Social and environmental factors modulate leucocyte profiles in free-living Greylag geese (*Anser anser*)

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Background. Blood parameters such as haematocrit or leucocyte counts are indicators of immune status and health, which can be affected, in a complex way, by exogenous as well as endogenous factors. Additionally, social context is known to be among the most potent stressors in group living individuals, therefore potentially influencing haematological parameters. However, with few exceptions, this potential causal relationship received only moderate scientific attention. **Methods.** In a free-living and individually marked population of the highly social and long-lived Greylag goose, *Anser anser*, we relate variation in haematocrit (HCT), heterophils to lymphocytes ratio(H/L) and blood leucocyte counts to the following factors: intrinsic (sex, age, raising condition, i.e. goose- or hand-raised), social (pair-bond status, pair-bond duration and parental experience) and environmental (biologically relevant periods, ambient temperature) factors. Blood samples were collected repeatedly from a total of 105 focal birds during three biologically relevant seasons (winter flock, mating season, summer). **Results.** We found significant relationships between haematological parameters and social as well as environmental factors. During the mating season, unpaired individuals had higher HCT compared to paired and family individuals and this pattern reversed in fall. Similarly, H/L ratio was positively related to pair-bond status in a seasonally dependent way, with highest values during mating and successful pairs had higher H/L ratio than unsuccessful ones. Also, absolute number of leucocytes tended to vary depending of raising condition in a seasonally dependent way. **Discussion.** Haematology bears a great potential in ecological and behavioural studies on wild vertebrates. In sum we found that HTC, H/L ratio and absolute number of leucocytes are modulated by social factors and conclude that they may be considered valid indicators of individual stress load.

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17 **Short title:** Leucocyte profiles in Greylag geese (*Anser anser*)

18 **Keywords:** Greylag geese, *Anser anser*, haematology, haematocrit, differential leucocyte count,

19 seasonal patterns, age, sex, social status

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22 **Abstract**

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24 immune status and health, which can be affected, in a complex way, by exogenous as well as

25 endogenous factors. Additionally, social context is known to be among the most potent

26 stressors in group living individuals, therefore potentially influencing haematological

27 parameters. However, with few exceptions, this potential causal relationship received only

28 moderate scientific attention.

29 **Methods.** In a free-living and individually marked population of the highly social and long-lived

30 Greylag goose, *Anser anser*, we relate variation in haematocrit (HCT), heterophils to

31 lymphocytes ratio(H/L) and blood leucocyte counts to the following factors: intrinsic (sex, age,

32 raising condition, i.e. goose- or hand-raised), social (pair-bond status, pair-bond duration and

33 parental experience) and environmental (biologically relevant periods, ambient temperature)

34 factors. Blood samples were collected repeatedly from a total of 105 focal birds during three

35 biologically relevant seasons (winter flock, mating season, summer).

36 **Results.** We found significant relationships between haematological parameters and social as

37 well as environmental factors. During the mating season, unpaired individuals had higher HCT

38 compared to paired and family individuals and this pattern reversed in fall. Similarly, H/L ratio

39 was positively related to pair-bond status in a seasonally dependent way, with highest values

40 during mating and successful pairs had higher H/L ratio than unsuccessful ones. Also, absolute

41 number of leucocytes tended to vary depending of raising condition in a seasonally dependent

42 way.

43   **Discussion.** Haematology bears a great potential in ecological and behavioural studies on wild

44   vertebrates. In sum we found that HTC, H/L ratio and absolute number of leucocytes are

45   modulated by social factors and conclude that they may be considered valid indicators of

46   individual stress load.

47

48 **Introduction**

49   Haematocrit (HCT) and differential leucocyte count are important diagnostic tools in gaining

50 information about an animal's condition and health and may be regarded as indicators of

51 individual responses to environmental and social conditions (Cooper 1975; Gavett & Wakeley,

52 1986; Hellgren, Vaughan & Kirkpatrick, 1989; Averbeck, 1992; Saino et al., 1997; Ots, Murumägi

53 & Hõrak, 1998; Bortolotti et al., 2009; Vinkler et al., 2010).

54

55   In wild birds HCT, i.e. the relative volume of red blood cells compared to the total blood

56 volume (Harrison & Harrison, 1986), has been used to indicate the animals' physical condition

57 (e.g. Saino et al., 1997; Ots, Murumägi & Hõrak, 1998) and is known to decrease in response to

58 stressful conditions (Dickens, Earle & Romero, 2009). However, HCT also varies with sex, age,

59 reproductive status, as well as geographic distribution (Rehder, Bird & Laguë, 1982; Rehder et

60 al., 1982; Dawson & Bortolotti, 1997a; Dawson & Bortolotti, 1997b; Bearhop et al., 1999; Potti

61 et al., 1999; Fair, Whitaker & Pearson, 2007).

62

63   The differential leucocyte count may be regarded as a proper proxy of individual immune

64 function (Dufva & Allander, 1995; Zuk, Johnsen & MacLarty, 1995; Johnsen & Zuk, 1998),

65 providing information on the relative occurrence of different leucocyte types. Absolute

66 leucocyte numbers generally scale negatively with body condition (as an indicator of health, see

67 Verhulst, Oosterbeek & Bruinzeel, 2002). In birds, heterophils are rather indicative of changes

68 in the environment (Gross & Siegel, 1983; Maxwell & Robertson, 1998). As lymphocyte

69 numbers decrease while heterophil numbers increase in response to stressful conditions, the

70   ratio of heterophils/lymphocytes (H/L ratio) is used as an indicator of physiological stress (e.g.

71   Gross & Siegel, 1983, 1986; McFarlane & Curtis, 1989; Maxwell, 1993; Vleck et al., 2000; Lebigre

72   et al., 2012). Finally, monocytes are long-lived phagocytic cells associated, together with other

73   leukocyte types (e.g. eosinophils, basophils), with defence against infections and bacteria (Davis

74   et al., 2008).

75

76   Social interactions are known to be among the most potent stressors in group living

77   individuals (von Holst, 1998; de Vries, Glasper & Detillion, 2003). Therefore, it may also

78   influence haematological parameters. However, with the exception of a few veterinary studies

79   (e.g. Zakari et al., 2014; Arfuso et al., 2016), this potential causal relationship (i.e. social context

80   and haematology) received only moderate scientific attention. In Black-capped Chickadees

81   (*Poecile atricapillus*), for example, dominant males had higher haematocrits compared to

82   subordinates (van Oort et al., 2007) and in yellow baboons (*Papio cynocephalus*) frequent

83   involvement in aggressive encounters was related with decreased lymphocyte count (Alberts,

84   Sapolsky & Altmann, 1992). On the other side, several studies suggest a relationship between

85   steroid hormones and haematocrit (e.g. a positive one with testosterone in White-plumed

86   Honeyeaters *Lichenostomus penicillatus* and American Kestrels *Falco sparverius*, Rehder,

87   Bird & Laguë, 1982; a negative one with corticosterone in both sexes of two species of

88   longspurs, *Calcarius ornatus* and *C.mccownii*, Lynn, Hunt & Wingfield, 2003; for a review see

89   Fair, Whitaker & Pearson, 2007), even though the exact mechanisms remain unclear.

90

91   We investigated variation in haematocrit and blood leucocytes in relation to endogenous

92   (age, sex) and exogenous factors (social status, season) in a free living and individually marked

93   population of the socially complex greylag geese, *Anser anser* (Lorenz, 1988; Kotrschal,

94   Hemetsberger & Weiß, 2006; Kotrschal, Scheiber & Hirschenhauser, 2010; Hemetsberger,

95   Scheiber & Weiß, 2013). Social context is known to be among the strongest modulators of the

96   physiological stress responses in greylag geese (e.g. Wascher, Scheiber & Kotrschal, 2008;

97   Wascher, Arnold & Kotrschal, 2008; Wascher et al., 2009; Kralj-Fiser et al., 2010), which in turn

98   are alleviated via emotional social support by partners (Frigerio et al., 2003; Scheiber et al.,

99   2005; Scheiber, Kotrschal & Weiß, 2009; Wascher et al., 2012). Across seasons, males and

100   females are faced with different demands (Kotrschal, Scheiber & Hirschenhauser, 2010);

101   consequently, physiological changes, such as levels of corticosterone, co-vary with seasonal

102   variation in behaviour (Hirschenhauser, Moestl & Kotrschal, 1999a; Hirschenhauser, Moestl &

103   Kotrschal, 1999b; Frigerio et al., 2004a). The reproductive season of greylag geese starts in

104   January. Testosterone and corticosterone levels increase over the season (Kotschal,

105   Hirschenahuser & Moestl, 1998; Hirschenhauser, Moestl & Kotrschal, 1999a), as do agonistic

106   encounters between individuals (Lorenz, 1988). With the beginning of the breeding season

107   (March to July) the flock disintegrates into pairs. As for most bird species, reproduction in geese

108   is energetically costly, especially for the females who lay and incubate the eggs (Raveling, 1979;

109   Thompson & Raveling, 1988). Parental geese show elevated corticosterone levels (Kotschal,

110   Hirschenahuser & Moestl, 1998) and synchronise their moult with the rearing period, so that

111   they start flying again together with their offspring, when the goslings are approximately 10

112   weeks old (Lorenz, 1988). In alignment with the parental phase (i.e. from hatching to fledging),

113   androgen levels reach their annual minimum in both sexes. The flock re-unites in August after

114   moulting, when androgens reach a second peak in unpaired males, while in paired individuals

115   the second peak is reached later in fall (Hirschenhauser, Moestl & Kotrschal, 1999a;

116   Hirschenhauser, Moestl & Kotrschal, 1999b). This hints at the close interplay between

117   physiology and social status in greylag geese, as suggested by further studies (e.g. stress

118   response and social allies: Sachser et al., 1998; heart rate in the context of sociality: Aureli,

119   Preston & de Waal, 1999; steroid hormones and social status: Wingfield, Hegner & Lewis, 1991;

120   Goymann et al., 2015).

121   We studied seasonal and individual variation in HCT and leucocyte counts. We tested

122   whether (a) individual factors, such as sex, age or raising condition, (b) social factors, such as

123   pair-bond status, (c) biologically relevant periods (mating season, after moult, autumn), or a

124   combination of the above, accounted for variation in haematological parameters. We

125   hypothesized that individuals of different sexes and social status (females and males, paired

126   versus unpaired) are faced with different social and energetic demands within the flock (e.g.

127   Metcalfe, Taylor & Thorpe, 1995; Senar et al., 2000), which in turn, will affect the immune

128   system and will be manifest in HCT and leucocyte count. Since the mating season is socially and

129   energetically more challenging than moult and the stable winter flock (Drent & Daan, 1980) we

130   predict that both haematological measurements will peak during this period, particularly in the

131   high-ranking parental individuals. Furthermore, we expect to find seasonal differences between

132   males and females, contingent with behavioural and physiological differences between the

133   sexes depending on an individual's social environment (Wingfield et al., 1991; Kotschal,

134   Hirschenahuser & Moestl, 1998; Hirschenhauser, Moestl & Kotrschal, 1999a Hirschenhauser,

135 Moestl & Kotrschal, 1999 b; Owens & Hartley, 1998; Wascher et al., 2012; Lees et al., 2015).

136 Additionally, as hand-raised individuals generally differ from parent-raised conspecifics in

137 glucocorticoid stress reactivity (Hemetsberger et al., 2010), we finally predict that hand-raised

138 geese will show higher values of both haematological parameters during socially challenging

139 periods than parent-raised ones.

140

141

142 **Material and methods**

143 *Ethical statement*

144   This study complies with all current Austrian laws and regulations concerning the work with

145 wildlife. Catching and blood sampling of focal individuals was permitted under Animal

146 Experiment License Nr. 66.006/0010-II/10b/2010 by the Austrian Federal Ministry for Science

147 and Research.

148

149 *Study area and focal animals*

150   The Konrad Lorenz Research Station (KLF) is situated at 550 m above sea level in the valley of

151 the river Alm in the Northern part of the Austrian Alps (47°48′E, 13°56′N). The non-migratory

152 flock of greylag geese we studied was introduced by Konrad Lorenz and co-workers in 1973

153 (Lorenz, 1988). The geese are unrestrained and generally spend the day close to the research

154 station where they are provided with supplemental food (pellets and grain) twice a day year-

155 round. At night, the birds roost on a lake approximately 10 km to the South (Almsee). As in wild

156 populations, natural predators (mainly red foxes, *Vulpes vulpes* and golden eagles, *Aquila*

157   *chrysaetos*) may account for losses up to 10 % of the flock per year (Hemetsberger, 2001,

158   Hemetsberger, 2002). All geese are marked with coloured leg rings and are habituated to the

159   close presence of humans. They neither show increased immunoreactive corticosterone

160   metabolites in their droppings nor heart rate increases when approached by familiar humans

161   (Scheiber et al., 2005; Wascher et al., 2012). Social behaviour and individual life-history data

162   have been monitored since 1973. Such long-term observations provide reliable information

163   about an individual's social relationships within the flock (i.e. paired or not). Approximately 20

164   % of the flock members are carefully hand-raised in the frame of specific research projects.

165   Details about the hand-raising tradition of the KLF are published elsewhere (Hemetsberger,

166   Scheiber & Weiss, 2013). During the period of data collection (autumn 2010 to spring 2013) the

167   number of geese in flock varied between 153 and 157 individuals.

168   Focal animals of the present study were 105 greylag geese of different age (at the time of

169   sampling ranging from 0.16 to 22.81 years, mean age ± SE = 5.06 ± 0.36), sex (67 males, 49

170   females), social status (68 paired individuals, 30 family individuals, 18 unpaired ones,) and

171   different raising condition (71 goose-raised and 45 hand-raised individuals).

172

173   *Data collection*

174   Data were collected during three phases, representing biologically relevant periods of the

175   year (Hemetsberger, Scheiber & Weiß, 2013): (1) in summer, after moulting; (2) in autumn

176   (winter flock) and (3) during the mating season before the beginning of the laying period

177   (approx. mid-March). Focal geese were caught by hand up to three times, once in each period

178   either by a familiar human observer who approached the geese from the back and picked them

179   up when being close enough or in a 'trapping enclosure', which the geese entered voluntarily

180   during feeding. In this way, chases or strong wing flapping were avoided. To control for diurnal

181   variation of the focal parameters, all geese were sampled in the morning between 0730 and

182   0930.

183   Weather data were provided by a weather station in Grünau (47° 51′E, 13° 57′N) operated

184   by Max Rauscher (www.gruenau.tv; last accessed 25 December 2014). Temperature data were

185   recorded every 5 min, for which we calculated daily means during our analysis.

186

187   *Blood samples*

188   A total number of 169 blood samples (N = 105 individuals, Table 1) were collected by

189   puncturing the tarsal vein with a sterile needle (24 µm diameter) and collecting blood in two

190   heparinized micro-haematocrit capillaries (75 mm). Furthermore, we measured tarsus, beak

191   and wing length, and body weight. The whole procedure lasted less than 7 minutes per

192   individual.

193

194   In order to determine an individual's blood cell count (Prinzinger, Misovic & Nagel, 2012)

195   one drop of blood was smeared onto a microscope slide, air-dried and stored until later

196   identification of leucocytes at the Clinical Pathology Platform of the University of Veterinary

197   Medicine in Vienna (Austria). Differential blood cells count provided information on absolute

198   leucocyte number/µl (LEUCO) and the relative occurrence of different leucocyte types

199   (heterophils, lymphocytes, monocytes, basophils and eosinophils, Prinzinger, Misovic & Nagel,

200   2012). Blood smears were Romanowsky-stained with Haemaquick (E. Lehmann GmbH, Salzburg

201   Austria) and microscopically evaluated. Thereafter, 100 white blood cells were differentiated

202   into heterophilic, eosinophilic or basophilic granulocytes, monocytes, and lymphocytes at

203   1,000x magnification using oil immersion. Results were provided in percentages.

204

205   The haematocrit capillaries were then sealed with plasticine at the bottom and centrifuged

206   at 8000 rpm for 5 min in order to determine the HCT. Volumes of red blood cell and plasma

207   respectively were measured on the capillaries to the nearest 0.5 mm with calipers. HCT was

208   then calculated as ratio as follow (Prinzinger, Misovic & Nagel, 2012): red blood cell

209   volume/(red blood cell volume + plasma volume). The individual arithmetic mean of the two

210   HCT values was then taken into further analyses.

211

212   *Statistical analysis*

213   We ran linear mixed effect models (LMMs) fitted with maximum likelihood using the nlme

214   package (Pinheiro et al., 2015; model formulation and computational methods are described in

215   Lindstrom & Bates 1990) in R (R Core Team 2015). Haematocrit, heterophils to lymphocytes

216   (H/L) ratio, leucocyte numbers and percentage of monocytes were considered each in turn as

217   response variable and four LMMs were calculated for each of the three data subsets. Subset (1)

218   included all 169 samples collected from 105 individuals. We tested 7 competing hypotheses: (a)

219   individual factors accounting for variation in haematological parameters with sex, age (*i.e.* days

220   of life) and raising condition (hand-raised, goose-raised) included as fixed factors; (b) social

221   factors (pair-bond status: unpaired, paired, family) explaining variation in haematological

222   parameters; (c) biologically relevant period (mating season, after moult, autumn) accounting

223 for variation in haematological parameters; (d) Social factors (pair-bond status) explaining

224 variation in a seasonally dependent way (the interaction between season and pair-bond status

225 was additionally included in the model); (e) individual factors (sex, age, raising-condition),

226 season and the interactions between season and individuals were included in the model; (f)

227 individual factors, pair-bond status and the interaction between individual factors and pair-

228 bond status; (g) null model. Subset (2) included only data from male-female pair bonded

229 individuals (68 samples from 50 individuals). We tested three hypotheses: (a) pair-bond

230 duration, defined as 'established' if the pair spent already one breeding season together or

231 'newly', if this was not the case or (b) parental experience, i.e. if the pair previously successfully

232 raised fledged offspring (yes/no), accounting for variation in haematological parameters; (c)

233 null model.

234

235 The third subset of data only included individuals from which morphological measurements

236 were collected besides blood samples (116 samples from 70 individuals). A model including the

237 body size index (BSI), calculated as ratio weight/tarsus (as indicator for body's structural size,

238 *sensu* Green 2001) as fixed factor, was tested against the null model. In order to account for

239 repeated measures for each observed individual, the individual identity was included as random

240 factor. We based our model selection on corrected Akaike's Information Criterion values (AICc).

241 We calculated the difference between the best model and each other possible model (ΔAICc)

242 and ranked the model combinations according to their ΔAICc, which provides an evaluation of

243 the overall strength of each model in the candidate set. Different competing models tested are

244 presented in Table 2. If multiple models qualified as the similarly good models, i.e. ΔAIC ≤ 2

245   (Burnham & Anderson, 2002; Burnham, 2004) we applied a model averaging approach, which

246   calculates model averaged parameters using the MuMIn package (version 1.15.6). In both data

247   subset (2) pair-bonded individuals and (3) morphological measures, for some of the parameters

248   the candidate models did not qualify as better as compared to the null model (Table 2) and are

249   therefore not presented in the results section.

250

251

252   **Results**

253   (1) *Full dataset:*

254   The best candidate model explaining variation in haematocrit included individual factors

255   (sex, age, raising condition), season as well as the interactions between season and individual

256   factors. HCT significantly increased with age in autumn but not during the mating season or

257   after moult (Table 3). The opposite pattern was found for percentage of monocytes, which

258   tended to decrease with age in autumn. The H/L ratio varied depending on pair-bond status in a

259   seasonally dependent way (Figure 1).

260

261   Leucocyte counts differed depending on pair-bond status in a seasonally dependent way.

262   Generally, leucocyte counts were highest after moult (family, mean ± SD: 11086.956 ±

263   4494.067) and in autumn (unpaired: 13125 ± 6300.51; paired: 13175 ± 4948.741; family:

264   9519.23 ± 3548.184) and decreased during the mating season (unpaired: 7933.333 ± 2651.303;

265   paired: 10500 ± 5154.748; family: 9200 ± 2111.194). This decrease was most pronounced in

266   unpaired individuals as compared to paired and family individuals. Further, leucocyte counts

267   tended to vary seasonally, depending on individual raising condition. In the winter leucocyte

268   counts were higher in hand-raised as compared to goose raised individuals (hand-raised:

269   13722.222 ± 5248.24; goose-raised: 10050 ± 3645.861) and this pattern reversed during the

270   mating season (hand-raised: 9468.75 ± 4870.810; goose-raised: 9715.909 ± 3901.993). In

271   summer (after moult) only hand-raised individuals were sampled (11086.956 ± 4494.067).

272

273   *(2) Pair-bonded individuals dataset*

274   HCT, leucocyte number, and percentage of monocytes were not significantly affected by

275   pair-bond duration or parental experience. H/L ratios were higher in pairs with an 'established'

276   pair-bond duration compared to those with a 'newly' formed one (established: 1.228 ± 0.593;

277   newly: 0.819 ± 0.418) and were higher in reproductively successful as compared to unsuccessful

278   pairs (Figure 2).

279

280   *(3) Morphological measures*

281   The body size index (BSI) was positively related to HCT, percentage of monocytes and H/L

282   ratio.

283

284   **Discussion**

285   Our results show that social and environmental factors interact with individual physiology in

286   a complex way. We found that in the free-living greylag geese investigated, haematocrit (HCT)

287   and differential leucocyte counts are seemingly contingent with a suite of endogenous (i.e. sex,

288   age, raising condition), social (i.e. pair-bond status, pair-bond duration and parental experience)

289   and environmental factors (i.e. biologically relevant seasons).

290

291   Interestingly, pair-bond status showed a seasonal dependent relationship with several

292   haematological parameters: unpaired individuals had the highest HCT during the mating season

293   and H/L ratio was significantly higher in individuals who successfully raised young as compared

294   to individuals which failed to fledge offspring that year. Our results hint at a complex

295   relationship between an individual's social status within the flock, the seasonal patterns of

296   corticosterone and individual behavioural investment in order to optimize the own fitness, as

297   discussed by Kotrschal, Hirschenhauser & Moestl (1998). In fact, during summer and winter, the

298   paired males with offspring had significantly higher corticosterone than both paired males

299   without offspring and singletons, whereas during the mating season, singletons had marginally

300   higher corticosterone than paired males. Furthermore, stress levels of all three male categories

301   were significantly higher during the mating season than during the rest of the year. Even

302   though we did not measure levels of corticosterone in the present study, a number of studies

303   confirm the close relationship between stress physiology and haematology (e.g. Gross & Siegel

304   1983; Dickens, Earle & Romero 2009).

305

306   Therefore we suggest that high HCT levels in unpaired individuals during the mating season

307   mirror social stress caused mainly by competition for a mate as well as by constrained access to

308   mates, whereas the haematological differences related to reproductive success might reflect

309   the costs of reproduction in successful geese. Such costs are one of the most significant

310   components of life-history trade-offs and the immune system has been proposed as an

311   important link between reproductive investment and survival (Sheldon & Verhulst 1996;

312   Deerenberg et al. 1997; French, Denardo & Moore 2007; French, Moore & Demas 2009;

313   Harshman & Zera 2007; Cox et al. 2010).

314

315   Although earlier avian studies found higher HCT levels in males than in females (e.g.

316   cormorant, *Phalacrocorax carbo*, Balasch et al. 1974; kestrel, *Falcus tinnunculus*, Rehder, Bird &

317   Laguë 1982; sparrowhawk, *Falcus sparverius*, Rehder & Bird 1983), our results are in line with a

318   recent meta-analysis based on 36 published studies did not provide evidence for sex differences

319   in HCT (Fair, Whitaker & Pearson 2007),).

320

321   Hence, the seasonal differences in relation to pair-bond status we found in our study may

322   reflect differences among greylag geese with different bonding status and energetics (De Graw,

323   Kern & King 1979; Jenni et al. 2006). High H/L ratio may reflect physiological stress produced by

324   competition for partners during the mating season and frequent agonistic interactions among

325   the unpaired individuals, which may not enjoy the stress buffering effect of emotional social

326   support by social allies, notably by a pair partner (Scheiber et al. 2005). On the other hand, high

327   H/L ratio may represent the costs of parental commitment for successfully breeding individuals.

328

329   Heterophils are the predominant immunological cell type within the Anseriformes (Lucas &

330   Jamroz 1961). They form the first line of cellular defence against invading microbial pathogens

331   (Maxwell & Robertson 1998). Findings in poultry suggest the H/L ratio to be a reliable indicator

332   of social stress (e.g. Gross & Siegel 1983; Vleck et al. 2000). This is supported by other studies in

333   birds, which have shown that H/L increases in response to a wide variety of stressors, including

334   long-distance migration (Owen & Moore 2006) and parasitic infection (Davis, Cook & Altizer

335   2004; Lobato et al. 2005; for a review see Davis, Maney & Maerz 2008).

336

337   We suggest that the decrease of the percentage of lymphocytes and the increase of

338   heterophils (i.e. high H/L ratio) may also reflect an increase in susceptibility to infections with

339   aging (Uciechowski & Rink 2015), which affects several haematological parameters (Maxwell et

340   al. 1990; Maxwell & Robertson 1998; Prinzinger, Misovic & Nagel 2012). In fact, glucocorticoids

341   and oestrogens may reduce T-cell production (i.e. low lymphocytes levels) whereas androgens

342   may increase susceptibility to infections via elevated heterophils levels (Nelson et al. 2002).

343   However, as we did not directly measure glucocorticoid hormones, we can only speculate about

344   the relation between haematology and health. Notwithstanding, our results confirm

345   haematology's great potential in studies of ecology and in wild vertebrates, as recently

346   suggested by Maceda-Veiga et al. (2015).

347

348   Finally, our results showed that raising condition did affect haematological parameters,

349   suggesting that goose-raised individuals could cope better with the stressful mating season

350   than hand-raised individuals, as the latter showed significantly lower levels of leucocytes as

351   compared to goose-raised geese. Although raising history does not seem to affect other

352   reproductive parameters such as number and weight of eggs laid, hatching success or number

353   of young fledged (Hemetsberger et al. 2010), this intrinsic aspect may deserve further attention

354   in the future when considering focal individuals.

355

356   In conclusion, our results indicate that the way social and intrinsic factors modulate

357   haematological parameters varied between seasons. Seasonal activities such as reproduction or

358   migration need to fine-tune physiology with weather conditions (e.g. Dorn et al. 2014; Frigerio

359   et al. 2004b; Romero, Reed & Wingfield 2000). By and large, the investigated haematological

360   parameters varied with individual behavioural investment and stress load. Therefore they may

361   be considered as valid indicators of social burden.

362

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367

368   **References**

369   Alberts SC, Sapolsky RM, Altmann J. 1992. Behavioral, endocrine, and immunological correlates of
370   immigration by an aggressive male into a natural primate group. *Hormones and Behavior*, 26: 167-178.

371   Arfuso F, Fazio F, Rizzo M, Marafioti S, Zanghì E, Piccione G. 2016. Factors affecting the haematological
372   parameters in different goat breeds from Italy. *Annals of Animal Science*, DOI: 10.1515/aoas-2015-0094.

373   Aureli F, Preston SD, de Waal FBM. 1999. Heart rate response to social interactions in free moving
374   Rhesus macaques: a pilot study. *Journal of Comparative Psychology* 113: 59-65.

375   Averbeck C. 1992. Haematology and blood chemistry of healthy and clinically abnormal Great
376   Blackbacked Gulls (*Larus marinus*) and Herring Gulls (*Larus argentatus*). *Avian Pathology*, 21: 215–223.

377   Balasch J, Palomeque J, Palacios L, Musquera S, Jimenez M. 1974. Hematological values of some great
378   flying and aquatic-diving birds. *Comparative Biochemistry and Physiology*, 49A: 137–145.

379 Bearhop S, Griffiths R, Orr K, Furness RW. 1999. Mean corpuscular volume (MCV) as a measure of
380 condition in birds. *Ecology Letters*, 2: 352–356.

381 Bortolotti GR, Mougeot F, Martinez-Padilla J, Webster LMI, Piertney SB. 2009. Physiological Stress
382 Mediates the Honesty of Social Signals. *PLoS ONE*, 4(3): e4983. doi:10.1371/journal.pone.

383 Burnham KP. 2004. Multimodel inference: Understanding AIC and BIC in model selection. *Sociological*
384 *Methods & Research*, *33*(2): 261–304.

385 Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: a practical information-*
386 *theoretic approach.* New York: Springer.

387 Cooper JE 1975. Haematological investigation in east African birds of prey. *Journal of Wildlife Diseases*,
388 11: 389–394.

389 Cox RM, Parker EU, Cheney DM, Liebl AL, Martin LB, Calsbeek R. 2010. Experimental evidence for
390 physiological costs underlying the trade-off between reproduction and survival. *Functional Ecology*, 24:
391 1262-1269. doi: 10.1111/j.1365-2435.2010.01756.x

392 Davis AK, Cook KC, Altizer S. 2004. Leukocyte profiles of House Finches with and without mycoplasmal
393 conjunctivitis, a recently emerged bacterial disease. *Ecohealth*, 1: 362-373.

394 Davis AK, Maney DL, Maerz JC. 2008. The use of leukocyte profiles to measure stress in vertebrates: a
395 review for ecologists. *Functional Ecology*, 22: 760-772.

396 Dawson RD, Bortolotti GR. 1997a. Are avian hematocrits indicative of condition? American kestrel as a
397 model. *Journal of Wildlife Management*, 61: 1297–1306.

398 Dawson RD, Bortolotti GR. 1997b. Variation in haematocrit and total plasma proteins of nestling
399 American kestrels (*Falco sparverius*) in the wild. *Comparative Biochemistry and Physiology A*, 117: 383–
400 390.

401 De Graw WA, Kern MD, King JR. 1979. Seasonal changes in the blood composition of captive and free-
402 living white-crowned sparrows. *Journal of Comparative Physiology*, 129B: 151-162.

403 Deerenberg C, Apanius V, Daan S., Bos N. 1997. Reproductive effort decreases antibody responsiveness.
404 *Proceedings of the Royal Society* B, 264: 1021–1029.

405 de Vries AC, Glasper ER, Detillion CE. 2003. Social modulation of stress responses. *Physiology and*
406 *Behavior*, 79: 399-407.

407 Dickens MJ, Earle KA, Romero LM. 2009. Initial transference of wild birds to captivity alters stress
408 physiology. *General and Comparative Endocrinology*, 160: 76–83.

409 Dorn S, Wascher CAF, Moestl E, Kotrschal K. 2014. Ambient temperature and air pressure modulate
410 hormones and behaviour in Greylag geese (*Anser anser*) and Northern bald ibis (*Geronticus eremita*).
411 *Behavioural Processes*, 108: 27–35.

412 Drent RH, Daan S. 1980. The Prudent Parent: Energetic Adjustments in Avian Breeding. *Ardea*, 68: 225-
413 258. DOI: http://dx.doi.org/10.5253/arde.v68.p225

414 Dufva R, Allander K. 1995. Intraspecific variation in plumage coloration reflects immune response in
415 Great Tit (*Parus major*) males. *Functional Ecology*, 9: 785–789.

416 Fair J, Whitaker S, Pearson B. 2007. Sources of variation in haematocrit in birds. *Ibis*, 149: 535-552.

French SS, Denardo DF, Moore MC. 2007. Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *American Naturalist*, 170: 79–89.

French SS, Moore MC, Demas GE. 2009. Ecological immunology: the organism in context. *Integrative and Comparative Biology*, 49: 246–253.

Frigerio D, Weiß B, Dittami J, Kotrschal K. 2003. Social allies modulate corticosterone excretion and increase success in agonistic interactions in juvenile hand-raised greylag geese (*Anser anser*). *Canadian Journal of Zoology*, 81: 1746–1754.

Frigerio D, Hirschenhauser K, Moestl E, Dittami J, Kotrschal K. 2004a. Experimentally elevated testosterone increases status signalling in male Greylag geese (*Anser anser*). *Acta Ethologica*, 7: 9-18.

Frigerio D, Dittami J, Moestl E, Kotrschal K. 2004b. Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). *General and Comparative Endocrinology*, 137: 29-36.

Gavett AP, Wakeley JS. 1986. Blood constituents and their relation to diet in urban and rural house sparrow. *Condor*, 88: 279-284.

Goymann W, Villavicencio, CP, Apfelbeck B. 2015. Does a short-term increase in testosterone affect the intensity or persistence of territorial aggression? — An approach using an individual's hormonal reactive scope to study hormonal effects on behaviour. *Physiology & Behavior*, 149, 310-316.

Green AJ. 2001. Mass/Length Residuals: Measures of Body Condition or Generators of Spurious Results? *Ecology*, 82: 1473–1483.

Gross WB, Siegel HS. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases*, 27: 972-979.

Gross WB, Siegel, PB. 1986. Effects of initial and second periods of fasting on heterophil/lym-phocyte ratios and body weight. *Avian Diseases*, 30: 345–346.

Harrison GJ, Harrison LR. 1986. *Clinical avian medicine and surgery*. WB Saunders, London.

Harshman LG, Zera AJ. 2007. The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution*, 22: 80–86.

Hellgren EC, Vaughan MR, Kirkpatrick RL. 1989. Seasonal patterns in physiology and nutrition of black bears in Great Dismal Swamp, Virginia-North Carolina. *Canadian Journal of Zoology*, 67: 1837-1850.

Hemetsberger J. 2001. Die Entwicklung der Gruenauer Graugansschar seit 1973. In: Kotrschal K, Mueller G, Winkler H, eds. *Konrad Lorenz und seine verhaltensbiologischen Konzepte aus heutiger Sicht*. Fuerth (Germany): Filander Verlag, 249–260.

Hemetsberger J. 2002. Populationsbiologische Aspekte der Grünauer Graugansschar *Anser anser*. PhD Thesis, University of Vienna, Vienna, Austria.

Hemetsberger J, Scheiber IBR, Weiß BM, Frigerio D, Kotrschal K. 2010. Influences of socially involved hand-raising on life history and stress responses in greylag geese. *Interdisciplinary Studies*, 11(3): 380-395.

Hemetsberger J, Scheiber IBR, Weiß BM. 2013. Greylag geese: from general principle to the Konrad Lorenz flock. In: Scheiber IBR, Weiß BM, Hemetsberger J, Kotrschal K, eds. *The social life of Greylag Geese – Patterns, Mechanisms and Evolutionary Function in an Avian Model System*. Cambridge University Press, Cambridge, UK, 3-25.

458   Hirschenhauser K, Moestl E, Kotrschal K. 1999a. Seasonal Patterns of Sex Steroids Determined from
459   Feces in Different Social Categories of Greylag Geese (*Anser anser*). *General and Comparative*
460   *Endocrinology*, 114: 67-79.

461   Hirschenhauser K, Moestl E, Kotrschal K. 1999b. Within-pair testosterone co-variation and reproductive
462   output in greylag geese (*Anser anser*). *Ibis*, 141: 577-586.

463   Jenni L, Mueller S, Spina F, Kvist A, Lindstroem L. 2006. Effects of endurance flight on hematocrits in
464   migration birds. *Journal of Ornithology* 147: 531-542.

465   Johnsen TS, Zuk M. 1998. Parasites, morphology, and blood characters in male red jungle fowl during
466   development. *Condor*, 100: 749–752.

467   Kotrschal K, Hirschenhauser K, Moestl E. 1998. The relationship between social stress and dominance is
468   seasonal in greylag geese. *Animal Behaviour*, 55: 171-176.

469   Kotrschal K, Hemetsberger J., Weiß BM. 2006. Homosociality in male greylag geese (*Anser anser*):
470   Making the best of a bad situation. In: Vasey P, Sommer V, eds. *Homosexual Behaviour in Animals: An*
471   *Evolutionary Perspective*. Cambridge: Cambridge University Press, 45-76.

472   Kotrschal K, Scheiber IBR, Hirschenhauser K. 2010. Individual performance in complex social systems. In:
473   Kappeler P, ed. *Animal Behaviour: Evolution & Mechanism*. Heidelberg: Springer Verlag, 121-148.

474   Kralj-Fiser S, Scheiber IBR, Kotrschal K, Weiß BM, Wascher CAF. 2010. Glucocorticoids enhance and
475   suppress heart rate and behaviour in time dependent manner in greylag geese (*Anser anser*). *Physiology*
476   *& Behavior*, 100: 394-400.

477   Lebigre C, Alatalo RV, Kilpimaa J, Staszewski V, Siitari H. 2012. Leucocyte counts variation and measures
478   of male fitness in the lekking Black Grouse. *Journal of Ornithology*, 153(1): 95-102.

479   Lees JJ, Nudds RL, Folkow LP, Stokkan K-A, Codd JR. 2015. Understanding sex differences in the cost of
480   terrestrial locomotion. *Proceedings of the Royal Society of London B*, doi:10.1098/rspb.2011.1334

481   Lindstrom MJ, Bates DM. 1990. Nonlinear mixed effects models for repeated measures data. *Biometrics*,
482   46: 673-687.

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

485   Lorenz K. 1988. *Hier bin ich – wo bist du? Ethologie der Graugans*. Piper Verlag, München, Germany.

486   Lucas AM, Jamroz J. 1961. *Atlas of Avian Hematology*. US Dep. of Agricolture, Washington D.C.

487   Lynn SE, Hunt KE, Wingfield JC. 2003. Ecological factors affecting the adrenocortical response to stress in
488   Chestnut-collared and McCown's longspurs (*Calcarius ornatus, Calcarius mccownii*). *Physiological and*
489   *Biochemical Zoology*, 76: 566–576.

490   Maceda-Veiga A., Figuerola J, Martínez-Silvestre A, Viscor G, Ferrari N, Pacheco M. 2015. Inside the
491   Redbox: Applications of haematology in wildlife monitoring and ecosystem health assessment. *Science*
492   *of the Total Environment*, 514: 322–332.

493   Maxwell MH. 1993. Avian blood leucocyte responses to stress. *World's Poultry Science Journal*, 49: 34-
494   43.

495   Maxwell MH, Robertson GW. 1998. The avian heterophil leucocyte: a review. *World's Poultry Science*
496   *Journal*, 54(02): 155-178.

497 Maxwell MH, Robertson, GW, Spence S, McCorquodale CC. 1990. Comparison of haematological values
498 in restricted and ad libitum-fed domestic fowls: white blood cells and thrombocytes. *British Poultry*
499 *Science*, 31: 399–405.

500 McFarlane JM, Curtis SE. 1989. Multiple Concurrent Stressors in Chicks: 3. Effects on Plasma
501 Corticosterone and the Heterophil:Lymphocyte Ratio. *Poultry Science*, 68(4): 522-527
502 doi:10.3382/ps.0680522.

503 Metcalfe NB, Taylor AC, Thorpe JE. 1995. Metabolic rate, social status and life-history strategies in
504 Atlantic salmon. *Animal Behaviour*, 49(2): 431-436.

505 Nelson RJ, Demas GE, Klein SL, Kriegsfeld LJ. 2002. *Seasonal Patterns of Stress, Immunefunction and*
506 *Desease*. Cambridge University Press.

507 Ots I, Murumägi A, Hõrak P. 1998. Haematological health state indices of reproducing great tits:
508 methodology and sources of natural variation. *Functional Ecology*, 12: 700–707.

509 Owen JC, Moore FR. 2006. Seasonal differences in immunological condition of three species of thrushes.
510 *Condor*, 108: 389–398.

511 Owens IPF, Hartley IR. 1998. Sexual dimorphism in birds: why are there so many different forms of
512 dimorphism? *Proceedings of the Royal Society London B*, 265: 397–407. doi:10.1098/rspb.1998.0308.

513 Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team. 2015. nlme: Linear and Nonlinear Mixed
514 Effects Models. R package version 3.1-121, http://CRAN.R-project.org/package=nlme, last retrieved
515 05.08.2015.

516 Potti J, Moreno J, Merino S, Frías O, Rodriguez R. 1999. Environmental and genetic variation in the
517 haematocrit of fledgling pied flycatchers. *Oecologia* 120: 1–8.

518 Prinzinger R, Misovic A, Nagel B. 2012. *Aviaere Haematologie – Das Vogelblut: Struktur, Funktion,*
519 *Diagnose und Parasiten*. Cuvillier Verlag, Goettingen, Germany.

520 R Core Team 2015. R: A language and environment for statistical computing. R Foundation for Statistical
521 Computing, Vienna, Austria. URL http://www.R-project.org/, last accessed 05.08.2015.

522 Raveling DG. 1979. The annual cycle of body composition of Canada geese with special reference to
523 control of reproduction. *Auk*, 96: 234-252.

524 Rehder NB, Bird DM. 1983. Annual profiles of blood packed cell volumes of captive American kestrels.
525 *Canadian Journal of Zoology*, 61: 2550–2555.

526 Rehder NB, Bird DM, Laguë PC. 1982. Variation in blood packed cell volume of captive American kestrels.
527 *Comparative Biochemistry and Physiology* 72A: 105–109.

528 Rehder NB, Bird DM, Laguë PC, Mackay C. 1982. Variation in selected hematological variables of captive
529 redtailed hawks. *Journal of Wildlife Diseases*, 18: 105–109.

530 Romero ML, Reed JM, Wingfield JC. 2000. Effects of weather on corticosterone response in wild free-
531 living passerine birds. *General and Comparative Endocrinology*, 118: 113–122.

532 Saino N, Cuervo JJ, Krivacek M, de Lope F, Møller AP. 1997. Experimental manipulation of tail ornament
533 size affects hematocrit of male barn swallows (*Hirundo rustica*). *Oecologia* 110: 186–190.

534 Sachser N, Duerschlag M, Hirzel D. 1998. Social relationships and the management of stress.
535 *Psychoneuroendocrinology*, 23: 891–904.

536 Scheiber IBR, Weiß BM. 2013. Beyond the pairbond: extended family bonds and female-centred clan
537 formation. In: Scheiber IBR, Weiß BM, Hemetsberger J, Kotrschal K, eds. *The social life of Greylag Geese*
538 *– Patterns, Mechanisms and Evolutionary Function in an Avian Model System*. Cambridge University
539 Press, Cambridge, UK, 105-118.

540 Scheiber IBR, Weiß BM, Frigerio D, Kotrschal K. 2005. Active and passive social support in families of
541 Greylag geese (*Anser anser*). *Behaviour*, 142: 1535-1557.

542 Scheiber IBR, Kotrschal K, Weiß BM. 2009. Benefits of family reunions: Social support in secondary
543 greylag goose families. *Hormones and Behavior*, 55: 133–138.

544 Sheldon BC, Verhulst S. 1996. Ecological immunology: costly parasite defences and trade-offs in
545 evolutionary ecology. *Trends in Ecology and Evolution*, 11: 317–321.

546 Senar JC, Polo V, Uribe F, Camerino M. 2000 Status signalling, metabolic rate and body mass in the
547 siskin: the cost of being a subordinate. *Animal Behaviour*, 59(1): 103-110.

548 Thompson SC, Raveling DG. 1988. Incubation behavior of Emperor geese compared with other geese:
549 Interactions of predation, body size, and energetics. *Auk*, 104: 707-716.

550 Uciechowski P, Rink L. 2015. Basophil, Eosinophil, and Neutrophil Functions in the Elderly. In: Massoud
551 A, Rezaei N, eds. *Immunology of aging*, Springer Verag.

552 van Oort H, Otter K, Fort KT, McDonell Z. 2007. Habitat, dominance, and the phenotypic quality of the
553 black-capped chickadees. *Condor*, 109: 88-96.

554 Verhulst S, Oosterbeek K, Bruinzeel LW. 2002. Haematological parameters, mass and moult status in
555 dunlins *Calidris alpina* preparing for spring migration. *Avian Science*, 2: 199-206.

556 Vinkler M, Schnitzer J, Munclinger P, Votýpka J, Albrecht T. 2010 Haematological health assessment in a
557 passerine with extremely high proportion of basophils in peripheral blood. *Journal of Ornithology*,
558 151(4): 841-849.

559 Vleck CM, Vertalino N, Vleck D, Bucher TL. 2000. Stress, corticosterone, and heterophil to lymphocyte
560 ratios in free-living Adélie penguins. *Condor*, 102: 392-400.

561 von Holst D. 1998. The concept of stress and its relevance for animal behaviour. *Advances in Behaviour*,
562 27: 1-131.

563 Wascher CAF, Scheiber IBR, Kotrschal K. 2008. Heart rate modulation in bystanding geese watching
564 social and non-social events. *Proceedings of the Royal Society of London*, 275(1643): 1653–1659.
565 doi:10.1098/rspb.2008.0146.

566 Wascher CAF, Arnold W, Kotrschal K. 2008. Heart Rate Modulation by Social Contexts in Greylag Geese
567 (*Anser anser*). *Journal of Comparative Psychology*, 122(1):100–107.

568 Wascher CAF, Scheiber IBR, Weiß BM, Kotrschal K. 2009. Heart rate responses to agonistic interactions
569 in greylag geese, *Anser anser*. *Animal Behaviour*, 77: 955-961.

570 Wascher CAF, Weiß BM, Arnold W, Kotrschal K. 2012. Physiological implications of pair-bond status in
571 greylag geese. *Biology Letters*, 8: 347-350. doi: 10.1098/rsbl.2011.0917.

572 Wingfield JC, Hegner RE, Lewis DM. 1991. Circulating levels of luteinizing hormone and steroid
573 hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver,
574 *Plocepasser mahali*. *Journal of Zoology*, 225: 43–58. doi:10.1111/j.1469-7998.1991.tb03800.x

575 Zakari FO, Ayo JO, Rekwot PI, Kawu MU. 2014. Effect of age, sex, physical activity and meteorological
576 factors on haematological parameters of donkeys (*Equus asinus*). *Comparative Clinical Pathology*, DOI
577 10.1007/s00580-014-2026-3

578 Zuk M., Johnsen TS, MacLarty T. 1995. Endocrine-immune interactions, ornaments and mate choice in
579 red jungle fowl. *Proceedings of the Royal Society of London B*, 260: 205 – 210.
580

581

582 **Authors' contributions**

583 DF coordinated the study, conducted data collection on the field, participated in data analysis

584 and wrote the manuscript; SCL participated in the design of the study and in the collection of

585 field data; JH participated in the design of the study and in the collection of field data; KK

586 participated in the design of the study and wrote the manuscript; CAFW participated in the

587 design of the study, carried out the statistical analyses, wrote the manuscript. All authors gave

588 final approval for publication.

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591

592 **Table and Figures Legends**

593 **Table 1:**

594 Details about the number of sampled individuals and the number of collected samples per

595 season and category (i.e. sex, social status, rearing condition).

596

597 **Table 2:**

598 Model selection of analyses that examined factors affecting (a) haematocrit (HCT), (b)

599 haeterophily to lymphocytes (H/L) ratio, (c) leucocyte count and (d) percentage of monocytes.

600 Individual identity was fitted as a random term. Three subsets of data have been tested: (1) The

601 full-dataset included all 169 samples collected from 105 individuals. Subset (2) included data

602 from male-female pair bonded individuals (68 samples from 50 individuals). (3) Only data from

603 individuals from which morphological measurements were collected was included (116 samples

604 from 70 individuals). LogLik: log-likelihood, AICc: second order Akaike's Information Criterion.

605 ΔAICc: the difference between the best model and each other possible model

606

607 **Table 3:**

608 Results of the full statistical models. Models for each response variable of all three data subsets

609 (1 = full dataset, 2 = paired individuals, 3 = body measures) are presented. Factors in bold are

610 significant ($p \leq 0.05$).

611

612 **Figure 1:**

613   Haematocrit in relation to season and pair-bond status. Gray circles represent individuals with

614   offspring (i.e. family); black circles represent paired individuals without offspring; plain circles

615   represent unpaired individuals. Circles indicate median, error bars represent interquartile

616   ranges (lower: 25th and upper: 75th percentile).

617

618   **Figure 2:**

619   Heterophil to lymphocyte (H/L) ratio in relation to individuals' reproductive success (both sexes

620   combined). Circles indicate median, error bars represent interquartile ranges (lower: 25th and

621   upper: 75th percentile).

622

623   **Table 1:**

624   Details about the number of sampled individuals and the number of collected samples per

625   season and category (i.e. sex, social status, rearing condition).

626

Season	Nr. of samples	Nr. of sampled individuals	Nr. of sampled individuals by sex		Nr. of samples by sex	Nr. of sampled individuals by rearing condition		Nr. of samples by	Nr of sampled individuals by social status		Nr. of samples by soc.stat.
After Molt	**23**	**23**	Males	10	10	goose-raised	23	23	single	0	0
			Females	13	13	hand-raised	0	0	paired	0	0
									family	23	23
Autumn	**82**	**73**	Males	37	40	goose-raised	37	37	single	8	8
			Females	36	42	hand-raised	36	45	paired	39	48
									family	26	26
Mating	**64**	**61**	Males	40	42	goose-raised	39	39	single	16	18
			Females	21	22	hand-raised	22	25	paired	30	31
									family	15	15

627

629   **Table 2:**

630   Model selection of analyses examining factors affecting (a) haematocrit (HCT), (b) haeterophily

631   to lymphocytes ratio (H/L ratio), (c) leucocyte count and (d) percentage of monocytes.

632   Individual identity was fitted as a random term. Three subsets of data have been tested: (1) the

633   full-dataset included all 169 samples collected from 105 individuals; subset (2) included data

634   from male-female pair bonded individuals (68 samples from 50 individuals); subset (3) included

635   only data from individuals from which morphological measurements were collected (116

636   samples from 70 individuals). LogLik: log-likelihood; AICc: second order Akaike's Information

637   Criterion; ΔAICc: difference between the best model and each other possible model.

638

Models	df	LogLik	AIC	ΔAICc
(1a) Full-dataset: HCT				
Sex, age, raising history, season, season*sex, season*age, season*raising-status	10	242.574	-463.757	0
Pair-bond status, season, season*pair-bond status	6	233.649	-454.78	8.976
Season	4	230.983	-453.722	10.034
Sex, age, raising history	6	219.471	-426.423	37.333
Pair-bond status	4	215.376	-422.508	41.248
Sex, age, raising history, pair-bond status, pair-bond status*sex, pair-bond status*age, pair-bond status*raising status	8	218.177	-419.454	44.302
Null model	3	207.589	-409.032	54.724
(1b) Full-dataset: H/L ratio				
Pair-bond status, season, season*pair-bond status	6	-196.356	405.23	0
Sex, age, raising history, season, season*sex, season*age, season*raising-status	10	-198.064	417.521	12.29
Season	4	-205.476	419.196	13.965
Sex, age, raising history	6	-207.444	427.406	22.176
Sex, age, raising history, pair-bond status, pair-bond status*sex, pair-bond status*age, pair-bond status*raising status	8	-205.462	427.824	22.593
Pair-bond status	4	-209.898	428.04	22.81
Null model	3	-213.424	432.994	27.763
(1c) Full-dataset: leucocyte count				

Pair-bond status, season, season*pair-bond status	6	-1650.015	3312.549	0
Sex, age, raising history, season, season*sex, season*age, season*raising-status	10	-1646.151	3313.715	1.166
Season	4	-1656.422	3321.087	8.538
Null model	3	-1660.982	3328.11	15.56
Sex, age, raising history	6	-1658.534	3329.586	17.036
Pair-bond status	4	-1660.977	3330.199	17.649
Sex, age, raising history, pair-bond status, pair-bond status*sex, pair-bond status*age, pair-bond status*raising status	8	-1657.008	3330.917	18.367
(1d) Full-dataset: percentage of monocytes				
Sex, age, raising history, season, season*sex, season*age, season*raising-status	10	-480.402	982.197	0
Pair-bond status, season, season*pair-bond status	6	-485.006	982.531	0.334
Season	4	-491.375	990.995	8.797
Sex, age, raising history	6	-491.792	996.104	13.906
Pair-bond status	4	-494.597	997.439	15.241
Null model	3	-496.611	999.369	17.171
Sex, age, raising history, pair-bond status, pair-bond status*sex, pair-bond status*age, pair-bond status*raising status	8	-493.017	1002.935	20.737
(2a) Pair-bonded individuals: HCT				
Null model	3	90.11	-173.846	0
Pair-bond duration	4	90.84	-173.046	0.8
Parental experience	4	90.276	-171.918	1.928
(2b) Pair-bonded individuals: H/Lratio				
Parental experience	4	-55.067	118.769	0
Pair-bond duration	4	-55.911	120.458	1.688
Null model	3	-58.245	122.865	4.095
(2c) Pair-bonded individuals: Leucocytes				
Null model	3	-665.648	1337.672	0
Parental experience	4	-665.424	1339.484	1.812
Pair-bond duration	4	-665.49	1339.616	1.944
(2d) Pair-bonded individuals: Monocytes				
Null model	3	-191.664	389.704	0
Pair-bond duration	4	-191.02	390.676	0.972
Parental experience	4	-191.293	391.222	1.518
(3a) Morphological measurements: HCT				
BSI	4	175.545	-342.731	0
Null model	3	146.71	-278.206	55.525
(3b) Morphological measurements: H/Lratio				
BSI	4	-146.399	301.158	0

Null model	3	-148.494	303.203	2.045
(3c) Morphological measurements: Leucocytes				
Null model	3	-1142.87	2291.954	0
BSI	4	-1142.073	2292.506	0.552
(3d) Morphological measurements: Monocytes				
BSI	4	-345.286	698.932	0
Null model	3	-349.29	704.794	5.862

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641 **Table 3:**

642 Results of the full statistical models. Models for each response variable of all three data subsets

643 (1 = full dataset, 2 = paired individuals, 3 = body measures) are presented. Factors in bold are

644 significant (p ≤ 0.05).

	Response variable	Estimate ± SE	t value	p
(1) Haematocrit				
	raising history	-0.078 ± 0.049	-1.574	0.118
	sex	0.021 ± 0.032	0.669	0.504
	age	0 ± 0	3.815	<0.001
	season	0.656 ± 0.011	5.715	<0.001
	season*raising history	0.03 ± 0.019	1.564	0.123
	season*sex	-0.02 ± 0.014	-1.425	0.159
	season*age	**0 ± 0**	**-3.572**	**<0.001**
(1) H/Lratio				
	pair-bond status	1.245 ± 0.346	3.588	<0.001
	season	**1.192 ± 0.23**	**5.177**	**<0.001**
	season*pair-bond status	**-0.563 ± 0.135**	**-4.528**	**<0.001**
(1) Leucocytes				
	pair-bond status	**-1.052 ± 0.293**	**3.545**	**<0.001**
	season	-0.555 ± 0.418	1.32	0.186
	raising history	**0.801 ± 0.384**	**2.057**	**0.039**
	sex	0.087 ± 0.253	0.339	0.734
	age	0.687 ± 0.409	1.644	0.1
	season*pair-bond status	**0.868 ± 0.265**	**3.212**	**0.001**
	season*raising history	-0.689 ± 0.372	1.812	0.069
	season*sex	-0.161 ± 0.252	0.623	0.532
	season*age	-0.691 ± 0.416	1.624	0.104
(1) Monocytes				
	raising history	-0.645 ± 0.375	1.698	0.089
	sex	0.053 ± 0.249	0.212	0.831
	age	0.516 ± 0.405	1.247	0.212
	season	0.374 ± 0.154	2.369	0.017
	pair-bond status	0.319 ± 0.29	1.085	0.277
	season*raising history	0.471 ± 0.362	1.276	0.201
	season*sex	-0.014 ± 0.246	0.056	0.955
	season*age	-0.807 ± 0.41	1.924	0.054
	season*pair-bond status	-0.004 ± 0.261	0.016	0.986
(2) H/L ratio				

	pair-bond duration	**0.268 ± 0.123**	**2.111**	**0.034**
	parental experience	**0.315 ± 0.123**	**2.493**	**0.012**
(3) HCT				
	BSI	**0 ± 0**	**10.281**	**<0.001**
(3) H/L ratio				
	BSI	**0.003 ± 0.001**	**2.048**	**0.046**
(3) Monocytes				
	BSI	**0.026 ± 0.008**	**3.248**	**0.002**

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647   **Figure 1**

648   Haematocrit in relation to season and pair-bond status. Gray circles represent individuals with

649   offspring (i.e. family); black circles represent paired individuals without offspring; plain circles

650   represent unpaired individuals. Circles indicate median, error bars represent interquartile

651   ranges (lower: 25th and upper: 75th percentile).

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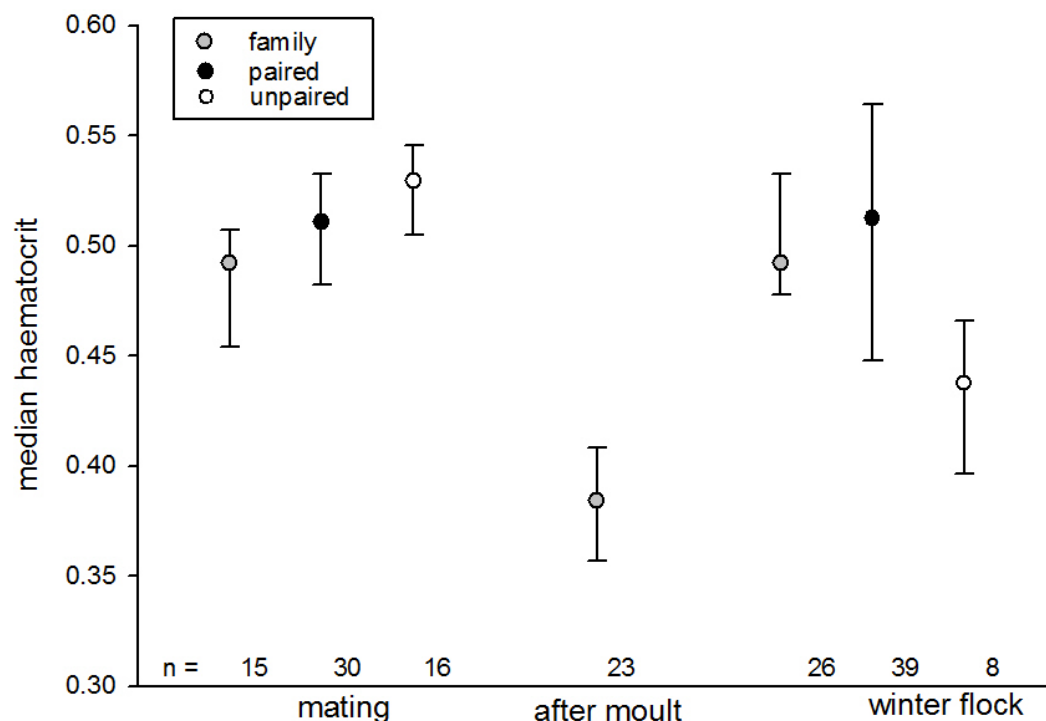
660 **Figure 2**

661 Heterophil to lymphocyte (H/L) ratio in relation to individuals' reproductive success. Circles

662 indicate median, error bars represent interquartile ranges (lower: 25th and upper: 75th

663 percentile).

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