**Dynamics of resting metabolic rate and innate immune response in malaria-infected Eurasian siskins**

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**Abstract**

Avian malaria, caused by parasites of the genus *Plasmodium*, is prevalent among wild bird populations worldwide and can have significant impact on avian health and populations. With the rise in global temperatures due to climate change, concerns have arisen about the spread of southern malaria species, that potentially can affect previously unexposed bird populations. We studied juvenile siskins infected with two distinct malaria parasites: *Plasmodium relictum* (SGS1 lineage) and *Plasmodium ashfordi* (GRW2 lineage). While the former is common in the Northern Palearctic, the latter is primarily found in Central and Southern Africa. We assessed the impact of these infections on siskins' physiological well-being using resting metabolic rate (RMR) and interleukin-6 (IL-6) levels. RMR reflects the energetic cost of disease, while IL-6 serves as a one of the inflammatory cytokines in the innate immune system's response to infection. Our experimental findings reveal distinct outcomes during the acute phase of SGS1 and GRW2 infections. The first one was marked by reduced RMR and IL-6 levels in siskins. A similar IL-6 pattern was observed in the GRW2 group initially, though it was not sustained. Additionally, GRW2-infected siskins showed distinct RMR dynamics compared to SGS1-infected birds. Our study did not conclusively demonstrate that tropical malaria has more severe effects on siskins. However, similarities with previous studies with SGS1 infected birds and variations in disease progression between the two experimental groups underscore the complexity of host-parasite interactions in avian malaria infections.

*Keywords*: Avian malaria, *Plasmodium relictum*, *Plasmodium ashfordi*, Experimental infection, Metabolic rate, Innate immunity.

**1. Introduction**

Each year, billions of birds undertake a remarkable journey from their breeding sites to wintering areas and back. The final autumn destination for most migrating Western Palearctic passerines is Central and Southern Africa, encompassing tropical and subtropical regions (Newton 2010; Shirihai and Svensson 2018). Due to their movement across various geographic areas during migration, migratory birds encounter a diverse range of blood-sucking insects along their routes. These insects may serve as vectors and can carry different species and strains of haemosporidian parasites, with transmission restricted to the distribution range of their competent vector. Specifically, migrating birds may become infected with malaria parasites of the *Plasmodium* genus (Plasmodiidae, Haemosporida), which are prevalent in the African region. Infected birds that manage to survive and return to their breeding grounds in the Northern Palearctic serve as reservoirs for new infections, as malaria parasites can persist in individuals for years (Bensch et al. 2007; Rooyen et al. 2013). This scenario presents a potential threat to birds belonging to resident or short-distance migratory species that have not co-evolved with malaria parasite species whose transmission extends beyond their habitats. It is well-known that *Plasmodium* parasites can be of high virulence when introduced to naïve populations that have not co-evolved with avian malaria species (Van Riper III et al. 1986). Furthermore, avian malaria outbreaks happen regularly in zoos, where captive birds (penguins particularly) are for the first time exposed to locally transmitting *Plasmodium* parasites (Cocumelli et al. 2021; González-Olvera et al. 2022; Meister et al. 2021).

Upon invading a vertebrate host organism, the parasite penetrates tissue cells of various organs, initiating multiplication. No parasites can be seen in the red blood cells during this period (so-called pre-patent period). This process precedes the subsequent stage, known as the acute phase, which is usually marked by a sharp increase of infected erythrocytes in the peripheral blood (Valkiūnas 2005). Similar to any other infection, avian malaria triggers the process of an immune response. In ecological immunology, a key hypothesis is that an organism's immune defense mechanism must strike a balance between effectiveness and energy expenditure (Demas and Nelson 2012). Activating and maintaining immune response consumes energy and can affect host metabolism (Eraud et al. 2005; Martin et al. 2003) and energy reserves (Bonneaud et al. 2003; Demas et al. 2003). The immune system is highly responsive and can become excessive and inappropriate and lead to immunopathology (Graham et al. 2005; Schulenburg et al. 2009; Sorci and Faivre 2009). Hence, despite its benefits of controlling parasite infections, immune defense comes at a high cost and a trade-off should exist between immunity and other energy-demanded physiological processes in the organism (Norris and Evans 2000; Owen-Ashley and Wingfield 2007; Sheldon and Verhulst 1996; Zuk and Stoehr 2002).

Directly measuring the energetic cost of immunity is challenging due to the immune system's complexity. However, assessing metabolic rate can provide valuable insights into the energetic costs associated with immune responses. In particular, measuring resting metabolic rate (RMR) during infection and comparing it with pre-infection baseline levels provides insights into the additional energy required for mounting and sustaining an immune response (Demas and Nelson 2012; Ricklefs et al. 1996). However, measuring the precise metabolic cost of immunity is challenging due to its intricate integration with other physiological systems within the organism (Lochmiller and Deerenberg 2000). That implies that RMR measured in our study reflected not only the immune response itself but also the energetic consequences of collateral damages caused by malaria, such as free hemoglobin and heme utilization, replenishment of destroyed red blood cells (RBCs) and tissue damages, and other related factors (Lochmiller and Deerenberg 2000; Sun et al. 2020).

Successful recovery following primary malaria infection and the development of acquired immunity depends on the first line of non-specific defense: innate immunity, specifically the activation of many pro- and anti-inflammatory cytokines (Gowda and Wu, 2018). IL-6 (interleukin 6) is one of the proinflammatory cytokines that is produced shortly after infection as a part of the induced innate immune response (Karel A. Schat 2014; Owen-Ashley and Wingfield 2007). IL-6 is one of the triggers of the acute phase response – the most energy demanding of all immune system defenses. The acute phase response generally requires a more immediate and intense energy expenditure due to its rapid response and systemic effects like fever and acute phase protein production (Owen-Ashley and Wingfield 2007). The IL-6 system is regarded as highly conserved in vertebrates and IL-6 demonstrates low species specificity in experiments (Zimmerman, 2014). While there is scarce information about the role of this cytokine in avian malaria models, a recent meta-analysis of IL-6 levels in malaria infected human patients and several studies on murine models suggest that IL-6 can serve as a marker for malaria severity (Carapau et al. 2007; Wilairatana et al. 2022; Wunderlich et al. 2012) and even could be used to differentiate malaria from other febrile disease in humans, though more studies are needed (Wilairatana et al. 2022).

In this study, we compared the impact of malaria infection on the physiological parameters of a common European passerine short-distance migrant – the Eurasian siskin (*Spinus spinus*) infected with two different avian haemosporidian parasites: *Plasmodium relictum* (lineage SGS1) and *Plasmodium ashfordi* (lineage GRW2). The first one is widespread in the Palearctic region, a generalist with a broad range of avian host species and high variability in developed levels of parasitemia depending on both host species and on host individuals (Martínez-de la Puente et al. 2021; Palinauskas et al. 2008; 2011). Experiments conducted with juvenile siskins revealed that during SGS1 malaria infection, they usually exhibit high levels of parasitemia (i.e., the quantity of infected erythrocytes) during the acute stage. However, considerable individual variation was observed, with some birds displaying a deficient number of infected erythrocytes (Mukhin et al. 2016; Palinauskas et al. 2008). Overall, this lineage is considered as severe and highly pathogenic, with a high potential for mortality in susceptible birds (Valkiūnas et al. 2018). *Plasmodium ashfordi* (GRW2) is also a generalist malarial parasite primarily transmitted in Africa. In Europe, this parasite has only been detected in adults of long-distance migrating birds after their return from wintering grounds in tropical Africa (Bensch et al. 2009). Experimental infections with *P. ashfordi* demonstrated its ability to develop high levels of parasitemia and lethality of its natural host, the great reed warbler (*Acrocephalus arundinaceus*)(Asghar et al. 2012), as well as in Eurasian siskins (Asghar et al. 2016).

Given the contradictory data on metabolic responses in birds during the parasitic infections (Robar et al. 2011), especially malaria, we cannot definitively predict whether relationship between RMR and parasitemia level will be positive or negative. RMR might increase in all infected juvenile siskins due to the development of an acute immune response against proliferating malaria parasites. This is supported by experiments involving the innate immune challenges in passerine birds (reviewed in Hasselquist and Nilsson (2012), but see Lee et al. (2005)). On the other hand, erythrocytic merogony of malarial parasites is characterized by the active destruction of erythrocytes and digestion of hemoglobin (Hb) by multiplying parasites. Progressing anemia could reduce the blood's oxygen-binding capacity, disrupting oxygen transportation and resulting in possible decrease of infected birds` RMR during the acute stage (Stager et al. 2021). This view is supported by Hayworth (1987) and aligns with the general knowledge of the *Plasmodium* parasite life cycle, although some studies have not confirmed it (Hahn, 2018; Stager, 2021). However, it is necessary to take into account that erythrocytes can transport much more oxygen than is required for basic maintenance. The maximum energy that birds can obtain from food and expend over long periods (i.e., without losing body mass) is about four times the BMR (Lindström & Kvist, 1995; Gavrilov, 2014). Therefore, at low or moderate levels of parasitemia, the remaining intact erythrocytes can not only support the basal metabolism but also handle the additional energy demands associated with immune responses, tissue repair, hematopoiesis, etc.

Given the energy demands associated with acute immune activation during infection, the destructive impact of *Plasmodium* parasites on oxygen-carrying erythrocytes, and the compensatory mechanisms in affected birds, we predict that resting metabolic rate (RMR) will initially increase in response to increasing parasitemia. This increase will continue up to a certain parasitemia threshold, beyond which the remaining healthy erythrocytes will be insufficient to sustain a high RMR. Additionally, the development of anemia and hypoxemia, exacerbated by lactic acidosis, further complicates the situation. As blood pH decreases, oxygen saturation of hemoglobin declines (Rigdon and Rostorfer 1946; Rostorfer and McGee 1946), and at elevated parasitemia levels, glycolysis becomes a critical energy source due to its oxygen-independent nature (Cumnock et al. 2018).

Regarding IL-6, we expect its levels to rise before or at the onset of the acute phase of malaria, and then decrease in birds that survive this crisis and develop a chronic infection. Consequently, we anticipate a positive correlation between IL-6 levels and RMR during the early stages of the disease, which may shift to a negative relationship as parasitemia peaks. Additionally, we expected different average responses to *P. relictum* SGS1 and *P. ashfordi* GRW2, with potentially more severe outcomes (possibly higher peak IL-6 and more pronounced change in RMR) for birds infected with the latter. This expectation is based on the distinct co-evolutionary histories of the host and these two parasite species.

**2. Materials and methods**

*2.1 Study site, host species*

The study was conducted at the Biological Station Rybachy of the Zoological Institute of the Russian Academy of Sciences (located at 55°09’N, 20°51’E), from July to October 2020. For our study, we selected siskins due to their status as a Palearctic bird species with a limited migratory range, which likely has not come into contact with parasites of African origin. Additionally, this species is common and abundant in the study area and proved itself a convenient candidate for experiments with malaria infection. Birds captured on their autumn migration were examined for the presence of haemosporidian parasites using microscopy and PCR-based diagnostic methods (see below).

Sixty juvenile siskins were randomly divided into three equal groups: one control and two experimental groups, referred to as SGS1, GRW2 and Control. Each bird was housed in an individual plastic cage within a vector-free room with a constant ambient temperature (+23°C) and light-dark photoperiod (L:D) as 17:7, mimicking the natural photoperiod on 1st July. Water and food were provided *ad libitum*.

*2.2 Experimental infections of birds and collection of blood*

We used two species of *Plasmodium* parasites for experimental infections: *P.* *relictum* (lineage SGS1) and *P. ashfordi* (lineage GRW2). The first one, SGS1, was initially isolated in 2018 from a naturally infected wild common rosefinch (*Carpodacus erythrinus*). Several juvenile siskins were infected from this bird, and since then they served as living donors of SGS1. The southern malaria strain, *P. ashfordi,* was collected from a wild wood warbler (*Phylloscopus sibilatrix*) during its spring migration in 2020. Its blood was cryopreserved according to Garnham (1966) and then thawed prior to the start of the experiment.

To multiply the parasites, a number of juvenile siskins (2 for SGS1, 3 for GRW2) were inoculated with infected blood obtained from SGS1 and GRW2 donors. For each experimental group, a mixture of infected donor blood, 3.7% sodium citrate (used as an anticoagulant) and 0.9% saline (all at a ratio of 4:1:5) was prepared as described by (Iezhova et al. 2005). Experimental birds received an injection of 150 μl of this mixture into their pectoral muscle. All control birds underwent the same procedure, with the exception that the inoculated blood originated from an uninfected donor.

Starting with the inoculation procedure and continuing every sixth day after, no more than two capillaries (approximately 150 μl) of blood were collected from the ulnar vein of each experimental bird. Two drops of this blood were used to prepare two smears, a fraction of blood was centrifuged at 10,000 rpm to separate plasma and red blood cells. Subsequently, plasma was aspirated and transferred into cryo-tubes, then stored at -196º in liquid nitrogen. The remained blood was washed from the capillaries and stored in SET-buffer (0.05 M Tris, 0.15 M NaCl, 0.5 M EDTA, pH 8.0) for molecular analyses, as described by Hellgren et al. (2004).

The blood smears were air-dried, fixed in absolute methanol, and stained with Giemsa according to the standard protocol (Valkiūnas 2005). These smears were then examined under a light microscope at 1000x magnification using oil immersion. The intensity of parasitemia was determined as a percentage by directly counting the number of parasites per 1000 erythrocytes, or per 10,000 erythrocytes if infections were light, as recommended by Godfrey Jr. et al. (1987).

*2.3 DNA extraction and PCR-based method*

We followed a standard ammonium-acetate protocol (Sambrook 1989) to extract total DNA from the collected blood samples. For PCR-based analysis, we followed nested-PCR protocol using primers specific to avian *Plasmodium* and *Haemoproteus* parasites (Hellgren et al. 2004). To control for false amplification, we used positive (DNA of *P. relictum*) and negative (nuclease-free water) controls. The parasite DNA amplification outcomes were assessed by running electrophoresis on a 2% agarose gel.

To determine the genetic lineages of used parasites, we sequenced fragments from both 5′ and 3′ ends using an ABI PRISM TM 3100 capillary sequencing robot (Applied Biosystems, USA). Obtained sequences were aligned using BioEdit software (Hall 1999) and identified using the BLAST-program in GenBank and the MalAvi database (Bensch et al. 2009).

*2.4 Measurements of metabolic rate*

To determine the maintenance of metabolic rate exhibited by healthy birds, we measured their basal metabolic rate (BMR) before inoculation. We designate it as BMR because it fulfills all the criteria for BMR measurement (McNab 1997). This trait represents the minimal energetic metabolism necessary to maintain normothermia in a resting inactive nonreproductive adult endotherm in the postabsorptive state, measured during the ρ-phase (for day-active species it is nighttime) and within the thermoneutral zone of ambient temperatures (McNab 1997). The term RMR is currently used extensively, as measuring RMR requires much less stringent conditions compared to BMR: the only necessary condition is that the animal must be at rest. The term RMR is commonly used in studies on energy expenditure in infected animals, including those infected with various parasites (Delahay et al. 1995; Devevey et al. 2008; Magnanou et al. 2006; Robar et al. 2011; Sun et al. 2020), including blood parasites (Hahn et al. 2018; Schall 1990; Stager et al. 2021).

Subsequently in the text, we consistently refer to the initial metabolic rate of birds as BMR, and post-inoculation metabolic rates as RMR. Both BMR and RMR were estimated by flow-through respirometry. The average duration between the capture of all birds and the initial measurement of BMR was 25 days. The average number of days between the capture and inoculation procedure was 31.1 days for all birds.

Birds were deprived of access to food for at least two hours before experiments to ensure that they were in a post-absorptive state during metabolic rate measurements. Each day, at about 21:00, we placed up to four birds into the individual polypropylene chambers with a volume of 1.2 liters. These chambers, with birds inside them, were then placed within a thermostat to maintain the ambient temperature of 27 °C, which is within the thermoneutral zone of siskins (Gavrilov 2014). Five separate membrane air pumps pushed the outside air through the chambers containing indicating silica gel, facilitating the removal of water vapor from the incoming air. The treated air was subsequently directed into the chambers with the birds, achieving a flow rate of approximately 350-400 ml/min. The air pumps were connected to the uninterruptible power supply system to prevent suffocation of birds in chambers while possible power outages.

Each our experimental group consisted of 20 birds. To avoid overlapping metabolic measurements, we staggered the inoculation procedure across different days for each group. The SGS1 group was inoculated first, followed by the GRW2 group four days later, and the Control group received their injections five days after the GRW2 group. The initial metabolic measurement for the SGS1 group was conducted on the tenth day post-inoculation, and subsequent measurements continued regularly until the end of the experiment.

To measure the metabolic rate of several birds throughout one night, we used an airflow-switching multiplexed system that automatically alternated between the chamber containing a bird and the empty reference chamber into the respirometer. Each bird was measured for 20 min in each cycle. The measurement of every two birds was followed by a 10-min measurement of the O2 concentration in the airflow from the reference (empty) chamber.

The air from the chambers was dried in a 50 ml tube containing Drierite® desiccant (USA) and then passed through the mass flow-meter of the FoxBox respirometer (Sable Systems, USA). A portion of the airflow was subsampled at a rate of 120 ml/min through the O2 gas analyzer of the FoxBox respirometer, using a Dwyer GFC-1106 flow controller (Dwyer Instruments, USA). The desiccant was replaced daily, as insufficiently dehumidified air can result in an underestimation of measured oxygen concentration (Melanson et al. 2010). To minimize the system`s washout time, the volume of all pathways downstream of the animal chambers, including the desiccant chamber, was minimized (Frappell et al. 1989; Lighton and Halsey 2011).

The fractional concentrations of O2 were recorded with a sampling interval of 4 seconds. In each of the 20-min measurements of each bird, we discarded the first five min as a wash-out time. To estimate the minimum oxygen consumption of each bird (VO2-min), we used minimum running average procedure (Withers 2001). Using this procedure, for each bird in each cycle, among all possible 5-min average VO2 values, we found the minimum one. Subsequently, among all such VO2-min (their number was equal to the number of cycles), we found the lowest one and used it as an estimate of BMR and RMR.

Before starting all experiments, the gas analyzer was zero-calibrated with 6.0 nitrogen. Before each measurement session, the gas analyzer was calibrated using atmospheric air. The volume of oxygen consumed by the birds (VO2) was calculated according to Eq. 1a in Koteja (1996), assuming a fixed respiratory quotient (RQ) of 0.8. This value was then converted into energy expenditure (kJ/day) using the energetic equivalent of 20.1 kJ per 1 L of oxygen consumed (Table 12-1 in Brody and Lardy (1946)). At about 7:00, birds were released from their chambers and weighed with an accuracy of 0.1 g. We used this morning’s body mass in all statistical analyses.

*2.5 Analysis of IL-6 level*

We determined IL-6 levels in birds` blood plasma by enzyme-linked immunosorbent assay using a commercial test system Chicken Interleukin 6, IL-6 ELISA Kit according to the manufacturer’s instructions (Puda Scientific Co., Ltd, China). This test system was developed for the quantitative measurement of IL-6 in serum, plasma and tissue homogenates of chickens. This is a “sandwich” type ELISA kit, its detection range is 0,1-32 pg/ml, and its sensitivity is 0.1 pg/ml. Optical density was measured on a Bio-Rad 680 microplate photometer (USA) at a wavelength of 450 nm. The ELISA Kit required a sample volume of 10 µL of plasma, which was diluted with 40 µL of Sample Diluent provided within the kit.

*2.5 Statistical analysis*

All statistical analyses, data processing and visualizations were performed with functions of the statistical programming language R v. 4.2.3 (R Core Team, 2023).

Due to the non-linear nature of our dataset, which violated both normality and equal variance assumptions, we opted to use a Generalized Additive Mixed Model (GAMM). The GAMM approach allowed us to appropriately model the non-linear relationships and handle the repeated measures for each bird throughout the experiment. Using GAMMs, we described the dynamics of parasitemia, RMR, IL-6 level. GAMMs are an extension of generalized additive models (GAM). As GAM, GAMMs incorporate smooth functions, which enable to model non-linear relationships between covariate and response variable, but the effect of grouping (random) factor was included as well (Wood 2017).

Within all our analysis the bird’s individual ring number (ID) was used as a random factor (Pedersen E. J. 2019). For each response variable, we fitted two models. The first one included one common smoother for all treatment levels (Control, SGS1 and GRW2), while the second model incorporated distinct smoothers, each corresponding to a different treatment. Both models within each response variable were compared using the Akaike information criterion (AIC). The model with the lowest AIC value was considered the final one.

In the GAMM applied for the description of RMR dynamics, we used to log10-transformed RMR as a response variable and as an explanatory variable we used the belonging to a specific experimental or control group and the DPI. Bird’s body mass (log10-transformed Mb) was included as a covariate. Smoothers were added for the DPI in order to account for the repeated measurements over time. For modeling the dynamics of IL-6 we used log10-transformed IL-6 level and as an explanatory variable we used belonging to a specific experimental or control group. For RMR and IL-6 level GAMMs were constructed based on Gaussian distribution. We did not include parasitemia as a predictor because the DPI predictor serves as a more consistent representation of infection progression. DPI provides a consistent measure of the infection timeline and helps avoid multicollinearity, which could occur if parasitemia were used directly due to its non-linear, bell-shaped pattern. By using DPI, we are better able to capture the dynamic changes in the response variable throughout the course of the infection. For modeling the dynamics of parasitemia, again, only belonging to a specific experimental or control group was used as explanatory variable. Since parasitemia, our response variable, cannot have negative values, we used a negative binomial distribution to model it using GAMM.

To assess the significant differences between our GAMMs for each studied physiological parameter, we employed a pairwise comparison of the estimated smoothers and their associated 95% confidence intervals (95 % CI). This approach enabled us to compare trends across different factor levels for all pairs of treatment groups over time. By examining the difference between trends, we could determine if any observed discrepancies were statistically significant. If the difference between trends for compared groups is negligible, it will be indistinguishable from zero, suggesting that the treatment (infection with one of the two lineages of parasites and the control group) did not induce a discernible change in response within either compared group. In contrast, when the shifts in trends occur, the CI excludes zero, and this allows us to draw conclusions about the differences between the groups without the necessity of calculating the P-value. For a more detailed description of the applied method see Mundo et al. (2022) and Simpson (2017).

For all models`, validity was assessed using residual and quantile-quantile plots. Function ‘appraise’ from the package “gratia” (Simpson 2023) was used for the diagnostic analysis. For GAM-based analysis we applied function ‘gam’ from the package “mgcv” (Wood 2017).

We checked the individual stability of RMR during the development of parasitemia by assessing its repeatability (Lessells and Boag 1987; Nakagawa and Schielzeth 2010). We estimated repeatability of RMR using linear mixed-effects models (LMM), fitted by the function rpt() from the “rptR” package (Stoffel et al. 2017). The individual ring number (ID) was set as a random effect and log10 (RMR) as a response factor. Since RMR highly depends on body mass (Mb), we estimated adjusted RMR repeatability (Nakagawa and Schielzeth 2010) using log10(Mb) as the covariate. Both the number of parametric bootstraps for estimation of standard error (SE) of repeatability (R) and the number of permutations for estimation of the P-value were set to 1E4.

*2.6* *Ethical statement*

Care and handling of animals was under current laws of Russia. Euthanasia of the experimental birds was permitted by the Forest and Nature Protection Agency of Kaliningrad Region, Russia (№ 26 of 13/06/2018), whose permits were based on the decisions made by the Specialized Committee of the Scientific Council of the Zoological Institute RAS and Russian Foundation for Basic Research. Experimental procedures were approved by the Ethical Committee of the Zoological Institute RAS (№ 2019-01-06 of 02/04/2019).

**3. Results**

*3.1 Parasitemia development*

All experimental birds were susceptible to the infections. The development of parasitemia in the SGS1 group was typical, with a prepatent period of about 6 days post inoculation (DPI) and a peak occurring on the 18th DPI, with a mean parasitemia 50.5% (±7.1%, here and thereafter SE are given). Minimal parasitemia during the acute stage was 0.1% and the maximum reached 90%. By the end of the experiment, all birds had less than 1% parasitemia except one bird with 3.5%. In the GRW2 group, the prepatent period exceeded 6 DPI, with the peak occurring with a slight delay on the 24th DPI with a mean parasitemia of 33.2% (±6.7). The acute stage was prolonged and by the end of the experiment in eight birds out of ten survived when parasitemia remained above 1%. The mortality rate for birds with the SGS1 was 50% (10 birds) and 45% (9 birds) for the GRW2 group. The control group experienced a mortality rate of 25% (5 birds).

GAMM fitted with two different smoothers for two parasite species (AIC = 1326) was better in comparison with GAMM including one common smoother for both species (AIC = 1362). The model parameters are given in Table 1.

No significant difference in the mean parasitemia level between the two parasite species was revealed in the fitted model (Table 1. parametric terms). The general patterns of parasitemia dynamics were similar for both species (Fig. 1, A). Although, in the case of SGS1, the parasitemia was significantly higher than that of GRW2 at the beginning of the disease development, at the end of the experiment, the parasitemia level of the second species became significantly higher than that of the first (Fig. 1, B).

*3.2 Analysis of metabolic rates` dynamics*

The mean value of BMR in siskins after the capture was 22.03 kJ/day with a mean body mass of 12.87 g. There were no significant differences in BMR observed between the experimental and control birds (ANOVA: P = 0.065) or between sexes (ANOVA: P = 0.639) prior to the inoculation procedure. Similarly, there was no significant difference in BMR between the experimental groups infected with SGS1 and GRW2 (ANOVA: P = 0.061).

In describing the RMR dynamics, the GAMM model with different smoothers (AIC = -753) was better than the model with common smoother for all groups (AIC = -700). This suggests that RMR dynamics differed for each siskin group (SGS1, GRW2 and Control). The smoothers for all groups were significantly curved (i.e., differed from the horizontal straight line, Table 2), indicating an unstable RMR level over the observation time within each group. (Fig. 2, A).

In the Control group, there was a slight increase in RMR during the first two weeks after the inoculation, whereas in both experimental groups, RMR exhibited a significant decrease.

In the SGS1 group, immediately after the inoculation, a decline in RMR was observed, followed by subsequent increase. By the end of the experiment, on average, RMR was higher than it was initially (Fig. 2, A). The RMR level in the SGS1 group was significantly lower than in the Control one during the period of 3-23 DPI, but later (32-50 DPI) the RMR level in the SGS1 group was higher than in the Control (Fig. 2, B).

In GRW2 birds, RMR at first decreased (Fig. 2, A): during 3-14 DPI it was significantly lower than in the Control group (Fig. 2, B). However, during the following days there were no significant differences between GRW2 and Control groups (Fig. 2. B).

The comparison of RMR dynamics in two inoculated groups (SGS1 vs GRW2, Fig. 2, B) reveals significantly higher RMR levels in GRW2 birds during 17-26 DPI. However, later for a short period (during 46-49 DPI) an opposite pattern was recorded.

*3.3* *Analysis of IL-6 level dynamics*

The best GAMM for IL-6 changes was, as for parasitemia and RMR, with different smoothers for each group of siskins (AIC = -353, for the model with common smoother AIC = -251). The parameters of the model are presented in Table 3.

After the inoculation procedure in the Control group of birds, the level of IL-6 began to decrease from its initial levels. It was the lowest on the 3rd week, after which it began to rise (Fig. 3, A). A similar pattern was revealed in the IL-6 dynamics between birds from the SGS1 group. The concentration of IL-6 decreased during several days after inoculation. However, at the end of the experiment, the average concentration was even higher than the initial level, due to some birds exhibiting several times higher levels of IL-6 from their zero-day values (Fig. 3, A). The IL-6 concentration in the SGS1 group was significantly higher than in the Control group at the beginning and near the end of the survey (Fig. 3, B). Nonetheless, during the middle of the observation period, the IL-6 concentration was significantly lower than in the Control group (Fig. 3, B).

The dynamics of IL-6 in the GRW2 group were more complex. It displayed two peaks: one during the first half of the observation period and another in the second half (Fig. 3, A). The IL-6 concentration in the SGS1 group was significantly higher than in the GRW2 group for a brief period at the beginning of the study (0-2 DPI) and during the last days of observations (45-53 DPI). From 6 to 22 DPI, IL-6 concentrations were significantly higher in the GRW2 group than in the SGS1 group, but no significant differences were found between groups 23-44 DPI.

*3.4 Analysis of repeatabilities*

The repeatabilities of mass-independent RMR in Control, SGS1 and GRW2 groups were R = 0.207±0.114 (P = 0.009), R = 0.359±0.136 (P = 0.007) and R = 0 (P = 0.997; there was singular fit in the model since the variance of the random effect was close to zero). The repeatabilities of log10(Mb) in Control, SGS1 and GRW2 groups were R = 0.464±0.122 (P < 0.001), R = 0.695±0.102 (P < 0.001) and R = 0.139±0.108 (P = 0.07), respectively.

**4. Discussion**

*4.1 Parasitemia development*

Both *P. relictum* SGS1 and *P. ashfordi* GRW2 avian malaria parasite species are considered generalists with a wide range of potential host species. According to published papers, both exhibit high levels of parasitemia during primary infections of juvenile siskins (Palinauskas et al. 2008; Videvall et al. 2017). Palinauskas et al. (2011) was the first to report susceptibility to infected blood inoculation and the formation of gametocytes of *P. ashfordi* in Northern Palearctic bird species, including *S. spinus* and *Loxia curvirostra*. The development of the *P. relictum* SGS1 parasite was more rapid than that of *P. ashfordi* GRW2. The acute stage started and ended earlier and more simultaneously in SGS1-infected birds than in GRW2. The long prepatent period of *P. ashfordi* in our study corresponds to the idea of a more extended prepatent period for most parasites of *Novyella* subgenus, to which *P. ashfordi* belongs (Garnham 1966; Palinauskas et al. 2011; Valkiūnas 2005). The prolonged prepatent period of *P. ashfordi* observed in our experiment aligns with findings from other studies and supports the notion of an extended prepatent period for most parasites of the *Novyella* subgenus, to which *P. ashfordi* belongs (Garnham 1966; Palinauskas et al. 2011; Valkiūnas 2005). In addition to the prolonged hidden stage in this group, high levels of parasitemia persisted until the end of our experiment on Day 54 post-infection (DPI). Previous studies with siskins infected with *P. ashfordi* commonly ended on DPI 30-33, during which authors observed a decline in parasitemia levels from its peak but noted that parasitemia remained high. However, whether this persistence is attributable to host-parasite interactions or specific characteristics of the parasite species, such as a late peak in parasitemia, remains unclear (Videvall et al. 2017).

*4.2 Oxygen consumption during different malaria infections in siskins*

A traditional view suggests that RMR should be positively linked to parasite loads (Bordes and Morand 2011). However, this statement still lacks experimental evidence, and available data contradict each other. Meta-analysis of research data that investigated the impact of parasitic load on the RMR of various animal groups indicated that, in most cases, the RMR of hosts increased after parasite infestation (Robar et al. 2011). Nonetheless, the overall effect of parasites was weak and not statistically significant. As the author suggests, the lack of consistent effect of parasites on hosts` energy metabolism in analyzed articles may be explained by different host-parasite systems used in described experiments (Robar et al. 2011)

We are aware of only three studies that focus on the impact of haemosporidian parasites on hosts` metabolic rates, and their results contradict each other. The first was conducted on two-year-old domestic canaries (*Serinus canaria*), where birds were infected with *Plasmodium relictum* of unknown genetic lineage. It was shown that during the peak of parasitemia, oxygen consumption decreased under both thermoneutral and low-temperature conditions (Hayworth et al. 1987). The study by Hahn et al. (2018) on infected great reed warblers (*Acrocephalus arundinaceus*) did not find any difference in RMR and maximal metabolic rate (MMR) between non-infected and experimentally infected birds with *Plasmodium relictum*  (lineage GRW4), during both acute and chronic stages. Hahn et al. (2018) concluded that low-level parasitemia (less than 1%) during avian malaria did not affect the aerobic performance of birds. A recent study by Stager et al. (2021) on the wild pink-sided junco *(Junco hyemalis mearnsi*) demonstrated that the presence of haemosporidian parasites (*Haemoproteus* or *Plasmodium*) did not correlate with any of the measured physiological indices, particularly with the RMR of the birds. The authors concluded that there was a minor cost of haemosporidian infection on either junco aerobic performance or energy budgets.

The results of our study reveal that oxygen consumption in immunologically naïve birds after malaria infection differs depending on the malaria parasite. In the *P. relictum* SGS1 group, the decrease of RMR coincided with the acute phase of parasitemia. This is similar to the result reported by Hayworth et al. (1987), who observed a significant decrease in oxygen consumption in canaries during the crisis period of *P. relictum* infection. Since the destruction of erythrocytes at this time is most pronounced, it is reasonable to assume that hematological parameters, such as hematocrit and hemoglobin, should be negatively affected by proliferating parasite (Hammond et al. 2000; Stager et al. 2021). Our study did not measure hematocrit level or hemoglobin concentration – parameters that reflect blood`s capacity to carry oxygen, because the amount of blood collected from each experimental bird was constrained. Several studies indicated a decrease in hematocrit in experimentally infected birds during the acute phase of the *Plasmodium* infection (Ilgūnas et al. 2019; LaPointe et al. 2012; Palinauskas et al. 2008; Paulman and McAllister 2005; Williams 2005), as well as in hemoglobin concentration (Krams et al. 2013; Palinauskas et al. 2022). Videvall et al. (2020) reported a negative correlation between parasitemia levels and the expression of genes involved in oxygen binding and transportation processes in siskins infected with *P. relictum* SGS1. Interestingly, in the earlier study with siskins and *P. ashfordi* GRW2, Videvall et al. (2015) observed a significant expression of genes responsible for metabolic functions and oxidation-reduction processes during both peak and decreasing parasitemia stages. Afterwards, during the late stage of malaria, when parasitemia decreased, catabolic processes became predominant compared to the peak of parasitemia. Comparing the result of these two studies, Videvall et al. noted similarities in functions of expressed genes in SGS1-infected (Videvall et al., 2020) and GRW2-infected siskins (Videvall et al., 2015). Authors also demonstrated that highly-virulent SGS1 induced a strong transcriptome response, while the low-virulent GRW4 – minor. According to this data and given the high levels of parasitemia observed for both P. relictum SGS1 and P. ashfordi GRW2 in our experiment, we conclude that both parasites are highly virulent and can substantially impact the measured physiological traits of experimental siskins.

The pathological consequences of malaria vary depending on host and parasite species, their interactions, environmental factors, host individual traits, and parasite isolates – all of which can affect the disease outcome (Cornet and Sorci 2014). In the study by Hahn et al. (2018), no correlation was observed between parasitemia intensity and birds' aerobic performance, while the peak parasitemia recorded in this study was lower than 1% of parasitized erythrocytes. In contrast, in Hayworth et. al. (1987) it ranged from 1.32% to 50%. Hayworth et. al. (1987) found a significant positive correlation between level of parasitemia at the peak and the relative decrease in oxygen consumption during low temperature conditions, though it was not significant in the thermoneutral zone. It appears evident that the higher the parasitemia the more detrimental impact it has on the bird’s health and would impact metabolic rate.

In the light of these considerations, it is curious that for siskins from the GRW2 group, our GAMM also showed a period of lowered RMR, albeit short. During that time period (6-12 DPI) the average level of parasitemia was relatively small (avg. 0.03 and 2.74%, respectively), indicating that acute phase had not appeared yet. But, starting on day 12 DPI, both RMR and parasitemia began to rise. We assume two possible explanations for this: a) the average parasitemia in the GRW2 group during the crisis was 33%, which is almost two-thirds of the parasitemia in the SGS1 group (50%). This lower parasitemia level in group GRW2 may not have caused sufficient erythrocyte destruction to reduce the metabolic rate. However, we cannot definitively claim that one parasitemia is less severe than the other, especially when both are so high. Additionally, it is noteworthy that relying solely on parasitemia levels within the bloodstream may not consistently offer an accurate assessment of malaria severity. For example, in the case of *P. elongatum* (subgenus *Huffia*), characterized by typically low-level parasitemia (generally below 1%), it can induce significant pathology (Valkiūnas et al. 2008). This arises from its impact on the erythropoietic system within the bone marrow, a consequence of the destruction of stem cells by exoerythrocytic stages (Palinauskas et al. 2016). b) the increase in RMR might indicate the activation of immune response or, at least, an increase in energy expenditure during the acute phase of an unfamiliar tropical malaria infection. This is suggested by the rise in RMR coincided with the peak parasitemia in birds GRW2 birds 24 DPI. After 30 DPI, the difference between the two experimental groups in RMR became statistically insignificant, with the tendency for birds from the SGS1 group to have a higher rate of metabolism. These two explanations do not contradict each other and may indeed be interrelated. It is possible that we are observing the effect hypothesized initially – that up to a certain level of parasitemia, healthy red blood cells can maintain the basal metabolic rate and even allow the bird to increase oxygen consumption to mount an immune response and resist the parasite. While our current data do not allow us to state this with certainty, future studies should aim to compare oxygen consumption levels and various indices of immune response among birds infected with the same parasite but exhibiting different parasitemia levels.

Interestingly, we observed an increase in oxygen consumption in the Control group after the inoculation of uninfected blood. This increase lasted for about 2 weeks, after which RMR decreased and, by the 5th week, became indistinguishable from the initial level. We see a possible reason for this in: a) stress, because with the beginning of the experiment, birds were regularly subjected to handling and blood taking procedures, which both may have caused a short term stress additional to chronic stress from captivity (Li et al. 2019; Thompson et al. 2015; Voss et al. 2010); b) immune response due to the inoculation with uninfected blood, since blood itself can cause an inflammatory response (Ellis et al. 2015; Garraud et al. 2016). We cannot exclude the possibility that these factors were acting simultaneously. a).

Despite the high peak of parasitemia in birds infected with *P. relictum* SGS1, repeatabilities of both body mass and mass-independent RMR during the course of the disease in this group were significant and even exceeded the corresponding repeatabilities in the Control group. However, both corresponding repeatabilities in birds infected with *P. ashfordi* GRW2 did not differ significantly from zero, suggesting that GRW2malaria infection causes more significant changes in bird physiology compared to SGS1.

*4.3 Il-6 level in the blood of infected siskins*

Some research suggests that in addition to the direct harm inflicted by the parasite, such as red blood cell destruction, the immune system's response plays a central role in malaria-induced damage (Artavanis-Tsakonas et al. 2003). This occurs predominantly during acute inflammation, caused by the immune system to eradicate the parasites. However, while inflammation aids in parasite clearance, heightened levels of pro-inflammatory cytokines can exacerbate immunopathology, contributing to severe tissue damage (Coban et al. 2018).

In the SGS1 group, siskins exhibited a consistent decline in IL-6 levels during the initial two weeks post-infection. However, towards the later stages of the experiment, it's noteworthy that IL-6 began to increase, reaching levels several times higher than those observed at the start of the experiment. In contrast to SGS1, birds infected with GRW2 parasite tended to increase their IL-6 level in plasma soon after the infection and up to 12 DPI. As shown in figure 1, the week between 12 DPI and 18 DPI was a period when parasites began to multiplicate rapidly. This coincided with the transition from the increase of IL-6 levels to its decrease in GRW2-infected siskins. It seems like birds in both groups tended to decrease IL-6 level in response to the rapid multiplication of the parasite. This aspect is particularly intriguing because, contrary to our findings, the majority of studies conducted on malaria-infected humans have linked elevated levels of IL-6 with malaria severity (reviewed in Wilairatana et al., 2022). Our observations appear to be somewhat similar to the findings from Henao's (2019) master's degree project, which also studied siskins infected with *Plasmodium relictum* SGS1. Henao's research demonstrated that the expression level of IL-15 on Day 8 post-infection (DPI) was negatively correlated with the rapidly increasing parasitemia levels. Specifically, birds with high parasitemia expressed less IL-15, approaching the expression levels observed in uninfected birds. This resemblance to our results for siskins from the SGS1 group is notable, although we observed a decrease in interleukin levels in both the Control and SGS1-infected groups. IL-15 is required for type 1 cytokine production, natural killer cells and dendritic cells responses, and, as it was demonstrated on *P.* *chabaudi* infected mice, for the synthesis of malaria-specific antibodies (Ing et al. 2005). The authors suggested that infected birds may express less IL-15 in order to control for hyperreactivity of immune response to the growing number of parasitic antigens (Henao 2019).

However, given the fact that IL-15 and IL-6 are different cytokines, there is a possibility that comparable mechanisms might be implicated in dampening their expression and diminishing their ultimate levels in the bloodstream. This suggests that the observed decrease in IL-6 in our study could potentially be linked to malaria-induced immunosuppression and/or activation of a tolerogenic immune response by the host itself, potentially to mitigate immunopathology (Calle et al. 2021; Henao 2019).

Analysis of birds` transcriptome response to malaria showed that expressed genes were associated with innate and adaptive immunity and their expression was active during the peak of parasitemia and less or not active during the malaria late stages (Paxton et al. 2023; Videvall et al. 2020). Paxton et al. (2023) showed that Hawaiʻi ‘amakihi (*Chlorodrepanis virens*) from a highly susceptible population that succumbed to *P. relictum* GRW4 in experiment, as well as those that recovered, exhibited different gene expression profiles at different stages of malaria. Birds that did not survive the infection had a dysregulation of the innate immune system, resulting in increased levels of gene expression at the middle and late stages of infection. In contrast, survivors showed the upregulation of genes of both innate and adaptive immunity at the peak of parasitemia. We cannot make a comprehensive assessment of gene expression solely based on IL-6 levels. However, in light of the findings by Paxton et al. (2023), it is remarkable that by the end of our experiment, a majority of birds infected with the GRW2 parasite had very low levels of IL-6, the lowest among all three groups of birds. In contrast, several birds from the SGS1 group that survived until the end of the experiment had several times higher levels of IL-6 in their blood plasma after they survived the critical moment at the peak parasitemia. Some research indicate that malaria might indirectly increase vulnerability to and complications of other diseases (Scott et al. 2011). Human patients with malaria are more prone to bacteremia (Lee and Coban 2018), and while anti-malaria interventions reduce malaria-related deaths, they also lead to a decline in overall morbidity and mortality, suggesting a broader influence on health (Aregawi et al. 2017). Our experiments involved wild birds. Despite implementing quarantine measures for all birds prior to the experiments, we were unable to completely rule out or diagnose concurrent infections that could potentially influence the results. We assume that the observed elevation of IL-6 in some birds from the SGS1 group may indicate the recrudescence of an unidentified chronic disease, which manifested following malaria-induced immune dysregulation. Additionally, this could also explain the mortality observed in the control group, where two out of five birds died on the 3rd and 9th days after inoculation with uninfected donor blood. Although the donor for the control group was not infected with malaria, it is possible that it carried another infection beyond our control.

Pro-inflammatory cytokines play a crucial role in eliminating malaria parasites and their up-regulation is associated with resistance mechanism within host immune response. Several works with deliberate suppression of inflammatory response in experimental animals infected with *Plasmodium* parasite have shown the reduction of the cost of infection, mortality and malaria virulence, while significant increase in parasitemia (see Sorci, 2013). Immunosuppression is considered as one of the tolerance mechanisms aimed to minimize the harm of inflammation, though the underlying machinery of it remains yet unexplored (Calle et al. 2021).

In the control birds, there was also a decrease of IL-6 levels right after the inoculation. This appears inconsistent with the idea that control birds experience prolonged immune activation due to a single injection of birds` blood free from malaria. Instead, it seems that the primary factor influencing changes in RMR and IL-6 levels in control siskins was the chronic stress arising from captivity and handling. Chronic stress can have immunosuppressive effects, contrary to the acute stress, which typically has immunoenhancing properties (reviewed in Dhabhar and Mcewen (1997), but see Martin et al. (2011)).

IL-6 is a multifunctional cytokine with roles in acute-phase immune response, immune regulation, and hematopoiesis (Heinrich et al. 1990; Van Snick 1990). Consequently, the regular procedure of blood sampling may influence IL-6 levels in the plasma of experimental birds during subsequent blood collection, potentially exacerbating the hemolytic effects of high parasitemia levels. To date, no studies have investigated the long-term effects of regular blood loss on interleukin serum concentrations in either mammals or birds. However, several studies have examined the impact of blood loss during surgical procedures on serum IL-6 levels in humans. These studies have shown a positive correlation between IL-6 levels and such factors such as operation duration, amount of blood loss, and extent of tissue injury, with peak levels typically observed within 48 hours post-surgery (Igarashi et al. 1996; Sakamoto et al. 1994). Regular blood sampling may also impact metabolic rate, in addition to serum IL-6 levels. However, there is limited research on the effects of blood loss on resting metabolic rate (RMR). Sun et al. (2020) simulated blood loss caused by hematophagous parasites in tree swallow nestlings (*Tachycineta bicolor*) and found no decrease in hemoglobin levels or changes in RMR due to blood loss alone. Interestingly, they observed a positive correlation between ectoparasite load and increased RMR, suggesting that continuous blood loss from feeding ectoparasites and the simultaneous production of red blood cells by parasitized birds may contribute to elevated RMR (Sun et al. 2020). In our study, we sampled no more than 150 μl of blood each six days (see Materials and methods). Though acute blood removal may have some effects on bird fitness, the research suggest that its impact is generally limited (Orzechowski, Shipley, Pegan, & Winkler, 2019). We cannot dismiss the potential adverse effects of this invasive procedure itself, nor its potential cumulative impact alongside parasite-induced anemia, on our measured parameters. Not all the highly parasitized, and therefore with high hemolysis level birds died during the experiment. The effect of blood sampling can be speculated through the control group of birds, as they had not experienced hemolysis from malaria parasite activity, only regular blood loss due to blood sampling. However, it still overlaps with effects from stress from captivity and handling, as discussed earlier.

Experimental birds infected with avian malaria parasites exhibited distinct dynamics of physiological parameters not only compared with control birds, but also between experimental birds where birds were infected with parasites of different *Plasmodium* species. SGS1 infected siskins had both reduced RMR and IL-6 levels during the acute stage of parasitemia, which is consistent with previous findings in other studies. Conversely, GRW2 infected birds demonstrated rather erratic shifts in both RMR and IL-6 levels, which were challenging to interpret in the context of parasitemia development. At the same time, the repeatability of mass-independent RMR and body mass was lower in GRW2 infected birds, than in SGS1 infected birds. We can confidently assert that different avian *Plasmodium* parasites can exert varying effects on the health parameters of their avian hosts. These disparities may also arise from the intricate co-evolutionary dynamics between the host and the parasite. Specifically, in the case of siskins, it is important to consider that they have likely co-evolved with the SGS1 parasite, which has adapted to their local environment. On the other hand, the GRW2 parasite originates from Africa and can be considered exotic for siskins. In the context of climate change and anthropological impacts, the transmission of tropical-origin parasites to northern latitudes has become a matter of concern. Should such transmission occur, it could lead to unpredictable outcomes for local bird populations. Furthermore, our findings with SGS1-infected siskins align closely with the limited existing research that has explored the effects of *P. relictum* parasites on aerobic performance and cytokine expression in avian hosts. Specifically, we observed similar patterns of cytokine expression and aerobic performance alterations as reported in previous studies. Our work helps to elucidate the complexities of the eco-physiological consequences of avian malaria infection and highlights the need for continued interdisciplinary research to fully comprehend the implications of these infections on avian health and population dynamics.

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Figure captions

Figure 1. Parasitemia (%) on different days post inoculation (DPI) of two Plasmodium species. Panel A represents the observed level of parasitemia (given in %) (dots) and predicted GAMM (lines, gray areas around the lines represent 95% CI). Panel B represents the difference between smoothers. Time periods when the difference between smoothers is significantly different from zero are marked by black rectangles.

Figure 2. RMR at different days post inoculation (DPI) in different birds’ groups. Panel A represents the observed RMR level (dots) and GAMM predictions (lines and 95% CI). Panel B represents the difference between smoothers. Time periods when the difference between smoothers is significantly different from zero are marked by black rectangles. The red shading reflects the level of parasitemia.

Figure 3. IL-6 at different days post inoculation (DPI) in different birds’ groups. Panel A represents the observed IL-6 concentration (dots) and GAMM predictions (lines and 95% CI). Panel B represents the difference between smoothers. Time periods when the difference between smoothers is significantly different from zero are marked by black rectangles. The red shading reflects the level of parasitemia.

Table 1. GAMM parameters characterizing the course of parasitemia after parasite inoculation.

| Parametric terms | | | | |
| --- | --- | --- | --- | --- |
| Term | Value | Std.Error | t | p |
| (Intercept) | 0.260 | 0.365 | 0.714 | 0.475 |
| Lineage (GRW2) | -0.221 | 0.564 | -0.391 | 0.695 |
| Smooth terms | | | |
| Term | edf | F | p |
| s(DPI): SGS1 | 6.939 | 234.997 | 0.000 |
| s(DPI): GRW2 | 6.286 | 119.558 | 0.000 |
| Random factor | 25.379 | 109.395 | 0.000 |

Table 2. GAMM parameters characterizing the course of RMR after parasite inoculation.

| Parametric terms | | | | |
| --- | --- | --- | --- | --- |
| Term | Value | Std.Error | t | p |
| (Intercept) | 0.869 | 0.108 | 8.026 | 0.000 |
| Lineage (SGS1) | -0.021 | 0.011 | -1.949 | 0.053 |
| Lineage (GRW2) | 0.007 | 0.011 | 0.654 | 0.514 |
| log10(Mass) | 0.444 | 0.097 | 4.559 | 0.000 |
| Smooth terms | | | |
| Term | edf | F | p |
| s(DPI): Control | 3.350 | 3.042 | 0.019 |
| s(DPI): SGS1 | 3.475 | 12.937 | 0.000 |
| s(DPI): GRW2 | 4.702 | 5.730 | 0.000 |
| Random factor | 32.139 | 2.115 | 0.000 |

Table 3. GAMM parameters characterizing the course of IL-6 concentration after parasite inoculation.

| Parametric terms | | | | |
| --- | --- | --- | --- | --- |
| Term | Value | Std.Error | t | p |
| (Intercept) | 1.102 | 0.018 | 61.756 | 0.000 |
| Lineage (SGS1) | 0.032 | 0.025 | 1.237 | 0.217 |
| Lineage (GRW2) | -0.081 | 0.026 | -3.143 | 0.002 |
| Smooth terms | | | |
| Term | edf | F | p |
| s(DPI): Control | 3.830 | 24.859 | 0.000 |
| s(DPI): SGS1 | 3.461 | 23.536 | 0.000 |
| s(DPI): GRW2 | 3.898 | 14.763 | 0.000 |
| Random factor | 24.938 | 0.908 | 0.000 |