Blood parasite infection causes marginal temporary costs in juvenile birds of prey

Tony Rinaud*[1], Meinolf Ottensmann[1], Tim Maximilian Rapp[1], Hugo Pereira[1], Kai-Philipp Gladow[1], Oliver Krüger[1], Nayden Chakarov[1]

[1] Department of Animal Behaviour, Bielefeld University, Bielefeld, Germany

*** Correspondence:**

Tony Rinaud

Department of Animal Behaviour

Bielefeld University

Morgenbreede 45

33615 Bielefeld

E-mail: tony.rinaud@uni-bielefeld.de

Orcid ID: https://orcid.org/0000-0003-3811-2270

Abstract

Physiological costs from parasites arise by host colonization and defence activation and can vary according to the interactions of host and parasite traits and states. Parasite-induced costs crucially differ between stages of infection but this is difficult to assess in wild vertebrates. To evaluate the effects of blood parasite infection in juvenile birds, we compared physiological measures of common buzzard nestlings (*Buteo buteo*) between stages of infection with *Leucocytozoon toddi*, a *Plasmodium*-like pathogen. We related proxies of infection damage to experimentally manipulated infection intensity. We expected infection costs to be higher at the onset of infection and during peak parasitemia, compared with hosts with decreasing parasitemia and uninfected ones. We found body condition to be initially negatively related to infection intensity, but this relationship disappeared by the late stages of infection. Furthermore, there was no difference in growth rate and other physiological measures among infection stages. This indicates negligible costs of parasitism and transient virulence of *Leucocytozoon* in the nestling stage of host. To diminish infection-driven mortality, juveniles may evolve to be particularly parasite-tolerant which further enhances parasite transmission in the population. Our results demonstrate the necessity of including infection courses, rather than point estimates in models of fitness costs of infection.

Keywords: Avian malaria, bird of prey, host-parasite interactions, physiology, infection costs

Background

Parasites are defined by exploitation of foreign resources, resulting in negative short- and long-term fitness effects on hosts (1–4). Parasite infections often directly affect host survival and reproduction through diverse lethal and sub-lethal effects on host physiology, behaviour and ecology (5–7). They can also exacerbate indirect effects of the abiotic environment (8,9), intra- or interspecific interactions (10), and other symbionts (11). Such combined detrimental effects may result in high fitness costs in juvenile animals, because the immaturity of juvenile defences can facilitate high intensities of infection (12–15). During early stages of host development, adaptive immunity has not fully matured yet. This may reduce the risk of pervasive autoimmunity but leaves juveniles more susceptible to, and slower at suppressing parasites compared with adults (16–18). Additionally, young hosts often rely on parental care and are less mobile (19), hence are not only susceptible but also particularly accessible for vectors until mobility is achieved. This provides a

particularly suitable ecological niche for parasites, whose probabilities of transmission and successful establishment in hosts are enhanced under these conditions. Correspondingly, some infections are particularly common and intense during "childhood" (12,20). Early life host-parasite interactions can therefore have important consequences for host survival and parasite transmission but are still not well understood in the wild. On the other hand, because severe fitness costs to juveniles in pre-reproductive age would lead to the dying out of susceptible host demes, a weaker juvenile immunity combined with high infection probabilities may also force parasites to become less virulent and evolve toward benign forms of symbiosis approaching commensalism.

In many endoparasites and infection models, upon host invasion, parasitemia (i.e. the proportion of infected host tissue) usually (1) first increases, (2) reaches a plateau/peak and then (3) decreases rapidly (Fig. 1) (21,22). When the adaptive immunity succeeds to mount a specific response, parasitemia decreases and (4) stabilizes at low chronic levels, usually followed in the long term by relapses or effective clearance (Fig 1) (21–23). Further on, we term these time periods of individual infections progressions "infection stages" (in contrast to tissue-specific parasite developmental stages). Due to tissue damage, parasite-driven resource depletion and inflammation, the physiological costs of infection can be expected to be immediate, i.e. to be highest during the pre-patent and acute stages of infection (i.e. shortly before and during peak parasitemia), rather than during chronic and low parasitemia when gradual recovery ensues (24–26). However, an open question remains whether this results in short-lived immediate costs, delayed costs of recovery, or both.

Vertebrate haemosporidian parasites mostly follow the model of infection progression outlined above but are known particularly because of several group members, which are significant disease and mortality agents. Five species of *Plasmodium* cause substantial pathology and mortality in humans, particularly in children. The sister clades *Haemoproteus* and *Leucocytozoon* have been suggested to play similar roles in wildlife (9, 21). Parasitic cells, merozoites released into the blood invade different types of blood cells (21,28) and blood parasitemia increases until reaching peak infection, which can last several days. While it has been shown that these parasites can cause tissue and organ dysfunction in hosts, the frequency and severity of these problems among wild hosts as well as factors which modulate them in nature are still unknown (21,24).

In this study, we performed a field experiment to examine the effects of blood parasite infection and used physiological measures as informative proxies of costs of infection in nestlings of wild common buzzards, *Buteo buteo*. In our study population, the prevalence of *Leucocytozoon toddi* before fledging of nestlings surpasses 50% (29,30). We recorded and manipulated the levels of parasitemia at two time points to evaluate how they affect physiological states and development and thus to reveal potential immediate infection- and immunity-related costs to hosts. We predicted immediate parasitic costs in nestlings, i.e. higher infection costs during challenging parasitemia stages (increasing and peak) compared with nestlings with decreasing parasitemia and uninfected ones. Additionally, we expected that, as the number of infected cells increases, the severity of costs will positively correlate with host parasitemia.

Methods

(a) Host-parasite system

Common buzzards (*Buteo buteo*) are accipitriform birds of prey that breed in temperate Eurasian forests, in tree nests at heights between 10 to 30 m. Common buzzards are long-lived (up to 29 years in the wild) and the most common accipitriform in Europe. They have the highest prevalence of blood parasites of the genus *Leucocytozoon* among sympatric raptor species (30). *Leucocytozoon toddi* (Eukaryota, Protista, Haemosporidiae, lineages MILANS04, MILVUS01, BUTBUT03) is by far the most common blood parasite in our study population of common buzzard (Chakarov, unpublished). The vector-borne transmission of *Leucocytozoon* has been suggested to mostly occur in a quasi-vertical direction (31). Black fly vectors (Simuliidae) may first suck blood from infected parents at the nest site and predominantly transmit the same genetic pool of parasites to their offspring (31). Prevalence of infection varies significantly across years, with a minimum of 13.6% in 2014, a maximum of 68.2% in 2020 and a mean of 44.2% from 2005-2020 in our long-term dataset on prevalence.

(b) Data collection

The study was performed in a 300-km² study area in North Rhine-Westphalia, Germany (8°25’ E and 52°06’ N) as part of a long-term study that started in 1989. From 2016 to 2020, 276 common buzzard nestlings (n = 32, 65, 71, 108 in 2016, 2018, 2019, 2020 respectively) were sampled. All

individuals were resampled on average eight days after first sampling (T_0 thereafter, mean ± s.d. = 8.36 ± 4.21 days, Fig. 1). At both sampling points, nestling age was estimated using a sex-specific polynomial regression between age and wing length, based on growth data for buzzards of known age (32). The average estimated age of nestlings at T_0 was 19.47 days (± s.d. 5.01) and was 27.84 days (± s.d. 5.36) at second sampling (T_1 thereafter), an age where most nestlings have already encountered *Leucocytozoon* and hence infections are microscopically visible (31). Blood smears were screened by microscopy for *Leucocytozoon toddi* infection at T_0 and T_1. Approximately 10.000 erythrocytes were scanned in thin blood smears and parasitemia at T_0 and T_1 was categorized as follows: not infected (no detectable parasites), parasitemia 1 (1–10 parasites per 10.000 erythrocytes), parasitemia 2 (>10–100 parasites per 10.000 erythrocytes); parasitemia 3 (>100–1000 parasites per 10.000 erythrocytes) and parasitemia 4 (>1000 parasites per 10.000 erythrocytes). Categorization of parasitemia allowed us to test for potential non-linear relationships between parasitemia and physiological parameters. Among the sampled nestlings, 66% (N=183) were infected during at least one of the two time points, whereas 34% (N=93) were not infected at both time points; only 23 nestlings naturally displayed decreasing parasitemia between both sampling points. An artificial treatment of decreasing parasitemia was achieved by giving 11mg of antimalarial medicine (Malarone™; Atovaquone and Proguanil Hydrochloride, GlaxoSmithKline, UK, range: 7-21 mg/kg) diluted in 0.5mL of distilled water to a random subset of sampled nestlings in 2018 (n = 14), 2019 (n = 41) and 2020 (n = 36). This uniform dose was non-toxic but higher than the usual weight-adjusted dosing, thus a uniform efficiency on parasites was assumed (33,34). Control nestlings represent two groups, nestlings that did not receive antimalarial medicine and nestlings that were orally given 0.5 mL of distilled water. Both groups did not differ in average physiological parameters nor in *Leucocytozoon* intensity, hence these individuals were pooled together in the control group, sampled in 2016 (n = 32), 2018 (n = 51), 2019 (n = 30) and 2020 (n = 72). Treated and control groups were on average 28.28 days (± s.d. 4.23) and 27.57 days (± s.d. 5.80) old at T_1, respectively. Similar treatments have been previously shown to substantially reduce the prevalence of *Plasmodium* in bird populations (5,34,35). According to the change in parasitemia between both sampling points, the 276 nestlings were separated into four groups (Fig. 1): (i) uninfected nestlings (no apparent infection at both samplings, n = 93), (ii) increasing infection (n = 92), (iii) peak infection (i.e., constant parasitemia

and >10 parasites per 10.000 erythrocytes at both samplings, n = 24) and (iv) decreasing infection (n = 67).

Across the study, 22 nestlings were resampled a third time. A consistent infection trend was observed for 77% of them, a when comparing the infection trend from first with either second or third sampling point (eight remained uninfected, six remained with increasing parasitemia, one remained at peak parasitemia and two remained with decreasing parasitemia), showing that two sampling events produce robust estimates of infection trend of *Leucocytozoon toddi* in more than two thirds of sampled individuals. The remaining five individuals (23%) of these 22 nestlings differed in their infection development between first and either second or third sampling. The stage of two nestlings changed from increasing parasitemia to stable parasitemia, one uninfected nestling changed into increasing parasitemia, another one changed from decreasing to peak parasitemia and one from decreasing parasitemia to stable, low parasitemia.

(c) Cost-indicative physiological parameters

At T_0 and T_1, we measured the body weight (to the nearest 5 g) with a spring scale, the wing length (to the nearest mm) with a wing ruler and the respiratory rate (duration of 30 breaths in seconds) of each nestling. The cloacal temperature (henceforth body temperature, measured with an electronic thermometer) was recorded in 2019 and 2020. The repeatability of the temperature measures was high: $R^2 = 0.91$ (CI = 0.81 – 0.96, $P < 0.001$), calculated from 27 paired measures taken 30s apart on both adults (> 2 calendar years, with unknown precise age) and nestling common buzzards (age: 20- 30 days, mean: 40.35°C, s.d. ± 0.39°C). To control for an ambient temperature effect, the average daily temperatures of the sampling days were obtained from the NASA POWER Project (36). Growth rate per day was calculated as the weight change between T_0 and T_1 divided by the difference in days separating the two measurements. The body condition index of a nestling was estimated for each sampling event as the residual variance of the sex-specific linear regression between weight and wing length (log-transformed), based on standard growth data of common buzzard nestlings (32). To account for state-dependent development of individual body condition, we used either body condition at T_1 or change in body condition (later ΔBC) between T_1 and T_0 as a proxy of costs in our models.

(d) Statistical analyses

We fitted a linear mixed model to estimate the effect of antimalarial treatment on the parasitemia of nestlings, adding year and interval between samplings as covariates and nest ID as a random factor. As suggested by Råberg et al. (2007), the comparison of slopes of a regression between host health and parasite load is a measure of tolerance, e.g. the steeper the slope, the steeper the health proxy decreases per unit of parasite load - the less tolerant the host. We hence estimated, at each infection stage (increasing, peak and decreasing parasitemia), the slope between a fitness-informative trait (ΔBC) and parasitemia. Slopes were compared among infection stages and to zero using the *emmeans* (38) package in R 4.0.2 (39).

To examine the relationship of different cost-related physiological parameters to (i) host infection stages and (ii) parasitemia, we used linear mixed models fitted by REML, implemented in lme4 (40). We fitted four models with breathing rate and body temperature at T_1 as well as body condition at T_1 and growth rate between T_0 and T_1 as the response variables, respectively. As fixed factors, we specified either (i) the infection stage or (ii) the parasitemia, as well as sampling interval (in days), year of sampling, sex and age. To account for a potential effect of the anti-malarial treatment on the response variables, we included treatment (antimalarial treatment versus control) as fixed factor in all models, although a previous analysis showed no effects of Malarone administration on body condition, growth rate and blood chemistry of the same population of buzzard nestlings (33). Numerical explanatory variables were standardized using a z-transformation (41). Nest ID was fitted as a random factor in all models to consider nestling relatedness. All models are described in detail in Supplementary Table S1.

Results

(a) Efficiency of the antimalarial treatment

The proportion of infected nestlings remained stable in the antimalarial treatment group, while in control nestlings it increased between T_0 and T_1 (Fig. 2a). The antimalarial treatment significantly reduced the parasitemia of treated nestlings (Treatment [Malarone]: est. = -1.77, *s.d.* = 0.28, *df* = 177, *t* = -6.45, *P* = 1.03e-9, Fig. 2b). Initial parasitemia in treated nestlings was higher than in control nestlings (permutation two sample t-test, mean ± *s.d.*: Control = 0.86 ± 1.34, Malarone =

1.58 ± 1.57, *perm.*: 999, t = -3.97, P = 0.002, Fig. 2b). This corresponded to an age difference between treated nestlings compared to control nestlings (*mean* = 21.4 days and 18.5 days, resp., t = 5.21, df = 225.44, P = 4.30e-07). At T_1, the age difference was not significant anymore (t = 1.31, df = 223.28, P = 0.191).

(b) Transient effect of parasitemia on host health throughout infection cycle

Overall, nestling Δ body condition (ΔBC, change between T_0 and T_1) correlated negatively with parasitemia (est. = -7.02, *s.d.* = 3.16, df = 274, t = -2.22, P = 0.027). This correlation changed, however, depending on the stage of infection - it was negative in nestlings with increasing (est. = -24.08, *s.d.* = 5.99, df = 177, t = -4.02, P < 0.001) and peak infections (est. = -41.80, *s.d.* = 23.47, df = 177, t = -1.78, P = 0.054) but did not correlate in nestlings with decreasing parasitemia (Fig. 3, Supplementary Table S2). Moreover, the slopes of ΔBC against parasitemia tended to be different between increasing and decreasing infection (Supplementary Table S2).

(c) Unchanged breathing rate across infection stages and intensities

Breathing rate did not correlate with infection stage or parasitemia (Table 1a & 2a respectively, fig. S4a). Heavier nestlings were breathing more slowly, regardless of their infection status and age (est. = -2.62, *CI* = -4.20 – -1.04, df = 252, t = -3.27, P = 0.001). The breathing rate of nestlings correlated negatively with ambient temperature (est. = -2.97, *CI* = -4.15 – -1.79, df = 126, t = -4.98, P < 0.001, Supplementary Table S3a). Breathing rates in 2018 and 2020 were significantly lower than in 2016 (Supplementary Table S3a). There was no significant effect of the antimalarial treatment on the breathing rate (Supplementary Table S3a).

(d) Lower body temperature at highest infection intensity

Body temperature did not differ among infection stages (Table 1a). However, body temperature decreased as parasitemia increased, due to a significantly lower body temperature of nestlings with acute parasitemia (level 4) compared with uninfected nestlings (est. = -0.78, *CI* = -1.34 – -0.23, df = 149, t = -2.79, P = 0.006, Table 2a, Fig. 4b). Body temperature of nestlings increased with ambient temperature and decreased with increasing time between both samplings. There was no effect of the antimalarial treatment on the body temperature (Supplementary Table S3a).

(e) Body condition decreased with higher infection intensities but not stages

Body condition did not differ among infection stages (Table 1b). Nestlings with acute peak infections (level 4) had a lower body condition compared with uninfected nestlings (Fig. 4c, est. = -43.99, *CI* = -82.92 – -5.05, *df* = 258, *t* = -2.22, *P* = 0.027, Table 2b). Nestlings at T_1 appeared to be leaner (i.e. apparent lower body condition) than at T_0. Nestlings had a significantly higher body condition in 2018, 2019 and 2020 compared to 2016 (Supplementary Table S3b). Age and the antimalarial treatment showed no effects on ΔBC (Supplementary Table S3b).

(f) Lower growth rate associated with highest infection intensities

Growth rate of nestlings did not differ between different infection stages after accounting for potential confounding factors, despite a non-significant reduction of growth in later infection stages (Table 1b, Supplementary Fig. S1d). Nestlings with highest parasitemia (level 4) tended to have lower growth rates than uninfected nestlings (Table 2b, Fig. 4d). Females had higher growth rates than males. Growth rate decreased with age of the nestlings, as they were approaching adult size. Growth rate tentatively appeared higher during the last year of the study and when the time interval between both samplings was longer (Supplementary Table S3b). Finally, there was no evidence for a difference in growth rate between antimalarial-treated and control nestlings.

Discussion

Host responses to parasite infections receive great interest because of their potentially crucial consequences for host fitness (42). However, to understand which responses are effective and adequate, specific measurements for a variety of host-parasite systems beyond the laboratory models are needed. Haemosporidian blood parasites are often considered pathogenic, even though the generality of evidence among hosts groups remains equivocal (43,44). Here, we recorded and experimentally manipulated infections of the main blood parasite in relatively slowly developing young hosts to assess infection-related changes in several physiological and developmental measures. Multiple samplings of the same individuals allowed us to estimate the stage along the course of infection for each of them. Some parasites have been shown to impair host body condition and growth rate, often through costs of defence deployment (11,45,46). In turn, these effects may lead to higher predation, starvation and inferiority of infected hosts in intra- and inter-

specific interactions (10). Altogether, consequences of parasite infections can impair the survival probability of infected hosts, but before labelling an ecological interaction as parasitism, costs have to be demonstrable (27,47).

Our results suggest that young common buzzards generally do not display parasite-induced costs during the course infection. Only in cases of acute infection (extraordinarily high levels of peak infection), nestlings appeared unable to completely maintain thermoregulation, body condition and growth rate. Substantial damage and population-wide mortality due to exceptionally high *Leucocytozoon* parasitemia have been reported in some populations of mostly non-coadapted hosts (18,21,48,49). However, our results indicate that while possible, costs of parasite exploitation and immune activation occur rarely in young birds of prey. While some species of blood parasites form large tissue stages (megalomeronts) which might be most strongly correlated with tissue damage and infection severity (50), others, including raptor-specific *Leucocytozoon*, typically do not appear to produce such stages (51). The moderate effects of *Leucocytozoon* on raptor nestlings are supported by recent studies showing that these parasites do not induce blood chemistry changes indicative of tissue damage (30). Future research across host-parasite associations is needed to test whether parasitic lineages lacking such tissue stages have a longer coadaptation history and are possibly as a result more benign for the hosts.

We found no difference in breathing rate among nestlings in different stages of infection and uninfected nestlings. This suggests that *Leucocytozoon* do not cause substantial anaemia in nestlings, which could reduce the physiological oxygen availability and lead to respiratory complications (26,52). Blood parasite genera differ in their life cycle with potential relevance in this aspect - *Plasmodium* multiply within red blood cells causing them to burst (schizogony) whereas *Leucocytozoon* and *Haemoproteus* only make infected blood cells weaker and/or more susceptible to immune defences (50). Both comparative and experimental studies are needed to discern if breathing rate remains unchanged only in young altricial hosts, which are not challenged through flight and other intense muscle use, or if this also applies to free-ranging hosts during peak infection. However, in certain host groups such as raptors (own observations), the majority of primary infection peaks are concentrated during the immobile nestling phase which may both open opportunities but also restrict the evolutionary paths parasites can take.

Thermoregulation failure as a typical inflammatory response is common in hosts infected by blood parasites (25,26). Such responses have been found only in bird-*Plasmodium* systems where fever and hypothermia appeared after parasite inoculation in captive birds (25,26). Indeed, buzzard nestlings with acute infections displayed lower body temperatures by on average 0.8°C, potentially reflecting hypothermia. However, no difference in body temperature was found among infection stages, suggesting that most hosts pass through all stages of infection without their thermoregulation being compromised.

As several different organs and tissue types may be targeted during infection with blood parasites, host body condition can be expected to deteriorate while parasites increasingly invade host cells (25,53). Nevertheless, body condition only appeared lower in nestlings experiencing acute infections, while there was no difference between infection stages. Thus, symptomatic costs of infection appear to be paid immediately and are only noticeable over rare and short periods when infections are extraordinarily intense. Nestling body condition predicts juvenile survival in large bird species (16). Consequently, if chicks should not be able to compensate for condition loss before independence, acute infections of nestlings may contribute to delayed increase of juvenile mortality.

Growth of infected nestlings did not differ compared to uninfected nestlings, even though it appeared to slow down during consecutive stages of parasitic infection, irrespective of age. Nestlings bearing a high parasitemia tended to display lower growth rates than other groups. Thus, our results suggest that parasitic costs are not apparent at every stage of infection (Fig. 3) but only at highest levels of parasitemia (Fig. 4), which occur in only few individuals. Furthermore, body condition, our best proxy for fitness-relevant host health, decreased with parasite load but this relationship turned around in later stages of infection (Fig. 3). This indicates immediate costs for hosts bearing intense infections and symptoms of infection occurring only during poorly controlled parasitemia. Differing slopes of host health to parasitemia (Fig. 3, table S2) between nestlings with increasing and decreasing parasitemia suggests resilience to infection, i.e. condition being regained after onset of adaptive immunity (after peak, already during decreasing parasitemia). Therefore, the overall effects of *Leucocytozoon toddi* infections in buzzards can be considered mild. Thus, the timing of infection in raptors and similar big birds may be adaptive, as parental care provides a buffer against potentially but rarely cost-intense, short periods of peak infection (21,54).

The apparent low virulence, together with the mostly absent physiological responses to infection suggest that host and parasite may have defused the co-evolutionary arms-race by reaching widespread transmission to the immunologically naïve and susceptible but tolerant nestling stage and thereby a different equilibrium to parasites of the genus *Plasmodium*. Unlike generalist parasites, parasites similar to raptor-specific *Leucocytozoon* may achieve very high prevalence among their main host species especially because of such relaxed selection pressure (30).

Recent research shows that individual survival within the same buzzard population is not explained by *Leucocytozoon* infection status or intensity (manuscript in review). Because survival is explained by body condition during the nestling phase but not by parasitemia directly, acute parasitic infections could decrease the long-term survival prospects of some individuals through lowered body condition of chicks (47). However, other environmental factors such as prey availability and weather conditions appear to be much more influential (55,56).

We show that year of sampling explains variation in all four physiological parameters. Body condition and growth rate are tightly linked to food availability, while annual fluctuations in field vole (*Microtus arvalis*) abundance as the main source of energy for common buzzards are known to have strong effects on their demography (55). Fluctuations in vole abundance also explain variation in the breeding success in different parts of the breeding range (57,58). In our study area, the proportion of voles among prey items corresponds to differences in body condition and growth rate of nestlings of the same cohorts (unpublished data). Future research should address specifically host-parasite relationships in the light of the highly dynamic food availability.

Overall, our long-term field experiment resulted in finding only few signs of infection costs on nestling physiology, suggesting overall low pathogenicity of specialist blood parasites in raptor nestlings. Potential negative effects were apparent rarely and transiently at highest infection peaks, but disappeared at later stages of the infection course. During most non-extreme infections, juveniles appear to be tolerant and robust hosts that enable parasite transmission without paying substantial fitness costs and avoid direct or indirect parasite-induced mortality. Our study demonstrates the necessity to understand in greater detail “childhood diseases” in the wild. We suggest exploration without prejudice of symbiotic and parabiotic relationships between microorganisms and the most probable host developmental stages at the time of first contact, as these interactions and their valence are most relevant for the long-term co-evolutionary dynamics.

Acknowledgments

We would like to thank Thomas Grünkorn who climbed trees to deliver us precious samples for this study. We are also grateful for all the field assistants that helped during fieldwork of 2016, 2018, 2019 and 2020.

Funding

This study was supported by the German Science Foundation (DFG) as part of the SFB TRR 212 "A Novel Synthesis of Individualisation across Behaviour, Ecology and Evolution: Niche Choice, Niche Conformance, Niche Construction (NC3)" [project number 396780709] and a research grant for the project "Short- and long-term consequences of malaria-like infections in birds" [project number 398434413].

CRediT

Conceptualization: TR, NC, MO; Data acquisition and curation: TR, MO, HP, KPG, TMR, OK, NC; Formal analysis: TR, MO; Funding acquisition: NC, OK; Investigation: TR, MO, NC; Methodology: TR, MO, NC; Project administration: NC, OK; Resources: NC, OK; Supervision: NC, OK; Validation: MO, NC; Visualization: TR, MO; Writing – original draft: TR, NC; Writing – review & editing: TR, TMR, MO, OK, NC.

Data, code and materials

The manuscript is available as a preprint on EcoevoRxiv under the license CC-By Attribution-ShareAlike 4.0 International (doi: 10.32942/osf.io/4tcqu).

Raw data and associated code for analyses are available on this anonymized repository (https://github.com/TonyRinaud/MS_physiology_infection). Supplementary materials are available on this repository (https://osf.io/4dhuv/).

Ethics

Sampling and drug application of common buzzards were performed in accordance with the relevant guidelines and regulations. This work followed the ARRIVE guidelines and was approved by the Animal Ethics Committee at Bielefeld University and permitted by the local authority Kreis Gütersloh, permit number: 4.5.2-723-Bussard and by the ethics committee of the Animal Care and Use Committee of the German North Rhine-Westphalia State Office for Nature, Environment and Consumer Protection (*Landesamt für Nature, Umwelt und Verbraucherschutz Nordrhein-Westfalen*) under permit numbers: 84-02.04.2014.A091, 84-02-04.2017.A147.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Stockdale JE, Dunn JC, Goodman SJ, Sheehan DK, Grice PV, Hamer KC. The protozoan parasite Trichomonas gallinae causes adult and nestling mortality in a declining population of European Turtle Doves, Streptopelia turtur. 2015;9.

2. Bermejo M, Rodriguez-Teijeiro JD, Illera G, Barroso A, Vila C, Walsh PD. Ebola Outbreak Killed 5000 Gorillas. Science. 2006 Dec 8;314(5805):1564–1564.

3. Simonsen L, Viboud C. A comprehensive look at the COVID-19 pandemic death toll. 2021;3.

4. Foley J, Clifford D, Castle K, Cryan P, Ostfeld RS. Investigating and Managing the Rapid Emergence of White-Nose Syndrome, a Novel, Fatal, Infectious Disease of Hibernating Bats: White-Nose Syndrome in Bats. Conserv Biol. 2011 Jan;no-no.

5. Knowles SCL, Palinauskas V, Sheldon BC. Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. J Evol Biol. 2010 Mar;23(3):557–69.

6. Poulin R. Evolution and phylogeny of behaviour manipulation of insect hosts by parasites. In: Parasitology [Internet]. 1998. Available from: https://books.google.de/books?id=4EDSLrvER_gC&lpg=SL19-PA3&ots=CfFKfZoQrw&dq=behaviour%20change%20parasite&lr&hl=fr&pg=SL19-PA3#v=onepage&q&f=false

7. Dunn JC, Goodman SJ, Benton TG, Hamer KC. Avian blood parasite infection during the non-breeding season: an overlooked issue in declining populations? BMC Ecol. 2013;13(1):30.

8. Brown AF, Pascoe D. Parasitism and Host Sensitivity to Cadmium: An Acanthocephalan Infection of the Freshwater Amphipod Gammarus pulex. J Appl Ecol. 1989;26(2):473–87.

9. Martinez J, Merino S. Host-parasite interactions under extreme climatic conditions. Curr Zool. 2011 Jun 1;57(3):390–405.

10. Hatcher MJ, Dick JTA, Dunn AM. How parasites affect interactions between competitors and predators. Ecol Lett. 2006 Nov;9(11):1253–71.

11. Ramsay C, Rohr JR. The application of community ecology theory to co-infections in wildlife hosts. Ecology [Internet]. 2021 Mar [cited 2022 Mar 25];102(3). Available from: https://onlinelibrary.wiley.com/doi/10.1002/ecy.3253

12. Ashby B, Bruns E. The evolution of juvenile susceptibility to infectious disease. Proc R Soc B Biol Sci. 2018 Jun 27;285(1881):20180844.

13. Cowman AF, Healer, Julie, Marapana, Danushka, Marsh, Kevin. Malaria: Biology and Disease. Cell. 2015;15.

14. Kubi C, Van Den Abbeele J, De Deken R, Marcotty T, Dorny P, Van Den Bossche P. The effect of starvation on the susceptibility of teneral and non-teneral tsetse flies to trypanosome infection. Med Vet Entomol. 2006 Dec;20(4):388–92.

15. Panter SN, Jones DA. Age-related resistance to plant pathogens. In: Advances in Botanical Research [Internet]. Elsevier; 2002 [cited 2021 Jan 18]. p. 251–80. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0065229602380327

16. Townsend AK, Wheeler SS, Freund D, Sehgal RNM, Boyce WM. Links between blood parasites, blood chemistry, and the survival of nestling American crows. Ecol Evol. 2018 Sep;8(17):8779–90.

17. World Health Organization. World malaria report 2015. World Health Organization. 2016.

18. Herman CM, Barrow JH, Tarshis IB. LEUCOCYTOZOONOSIS IN CANADA GEESE AT THE SENEY NATIONAL WILDLIFE REFUGE. J Wildl Dis. 1975 Jul;11(3):404–11.

19. Grew R, Ratz T, Richardson J, Smiseth PT. Parental care buffers against effects of ambient temperature on offspring performance in an insect. Ridley A, editor. Behav Ecol. 2019 Sep 28;30(5):1443–50.

20. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. Proc R Soc B Biol Sci. 2015 Dec 22;282(1821):20143085.

21. Valkiūnas G. Avian malaria parasites and other haemosporidia. Boca Raton: CRC Press; 2005. 932 p.

22. Valkiūnas G, Atkinson CT. Introduction to life cycles, taxonomy, distribution, and basic research techniques. Avian Malar Relat Parasites Trop Ecol Evol Syst. 2020;45–80.

23. Snounou G, Jarra W, Viriyakosol S, Wood J, Brown K. Use of a DNA probe to analyse the dynamics of infection with rodent malaria parasites confirms that parasite clearance during crisis is predominantly strain- and species-specific. Mol Biochem Parasitol. 1989 Nov;37(1):37–46.

24. Valkiūnas G, Iezhova TA. Exo-erythrocytic development of avian malaria and related haemosporidian parasites. Malar J. 2017;24.

25. Williams RB. Avian malaria: clinical and chemical pathology of *Plasmodium gallinaceum* in the domesticated fowl *Gallus gallus*. Avian Pathol. 2005 Feb;34(1):29–47.

26. Hayworth AM, van Riper C, Weathers WW. Effects of Plasmodium relictum on the Metabolic Rate and Body Temperature in Canaries (Serinus canarius). J Parasitol. 1987 Aug;73(4):850.

27. Puente JM de la, Merino S, Tomás G, Moreno J, Morales J, Lobato E, et al. The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. Biol Lett. 2010 Oct 23;6(5):663–5.

28. Zhao W, Liu J, Xu R, Zhang C, Pang Q, Chen X, et al. The Gametocytes of *Leucocytozoon sabrazesi* Infect Chicken Thrombocytes, Not Other Blood Cells. Culleton R, editor. PLOS ONE. 2015 Jul 28;10(7):e0133478.

29. Chakarov N, Pauli M, Krüger O. Immune responses link parasite genetic diversity, prevalence and plumage morphs in common buzzards. Evol Ecol. 2017 Feb;31(1):51–62.

30. Wiegmann A, Springer A, Rinaud T, Ottensmann M, Legler M, Krüger O, et al. The prevalence of Leucocytozoon spp. in nestlings of three wild raptor species including implications on haematological and blood chemistry values. Int J Parasitol Parasites Wildl. 2021 Dec;16:236–43.

31. Chakarov N, Linke B, Boerner M, Goesmann A, Krüger O, Hoffman JI. Apparent vector-mediated parent-to-offspring transmission in an avian malaria-like parasite. Mol Ecol. 2015 Mar;24(6):1355–63.

32. Bijlsma RG. Roofvogels: handleiding veldonderzoek. 1998.

33. Wiegmann A, Rinaud T, Ottensmann M, Krüger O, Springer A, Legler M, et al. Tolerability of Atovaquone/Proguanil Application in Common Buzzard Nestlings. Vet Sci [Internet]. 2022;9(8). Available from: https://www.mdpi.com/2306-7381/9/8/397

34. Palinauskas V, Valkiūnas G, Križanauskienė A, Bensch S, Bolshakov CV. Plasmodium relictum (lineage P-SGS1): Further observation of effects on experimentally infected passeriform birds, with remarks on treatment with Malarone™. Exp Parasitol. 2009 Oct;123(2):134–9.

35. Schoenle LA, Kernbach M, Haussmann MF, Bonier F, Moore IT. An experimental test of the physiological consequences of avian malaria infection. Ardia D, editor. J Anim Ecol. 2017 Nov;86(6):1483–96.

36. Sparks A. nasapower: NASA-POWER Data from R [Internet]. 2022. Available from: https://CRAN.R-project.org/package=nasapower

37. Råberg L, Sim D, Read AF. Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. Science. 2007 Nov 2;318(5851):812–4.

38. Lenth RV. emmeans: Estimated Marginal Means, aka Least-Squares Means [Internet]. 2022. Available from: https://CRAN.R-project.org/package=emmeans

39. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.R-project.org

40. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models using lme4. ArXiv14065823 Stat [Internet]. 2014 Jun 23 [cited 2020 Nov 3]; Available from: http://arxiv.org/abs/1406.5823

41. Schielzeth H. Simple means to improve the interpretability of regression coefficients: *Interpretation of regression coefficients*. Methods Ecol Evol. 2010 Jun;1(2):103–13.

42. Dadam D, Robinson RA, Clements A, Peach WJ, Bennett M, Rowcliffe JM, et al. Avian malaria-mediated population decline of a widespread iconic bird species. R Soc Open Sci. 2019 Jul 26;6(7):182197.

467 43. Granthon C, Williams DA. Avian Malaria, Body Condition, and Blood Parameters In Four Species of
468 Songbirds. Wilson J Ornithol. 2017 Sep;129(3):492–508.

469 44. Hahn S, Bauer S, Dimitrov D, Emmenegger T, Ivanova K, Zehtindjiev P, et al. Low intensity blood
470 parasite infections do not reduce the aerobic performance of migratory birds. Proc R Soc B Biol Sci.
471 2018 Jan 31;285(1871):20172307.

472 45. Blanco G, Puente JDL, Corroto M, Baz A, Colás J. Condition-dependent immune defence in the
473 Magpie: how important is ectoparasitism? Biol J Linn Soc. 2001 Feb;72(2):279–86.

474 46. Møller AP, Christe Ph, Erritzøe J, Mavarez J, Moller AP, Erritzoe J. Condition, Disease and Immune
475 Defence. Oikos. 1998 Nov;83(2):301.

476 47. Pigeault R, Cozzarolo CS, Choquet R, Strehler M, Jenkins T, Delhaye J, et al. Haemosporidian
477 infection and co-infection affect host survival and reproduction in wild populations of great tits. Int J
478 Parasitol. 2018 Dec;48(14):1079–87.

479 48. Valkiūnas G, Iezhova TA. Insights into the Biology of Leucocytozoon Species (Haemosporida,
480 Leucocytozoidae): Why Is There Slow Research Progress on Agents of Leucocytozoonosis?
481 Microorganisms. 2023;11(5):1251.

482 49. Jia T, Huang X, Valkiūnas G, Yang M, Zheng C, Pu T, et al. Malaria parasites and related
483 haemosporidians cause mortality in cranes: a study on the parasites diversity, prevalence and
484 distribution in Beijing Zoo. Malar J. 2018;17:1–11.

485 50. Atkinson, T., C., Van Riper III, C. Bird-Parasite Interactions. J.E Loye&M. Zuk. Vol. Pathology and
486 epizootiology of haematozoa. Oxford Ornitohlogy Series; 1991. 48 p.

487 51. Himmel T, Harl J, Kübber-Heiss A, Konicek C, Fernández N, Juan-Sallés C, et al. Molecular probes for
488 the identification of avian Haemoproteus and Leucocytozoon parasites in tissue sections by
489 chromogenic in situ hybridization. Parasit Vectors. 2019;12(1):1–10.

490 52. Wale N, Jones MJ, Sim DG, Read AF, King AA. The contribution of host cell-directed vs. parasite-
491 directed immunity to the disease and dynamics of malaria infections. Proc Natl Acad Sci. 2019 Oct
492 29;116(44):22386–92.

493 53. Commichau C, Jonas D. A disease of ducklings caused by Leucocytozoon simondi with special
494 reference to histopathological diagnosis. Zentralblatt Für Veterinärmedizin Reihe B. 1977;24(8):662–
495 7.

496 54. Shutler D, Ankney CD, Mullie A. Effects of the blood parasite Leucocytozoon simondi on growth
497 rates of anatid ducklings. 1999;77:6.

498 55. Sundev G, Yosef R, Birazana O. Brandt's Vole density affects nutritional condition of Upland Buzzard
499 Buteo hemilasius on the Mongolian Grassland Steppe. 2009;86:9.

500 56. Kostrzewa A, Kostrzewa R. The relationship of spring and summer weather with density and
501 breeding performance of the Buzzard Buteo buteo, Goshawk Accipiter gentilis and Kestrel Falco
502 tinnunculus. Ibis. 2008 Apr 3;132(4):550–9.

503 57. Chakarov N, Krüger O. Mesopredator Release by an Emergent Superpredator: A Natural Experiment
504 of Predation in a Three Level Guild. Peter HU, editor. PLoS ONE. 2010 Dec 6;5(12):e15229.

505 58. Panek M. Does Habitat Diversity Modify the Dietary and Reproductive Response to Prey
506 Fluctuations in a Generalist Raptor Predator, the Eurasian Buzzard Buteo buteo? Birds. 2021 Mar
507 1;2(1):114–26.

508

Tables

Table 1: Results of four linear mixed models described in Supplementary Table S1, testing the effect of infection stages on the cost-related physiological parameters of Common Buzzard nestlings. a) Breathing rate and body temperature as response variables, b) Body condition and growth rate as response variables. Full model results are presented in Supplementary Table S3.

a. *Predictors*	**Breathing rate at T_1**					**Body temperature at T_1**				
	Estimates	*CI*	*df*	*t*	*p*	*Estimates*	*CI*	*df*	*t*	*p*
Intercept	36.49	31.35 – 41.64	174	13.99	**<0.001**	39.78	39.35 – 40.21	131	181.72	**<0.001**
Increasing parasitemia	-0.54	-3.00 – 1.91	256	-0.44	0.664	-0.13	-0.48 – 0.21	145	-0.78	0.435
Peak parasitemia	-1.08	-4.80 – 2.65	251	-0.57	0.570	-0.13	-0.58 – 0.32	142	-0.58	0.562
Decreasing parasitemia	-1.45	-4.19 – 1.28	256	-1.05	0.295	0.06	-0.28 – 0.41	151	0.35	0.724
…	…	…		…	…	…	…		…	…
Random Effects	135 Nests					77 Nests				
Observations	269					178				
Marginal / Conditional R^2	0.180 / 0.397					0.303 / 0.712				

b. *Predictors*	**Body condition at T_1**					**Growth rate**				
	Estimates	*CI*	*df*	*t*	*P*	*Estimates*	*CI*	*df*	*t*	*P*
Intercept	-46.28	-92.83 – -0.27	186	-1.96	0.051	18.73	11.86 – 25.60	190	5.38	**<0.001**
Increasing parasitemia	1.16	-21.22 – 23.53	263	0.10	0.919	-0.93	-4.36 – 2.51	265	-0.53	0.596
Peak parasitemia	-21.42	-55.19 – 12.34	249	-1.25	0.213	-2.09	-7.32 – 3.14	259	-0.79	0.433
Decreasing parasitemia	-3.81	-28.81 – 21.20	258	-0.30	0.765	-2.79	-6.64 – 1.07	265	-1.42	0.156
…	…	…		…	…	…	…		…	…
Random Effects	139 Nests					139 Nests				
Observations	276					276				
Marginal / Conditional R^2	0.230 / 0.530					0.232 / 0.452				

Table 2: Results of four linear mixed models described in Supplementary Table S1, testing the effect of parasitemia at T_1 on cost related physiological parameters in common buzzard nestlings. a) Breathing rate and body temperature as response variables, b) Body condition and growth rate. Full model results are presented in Supplementary Table S4.

a. *Predictors*	**Breathing rate at T_1**					**Body temperature at T_1**				
	Estimates	CI	df	t	P	Estimates	CI	df	t	P
Intercept	36.13	31.01 – 41.25	167	13.93	**<0.001**	39.88	39.47 – 40.30	129	190.38	**<0.001**
Parasitemia 1	0.93	-1.71 – 3.58	251	0.69	0.488	0.03	-0.30 – 0.37	142	0.19	0.851
Parasitemia 2	-0.70	-3.63 – 2.24	250	-0.47	0.640	0.01	-0.32 – 0.34	147	0.06	0.952
Parasitemia 3	-1.22	-3.88 – 1.44	254	-0.90	0.367	-0.16	-0.53 – 0.21	151	-0.85	0.398
Parasitemia 4	0.33	-4.09 – 4.75	253	0.15	0.882	-0.78	-1.34 – -0.23	149	-2.79	**0.006**
…	…	…		…	…	…	…		…	…
Random Effects	135 Nests					77 Nests				
Observations	269					178				
Marginal / Conditional R^2	0.184 / 0.402					0.329 / 0.709				

b. *Predictors*	**Body condition at T_1**					**Growth rate**				
	Estimates	CI	df	t	P	Estimates	CI	df	t	P
Intercept	-40.16	-86.25 – 28.09	179	-1.72	0.087	18.77	11.97 – 25.57	182	5.45	**<0.001**
Parasitemia 1	7.36	-16.29 – 31.01	248	0.61	0.540	0.53	-3.15 – 4.21	259	0.28	0.777
Parasitemia 2	3.87	-22.53 – 30.26	246	0.29	0.773	-1.74	-5.85 – 2.37	258	-0.83	0.405
Parasitemia 3	-8.59	-32.88 – 15.71	263	-0.70	0.497	-2.32	-6.05 – 1.42	263	-1.22	0.223
Parasitemia 4	-43.99	-82.92 – -5.05	258	-2.22	**0.027**	-5.26	-11.29 – 0.76	263	-1.72	0.087
…	…	…		…	…	…	…		…	…
Random Effects	139 Nests					139 Nests				
Observations	276					276				
Marginal / Conditional R^2	0.238 / 0.539					0.241 / 0.458				

Figures

Figure 1: Theoretical dynamics of parasitemia in a host. The y-axis is parasitemia score. The x-axis represents time with the different phases of parasitemia. The corresponding infection stages at resampling (1 = uninfected, 2 = increasing, 3 = peak and 4 = decreasing) are shown as vertical colour bands. The top right diagram shows the average interval between first (T_0) and second (T_1) sampling. The red points represent the time points used in models testing for costs of infection on physiological parameters.

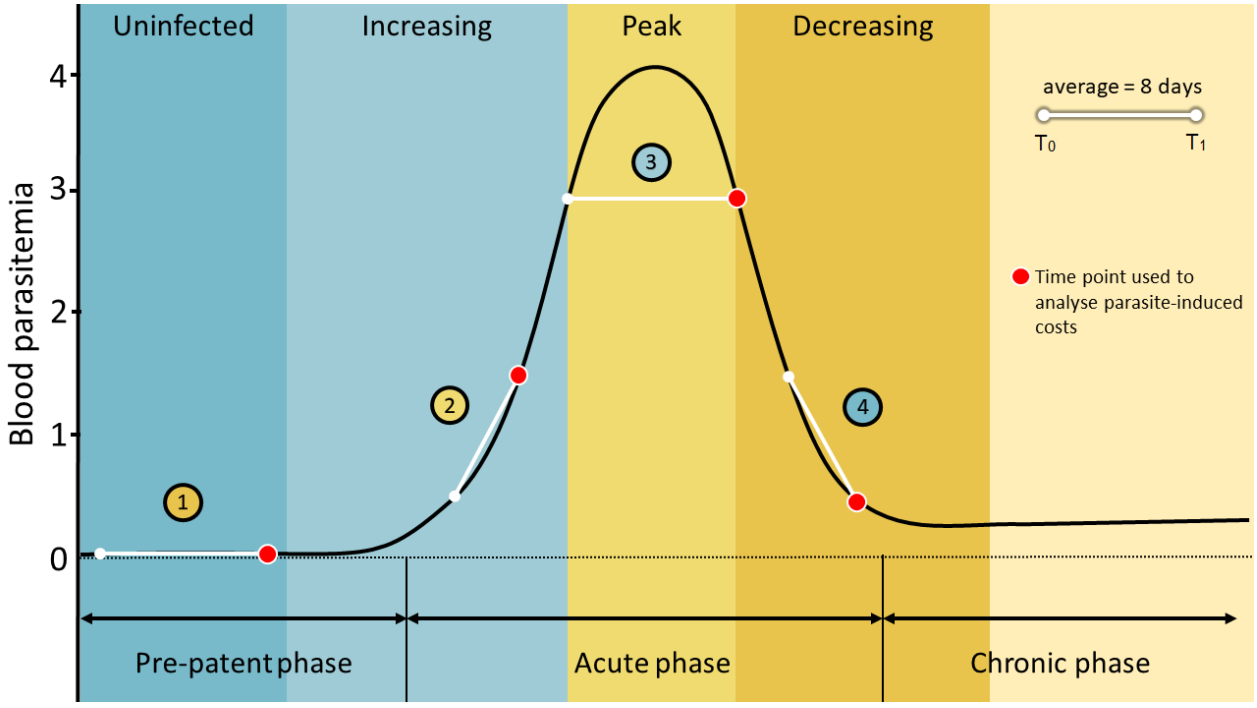
Figure 2: a) First-to-second sampling count of uninfected and infected nestlings between treatments (control versus antimalarial treatments). b) First-to-second sampling mean parasitemia between control and Malarone-treated nestlings. Error bars represent standard errors.

Figure 3: Relationship between body condition change (proxy of host health) and parasitemia at second sampling (parasitic load as a score from 1 to 4) from the raw dataset. Reaction norms are displayed for each of the infection stages (blue: increasing, yellow: peak and red: decreasing parasitemia). Comparisons of reaction norms among infection stages and in comparison to a null slope are presented in Supplementary Table S2. A ΔBC value of 0 means no change of body condition between T_0 and T_1. P-values of comparisons of regression slopes to zero are displayed in boxes (see Table S2).

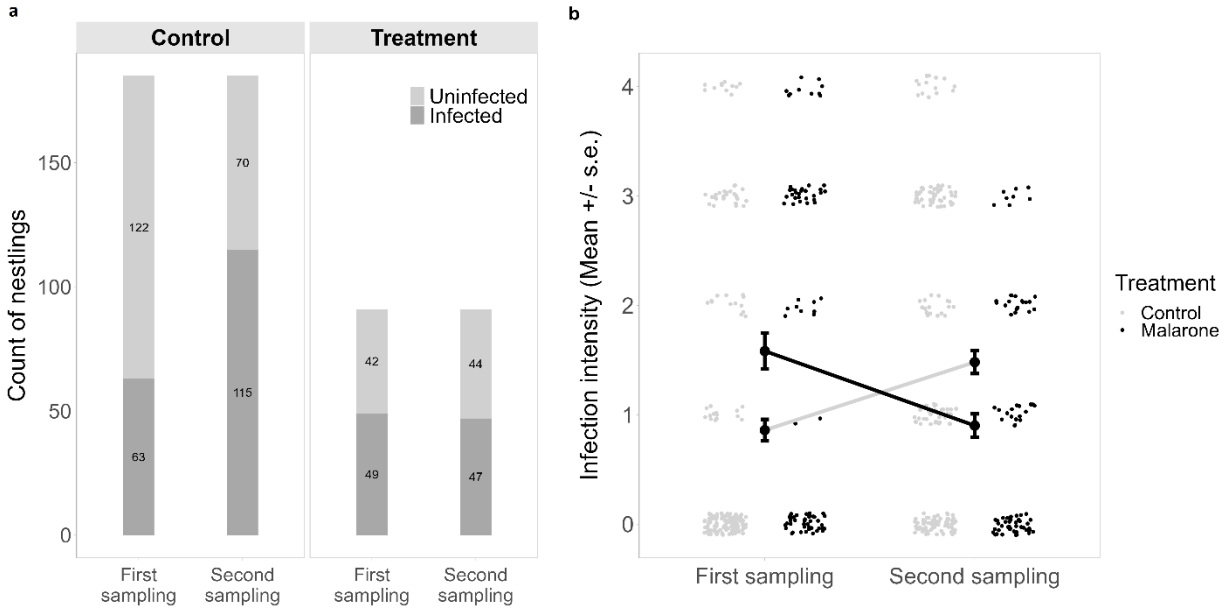
Figure 4: Comparison of predicted resistance-related physiological parameters among parasitemia (ranging from 0, uninfected, to 4, highest parasitemia) from each of the four linear models. Points are predicted means, coloured boxes the standard errors and 95% CI as the error bars. The black dashed lines represent the predicted mean value across groups of a given parameter. a) comparison

538 of breathing rate, b) comparison of body temperature, c) comparison of body condition and d)
539 comparison of growth rate among infection intensity groups at T_1.

Figure 1

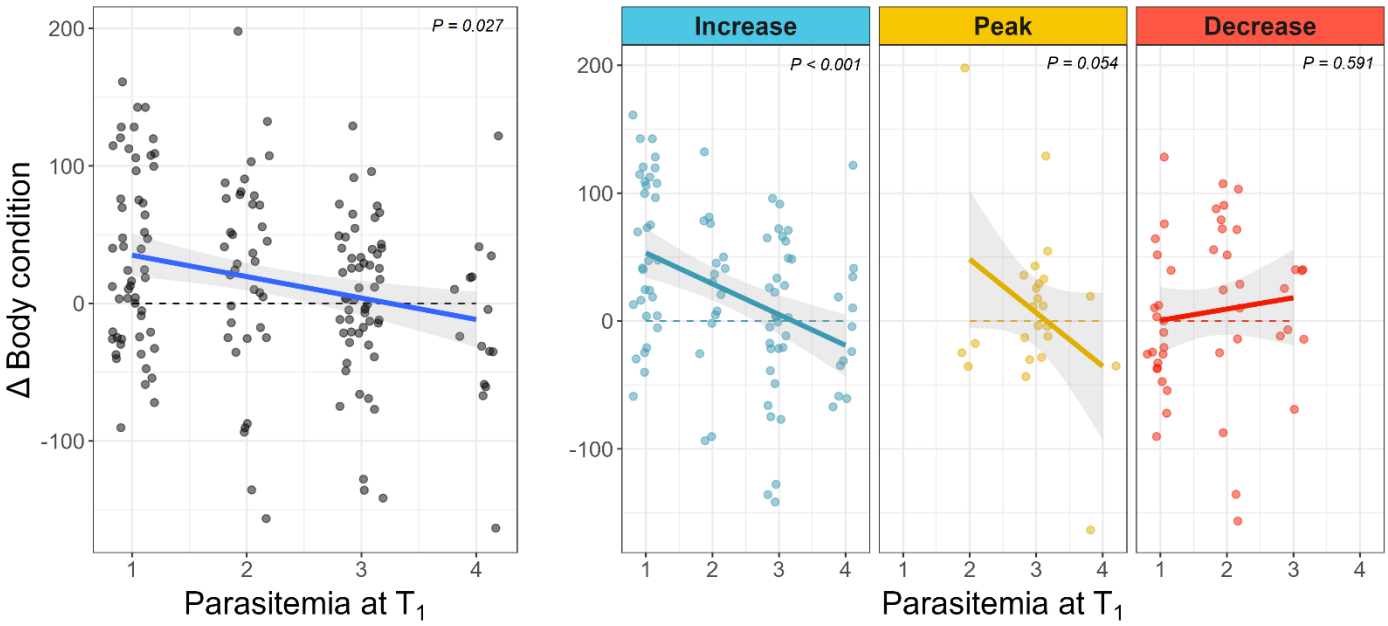

Uninfected
Increasing
Peak
Decreasing
Blood parasitemia
0
1
2
3
4
1
2
3
4
average = 8 days
T0
T1
Time point used to analyse parasite-induced costs
Pre-patent phase
Acute phase
Chronic phase

542 **Figure 2**



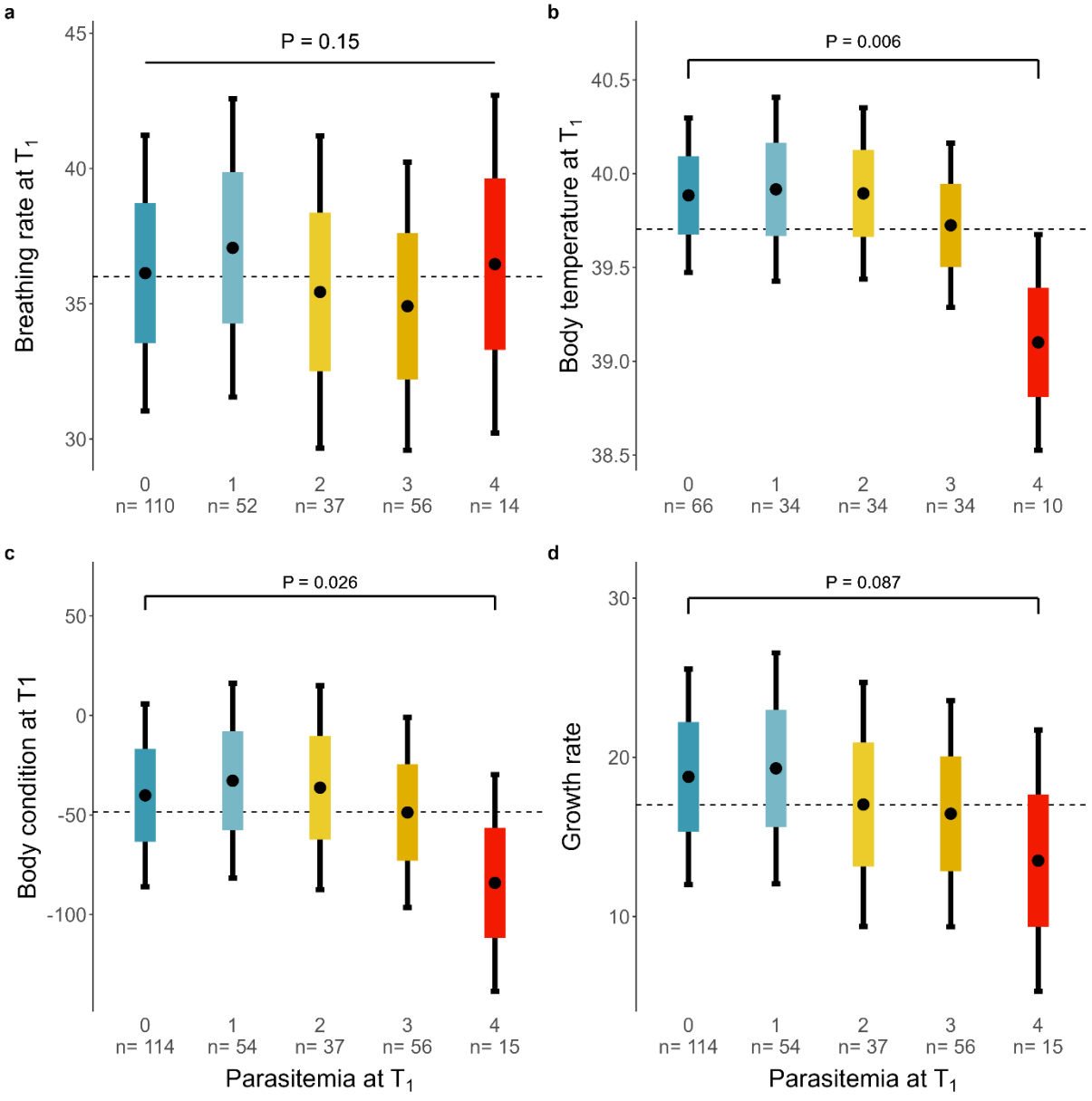
543

544 **Figure 3**



5

546 **Figure 4**



547