Haematological variables are good predictors of recruitment in nestling pied flycatchers (*Ficedula hypoleuca*)¹

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Abstract: The number of different types of circulating leucocytes may provide information about the health state of birds in the wild. We counted the number and proportions of circulating leucocytes in blood smears of nestling pied flycatchers (*Ficedula hypoleuca*) shortly before fledging. We studied the relationship between these haematological measures and environmental factors like parasitism, body mass, hatching date, and brood size. The heterophils-to-lymphocytes ratio was higher in nestlings whose nests suffered from mite infestation and in lighter individuals, heterophils being the cells that responded preferentially to malnutrition. Recruited birds had lower lymphocyte and heterophil counts when nestlings than non-recruited ones. Our results show that heterophil count is a better predictor of local recruitment than other variables widely used for nestlings as survival predictors, like body mass or hatching date, supporting the hypothesis that low heterophil counts reflect a good individual health state in nestlings.

Keywords: health state, leucocytes, nestlings, parasitism, pied flycatchers, recruitment.

Résumé : Le nombre des différents types de leucocytes en circulation dans le sang peut fournir de l'information au sujet de l'état de santé des oiseaux dans la nature. Nous avons compté le nombre et déterminé les proportions de leucocytes circulant dans des frottis sanguins provenant d'oisillons gobemouches noirs (*Ficedula hypoleuca*) peu de temps avant leur premier envol. Nous avons de cette façon étudié la relation entre ces mesures hématologiques et des facteurs environnementaux, comme le parasitisme, la masse corporelle, la date de l'éclosion et la taille de la couvée. Le rapport entre les leucocytes neutrophiles et les lymphocytes est plus élevé chez les oisillons dont les nids sont infestés par des mites et chez les individus les plus légers, les leucocytes neutrophiles étant les cellules sanguines qui se manifestent le plus lors d'une malnutrition. Les oiseaux qui ont recruté dans la population avaient des numérations de lymphocytes et de leucocytes neutrophiles moins élevées au stade de jeunes au nid que ceux qui n'ont pas recruté. Ainsi, la numération des leucocytes neutrophiles serait un meilleur prédicteur du recrutement local que d'autres variables fréquemment utilisées, comme la masse corporelle ou la date d'éclosion. En somme, nous appuyons l'hypothèse que les numérations de leucocytes neutrophiles constituent de bons indicateurs de santé chez les oisillons.

Mots-clés : état de santé, gobemouche noir, leucocytes, oisillons, parasitisme, recrutement.

Nomenclature: Baker, 1956; Moss, 1968; Bennett, Peirce & Earle, 1994.

Introduction

The immune system is a defence mechanism that protects animals from pathogen attacks. Immune responses are mediated by a variety of cells that originate in the bone marrow and differentiate into specific cell types in primary lymphoid organs (Janeway *et al.*, 1999). White blood cell circulation is crucial to the performance of the surveillance as well as effector functions of the immune system. Because immune cells move between tissues through the bloodstream, the numbers and proportions of leucocytes in circulation provide an important representation of the state of leucocyte distribution in the body (Dhabhar *et al.*, 1995). The major classes of avian white blood cells include B and T lymphocytes, heterophils, basophils, monocytes, macrophages, eosinophils, and

other auxiliary cells. Lymphocytes and heterophils are the most abundant types of leucocytes of avian blood (Campbell, 1995). Lymphocytes have high antigenic specificity given by the diversity of antigenic receptors on their surface (Janeway *et al.*, 1999), while the phagocytic heterophils are the most important components of non-specific immunity (Maxwell, 1993).

The relationship between leucocytes and stressors responsible for the induction of physiological stress in birds has been intensively studied in the last decades. Since Davidson, Rowell, and Rea (1983) and Gross and Siegel (1983) described a ratio calculated from the proportions of heterophils (H) and lymphocytes (L) in domestic fowl as a measure of stress, the H/L ratio has become widely accepted as a reliable and accurate physiological indicator of stress (Maxwell & Robertson, 1998). In this sense, nestling levels of physiological stress may reflect the variable conditions to which they are exposed with respect to nutrition during growth (Hoi-Leitner *et al.*, 2001).

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The development of the immune system in altricial birds has been little studied (Apanius, 1998b). Altricial nestlings have a well-developed innate immune system, but do not have the full lymphocyte repertoire. In fact, the phagocytic system has been suggested to be the major effector mechanism of the neonate (Klasing & Leshchinsky, 1999). Several factors may affect levels of different types of leucocytes in altricial nestlings. The level of nutrition in the nest may affect the development of the immune system, given that substrates like amino acids and lipids are required for the initial leucopoiesis needed to supply hatchlings with leucocytes (Klasing & Leshchinsky, 1999). Accordingly, nestling immune function appears to be sensitive to growth conditions (Hörak *et al.*, 1999). Also, nutrition may be linked to brood size, and nestling size or dominance rank within broods. Thus, Moreno *et al.* (2002a) found a strong correlation between H/L and nestling mass in pied flycatchers (*Ficedula hypoleuca*) and Martínez-Padilla *et al.* (2004) have shown an association of stress to within-brood rank in nestling European kestrels (*Falco tinnunculus*). Moreover, experimental studies by Ilmonen *et al.* (2003) and Hörak *et al.* (1999) showed an influence of brood size manipulation on leucocyte profiles. Also, parasites may induce increased circulation of certain types of leucocytes (Ots & Hörak, 1998), as the host can regulate the degree of infection through the activities of its immune system (Wakelin, 1984). Merino *et al.* (2000) showed that blood parasites have pathogenic effects on their hosts in the wild, while ectoparasites have been reported to decrease growth of nestling pied flycatchers (Merino & Potti, 1995b; Merino *et al.*, 1998).

Total leucocyte count has been interpreted either as a signal of increased immunocompetence (Gustafsson *et al.*, 1994; Dufva & Allander, 1995) or as the opposite (Apanius, 1998a). However, the abundance of leucocytes in the blood stream may be subjected to correlations with nestling fitness parameters without any prior assumption about expected trends and without presuming associations with immunocompetence.

The variables most frequently connected with survival in the ornithological literature are body mass and hatching date. Individuals with a higher mass at fledging have been shown to have a higher probability of survival (Perrins, 1965; Tinbergen & Boerlijst, 1990; Magrath, 1991; Both, Visser & Verboven, 1999; Monrós, Belda & Barba, 2002). In the same way, hatching date has been proposed as a predictor of post-fledging survival in many studies (Perrins, 1965; Cooke, Findlay & Rockwell, 1984; Harris, Halley & Wanless, 1992), while in others its effect remains unclear (Monrós, Belda & Barba, 2002). However, body mass and hatching date could in fact be mere correlates of the real condition or health factor driving survival probability after fledging. It is known that a wide array of pathogens and parasites affect survival of their avian hosts (Janeway *et al.*, 1999). If haematological indices reflect infection status, we could expect them to be associated with nestling survival prospects after leaving the nest. Møller and Saino (2004) have shown that immune function parameters are associated with survival of adults and juveniles. However, no study has yet tried to link offspring recruitment probability to leucocyte counts.

In this study we analyze leucocyte counts in peripheral blood of nestling pied flycatchers in relation to nutrition, parasitism, and survival prospects. Ots, Murumägi, and Hörak (1998) studied the sources of error of the leucocyte and parasite counts and obtained high repeatabilities for all measures. Leucocyte counts in blood smears require only a single capture and therefore are useful for evaluating the health status of wild birds because large samples can be obtained while reducing the stress involved in multiple captures (Moreno *et al.*, 2002a; Moreno, 2003). Firstly, we aimed to determine which haematological variables were affected by parasitism, body mass, hatching date, and brood size. We tried to obtain the models best explaining variation in leucocyte counts. Our second objective was to determine whether local recruitment was better predicted by leucocyte counts than by body mass and hatching date. We assumed that local recruitment is an unbiased estimate of survival and discuss this assumption below.

Given our present ignorance about patterns of natural variation of haematological parameters in wild populations and their relationship with immune function or health status, observed associations will contribute to an improved understanding of the functional meaning of haematology in the wild. Leucocyte counts from blood smears are easy to obtain in the field and do not involve prolonged manipulation of wild animals. Our study aims at clarifying the predictive value of haematological variables in relation to nestling fitness.

Methods

The pied flycatcher is a small (12–13 g) hole-nesting passerine of European woodlands. It is a summer visitor that adapts readily to breeding in artificial nest-boxes. Egg laying in the population being studied typically begins in late May, and clutch sizes range from two to seven eggs, with a mode of six eggs (mean = 5.73). The female incubates alone and receives part of her food from her mate. Young are brooded by the female only up to the age of 7 d (Sanz & Moreno, 1995). Both sexes feed the young (Moreno *et al.*, 1995). Young fledge within 14–16 d of hatching. This occurs in the second half of June in our study area. There are no second clutches in our study population.

The study was conducted in 2000 in a deciduous forest of Pyrenean oak (*Quercus pyrenaica*) at 1,200 m a.s.l. in the vicinity of La Granja, Segovia province, central Spain (40° 48' N, 4° 01' W). A study of a population breeding in nest-boxes in this area has been conducted since 1991. Nest-boxes (125 × 117 mm bottom area) are cleaned every year after the breeding season. Every year, the nest-boxes are checked for occupation by pied flycatchers, and the dates of clutch initiation, clutch sizes, and number of fledged young are determined. Nestlings are weighed 13 d after hatching (hatching day = day 0) to the nearest 0.1 g with a Pesola spring balance (Pesola AG, Baar, Switzerland). All chicks are ringed with numbered aluminium bands (Dirección General de Conservación de la Naturaleza [DGCN] bands, ringing permit by regional authorities). The recruitment of ringed

chicks as breeders to the population was estimated during the three subsequent years, as a large fraction of recruits do not turn up until their second or third year of life (Potti & Montalvo, 1991).

To quantify haemoparasites and count leucocytes, a blood smear was obtained from the brachial vein of nestlings. A drop of blood was smeared on individually marked microscope slides, air-dried, fixed in absolute ethanol, and stained with Giemsa for 45 min. Slides were examined under microscope (1,000x magnification with oil immersion) to estimate the proportion of different types of leucocytes (Merino *et al.*, 2001). Examination was ended when 100 leucocytes had been found, excluding thrombocytes, which usually are irregularly distributed. Fields with similar densities of erythrocytes were scanned for all individuals. The number of leucocytes per 10,000 erythrocytes was calculated by counting the number of erythrocytes per field and multiplying by the number of fields viewed to count 100 leucocytes. Leucocyte counts were highly repeatable (14 blood smears were scanned twice, $r = 0.83, 0.89, 0.72$ for lymphocytes, heterophils, and basophils respectively, all $P < 0.001$). The other counts were not repeatable and will not be discussed further.

Smears were microscopically examined for extraerythrocytic haemoparasites under 200x magnification. To control for possible non-random distributions of parasites, half of each symmetrical smear was entirely scanned. Quantification was made by transformation of parasites observed to parasites per 100 fields (Merino & Potti, 1995a). Intraerythrocytic haematozoa were detected and quantified under oil immersion (1,000x magnification) by counting the number of parasites per 2,000 erythrocytes (Godfrey, Fedynich & Pence, 1987). The person examining blood samples (E.L.) had no previous information about the individual birds except ring number.

The main ectoparasite of pied flycatcher nestlings is the mite *Dermanyssus gallinoides* (Merino & Potti, 1995b; 1996). Mite parasitism was scored semi-quantitatively by an infestation score. Infestation was scored as 0 (no mites detected either on nest-box, nest material, or nestlings when handled on day 13), 1 (nests with scattered mites detected when handling chicks), 2 (nests with many mites running over the nestlings when handled [hundreds of mites]), and 3 (nests with many mites at the nest entrance, running over the nest-box and nestlings when handled, and crawling over the hands of the researcher [thousands of mites]). Merino and Potti (1995b) have validated this relative measure of mite infestation.

A sample of 71 broods and 303 nestlings with all haematological data available was used for this study. Repeatability analyses (Lessells & Boag, 1986) of leucocytes per 10,000 erythrocytes were carried out to assess the variability between and within broods. In every tested parameter, variability between broods was significantly higher than variability within broods ($r = 0.121, 0.290, 0.252, 0.149$ for lymphocytes, heterophils, basophils, and total leucocytes respectively, all $P < 0.01$). Four different mixed ANOVA models with lymphocytes, heterophils, H/L, and total leucocytes as the dependent variables were performed (degrees of freedom of the error term computed using the Satterthwaite method). In order to avoid

pseudoreplication, brood was included as a random factor. Log-transformed values of all haematological parameters were used in statistical analyses to obtain normal distributions. Every haematological measure was related to the following variables: nestling body mass, hatching date, brood size, *Trypanosoma* intensity of infection, and mite score. The sequential Bonferroni correction (Rice, 1989) was applied to the analyses testing influence of stressors on leucocyte profiles.

It has been shown in previous studies of nestling passerines that the recruitment of pied flycatcher fledglings does not depend on brood of origin (Moreno *et al.*, in press). The fates of individual nestlings with respect to recruitment will therefore be considered as statistically independent observations. Recruitment of individual nestlings (recruited and non-recruited) was included in GLZ analyses based on binomial distributions and logistic link functions (STATISTICA, StatSoft Inc., Tulsa, Oklahoma, USA) and was related to mite score, *Trypanosoma* intensity of infection, body mass, brood size, hatching date, and leucocyte counts. Separate analyses were run for each of the four haematological variables to test their suitability as predictors of local recruitment when compared with ecological variables. The program gives the goodness of fit of all possible models including from one to six predictor variables. The Akaike information criterion (AIC) and the likelihood ratio chi-square (ΔD) were used to select the models with the highest parsimony.

Paired *t*-tests were performed to confirm the robustness of the analyses by comparing all variables between recruits and their non-recruited siblings.

If not stated otherwise, all means are presented with SE. Non-normal distributions are represented by medians and percentiles.

Results

The mean values of leucocyte proportions were $54.4 \pm 0.76\%$ for lymphocytes, $28.6 \pm 0.85\%$ for heterophils, and $13.98 \pm 0.14\%$ for basophils ($n = 303$). The mean value of the heterophil/lymphocyte ratio was 0.62 ± 0.03 . This value was significantly higher than the H/L of adult females from the same population (Moreno *et al.*, 2002b, $t = 2.76$, $P < 0.01$). The median value of lymphocytes per 10,000 erythrocytes was 9.44 (lower and upper quartiles: 6.22 and 13.67), the median of heterophils per 10,000 erythrocytes was 4.06 (2.43-6.41), the median of basophils per 10,000 erythrocytes was 2.06 (1.01-3.90), and the median of total leucocytes was 17.01 (11.96-24.60). Prevalence of infection by *Trypanosoma* spp. was 47.8% ($n = 303$), and the mean *Trypanosoma* intensity for infected individuals was 11.9 ± 1.33 parasites per 100 fields ($n = 145$). The number of nestlings infected by *Trypanosoma* per brood did not fit a Poisson distribution ($P = 0.005$), so it can be concluded that the probability of infection is contagious within broods. Five chicks were infected with *Haemoproteus* spp. Mites were detected in 38% of broods ($n = 67$), with a modal score of 2.

The heterophil/lymphocyte (H/L) ratio increased with mite score ($F_{3, 60} = 4.71$, $P = 0.005$). This result was

marginally nonsignificant after Bonferroni correction (corrected significance level: $P = 0.0028$). Likewise, the H/L ratio was highly correlated with body mass, decreasing when body mass increased ($F_{1, 95} = 15.76$, $P < 0.001$). The total number of heterophils per 10,000 erythrocytes decreased significantly with body mass ($F_{1, 91} = 10.73$, $P = 0.0015$) and was not significantly related to mite score ($F_{3, 60} = 3.37$, $P = 0.024$, corrected significance level: $P = 0.0028$). Total leucocyte count was not related to any of the variables considered. No haematological variable was found to be significantly affected by brood size and *Trypanosoma* infection intensity (analyses not shown).

The haematological parameters significantly related to individual local recruitment probability were total number of leucocytes per 10,000 erythrocytes ($P = 0.002$), number of lymphocytes per 10,000 erythrocytes ($P = 0.032$), and number of heterophils per 10,000 erythrocytes ($P = 0.006$) (Figure 1), while the H/L ratio was not significantly related to local recruitment ($P = 0.27$). When including all ecological variables together with each haematological parameter in logistic regression models, only the heterophil count by itself predicted local recruitment better than complex models including haematological variables and mite score (Table I). Within-brood comparisons through paired *t*-tests confirmed that recruits had lower heterophil and total leucocyte counts than their siblings (Table II).

Discussion

Leucocyte counts are a monitoring technique broadly used by ecologists (Norris & Evans, 2000). This measure provides information about the status of certain components of an individual's immune system at the time it was sampled, concerning not only immune cell distribution but also total number of circulating leucocytes. Hôrak *et al.* (1999) have analyzed the sources of variation in leucocyte counts in altricial nestlings. Altricial nestlings should have a well-developed innate immune response based on macrophages and granulocytes and a developing and less active lymphocyte response, according to the information on avian chicks mostly dealing with domestic precocial species (Klasing & Leshchinsky, 1999). Thus, we should expect lymphocyte variation to be less responsive to some environmental factors.

In our study, ectoparasites and nutritional state were associated to haematological parameters. Although the results concerning the association of mite score with leucocyte profiles have to be considered with caution (it was marginally nonsignificant after Bonferroni correction), the tendency in white blood cell profiles detected in individuals from broods suffering from mite infestation might be consistent with immune responses expected against ectoparasitic arthropods (Wakelin, 1984). Mites can be found in nests and on the skin of pied flycatchers (Merino & Potti, 1995b; 1996), so when the mites feed on blood, the first cells to mobilize to the attachment site are heterophils, followed by basophils, mast cells, and eosinophils (Wakelin, 1984). Therefore, the increase in H/L ratio detected may be the consequence of an increased proliferation and differentiation of heterophils produced in order to enhance the inflammatory reaction to

FIGURE 1. Mean ($\pm$ SE) total leucocyte count per 10,000 erythrocytes (a), heterophil count per 10,000 erythrocytes (b), and lymphocyte count per 10,000 erythrocytes (c) in recruited and non-recruited birds.

mites. Also, ectoparasites may affect leucocyte traffic by means of their demonstrated harmful effect on nestling condition (Merino & Potti, 1995b; Merino *et al.*, 1998; Moreno *et al.*, 1999; Potti *et al.*, 1999; Moreno *et al.*, 2002b), so nestling condition may explain part of the effect of mites. This effect has not been detected in adult pied flycatchers (Moreno *et al.*, 2002b), which suggests that nestlings are more vulnerable to mite pathogenicity.

TABLE I. Generalized linear models based on binomial distributions and logistic link functions of individual local recruitment in relation to each haematological variable together with the following ecological variables: mite score, brood size, body mass, hatching date, and *Trypanosoma* infection intensity. Single-effect models and complex models with higher parsimony (lower AIC) than the best single-effect model are presented. Models with the highest parsimony are in bold characters.

Effects	df	AIC	D	P
Heterophils	1	113.926	7.058	0.008
Mite score	1	115.365	5.620	0.017
Body mass	1	118.178	2.805	0.094
Hatching date	1	120.375	0.610	0.435
<i>Trypanosoma</i>	1	120.932	0.052	0.820
Brood size	1	120.717	0.267	0.604
Effects	df	AIC	D	P
Lymphocytes	1	117.428	3.556	0.059
Mite score	1	115.365	5.620	0.017
Body mass	1	118.179	2.805	0.094
Hatching date	1	120.375	0.610	0.435
<i>Trypanosoma</i>	1	120.932	0.052	0.820
Brood size	1	120.717	0.267	0.604
Lymphocytes + mites	1	113.747	9.237	0.010
Effects	df	AIC	D	P
Leucocytes	1	113.276	7.707	0.005
Mite score	1	115.365	5.620	0.017
Body mass	1	118.179	2.805	0.094
Hatching date	1	120.375	0.610	0.435
<i>Trypanosoma</i>	1	120.932	0.052	0.820
Brood size	1	120.717	0.267	0.604
Leucocytes + mites	2	110.173	12.566	0.002
Effects	df	AIC	D	P
H/L	1	119.742	3.241	0.198
Mite score	1	115.365	5.620	0.017
Body mass	1	118.179	2.805	0.094
Hatching date	1	120.375	0.610	0.435
<i>Trypanosoma</i>	1	120.932	0.052	0.820
Brood size	1	120.717	0.267	0.604

Furthermore, the mean adult female H/L ratio was significantly lower than that of nestlings (Moreno *et al.*, 2002b).

As expected, body mass was significantly correlated with H/L ratio, with lighter individuals having higher values of H/L. This effect was mediated by an increased traffic of heterophils. Moreno *et al.* (2002a) found that mean nestling body mass in broods was related to mean within-brood values for H/L ratio and other stress parameters like heat shock protein levels. Gross and Siegel (1986) and Tripathi and Bhati (1997) found that birds in captivity respond to periods of food deprivation and malnutrition by increasing heterophil numbers and correspondingly decreasing lymphocyte numbers. This stress profile agrees with the one found by Dhabhar *et al.* (1995), in which stress induced in rats increased plasma corticosterone and was related to increased traffic of granulocytes in blood circulation and sequestration of lymphocytes in lymphoid tissue. The effect of stressors on H/L ratio has been widely studied in birds (Hörak, Ots & Murumägi, 1998; Moreno *et al.*, 1998; Vleck *et al.*, 2000; Moreno *et al.*, 2002a,b,c; Owen & Sogge, 2002), but few studies have analyzed this parameter in nestlings (Hörak *et al.*, 1999; Moreno *et al.*, 2002a; Ilmonen *et al.*, 2003). The fact that in our study nestlings respond to food stress only by showing a higher number of circulating heterophils

may reflect the higher degree of activation of the innate immune system supposed in altricial nestlings (Klasing & Leshchinsky, 1999). Ilmonen *et al.* (2003) also detected heterophilia and H/L ratio increments in pied flycatcher chicks in experimentally enlarged broods, which agrees with our results of nestling response to stress during growth. The absence of significant variation in lymphocyte counts may be due to our lack of data on viral and bacterial infections, which would probably explain patterns not detected in this study.

We have used local recruitment rate as a substitute measure of post-fledging survival. It has been shown that recruitment rates in Spanish pied flycatcher populations are higher than in other areas (Potti & Montalvo, 1991; Sanz *et al.*, 2003). As female local return rates are significantly lower than the calculated survival rates in our study area (Sanz, 2001), recruitment may be underestimated in the present study. However, the important question is whether the variables included in our analysis (body mass, hatching date, and leucocyte count) are related or not to differential dispersal (Monrós, Belda & Barba, 2002). Nilsson (1989) found that late-hatched nestlings may be more prone to move away from their natal area. In addition, Potti and Montalvo (1991) saw a date-dependent local recruitment rate in another Spanish pied flycatcher population, although they did not find any correlation between short-range natal dispersal distance and hatching date or body condition. In the related collared flycatcher (*Ficedula albicollis*), hatching date and condition did not affect natal dispersal distance (Pärt, 1990). We assume that long-distance dispersal rates vary in the same way as short-range dispersal, and therefore we surmise that no bias due to effects of body mass and hatching date on dispersal is introduced by using local recruitment rates as a parameter of real survival. There are no hypotheses connecting health state with natal dispersal, so we assume that there is no bias in relating local recruitment to leucocyte counts.

Total leucocyte count has traditionally had a controversial interpretation. High values of leucocyte count have been considered as a signal of good health state (Gustafsson *et al.*, 1994; Dufva & Allander, 1995), but this increase in white blood cells may be due to an increased traffic of granulocytes in stress situations (Apanius, 1998a). Furthermore, low leucocyte counts may reflect not only low circulating levels of granulocytes, but also low lymphocyte numbers, which could indicate a lack of current infections requiring a specific immune response from the host (Norris & Evans, 2000) or even low susceptibility to infections. Our results support the hypothesis that low leucocyte counts reflect a good individual health state, showing that recruited birds indeed had a low leucocyte count when nestlings, mediated by low heterophil and lymphocyte counts. However, the logistic regression analyses including all ecological variables together with each haematological parameter revealed that most haematological variables only predicted local recruitment when considered together with mite score in complex models. Only the heterophil count by itself was a better predictor in single-effect models than in complex models together with mite score. Thus, mite parasitism has a clear effect

TABLE II. Significance level from paired *t*-tests under the null hypothesis that the mean difference within-broods between recruited and non-recruited individuals equals 0. Mean values are expressed with their SD. *n* = 16 broods.

	Non-recruited	Recruited	<i>t</i>	<i>P</i>
Log (lymphocytes/10,000 erythrocytes ⁻¹)	1.008 ± 0.18	0.91 ± 0.22	1.70	0.11
Log (heterophils/10,000 erythrocytes ⁻¹)	0.67 ± 0.17	0.57 ± 0.20	2.79	0.014
Log (leucocytes/10,000 erythrocytes ⁻¹)	1.22 ± 0.16	1.07 ± 0.23	2.62	0.02
Log (H/L)	-0.41 ± 0.31	-0.43 ± 0.27	0.32	0.75
Body mass (g)	14.57 ± 0.68	14.43 ± 0.64	0.93	0.34

on recruitment probability of nestlings, greatly improving the predictive capacity of haematological variables other than the heterophil count. This suggests that the heterophil count may be used as an independent indicator of nestling health and fitness in the wild. Recruitment within broods was also related to these measures: recruited nestlings had lower leucocyte and heterophil counts than their non-recruited siblings. Lymphocyte counts did not differ significantly between recruits and their non-recruited siblings, which suggests that recruitment is not associated as strongly with this parameter as with heterophil counts. Although Ots, Murumägi, and Hõrak (1998) showed that haematological measures had moderate or scant persistence in time, our data suggest that the nestling heterophil count is a good predictor of recruitment probability. Chicks in good health may face up better to pathogens when juveniles, and may avoid preferential predation upon weak individuals (Temple, 1986; Møller & Erritzøe, 2000). Considering that body mass is related to these haematological measures, it is possible that using body mass as a survival predictor in fact hides the underlying effect of health state as expressed through leucocyte counts.

Our results show that heterophil count is a better predictor of local recruitment than body mass or hatching date, apart from other ecological variables. Studies by Wilson and Wilson (1978) related survival of captive red grouse (*Lagopus lagopus scoticus*) with low heterophil counts in peripheral blood, while Hõrak and Ots (1998) did not find relationships between yearling haematological indices and their future survival prospects. This is the first study to our knowledge that relates nestling leucocyte counts to their subsequent probability of survival in the wild, although Christe *et al.* (2001) also found that immune parameters predicted local recruitment. Further studies should perform a battery of physiological tests to assess the effect of health state on recruitment probability of nestlings.

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