



HAL
open science

Haematological health assessment in a passerine with extremely high proportion of basophils in peripheral blood

Michal Vinkler, Jan Schnitzer, Pavel Munclinger, Jan Votýpka, Tomáš Albrecht

► **To cite this version:**

Michal Vinkler, Jan Schnitzer, Pavel Munclinger, Jan Votýpka, Tomáš Albrecht. Haematological health assessment in a passerine with extremely high proportion of basophils in peripheral blood. *Journal für Ornithologie = Journal of Ornithology*, 2010, 151 (4), pp.841-849. 10.1007/s10336-010-0521-0 . hal-00582602

HAL Id: hal-00582602

<https://hal.science/hal-00582602>

Submitted on 2 Apr 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Title:

2 **Haematological health assessment in a passerine with extremely high proportion of**
3 **basophils in peripheral blood**

4

5 Authors:

6 Michal Vinkler (1, 3), Jan Schnitzer (1), Pavel Munclinger (1), Jan Votýpka (2) and Tomáš

7 Albrecht (1, 3)

8

9 Addresses:

10 1) Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7,
11 Prague, CZ-128 44, Czech Republic

12 2) Department of Parasitology, Faculty of Science, Charles University in Prague, Viničná 7,
13 Prague, CZ-128 44, Czech Republic

14 3) Institute of Vertebrate Biology, v.v.i., Academy of Sciences of the Czech Republic,
15 Studenec 122, CZ-675 02, Czech Republic

16

17 Author for correspondence:

18 Michal Vinkler, Department of Zoology, Faculty of Science, Charles University in Prague,
19 Viničná 7, Prague, CZ-128 44, Czech Republic

20 e-mail: vinkler1@natur.cuni.cz

21 Tel.: +420 221 95 18 39

22 Fax: +420 221 95 18 41

23

24 Word count: 6381

25

26 **Abstract**

27 Haematological methods are widely utilized among avian ecologists as a means for individual
28 health assessment. However, the technical simplicity of some of the tests may easily lead to
29 oversimplification of the evaluation. Here we show in the Scarlet rosetfinch (*Carpodacus*
30 *erythrinus*) that haematological parameters other than the widely used heterophil/lymphocyte
31 (H/L) ratio may be important to investigate. We give the full description of seven basic
32 haematological traits (leukocyte differential count, immature erythrocyte count, haematocrit,
33 mean cell volume, total red and white blood cell count and blood parasite occurrence). Most
34 remarkably, the examination of 178 adults and 155 nestlings has revealed that this species has
35 an extraordinarily high proportion of basophils in the peripheral blood (on average about 42%
36 and 56% respectively). Although the high basophil count is a general trait even in healthy
37 individuals of this species, the proportion of these cells is condition-dependent and is further
38 increased by *Haemoproteus* infection. Our results also suggest that the immature erythrocyte
39 count in the peripheral blood is a good predictor of the nestlings' growth rate. We conclude
40 that the rosetfinch haematology differs strikingly from other avian species with known values
41 of basic haematological parameters. We therefore emphasize the importance of a general
42 haematological examination, based on material obtained by an appropriate method (e.g. for
43 smear preparation, we recommend using differential staining and avoiding prior methanol
44 fixation).

45

46

47 **Key words**

48 Avian immunity, basophilic granulocyte, hematology, hematocrit, leucocyte differential
49 count, packed cell volume, polychromatic erythrocyte.

50

51 **Introduction**

52 Health has a major effect on body condition and vigour, which consequently determines
53 individual fitness. Adoption of appropriate methods that enable a reliable estimation of health
54 is therefore of crucial importance to most ecological and evolutionary research. Although
55 there are various ways to investigate health, the most widely used method for health
56 assessment is the basic haematological survey (Ardia and Schat 2008). The main procedure
57 for a haematological survey is to determine the cellular composition of blood. In avian
58 peripheral blood, there are five morphologically distinguishable types of leukocytes present:
59 lymphocytes, heterophils, basophils, eosinophils and monocytes (Lucas and Jamroz 1961).
60 Normal values of cellular proportions may differ among species (Campbell and Ellis 2007;
61 Davis 2009) and may also vary between free-living and captive-held birds (Ewenson et al.
62 2001). In most bird species with published values of haematological traits, however, only
63 lymphocytes and heterophils are detected in sufficient numbers to enable reliable
64 interindividual comparison (the combined number of lymphocytes and heterophils typically
65 accounts for 85-95% of all leukocytes in a blood smear; Davis 2009). Several studies have
66 documented that the ratio of lymphocytes and heterophils (H/L) may reliably indicate stress
67 (El Lethey et al. 2003; Davis et al. 2008) as well as infection status for some diseases (Davis
68 et al. 2004; Chakarov et al. 2008; Fokidis et al. 2008; Norte et al. 2009a). These findings have
69 led avian ecologists to use the H/L ratio as general measurement of health status, which is
70 quick, repeatable, and easy to obtain (Ots et al. 1998; Ardia and Schat 2008).

71

72 Despite the growing number of haematological studies in birds (e.g. see Moreno et al. 1998;
73 Hauptmanová et al. 2002; Uhart et al. 2003; Sargent et al. 2004; Friedl and Edler 2005; Davis
74 et al. 2004; Mercurio et al. 2008) data on blood cellular composition are still only available
75 for a limited number of passerine species. In contrast to the known variability (e.g.

76 interspecific or linked to age and sex) and fluctuations (e.g. due to stress, season or health
77 changes) in heterophil and lymphocyte counts, the variability among other cell types is often
78 considered as only minor and rare (Maxwell 1981; Dufva and Allander 1995; Scope et al.
79 2002; Dubiec et al. 2005; Lobato et al. 2005; Davis 2005, Norte et al. 2009a; Norte et al.
80 2009b). Nevertheless, certain illnesses induce quantitative changes in the number of
81 basophils, eosinophils and monocytes that are comparable to the changes observed in
82 lymphocytes and heterophils (see e.g. Fudge 1989; Campbell and Ellis 2007).

83

84 Detailed studies of the composition of avian blood cells can offer more information than is
85 usually considered. Aside from the common methods of estimating proportions of leukocytes
86 and quantities of blood-borne cells (described either as absolute counts or as haematocrit; Ots
87 et al. 1998), determining the blood cell dynamics may be equally valuable. For instance,
88 immature erythrocyte count can reveal the presence of anaemic diseases (Campbell and Ellis
89 2007) and there is some toxicological evidence suggesting potential importance of this trait
90 for wildlife studies (see Yamato et al. 1996; Belskii et al. 2005; Carleton 2008). However, to
91 our knowledge there has been no effort in avian ecology to examine the health-predictive
92 potential of peripheral blood immature erythrocyte count in free-living birds.

93

94 In this study we investigated the basic haematological traits in the Scarlet rosefinch
95 (*Carpodacus erythrinus*). The Scarlet rosefinch is a small sexually dichromatic passerine
96 belonging to the subfamily Carduelinae (Cramp et al. 1994). A remarkable feature of this
97 species is the especially long distance and direction of its migration. The wintering grounds
98 are located in the southern part of Asia (Cramp et al. 1994), lying in a distance of
99 approximately 5 500 km from the breeding grounds in central Europe. Thus, the energetically
100 costly migration may be expected to pose a powerful selection on health related traits in this

101 species (Albrecht et al. 2007). Here we present data on the leukocyte differential count, total
102 counts of leukocytes and erythrocytes, haematocrit, blood parasites, and the proportion of
103 immature blood cells. Most of the traits are reported in adults as well as in seven-day-old
104 hatchlings. Chosen haematological traits were correlated with morphological condition-
105 dependent traits and blood borne parasite infection status to assess their convenience for
106 general health prediction in this species.

107

108

109 **Materials and methods**

110 *Field procedures*

111 The research was carried out on a Scarlet rosefinch population breeding in the Vltava river
112 valley, Šumava National Park, southern Bohemia, Czech Republic (N 48°48'–48°50', E
113 13°55'–13°57', ~730 m above sea level; for more detailed description see Albrecht 2004).
114 This population numbers at least 200 breeding pairs (Šťastný et al. 2006) and represents the
115 western edge of the breeding grounds for this species (Risberg and Stjernberg 1997). In five
116 successive seasons (2004-2008) we examined the basic haematological traits of 178 Scarlet
117 rosefinch adults (110 males, 68 females) and 155 nestlings (not all traits were examined in
118 every individual).

119 In each season samples were collected during the pre-breeding (second half of May) and
120 breeding (second half of June) periods. Adult birds were captured into standard ornithological
121 mist nets, either with the lure (stuffed male Scarlet rosefinch skin) or by chance in the
122 surrounding of their putative breeding spot. After capture, each bird was placed into a fabric
123 bag, weighed using a spring balance (MicroLine 20060, 60g, d=0.5g; Pesola AG, Baar,
124 Switzerland) and a sample of blood (about 20-70 μ l) was collected by puncturing the brachial
125 vein. Thereafter the tarsus length was measured by digital calliper (accuracy 0.01mm) as a

126 general estimate of individual's size (Senar and Pascual 1997). Individual weight was later
127 divided by tarsus length and this weight standardized on size (hereafter referred as mass) was
128 used for further analyses as a condition indicator. When all required information was
129 collected the bird was tagged with a standard steel ring from the Czech ringing station (N
130 MUSEUM PRAHA) and released. The manipulation time was about twenty minutes in total.

131

132 Every year during the breeding season the Scarlet rosetfinch nests were systematically
133 searched. At the day of hatching each nestling was individually marked with a permanent
134 marker and the hatching date was recorded. On day 7 post hatch (i.e., determined individually
135 for each nestling within the nest according to its hatching date), a blood sample (ca. 10-30 μ l)
136 was collected from each nestling. Thereafter the nestling was tagged with a darkened steel
137 ring, weighted and the tarsus length was measured. On the next day the nestling was
138 measured again to obtain the measure of nestling growth (tarsus length on day 8 post hatch
139 minus the length on day 7 post hatch). Sampling of nestlings was performed in a hidden place
140 about 20m away from the nest to minimise the risk of attracting a predator. No nestling spent
141 more than 30 minutes out of the nest.

142

143 *Leukocyte differential count*

144 In all adults as well as in all nestlings a drop of blood was used immediately after collection
145 for the preparation of a blood smear. The air-dried smears were not fixed in methanol (as
146 usual in mammalian haematology) before staining because methanol fixation decreases the
147 stainability of basophil granules, which impairs correct identification of basophils (Robertson
148 and Maxwell 1990, personal experience; see also Dubiec et al. 2005). After transportation to
149 laboratory all smears were stained by Pappenheim's panoptical method: 3 minutes in
150 concentrated May-Grünwald staining solution (Merck & Co., Inc. Whitehouse Station, NJ,

151 USA), 2 minutes in the same solution diluted 1:1 with distilled water and 15 minutes in
152 Giemsa's solution (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:40 with distilled water,
153 then washed with water and air dried. This differential staining method enables the
154 recognition of all basic blood cell types under a light microscope. All smears were scanned
155 with Olympus CX-31 microscope (Olympus Corporation, Tokyo, Japan) under magnification
156 of 1000x to count the proportions of lymphocytes, heterophils, eosinophils, basophils,
157 monocytes and immature leukocytes within a sample of 110-140 leukocytes in the smear.
158 Being morphologically similar to leukocytes described from other avian species individual
159 leukocyte types were identified according to Lucas and Jamroz (1961) and Campbell and Ellis
160 (2007). The immature stages of all leukocyte types found in the peripheral blood were
161 assigned to a single category due to the potentially high risk of incorrect identification of the
162 cell type. The repeatability of measuring the leukocyte frequencies was calculated based on a
163 sample of 27 adult individuals for which counts were taken two separate times. It was $r=0.87$
164 for lymphocytes, $r=0.91$ for heterophils, $r=0.89$ for basophils, $r=0.71$ for eosinophils, $r=0.32$
165 for monocytes and $r=0.33$ for immature cells. The low values for repeatability in monocytes
166 and immature cells were caused by their low frequencies among blood leukocytes (on average
167 less than 5% in both cell types).

168

169 *Erythrocyte differential count*

170 In 20 randomly chosen adults and 20 nestlings, we measured the differential count of
171 immature erythrocytes. In each individual blood smear 5 randomly chosen monolayer fields
172 were photographed (camera E-410; Olympus Corporation, Tokyo, Japan) at 100x objective
173 magnification (ca. 1000-2000 cells). Copies of all images were transformed into black and
174 white 1 bit format with resolution 80 dpi by Corel PHOTO-PAINT X3 software (Corel
175 Corporation, Ottawa, Canada) so that only the uniformly black nuclei remained visible.

176 Where necessary, the neighbouring nuclei were manually separated and potential dirt was
177 deleted. The total number of cells per image was automatically counted from these adjusted
178 images by the particle analyser of ImageJ software (Rasband W.S. ImageJ, U.S. National
179 Institutes of Health, Bethesda, MD, USA, <http://rsb.info.nih.gov/ij/>, 1997-2008). The original
180 photographs were then used to manually count the immature erythrocytes, thrombocytes and
181 leukocytes within the images using ImageJ cell counter. Within erythrocytes we distinguished
182 among normal mature erythrocytes, immature polychromatic erythrocytes and hypochromatic
183 cells (for detailed description see Campbell and Ellis 2007). The number of reticulocytes has
184 not been determined as this would require a special type of staining (Campbell and Ellis
185 2007). In samples from the adults, but not the nestlings, it is not clear whether the
186 hypochromatic erythrocytes represent immature ontogenetic stages or rather aberrant cells
187 (Campbell and Ellis 2007). Therefore only polychromatic cells were counted as immature
188 erythrocytes in adults ($r=0.89$) while in nestlings a join category of polychromatic and
189 hypochromatic cells was created ($r=0.95$).

190

191 As the onset of degenerative processes in thrombocytes may take place rapidly after the blood
192 collection and variably among individuals (Lucas and Jamroz 1961), no thrombocyte counts
193 were recorded.

194

195 *Haematocrit*

196 The haematocrit (packed cell volume) values were recorded in 56 adults. Blood samples were
197 centrifuged in heparinised capillary tubes for 5 min. at 11000 rpm. Then the proportion of
198 cells in the total volume of blood was measured and recorded as a percentage. The
199 repeatability of the measurement was $r=0.97$, estimated based on a sample of 17 individuals in
200 which two capillary tubes of blood were collected. The haematocrit measurement was not

201 carried out in nestlings as it requires the collection of an additional 40-50 μ l of blood, which
202 exceeds the volume of blood that can be safely taken.

203

204 *Total leukocyte and erythrocyte count*

205 In 20 adult rosefinches captured in 2008, we examined the total red blood cells count (i.e.
206 number of erythrocytes per volume unit of blood, hereafter TRBC), and the total white blood
207 cells count (i.e. number of leukocytes per volume unit of blood, hereafter TWBC). A blood
208 sample of 15 μ l was diluted in 2985 μ l of Natt and Herrick's solution (for composition
209 description see Campbell and Ellis 2007) and left for several hours in a field refrigerator to
210 stain the cells. Thereafter the numbers of cells were counted in the Bürker's counting chamber
211 (100 large squares for leukocytes and 20 rectangles for erythrocytes were scanned). Neither
212 TRBC nor TWBC was investigated in nestlings to avoid any potentially harmful effect of
213 larger blood volume losses on their ontogeny. Haematocrit and erythrocyte count values were
214 used for calculation of the mean cell volume (MCV) as a quotient of these two multiplied by
215 10 (Campbell and Ellis 2007). Since 16 out of the 20 samples that we measured TRBC and
216 TWBC came from males, we investigated the relationship of these parameters to condition
217 only for this sex.

218

219 Total and differential counting of erythrocytes and leukocytes as well as all other
220 haematological examinations were performed by one person only (MV) to minimise any
221 potential variability among the measurements.

222

223 *Parasite prevalence*

224 The method for blood parasite load assessment has been previously described in detail
225 elsewhere (see Votýpka et al. 2003) and so here we report it only briefly. The blood smears

226 were examined with a light microscope at 200x magnification for 5 minutes (approximately
227 equivalent to the observation of 50 microscopic fields). Each smear was thereafter examined
228 for another 10 minutes at 1000x magnification (equivalent to 100 microscopic fields;
229 minimally 10 000 erythrocytes). When no parasites were detected after this time, the smear
230 was considered as negative.

231

232 *Statistics*

233 For comparisons of sexes and age classes the Two-sample t-test was performed where
234 possible (i.e. when the variable had a normal distribution or when normality was achieved by
235 transformation). However, as cell frequencies often did not possess Gaussian distribution,
236 nonparametrical Wilcoxon rank-sum test had to be adopted. Correlations were tested by
237 Pearson's product-moment correlation. Generalized linear models were used for the
238 haematological-condition analyses. In these models the distribution was approximated to
239 Gaussian (e.g., in the analyses of basophils in adults and immature erythrocytes) or to
240 binomial (e.g., in the analysis of H/L ratio in adults). We used generalized linear mixed effect
241 approach to test the influence of nestling condition on its H/L ratio and basophil count. In this
242 analysis, the identity of nests was treated as a random effect. Minimal adequate models, i.e.
243 models with all terms significant (Crawley 2002) were obtained by backward eliminations of
244 particular terms in candidate models using likelihood ratio approach starting with the most
245 complex terms (Crawley 2002). The significance of a particular term adjusted for the effects
246 of other terms was based on the change in deviance between the full and reduced models,
247 distributed as χ^2 with degrees of freedom (df) equal to the difference in the degrees of
248 freedom between the models with and without the term in question. F statistics rather than χ^2
249 statistics were used in cases of overdispersion. All presented significance values are based on
250 the Type III Sum of squares. In all cases the reliability of the sample distribution

251 approximation to the chosen distribution was tested by One-sample Kolmogorov-Smirnov
252 goodness-of-fit test. The significance level was set to $p=0.05$. Repeatability was assessed
253 according to Lessells and Boag (1987). All statistical analyses were performed using S-PLUS
254 6.0 (Lucent Technologies, Inc, USA) and R 2.8.1. (<http://www.r-project.org/>) softwares.

255

256

257 **Results**

258 *Basic description of the Scarlet rosefinch haematology*

259 The leukocyte differential count in adult individuals is summarized in Table 1. In the adult
260 rosefinches, haematocrit values ranged from 40 % to 65 % (median 56 %, $n=56$), the TRBC
261 counts varied from $5.21 \times 10^6/\mu\text{l}$ to $7.91 \times 10^6/\mu\text{l}$ (median $6.49 \times 10^6/\mu\text{l}$, $n=20$) and TWBC counts
262 ranged from $4.50 \times 10^3/\mu\text{l}$ to $22.00 \times 10^3/\mu\text{l}$ (median $8.00 \times 10^3/\mu\text{l}$, $n=20$). The MCV values were
263 between 68.07 fl/cell and 102.00fl/cell (median 86.72 fl/cell, $n=20$). Immature erythrocytes
264 accounted for 0.83-5.70% of all erythrocytes (median 2.86%, $n=20$). The only common blood
265 parasite detected in adult birds was *Haemoproteus* sp. (24.7 % of samples, $n=178$).
266 *Leucocytozoon* sp. was found in only one individual and seven birds were infected by avian
267 filariae.

268

269 The description of leukocyte differential count in rosefinch nestlings is given in Table 2. In
270 the nestlings, a range from 5.98% to 38.20% of all erythrocytes were classified as immature
271 (median 22.59%, $n=20$). No blood parasites were detected in nestlings.

272

273 Clear differences exist between adults and nestlings in all parameters of leukocyte differential
274 count ($n_{\text{nestlings}}=155$, $n_{\text{adults}}=178$, Wilcoxon rank-sum test for lymphocytes $Z=-9.81$, for
275 heterophils $Z=-8.62$, for basophils $Z=8.86$ and for eosinophils $Z=11.05$, p in all cases

276 <<0.001) as well as in the percentage of immature red blood cells (Exact Wilcoxon rank-sum
277 test for polychromatic cells $n_{\text{nestlings}}=10$, $n_{\text{adults}}=10$, $W=148$, $p<0.001$). Significant sex
278 differences in adults were found only in some parameters of leukocyte differential count
279 (Two-sample t-test for the eosinophil count after arcsin transformation $t=2.83$, $n=178$,
280 $p=0.005$; see also H/L ratio in condition analysis) and in the representation of immature
281 polychromatic erythrocytes among the red blood cells (Two-sample t-test $t=2.11$, $n=20$, $p=$
282 0.049). No sex differences were determined in the values for haematocrit, TWBC, TRBC or
283 MCV.

284

285 *Haematological parameters and condition*

286 As most counts of individual leukocyte types are to some extent intercorrelated, we only
287 analysed factors in association with H/L ratio (a commonly investigated parameter) and
288 basophil count (a major leukocyte type in Scarlet rosetfinch) between which the correlation
289 was insignificant (Pearson's product-moment correlation $t = -1.81$, $df = 161$, $p\text{-value} = 0.072$,
290 $\text{correl. coef.} = -0.14$). In adults the H/L ratio was significantly dependent on year ($p<0.001$),
291 period of capture ($p<0.001$), individual sex ($p=0.021$), mass ($p=0.003$, in interaction with
292 period of capture $p=0.028$) and size in interaction with sex ($p=0.023$). In contrast, the basophil
293 count in adults did not vary significantly between years and was independent of sex or
294 individual size. Basophil count was significantly associated with individual mass (predicting
295 body condition, $p=0.021$, Fig. 1), period of capture ($p<<0.001$) and infection by blood
296 parasites of the *Haemoproteus* genus ($p=0.020$). Minimal adequate models for H/L ratio and
297 basophil count in adults are given in Tables 3 and 4.

298

299 We have found no significant minimal adequate model in the case of basophil count for
300 nestlings (the model comprising interaction of hatching date and nestling mass was

301 insignificant as a whole, $p=0.057$, $df=3$, $\chi^2=7.52$, $n=151$). However, the H/L ratio was
302 significantly associated with nestlings size ($p=0.018$) and mass ($p=0.041$; Table 5).

303

304 Although there was no interaction of immature erythrocyte count in adults with any condition
305 indicator *per se*, we have found a significantly negative association between the percentage of
306 immature erythrocytes and individual size ($p=0.020$, value \pm SE = -1.417 ± 0.556 , $df = 1/18$,
307 $F=6.49$, $n=20$; Fig. 2). Values for haematocrit ($n=56$), TRBC ($n=16$), TWBC ($n=16$) and
308 MCV ($n=16$) in adults were not correlated with individual size or mass (in all cases $p>0.20$).
309 In nestlings, we have found a significantly positive association between immature erythrocyte
310 count and growth ($p=0.014$, value \pm SE = 15.943 ± 5.835 , $df = 1/18$, $F=7.47$, $n=20$; Fig. 3).

311

312

313 **Discussion**

314 Our results have shown two interesting points: first, the basophil count may be an important
315 parameter to assess in studies utilizing haematological methods; and second, immature cell
316 count seems to serve as a useful predictor of growth and developmental speed in nestlings.

317

318 In the peripheral blood of Scarlet rosefinches we have found an unusually high proportion of
319 basophils, reaching up to 86% of all leukocytes in adults and 91% in nestlings (medians 40%
320 and 56%, respectively). Analysis of the association of leukocyte differential count with
321 condition has revealed that, at least in adults, the basophil count serves as a better indicator of
322 condition and health than the H/L ratio. While H/L ratio was dependent on factors that have
323 no or only limited relationship to condition (such as year, sex or size), the basophil count was
324 linked to individual mass (predicting condition) and *Haemoproteus* infection (indicating

325 individuals' health). In nestlings, however, not the basophil count but the H/L ratio correlated
326 with condition-related traits (especially with individual mass).

327

328 To date, the function of basophils is only poorly understood in birds. This cell type seems to
329 be involved in acute inflammatory defence and immediate hypersensitivity reactions (Daloia
330 et al. 1994; Maxwell and Robertson 1995; Campbell and Ellis 2007). As avian basophil
331 granules are extremely water soluble, they may be easily damaged during the staining process
332 (Lucas and Jamroz 1961), which impairs the detection of these cells. This might be the reason
333 why precise data on the variability of basophil levels in peripheral blood are limited in free-
334 living birds (but see Davis 2009). Nevertheless, there is some clear evidence suggesting that
335 the proportion of basophils among blood-borne leukocytes is much higher in some avian
336 species than the normal physiological values of most mammals (Maxwell and Robertson
337 1995). A good example of natural variability in basophil counts was given by Friedl and Edler
338 (2005), who found that the percentage of basophils ranges from 0% to 24% in the Red bishop
339 (*Euplectes orix*). High levels of basophils in peripheral blood were also reported in some
340 other passerine species (e.g. Pine siskin, *Carduelis pinus*, or Pied flycatcher, *Ficedula*
341 *hypoleuca*; Davis 2009). Even in non-passerines such as Puna ibis (*Plegadis ridgewayi*; Coke
342 et al. 2004), the Common pheasant (*Phasianus colchicus*; Lucas and Jamroz 1961), and some
343 strains of the domestic chicken (Maxwell and Robertson 1995), normal basophil levels in
344 adults may exceed 10%. Much lower basophil counts (0-5%) were detected in most finches,
345 other than Scarlet rosefinches (Campbell and Ellis 2007; Davis 2009). Nevertheless, in the
346 House finch (*Carpodacus mexicanus*), a species that is closely related to the Scarlet rosefinch,
347 the basophil granulocytes are more frequent than heterophils in the peripheral blood of free-
348 living individuals (Davis et al. 2004; Davis 2005). Thus our results are unusual due to the
349 extreme number of basophiles but not inconsistent with the former findings.

350

351 Although basophils are known to be released into blood circulation in higher numbers due to
352 stress (Maxwell 1993; Altan et al. 2003; Campbell and Ellis 2007; Bedáňová et al. 2007), it is
353 unlikely that our results are caused by stressful manipulation of the birds. In the field, all
354 blood samples were collected within 30 minutes after the capture and prior to any further
355 manipulation of the individual. Davis (2005) previously reported that a handling time less
356 than 1 hour does not influence the leukocyte differential count in peripheral blood of the
357 House finch. Moreover, Scope et al. (2002) have shown that there are no significant changes
358 in peripheral blood basophil numbers even 3 hours after the stressful event. Acute stress is
359 known to increase the H/L ratio (Lazarevic et al. 2000; Ewenson et al. 2001; Ruiz et al. 2002;
360 Scope et al. 2002; El Lethy et al. 2003; Bedáňová et al. 2007; Davis et al. 2008) and we did
361 not find unusually high proportions of heterophils in our smears. Furthermore, a high
362 proportion of basophils was similarly recorded in blood from nestlings for which no
363 potentially stressful capturing was performed. We propose that the high levels of basophils
364 are present in this passerine species with long-distance migration as a part of a particular
365 immunological anti-parasite defence. Levels of basophils are known to reflect infection status
366 of some diseases (Falcone et al. 2001). Also our results show higher basophil levels in birds
367 suffering from *Haemoproteus* infection and those in worse condition (having a lower body
368 mass). This is in concordance with findings of earlier studies reporting that *Haemoproteus*
369 infection alters the health state as well as blood parameters (Ots and Hōrak 1998) including
370 basophil levels (Garvin et al. 2003).

371

372 In our study we have shown that the immature erythrocytes are reasonably common in Scarlet
373 rosefinch peripheral blood circulation (in nestlings much more than in adults). Even in our
374 limited samples we were able to detect clear association between the immature erythrocyte

375 count and size in adults and with growth rate in nestlings. These results suggest that immature
376 erythrocyte count may represent an important haematological trait related to individual
377 development and energetic costs.

378

379 A large body of convincing evidence from both birds and mammals suggests that a high
380 percentage of immature erythrocytes among the red blood cells is indicative of disease,
381 toxicosis or anaemia (Constantino and Cogionis 2000; Campbell and Ellis 2007). Still, the
382 occurrence of small quantities of immature erythrocytes in peripheral blood is a normal state
383 in birds (Campbell and Ellis 2007). In this study, we observed no clinical signs of illness
384 among the individuals investigated. All birds had healthy appearance and their locomotive
385 activity was normal. The values for haematocrit and the percentage of polychromatic
386 erythrocytes among the red blood cells obtained in our sample of adults also did not indicate
387 any abnormality when compared to the values published for healthy captive-held passerines
388 (Campbell and Ellis 2007). On the other hand, both TRBC and TWBC had higher levels than
389 those described for captive-held passerines. We assume that high TRBC and perhaps also
390 TWBC levels may represent ecological adaptation in this species. Greater amounts of
391 erythrocytes may be required during the long distance migration to support the high oxygen
392 demands of the tissues under intense physical effort. Although the numbers of leukocytes in
393 our sample had a much greater range than the values known for finches bred in captivity, in
394 most cases our values lie within the normal values published. The sample was, unfortunately,
395 too small to try to explain the reason for elevated leukocyte levels in some of the individuals.
396 Nonetheless, as the high levels of immature erythrocytes in peripheral blood indicate the
397 presence of blood-regenerative processes (Yamato et al. 1996; Campbell and Ellis 2007;
398 Carleton 2008), we hypothesise that this trait may be related to the rate of blood cell
399 formation and thus possibly also to metabolic rate in healthy individuals.

400

401 **Conclusion**

402 This study has clearly highlighted the necessity of the basic examination of the cellular
403 composition of peripheral blood prior to simplification of the health-evaluating method.
404 Contrary to most mammals and poultry, some avian species may circulate in their peripheral
405 blood relatively high proportions of leukocytes of other types than lymphocytes and
406 heterophils, e.g. basophils. Researchers in ecology should be aware of this fact. We suggest
407 that basophil count may represent a valuable indicator of health in some species. We do not
408 recommend blood smear staining solely with Giemsa's staining solution (e.g. suitable for
409 parasite detection) even in the cases with high proportions of heterophils in the blood films.
410 This method severely impairs the discrimination of leukocytes, which makes the results less
411 reliable. Moreover, when differential staining is adopted, the erythrocyte differential count
412 may be also estimated. Our results indicate that immature erythrocyte count may serve as a
413 suitable tool for estimating development. In avian haematology we also advise forgoing
414 fixation with methanol prior staining. Methanol fixation decreases the visibility of granules in
415 basophils and thus worsens their detection (Robertson and Maxwell 1990; basophils without
416 clearly visible granules can resemble lymphocytes and thus bias even the resulting H/L ratio).
417 However, notwithstanding these potential methodological pitfalls, if appropriate technique is
418 adopted, haematological methods represent a useful and reliable tool for the estimation of
419 health status.

420

421 **Zusammenfassung**

422 **Hämatologische Untersuchung des Gesundheitszustands in Singvögeln mit extrem**
423 **hohem Anteil basophiler Blutkörperchen in peripherem Blut**

424

425 Hämatologische Methoden werden von Ökologen häufig für die Untersuchung des
426 individuellen Gesundheitszustandes von Vögeln verwandt. Die technische Einfachheit einiger
427 dieser Tests kann jedoch zu einer starken Vereinfachung der Evaluation derselben führen.
428 Hier zeigen wir an Karmingimpeln (*Carpodacus erythrinus*) die Bedeutsamkeit anderer
429 hämatologischer Parameter als das häufig genutzte Verhältnis Heterophiler Zellen zu
430 Lymphozyten (H/L ratio). Wir beschreiben sieben hämatologische Charakteristika im Detail
431 (Leukozyten Differentialblutbild, unreife Erythrozytenzahl, Hämatokrit, das mittlere
432 Zellvolumen, die Gesamtzahl der roten und weißen Blutkörperchen und das Auftreten von
433 Blutparasiten). Besonders aufgefallen bei der Untersuchung von 178 Adulten und 155
434 Nestlingen war ein außergewöhnlich hoher Anteil Basophiler im peripheren Blut. Obwohl der
435 große Anteil Basophiler ein generelles Charakteristikum auch in gesunden Individuen dieser
436 Art ist, war der Anteil dieser Zellen abhängig von der Kondition und nahm bei Infektion
437 durch *Haemoproteus* zusätzlich zu. Unsere Ergebnisse lassen auch vermuten, dass die Anzahl
438 immaturer Erythrozyten im peripheren Blut ein guter Indikator für die Wachstumsrate von
439 Nestlingen ist. Wir schlussfolgern, dass das Blutbild von Kardinalgimpeln auffällig anders ist
440 als das von anderen Vogelarten mit bekannten Baseline-Werten hämatologischer Parameter.
441 Deshalb betonen wir die Notwendigkeit hämatologischer Voruntersuchungen, gründend auf
442 Daten erfasst mit einer angemessenen Methode (z. B. für Blutausstriche empfehlen wir
443 unterschiedliche Färbungen und die Vermeidung von vorhergehender Fixation durch
444 Methanol).

445

446

447 **Acknowledgement**

448 We are grateful to Professor Drahoslav Pravda and Professor Eva Straková for their remarks
449 to the haematological methods adopted in this study. We would also like to appreciate the

450 help from Martin Lundák, Lubor Červa, Jaroslav Jelínek, František Zicha, Pavel Jaška, Hana
451 Mrkvičková, Radka Poláková and Marta Promerová with the field work and we wish to
452 acknowledge Zdena Csiebreiová for technical assistance. Our gratitude belongs last but not
453 least to Alison Golinski, Martina Pokorná and Dagmar Vinklerová for their comments to the
454 manuscript. This work was supported by the Grant Agency of Charles University (project
455 191/2004/B-Bio), the Grant Agency of the Academy of Sciences of the Czech Republic
456 (project IAA600930608), the Grant Agency of the Czech Republic (project 206/06/0851) and
457 the Ministry of Education of the Czech Republic (project MSMT No. 0021620828). T.A. was
458 partially supported by the Research Centrum project No. LC06073. The research was
459 approved by Ethical Committee of the Institute of Vertebrate Biology, Academy of Sciences
460 of the Czech Republic and was carried out in accordance with the current laws of the Czech
461 Republic.

462

463

464 **References**

465 Albrecht T (2004) Edge effect in wetland-arable land boundary determines nesting success of
466 scarlet rosefinches (*Carpodacus erythrinus*) in the Czech Republic. *Auk* 121:361-371

467 Albrecht T, Schnitzer J, Kreisinger J, Exnerova A, Bryja J et al. (2007) Extrapair paternity
468 and the opportunity for sexual selection in long-distant migratory passerines. *Behav Ecol*
469 18:477-486

470 Altan O, Pabuccuoglu A, Altan A, Konyalioglu S, Bayraktar H (2003) Effect of heat stress on
471 oxidative stress, lipid peroxidation and some stress parameters in broilers. *Br Poult Sci*
472 44:545-550

473 Ardia DR, Schat KA (2008) Ecoimmunology. In: Davison F, Kaspers B, and Schat KA (eds)
474 Avian Immunology. Academic Press, Elsevier, London, pp 421-441

475 Bedáňová I, Voslářová E, Večerek V, Pištěková V, Chloupek P (2007) Haematological
476 profile of broiler chickens under acute stress due to shackling. Acta Vet BRNO 76:129-135

477 Belskii EA, Lugas'kova NV, Karfidova AA (2005) Reproductive parameters of adult birds
478 and morphophysiological characteristics of chicks in the pied flycatcher (*Ficedula*
479 *hypoleuca* Pall.) in technogenically polluted habitats. Russ J Ecol 36:329-335

480 Campbell TW, Ellis CK (2007) Avian and Exotic Animal Hematology and Cytology, 3rd edn.
481 Blackwell Publishing Professional, Ames

482 Carleton RE (2008) Ectoparasites affect hemoglobin and percentages of immature
483 erythrocytes but not hematocrit in nestling Eastern Bluebirds. Wilson J Ornithol 120:565-
484 568

485 Chakarov N, Boerner M, Krüger O (2008) Fitness in common buzzards at the cross-point of
486 opposite melanin-parasite interactions. Funct Ecol 22:1062-1069

487 Coke RL, West GD, Hoover JP (2004) Hematology and plasma biochemistry of captive puna
488 ibis (*Plegadis ridgewayi*). J Wildl Dis 40:141-144

489 Constantino BT, Cogionis B (2000) Nucleated RBCs - Significance in the peripheral blood
490 film. Laboratory Medicine 31:223-229

491 Cramp S, Perrins CM, Brooks DJ (1994) The Birds of the Western Palearctic. Oxford
492 University Press, Oxford

493 Crawley MJ (2002) Statistical Computing: An Introduction to Data Analysis using S-Plus.
494 Wiley, Chichester

495 Daloia MA, Samour JH, Howlett JC, Bailey TA, Naldo J (1994) Haemopathological
496 Responses to Chronic Inflammation in the Houbara-Bustard (*Chlamydotis-Undulata-*
497 *Macqueenii*). Comparative Haematology International 4:203-206

498 Davis AK (2005) Effect of handling time and repeated sampling on avian white blood cell
499 counts. J Field Ornithol 76:334-338

500 Davis, AK (2009) The Wildlife Leukocytes Webpage: The ecologist's source for information
501 about leukocytes of wildlife species. www.wildlifehematology.uga.edu

502 Davis AK, Cook KC, Altizer S (2004) Leukocyte profiles of House Finches with and without
503 mycoplasmal conjunctivitis, a recently emerged bacterial disease. *EcoHealth* 1:362-373

504 Davis AK, Maney DL, Maerz JC (2008) The use of leukocyte profiles to measure stress in
505 vertebrates: a review for ecologists. *Funct Ecol* 22:760-772

506 Dubiec A, Witek M, Cichon M (2005) Seasonal decline in leukocyte concentrations and
507 reproductive output in female Great Tits (*Parus major*). *Auk* 12:27-34

508 Dufva R, Allander K (1995) Intraspecific variation in plumage coloration reflects immune-
509 response in Great Tit (*Parus-Major*) males. *Funct Ecol* 9:785-789

510 El Lethy H, Huber-Eicher B, Jungi TW (2003) Exploration of stress-induced
511 immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen
512 responses. *Vet Immunol Immunopathol* 95:91-101

513 Ewenson EL, Zann RA, Flannery GR (2001) Body condition and immune response in wild
514 zebra finches: effects of capture, confinement and captive-rearing. *Naturwissenschaften*
515 88:391-394

516 Falcone FH, Pritchard DI, Gibbs BF (2001) Do basophils play a role in immunity against
517 parasites? *Trends Parasitol* 17:126-129

518 Fokidis HB, Greiner EC, Deviche P (2008) Interspecific variation in avian blood parasites and
519 haematology associated with urbanization in a desert habitat. *J Avian Biol* 39:300-310

520 Friedl TP, Edler R (2005) Stress-dependent trade-off between immunological condition and
521 reproductive performance in the polygynous red bishop (*Euplectes orix*) . *Evol Ecol*
522 19:221-239

523 Fudge AM (1989) Avian hematology: identification and interpretation. *Proceedings*
524 *Association of Avian Veterinarians* 284-292

525 Garvin MC, Homer BL, Greiner EC (2003) Pathogenicity of *Haemoproteus danilewskyi*,
526 Kruse, 1890, in blue jays (*Cyanocitta cristata*). J Wildl Dis 39:161-169

527 Hauptmanová K, Literák I, Bártová E (2002) Haematology and leucocytozoonosis of great tits
528 (*Parus major* L.) during winter. Acta Vet Brno 71:199-204

529 Lazarevic M, Zikic D, Uscebrka G (2000) The influence of long term sound stress on the
530 blood leukocyte count, heterophil/lymphocyte ratio and cutaneous basophil hypersensitive
531 reaction to phytohemagglutinin in broiler chickens. Acta Vet Beogr 50:63-75

532 Lessells CM, Boag PT (1987) Unrepeatable Repeatabilities - A Common Mistake. Auk
533 104:116-121

534 Lobato E, Moreno J, Merino S, Sanz JJ, Arriero E (2005) Haematological variables are good
535 predictors of recruitment in nestling pied flycatchers (*Ficedula hypoleuca*). Ecoscience
536 12:27-34

537 Lucas AM, Jamroz C (1961) Atlas of Avian Hematology. United States Department of
538 Agriculture, Washington

539 Maxwell MH (1981) Leucocyte diurnal rhythms in normal and pinealectomized juvenile
540 female fowls. Res Vet Sci 31:113-115

541 Maxwell MH (1993) Avian blood leukocyte responses to stress. Worlds Poult Sci J 49:34-43

542 Maxwell MH, Robertson GW (1995) The avian basophilic leukocyte: A review. Worlds Poult
543 Sci J 51:307-325

544 Mercurio DDG, Marte BRG, Cruzana BC (2008) Hematological values of chestnut mannikin
545 (*Lonchura malacca*) caught in Laguna. Philippine Journal of Veterinary Medicine 45:63-66

546 Moreno J, de Leon A, Fargallo JA, Moreno E (1998) Breeding time, health and immune
547 response in the chinstrap penguin *Pygoscelis antarctica*. Oecologia 115:312-319

548 Norte AC, Araujo PM, Sampaio HL, Sousa JP, Ramos JA (2009a) Haematozoa infections in a
549 Great Tit *Parus major* population in Central Portugal: relationships with breeding effort and
550 health. *Ibis* 151:677-688

551 Norte AC, Ramos JA, Sousa JP, Sheldon BC (2009b) Variation of adult Great Tit *Parus*
552 *major* body condition and blood parameters in relation to sex, age, year and season. *J*
553 *Ornithol* 150:651–660

554 Ots I, Hõrak P (1998) Health impact of blood parasites in breeding great tits. *Oecologia*
555 116:441-448

556 Ots I, Murumagi A, Hõrak P (1998) Haematological health state indices of reproducing Great
557 Tits: methodology and sources of natural variation. *Funct Ecol* 12:700-707

558 Risberg L, Stjernberg T (1997) Scarlet Rosefinch (*Carpodacus erythrinus*). In: Hagemeyer
559 WJM and Blair MJ (eds) *The EBCC Atlas of European Breeding Birds*. T and A D Poyser,
560 London, pp 732-733

561 Robertson GW, Maxwell MH (1990) Modified staining techniques for avian blood-cells. *Br*
562 *Poult Sci* 31:881-886

563 Ruiz G, Rosenmann M, Novoa FF, Sabat P (2002) Hematological parameters and stress index
564 in rufous-collared sparrows dwelling in urban environments. *Condor* 104:162-166

565 Scope A, Filip T, Gabler C, Resch F (2002) The influence of stress from transport and
566 handling on hematologic and clinical chemistry blood parameters of racing pigeons
567 (*Columba livia domestica*). *Avian Dis* 46:224-229

568 Senar JC, Pascual J (1997) Keel and tarsus length may provide a good predictor of avian body
569 size. *Ardea* 85:269-274

570 Sargent N, Rogers T, Cunningham M (2004) Influence of biological and ecological factors on
571 hematological values in wild Little Penguins, *Eudyptula minor*. *Comp Biochem Physiol A-*
572 *Mol Integr Physiol* 138:333-339

573 Šťastný K, Bejček V, Hudec K (2006) Atlas hnízdního rozšíření ptáků v České republice.
574 Aventinum, Praha

575 Uhart MM, Quintana F, Karesh WB, Braselton WE (2003) Hematology, plasma biochemistry,
576 and serosurvey for selected infectious agents in southern giant petrels from Patagonia,
577 Argentina. J Wildl Dis 39:359-365

578 Votýpka J, Šimek J, Tryjanowski P (2003) Blood parasites, reproduction and sexual selection
579 in the red-backed shrike (*Lanius collurio*). Ann Zool Fenn 40:431-439

580 Yamato O, Goto I, Maeda Y (1996) Hemolytic anemia in wild seaducks caused by marine oil
581 pollution. J Wildl Dis 32:381-384

582

583

584

585

586

587

588

589

590

591

592

593

594

595

596

597

598 **Tables**

599 Table 1: Leukocyte differential count in scarlet rosefinch adults (n=178).

Cell type	Range (%)	Mean (%) ± SD	Median (%)
Lymphocytes	3.60 - 73.39	28.28 ± 13.04	25.67
Immature leukocytes	0.00 - 5.50	0.67 ± 0.99	0.00
Heterophils	0.00 - 71.88	18.73 ± 11.85	17.67
Basophils	13.33 - 85.60	41.93 ± 14.89	39.60
Eosinophils	0.00 - 27.82	6.60 ± 5.43	5.42
Monocytes	0.00 - 15.13	3.78 ± 2.99	3.25

600

601

602 Table 2: Leukocyte differential count in Scarlet rosefinch nestlings (n=155).

Cell type	Range (%)	Mean (%) ± SD	Median (%)
Lymphocytes	4.59 - 41.38	15.49 ± 6.63	14.17
Immature leukocytes	0.00 - 18.58	2.13 ± 2.50	1.59
Heterophils	0.00 - 25.86	9.12 ± 4.49	8.53
Basophils	7.76 - 90.99	55.86 ± 10.80	55.73
Eosinophils	0.83 - 33.88	15.48 ± 7.18	14.63
Monocytes	0.00 - 10.74	1.92 ± 1.90	1.57

603

604

605 Table 3: Minimal adequate model for H/L ratio in adults, n=163, df=10/162, F=4.62,

606 $p < 0.001$. Slope values are given only for continuous variables.

Variable	Slope ± SE	df	F	p
Year		4/152	5.28	<0.001
Sex		2/152	3.96	0.021
Period of capture		2/152	8.84	<0.001
Size	-0.085 ± 0.040	2/152	2.89	0.058
Mass	2.081 ± 0.375	2/152	5.98	0.003
Sex : size	0.252 ± 0.037	1/152	5.29	0.023
Period of capture : mass	2.453 ± 0.378	1/152	4.95	0.028

607

608

609 Table 4: Minimal adequate model for basophil count in adults, n=163, df=3/159, F=9.30,

610 $p < 0.001$.

Variable	Slope ± SE	df	F	p
Mass	-0.427 ± 0.183	1/159	5.42	0.021
Period of capture	-0.049 ± 0.012	1/159	16.87	<<0.001
<i>Haemoproteus</i> inf.	0.031 ± 0.013	1/159	5.49	0.020

611

612

613 Table 5: Minimal adequate model for H/L ratio in nestlings, n=151 nestlings in 43 nests, df=2,

614 Chi=15.89, p<0.001. Model is based on GLMM modelling with nests treated as random

615 effect.

Variable	Slope \pm SE	df	Chi	p
Size	-0.133 \pm 0.051	1	5.62	0.018
Mass	-1.189 \pm 0.581	1	4.16	0.041

616

617

618

619

620

621

622

623

624

625

626

627

628

629

630

631

632

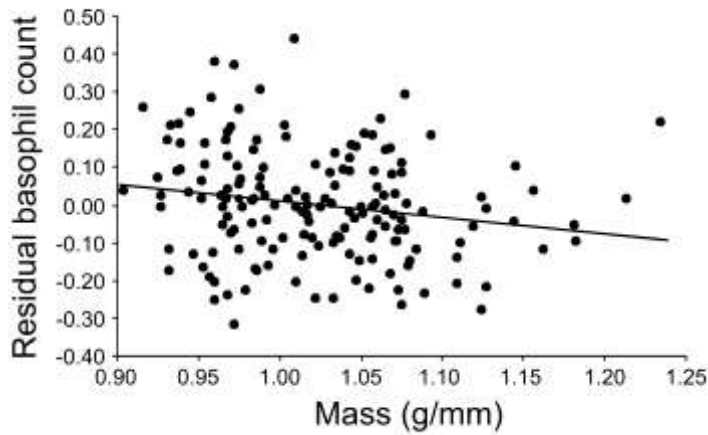
633

634

635

636 **Figures**

637 Fig. 1: The association of individual mass with basophil count in adult Scarlet rosefinches
638 (n=163). Differential basophil count is given as residuals of the arcsin transformed values
639 after controlling for period of capture and *Haemoproteus* infection (for the minimal adequate
640 model see Table 4); mass (individual weight divided by tarsus length) is given in g/mm.

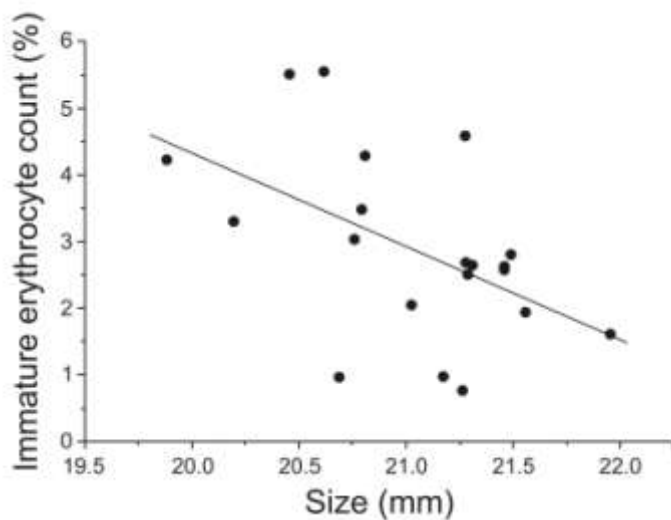


641

642

643 Fig. 2: Association of the immature erythrocyte percentage and individual size in adult Scarlet
644 rosefinches (n=20). For details to the immature erythrocyte percentage see methods; size is
645 given as individual tarsus length in mm.

646

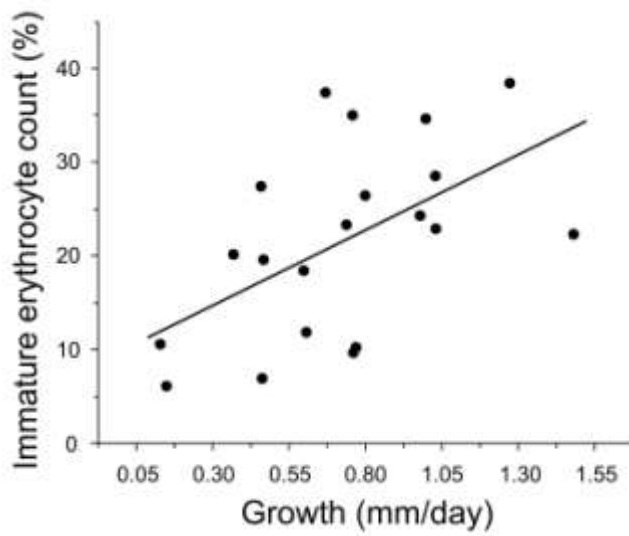


647

648

649 Fig. 3: Association of immature erythrocyte count and growth in Scarlet rosefinch nestlings
650 (n=20). For details to the immature erythrocyte percentage see methods; nestling growth is
651 given as individual tarsus length in mm on day 8 post hatch minus the length on day 7 post
652 hatch.

653



654

655