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 from 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 for the hosts at the onset of infection and during peak parasitemia than hosts with decreasing parasitemia and uninfected ones. We found body condition to be initially negatively correlated 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 the host. To diminish infection-driven mortality, juveniles may evolve to be particularly parasite-tolerant, further enhancing 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 the 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 exert indirect effects by exacerbating the adverse impacts of the abiotic environment [8,9], or influencing intra- or interspecific interactions [10], and other symbionts [11]. Such combined detrimental effects may impose particularly high fitness costs on juvenile animals [12–15]. During the 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 intense infections than adults [16–18]. Additionally, young hosts often rely on parental care and are less mobile [19]; which makes them particularly accessible to vectors. 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. 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. However, such costs in developmental stages remain poorly understood in the wild, mainly due to the hardships of longitudinal samplings in wild juveniles.

Regardless of the severity of parasitic damages, parasitemia (i.e. the proportion of infected host tissue) usually (1) first increases following host invasion, (2) reaches a plateau/peak and then (3) decreases rapidly (Fig. 1) [21,22]. When the adaptive immunity succeeds in mounting 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 infection progressions "infection stages" (in contrast to tissue-specific parasite developmental stages). The physiological costs of infection, tissue damage, parasite-driven resource depletion and inflammation, are expected to be immediate, i.e. most pronounced during the pre-patent and acute stages of infection - shortly before and during peak parasitemia -, rather than during chronic infection when parasitemia is low and gradual recovery ensues [24–26]. However, an open question remains whether the physiological costs of the infection are short-lived and immediate, delayed and associated with recovery, or a combination of both.

Vertebrate haemosporidian parasites, one of the parasites that mostly follow the model of infection progression outlined above is mostly known 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 it reaches 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*. 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 [29]. In our study population, the prevalence of *Leucocytozoon toddi* before the 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. *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 substantially 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 monitoring 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 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 neither time points; only 23 nestlings naturally displayed decreasing parasitemia between both sampling points. An artificial treatment of decreasing parasitemia was achieved by giving 11 mg 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).

To test whether two sampling events produce robust estimates, we resampled a small subset of individual for the third time. Between 2016 and 2020, 17 nestlings (4, 5, 2, 6 respectively for 2016, 2018, 2019, 2020) were resampled for a third time. In 2022, three additional nestlings were sampled three time and were included in this analysis. All twenty nestlings were resampled at an average of 6.1 days (s.d. ± 3.6 days) after second sampling.

(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]. The 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 the 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 a 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) Robustness of infection stages

Across the study, 20 nestlings were resampled a third time (Fig. S6). A consistent infection trend was observed for 65% of them when comparing the infection trend from the first with either the second or third sampling point. Five remained uninfected, six remained with increasing parasitemia, one remained at peak parasitemia, and one remained with decreasing parasitemia. The last seven individuals (35%) of these 20 nestlings slightly differed in their infection development between the first and either second or third sampling. The trend of two nestlings changed from increasing to stable parasitemia, one uninfected nestling changed into increasing parasitemia, two nestling with stable parasitemia changed respectively to increasing and decreasing parasitemia, another one changed from decreasing to peak parasitemia and one from decreasing parasitemia to stable, low parasitemia.

(c) 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).

(d) 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).

(e) Lower body temperature at highest infection intensity

Body temperature did not differ among infection stages (Table 1A). However, body temperature was significantly lower in the nestlings with acute parasitemia (level 4) compared with uninfected ones (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).

(f) 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).

(g) Lower growth rate associated with highest infection intensities

The 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 the highest parasitemia (level 4) tended to have lower growth rates than uninfected nestlings (Table 2B, Fig. 4D). Females had higher growth rates than males. The growth rate decreased with the age of the nestlings, as they were approaching adult size. The 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

We found that a proxy of host health, body condition decreased with higher parasitemia levels. This finding was shown to be infection stage-dependent, as this negative correlation turned positive at the outset of the infection course (decreasing parasitemia, Fig. 3B). The infection stages had no effect on the physiological parameters considered, but body temperature, body condition

and growth rate were found significantly lower only at the highest infection intensity (level 4, Fig. 4)

Our results suggest that young common buzzards generally do not display parasite-induced costs during the course of 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 [18,21,42,43]. 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 [44], others, including raptor-specific *Leucocytozoon*, typically do not appear to produce such stages [45]. 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 [29]. Future research across host-parasite interactions is needed to test whether parasitic lineages lacking such tissue stages have a longer coadaptation history and are consequently 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 [26,46]. 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 [44]. 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 [25,47]. Nevertheless, body condition only appeared worst 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. In large bird species, nestling body condition predicts juvenile survival (16, Ottensmann et al., in review). Consequently, acute infections of nestlings may contribute to delayed increase of juvenile mortality, under the condition that chicks are not able to compensate for condition loss before independence.

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 the highest levels of parasitemia (Fig. 4), which occur in only a few individuals. Furthermore, body condition, our best proxy for fitness-relevant host health, decreased with parasite load but this relationship turned around in the 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 [21,48].

307 The apparent low virulence, together with the mostly absent physiological responses to infection
308 suggest that the host and parasite may have defused the co-evolutionary arms-race by reaching
309 widespread transmission to the immunologically naïve and susceptible but tolerant nestling stage
310 and thereby, a different equilibrium to parasites of the genus *Plasmodium*. Unlike generalist
311 parasites, parasites similar to raptor-specific *Leucocytozoon* may achieve very high prevalence
312 among their main host species, especially because of such relaxed selection pressure [29].

313 Recent research shows that individual survival within the same buzzard population is not explained
314 by *Leucocytozoon* infection status or intensity (manuscript in review). Because survival is
315 explained by body condition during the nestling phase but not by parasitemia directly, acute
316 parasitic infections could decrease the long-term survival prospects of some individuals through
317 lowered body condition of chicks [49]. However, other environmental factors, such as prey
318 availability and weather conditions appear to be much more influential [50,51].

319 We show that the year of sampling explains variation in all four physiological parameters. Body
320 condition and growth rate are tightly linked to food availability, while annual fluctuations in field
321 vole (*Microtus arvalis*) abundance as the main source of energy for common buzzards are known
322 to have strong effects on their demography [50]. Fluctuations in vole abundance also explain
323 variation in the breeding success in different parts of the breeding range [52,53]. In our study area,
324 the proportion of voles among prey items corresponds to differences in body condition and growth
325 rate of nestlings of the same cohorts (unpublished data). Future research should specifically
326 address host-parasite relationships in light of the highly dynamic food availability.

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

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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

513 of breathing rate, **B)** comparison of body temperature, **C)** comparison of body condition and **D)**

514 comparison of growth rate among infection intensity groups at T_1.