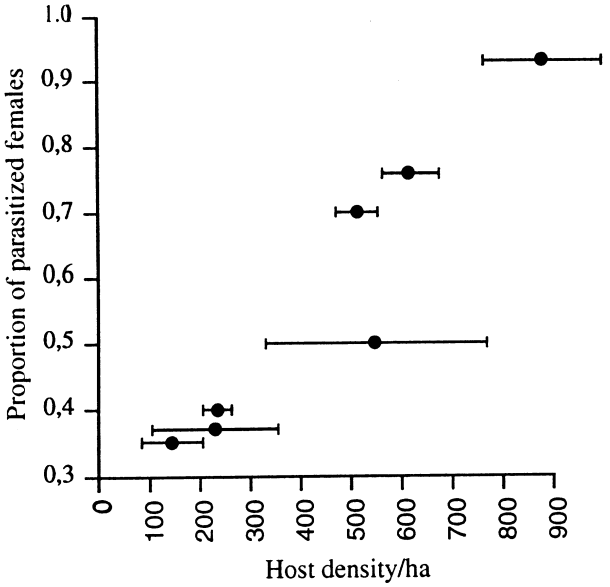


**Figure 5** Relationship between the prevalence of haemogregarines and the estimated natural population density of *L. vivipara*. Each point represents one population. Density estimates = number of individuals/ha and confidence interval (1.96 × SD).

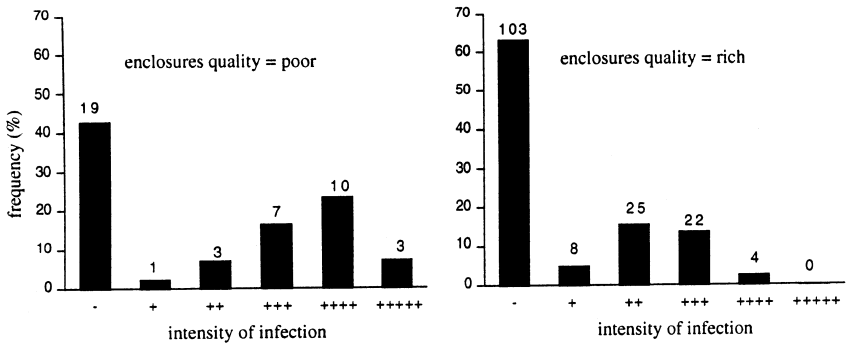


**Figure 4** Frequency (%) of *L. vivipara* in "poor" and "rich" seminatural enclosures remaining alive 9 months after the establishment of 20 individuals per enclosure (eight enclosures of poor quality and eight enclosures of rich quality). Abcisse represents the intensity of natural infection by haemogregarines: –, noninfected; +, very rarely infected; ++, rarely infected; +++, moderately infected, ++++, strongly infected; and +++++, very strongly infected (for details see *Materials and Methods*). The numbers above the bars represent the number of individuals

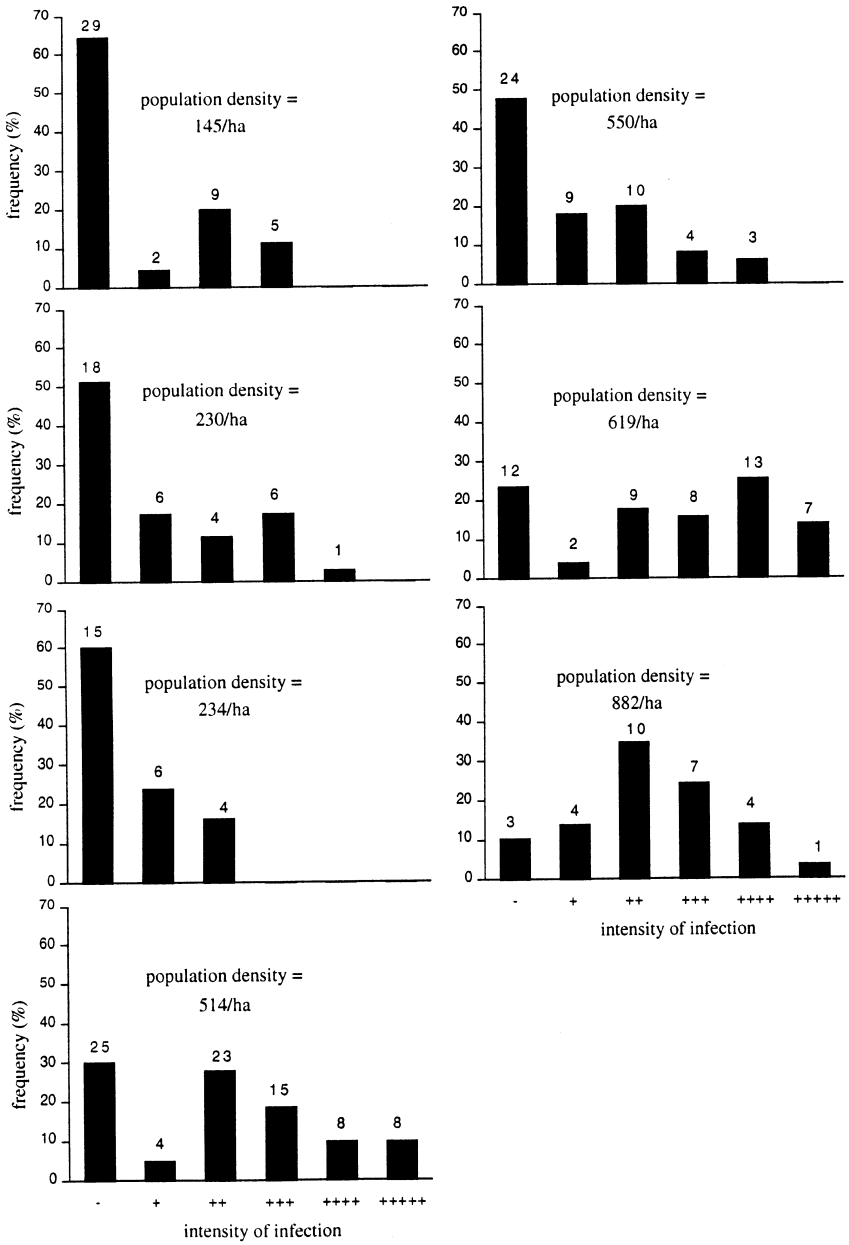


**Figure 6** Frequency (%) of female *L. vivipara* naturally infected with haemogregarines in seven populations of different density (number of individuals/ha). Abcisse represents the intensity of infection by haemogregarines: –, noninfected; +, very rarely infected; ++, rarely infected; +++, moderately infected; ++++, strongly infected; and +++++, very strongly infected (for details see *Materials and Methods*). The numbers above the bars represent the number of individuals sampled for the assessment of parasite load.

## DISCUSSION

Before our detailed discussion, we summarize the main results: (i) under laboratory conditions, stress factors affected corticosterone levels and parasite load; (ii) when stress level was manipulated by imposing the same population density in enclosures of different quality, the individuals remaining alive after 9 months in the poor quality enclosures were more frequently parasitized and had a higher parasite load per individual than those remaining alive in the rich quality enclosures; and (iii) in natural populations, habitat had no significant influence on parasitaemia and parasite prevalence, but we found that both parasite prevalence and intensity of parasitaemia increased with increased host population density.

### Effect of experimental stress on parasite load

Our results demonstrate that environmental stress can have a strong effect on the prevalence and intensity of parasitism in hosts. Lizards subjected to several stressors (predation risk, food competition) showed a greater increase in parasitaemia than controls. Furthermore, parasitaemia in experimentally infected lizards subjected to crowding was significantly higher than in noncrowded lizards. In addition, we found an increase in plasma corticosterone

in stressed lizards, thus indicating the effectiveness of the stress treatment. In the stress experiment, as noted previously, pre-experimental corticosterone levels of the high stress group were significantly greater than those of the low stress group. Animals in the high stress group could have been more immunosuppressed prior to the experiment, and this precondition could have influenced the differences in development of parasitic infections. This possibility does not invalidate the results of our experiment, however, as the increase in plasma corticosterone as a result of the experimental treatment was greater in the high stress than in the low stress group.

Physiological costs of parasitism have been demonstrated previously in *L. vivipara* (Oppliger *et al.* 1996a; Oppliger & Clobert 1997). For example, infection with haemogregarines reduces haemoglobin concentration, reduces locomotor performance, and lowers the rate of tail regeneration. Therefore, the increase in parasite susceptibility induced by environmental stress is likely to have important consequences for these lizards. Direct effects of social stress on susceptibility to parasitic infection have also been demonstrated in laboratory mice (Barnard *et al.* 1993, 1994, 1996; Smith *et al.* 1996). Several recent studies have shown a trade-off between reproductive effort (thus, nutritional status and, presumably, stress level) and defense against parasites (Festa-Bianchet 1989; Richner *et al.* 1995; Oppliger *et al.* 1996b; see also review by Sheldon & Verhulst 1996). The biochemical mechanisms underlying this trade-off remain poorly understood, but it has been suggested that immune responses to parasitism are costly and may be reduced during periods of stress. A negative interaction between the endocrine and immune system was found in several studies (Grossman 1988; see also review by Finch & Rose 1995). In particular, it has been shown that corticosterone is intimately linked to immune function (Besedovsky *et al.* 1986). Schall (1996) has shown that in a lizard species, infection itself can cause increased production of stress hormones. Corticosterone is secreted under stress situations and may be the common mechanism that links all stressors (malnutrition, reproductive effort, social interactions) to immunosuppression. The adaptive role of such a mechanism could be explained in the framework of resource reallocation (Folstad & Karter 1992; Wedekin & Folstad 1994): Because of the benefits of the stress response (e.g. a quick response to any given danger or conflict), animals may be forced to reallocate resources among functions with competing demands and consequently deprive the immune system of essential metabolites.

An important implication of our experiments is that any disturbance of wild animal populations could be sufficiently stressful to suppress the immune response and therefore modify the intensity of diseases and the survivorship of the host population. This is potentially very important, especially because we know that parasites can act as selective agents (Combes 1995; Begon *et al.* 1996). Results are also intersting in the context of conservation biology, because for instance habitat destruction usually affects density and the levels of infection (Holmes 1996).

Our experimental stress study was carried out under controlled laboratory conditions. To be more realistic, experiments need to be conducted under more natural conditions.

### Effect of habitat quality on blood parasitism

The experiment under seminatural conditions showed an effect of habitat quality on both the intensity and the prevalence of parasitism. Indeed, when we manipulated environmental stress by using enclosures of poor and rich quality, we observed positive correlations between habitat quality and (i) the prevalence of blood parasites and (ii) the intensity of parasitaemia. This relationship was noted 9 months after the establishment of lizards in the enclosures. The observed effect was two-fold. Lizard density decreased in poor quality enclosures, and we observed an increase in parasite prevalence and parasitaemia when compared with the rich quality enclosures. There are two possible explanations for these observations. First, survivorship may have been greater in parasitized than nonparasitized individuals inside poor quality enclosures. Second, increased environmental stress may have led to higher levels of parasitism and mortality in low quality compared with high quality enclosures. Better survival of parasitized compared with nonparasitized lizards seems unlikely. We therefore suggest that the level of stress encountered in poor quality enclosures led to immunosupression in these lizards. Environmental stress was certainly not induced by competition for food because we provided sufficient crickets. We believe that animals were probably stressed by competition for other important limiting factors (shelters, basking sites, water sources). In this scenario, parasitism may not be the primary cause of population regulation, but it may well contribute to population limitation as a secondary factor. Stress induced by density could decrease the immune response and increase parasite numbers, with potential deleterious effects for the host individuals (Oppliger *et al.* 1996a; Sorci *et al.* 1996; Oppliger & Clobert 1997).

### Correlation between density and parasitism

Field observations showed that both parasite load per individual (parasitaemia) and parasite prevalence (proportion of infected lizards) significantly increased with the population density of the host. One possible explanation is that the probability of parasite transmission (Sorci *et al.* 1997)

increases with the density of hosts, leading to an increase in parasite prevalence. Theoretical frameworks for host–parasite systems assume that the probability of an uninfected host becoming infected is density or frequency dependent (May & Anderson 1979; Anderson & May 1979; Getz & Pickering 1993). Density dependence may play a role in our field sites; however, this would not apply to our laboratory experiment, which showed the same link between density and parasite prevalence in the absence of parasite vectors.

The demographic consequence of the link between parasite prevalence and host density must be interpreted with caution. In populations of *L. vivipara*, density does not vary much from one year to the next (Massot *et al.* 1992) and no population cycles have been observed. Furthermore, factors such as the length of the activity season, food availability, habitat characteristics, or predation rate (Massot *et al.* 1992), appear to be more important than this blood parasite in determining the density of lizards in the populations we have studied. However, this does not imply that blood parasites do no affect these populations. Indeed, theoretical and empirical studies have addressed ways in which parasites could modify host life-history strategies (Minchella & Lo Verde 1985; Hochberg *et al.* 1992; Forbes 1993; Perrin *et al.* 1996; Sorci *et al.* 1996; Møller 1997). Moreover, empirical data have shown that maternal ectoparasite load of common lizards was associated with certain offspring life-history traits (Sorci & Clobert 1995). Haemogregarines may influence age-dependent host mortality in common lizard populations (Sorci 1996) and thus act as a "senescence" factor (Michalakis *et al.* 1992). In this case, we would expect to observe modifications in life-histories among populations of *L. vivipara* in response to the intensity of parasite prevalence. For example, females from dense populations (with high parasite prevalence) might be predicted to reduce the impact of parasites by investing more in reproduction during the earlier life stages compared with females in sparser populations. In return, old females from dense populations should suffer higher mortality rate than females from less dense populations (Sorci *et al.* 1996). Therefore, we should observe different life-history strategies (trade-off between earlier reproductive investment and survival) varying with population density. As yet, no data concerning life-history traits of the studied populations have been collected, and research along this line is in progress.

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## BIOSKETCH

Anne Oppliger's research inrerests include host–parasite interactions, sexual selection, and sperm competition.

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