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1 **Age and spatio-temporal variations in food resources**
2 **modulate stress-immunity relationships in three populations**
3 **of wild roe deer**

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22

23 **Abstract**

24 Living in variable and unpredictable environments, organisms face recurrent stressful situations.
25 The endocrine stress response, which includes the secretion of glucocorticoids, helps organisms to
26 cope with these perturbations. Although short-term elevations of glucocorticoid levels are often
27 associated with immediate beneficial consequences for individuals, long-term glucocorticoid
28 elevation can compromise key physiological functions such as immunity. While laboratory works
29 highlighted the immunosuppressive effect of long-term elevated glucocorticoids, it remains largely
30 unknown, especially in wild animals, whether this relationship is modulated by individual and
31 environmental characteristics. In this study, we explored the co-variation between baseline cortisol
32 levels, assessed non-invasively using faecal cortisol metabolites (FCMs), and 12 constitutive
33 indices of innate, inflammatory, and adaptive immune functions, in wild roe deer living in three
34 populations with contrasting environmental conditions. Using longitudinal data on 564 individuals,
35 we further investigated whether age and spatio-temporal variations in the quantity and quality of
36 food resources affect the relationship between FCMs and immunity. Negative covariation with
37 glucocorticoids was evident only for innate and inflammatory markers of immunity, while adaptive
38 immunity appeared to be positively or not linked to glucocorticoids. In addition, the negative
39 covariations were generally exacerbated, or revealed, in individuals facing harsh environmental
40 constraints and in old individuals. Therefore, our results highlight the importance of measuring
41 multiple immune markers of immunity in individuals from contrasted environments to unravel the
42 complex relationships between glucocorticoids and immunity in wild animals. Our results also help
43 explain conflicting results found in the literature and could improve our understanding of the long-
44 term consequences of elevated glucocorticoid levels on disease spread and population dynamics.

45 **Keywords**

46 stress hormones, ecophysiology, innate immunity, adaptive immunity, faecal glucocorticoid
47 metabolites, trade-off

48 **1. Introduction**

49 The neuroendocrine stress response helps animals to cope with recurrent stressful situations in
50 natural environments (Sapolsky et al., 2000; Wingfield and Romero, 2001). Exposure to stressors
51 stimulates the hypothalamic-pituitary-adrenal (HPA) axis and leads, among others, to an increase in
52 the secretion of glucocorticoids by the adrenocortex (Sapolsky et al., 2000; Sheriff et al., 2011).
53 Glucocorticoids, which contribute to the control of an individual's energy balance through
54 acquisition, storage and mobilization, also coordinate the body's overall response to stressors
55 through metabolic changes that depend on their concentration and duration of secretion. (Hau et al.,
56 2016). This hormonal cascade is then subjected to negative feedback from glucocorticoids on their
57 own secretion (Romero, 2004; Sheriff et al., 2011), facilitating a return to a baseline level that
58 ensures maintenance of daily activities according to the current life-history stage (Möstl and Palme,
59 2002; Wingfield and Sapolsky, 2003). While short-term elevation of glucocorticoid levels promotes
60 survival (Sapolsky et al., 2000; Breuner et al., 2008), chronic elevation of glucocorticoids may alter
61 physiological functions and ultimately compromise both survival and reproductive success on the
62 long-run (Boonstra, 2005). Among these functions, the potential immunosuppressive action of
63 glucocorticoids is one of the most discussed effects of chronic stress (Martin, 2009).

64 Immunity is a key physiological function of vertebrates: it includes innate and adaptive components
65 (Stanley, 2002), each comprising cellular and humoral effectors (Stanley, 2002). While innate
66 immunity sets up rapidly (within hours) and is mostly non-specific, adaptive (memory-based)
67 immunity deals with repeated infections and selectively eliminates pathogens (Lee, 2006). Like any
68 other physiological function, the maintenance and functioning of the immune system requires

69 energy (Lee, 2006; Martin, 2009). Hence, immunity has been hypothesised to trade-off against
70 other energy demanding physiological functions (Martin, 2009). Glucocorticoids may mediate these
71 trade-offs, with elevation of these hormones redirecting the energy away from immunity towards
72 functions promoting immediate survival but with deleterious effects in the long term (Lee, 2006;
73 Martin, 2009). However, costs vary depending on the stage (development, maintenance, or
74 activation) and component of immunity (Klasing, 2004, Lee, 2006). The adaptive immune response
75 is thought to have a lower activation energy cost than the innate immune response, the
76 inflammatory response entailed by innate response being particularly energy demanding (McDade,
77 2016). Therefore, differential effects of trade-off mediated by glucocorticoids can be expected
78 between the components of immunity.

79 So far, most empirical studies documenting a detrimental effect of chronic stress on immune
80 functions have been conducted on laboratory or domestic animals (e.g. Dhabhar et al., 1994;
81 Dhabhar et al., 1995; Wada et al., 2010; Moazzam et al., 2012) and much less studies have been
82 conducted in wild populations (e.g. Bourgeon and Raclot, 2006; Brooks and Mateo, 2013;
83 Josserand et al., 2020). In wild populations, exposure to fluctuating environmental conditions is
84 more intense than in captivity in terms of resources, variation of temperature, predator risk or
85 disease threats for instance, which could lead to different outcomes regarding the relationship
86 between glucocorticoids and immunity. In addition, a large body of literature shows that diverse
87 internal and external factors could influence both immunity (Lee, 2006; Martin, 2009) and
88 glucocorticoid secretion (Hau et al., 2016). For instance, poor environments (in terms of resource
89 quantity or quality) can lead to both increased glucocorticoid levels (Fokidis et al., 2012; Carbillet
90 et al., 2020) and decreased concentrations of immune parameters (Dhabhar et al., 1994; Dhabhar et
91 al., 1995), without any causal relationships. It has also been shown that baseline glucocorticoid
92 levels are related to age (Sapolsky et al., 1983) as are several immune parameters (e.g.
93 immunosenescence, see Nussey et al., 2012; Cheynel et al., 2017). To date, the contribution of these

94 individual and environmental factors has not been investigated and their role in modulating the
95 relationship between immunity and glucocorticoids remains poorly understood.

96 The aim of this study was to analyse whether the relationship between glucocorticoid levels and
97 immunity is affected by both individual and environmental factors in three wild populations of roe
98 deer (*Capreolus capreolus*) living in habitats with contrasting environmental conditions. Baseline
99 glucocorticoid levels were assessed non-invasively by measuring faecal cortisol metabolites
100 (FCMs), which reflect overall circulating glucocorticoid levels that an individual has experienced
101 over a particular time-period which is species-specific (Palme et al., 2005; Sheriff et al., 2011;
102 Palme, 2019). The immune function was assessed the same years than FCMs by measuring twelve
103 immune parameters encompassing the innate (neutrophils, eosinophils, basophils, monocytes,
104 hemagglutination, hemolysis), inflammatory (alpha-1-globulin, alpha-2-globulin, beta-globulin,
105 haptoglobin) and adaptive (lymphocytes, gamma-globulin) immunity (Cheynel et al. 2017).

106 Based on current knowledge, we expected that individuals with higher baseline glucocorticoid
107 levels would exhibit weaker overall immunity than those with lower baseline glucocorticoid levels.
108 More precisely, we expected (prediction 1 that high levels of FCMs would be associated with low
109 levels of parameters measuring innate (cellular and humoral) immunity (neutrophils, eosinophils,
110 basophils, monocytes, hemagglutination, hemolysis), as well as inflammation (alpha-1-globulin,
111 alpha-2-globulin, beta-globulin and haptoglobin) due to their high activation cost (Dhabhar et al.,
112 2012; Brooks and Mateo, 2013; McDade et al., 2016). On the opposite, we expected a weaker
113 relationship between FCMs and cellular and humoral adaptive immune parameters (lymphocyte and
114 gamma-globulin concentrations) due to their lower activation cost (Klasing, 2004, Lee, 2006). In
115 addition, we expected (prediction 2) that the negative relationship between FCMs and immune
116 parameters would appear, or be exacerbated, in individuals facing poor environmental conditions
117 such as low availability in food resources, compared to individuals living in favourable
118 environments (Hau et al., 2016). Finally, we expected (prediction 3) that the negative relationship
119 between FCM levels and immune parameters would appear, or be exacerbated, in older individuals

120 compared with younger ones, due to impairment of both the adrenocortical stress response
121 (Sapolsky et al., 1983) and the immune response in old individuals (Cheynel et al., 2017).

122 **2. Material and methods**

123 **2.1 Study populations**

124 This study was conducted on three wild populations of roe deer living in contrasting habitats.
125 First, the Trois-Fontaines population is located in an enclosed forest (1,360 hectares) in the north-
126 east of France (48°43' N, 4°55' E). The climate is continental, and the soil is particularly rich,
127 making it a very productive forest offering a homogeneous and high-quality resources habitat for
128 roe deer (Pettorelli et al., 2006). Second, the Chizé population is located in an enclosed forest
129 (2,614 hectares) in western France (46°50' N, 0°25' W). The climate is temperate oceanic, with
130 Mediterranean influences. Due to poor soil quality and frequent summer droughts, the forest
131 productivity is low compared to Trois-Fontaines (Pettorelli et al., 2006), making it a relatively poor-
132 quality habitat for roe deer in terms of resources (Gaillard et al., 1993). At a finer scale, three
133 sectors are distinguished in this population according to the quantity and quality of resources
134 (Pettorelli et al., 2001). Sector 1 is composed of oaks (*Quercus spp.*) and hornbeams (*Carpinus*
135 *betulus*) and is considered to be of better quality than the other two sectors, sector 2 is composed of
136 oaks and Montpellier maples (*Acer monspessulanum*) and is considered to be of intermediate
137 quality, and sector 3, composed of beeches (*Fagus sylvatica*) is the sector of worse quality.
138 Pettorelli and colleagues (2003) reported that these differences in habitat composition result in
139 differences in roe deer body mass, with juveniles in sector 1 being on average 2 kg heavier than
140 those in sector 3. Finally, the Aurignac population is located in an agricultural landscape (10,000
141 hectares), in south-western France (43°13' N, 0°52' E) and is part of a Long-Term Socio-Ecological
142 Research platform called ZA PYGAR. This site is exposed to an oceanic climate, with summer
143 droughts (Hewison et al., 2007). It provides a highly heterogeneous environment with a fragmented
144 landscape composed of forests, grassland and cultivated fields (see Martin et al., 2018 for details).

145 This study site provides overall high-quality habitat for roe deer (as Trois-Fontaines) but can also be
146 divided into three sectors according to resource quality and habitat openness (see Morellet et al.,
147 2009 for details). The most open habitats (sector 1) offer more important and high-quality food
148 resources for roe deer during most of the year (Abbas et al., 2011), but can also be a source of
149 higher exposure to stressors (Bonnot et al., 2013), such as road and human dwellings leading to
150 higher FCM levels (Carbillet et al., 2020), compared to the partially wooded area (sector 2) and
151 woodland (sector 3). Hewison and colleagues (2009) have shown a beneficial effect of landscape
152 openness on roe deer reproduction, demographic performance, and juveniles body mass, with
153 juveniles in sector 3 weighing on average 2.0 kg less than in sector 2, and 3.1 kg less than in sector
154 1 (Hewison et al., 2009).

155 **2.2 Data collection**

156 As part of long-term capture-mark-recapture programs initiated in 1975, 1977 and 1996, in Trois-
157 Fontaines, Chizé and Aurignac respectively, 6 to 12 days of drive net captures are organised
158 between December and March each year. At each capture session, between 30 and 100 beaters push
159 roe deer towards nets deployed over 4 km. Once captured, each animal is marked, weighed (to the
160 nearest 100 g), sexed, and age is determined by tooth eruption patterns in Aurignac (Hewison et al.,
161 1999), with 2 age classes: juveniles (< 1 year) and adults (> 1 year). For the Trois-Fontaines and
162 Chizé populations, the exact age (in years) is known since the individuals were all captured and
163 marked during their first year of life when the age can be estimated without error. Blood samples
164 are taken from the jugular vein (up to 1 mL/kg) since 2009 in Aurignac and since 2010 in Trois-
165 Fontaines and Chizé. Part of the collected blood is transferred to a dry tube, centrifuged, and the
166 serum conserved at -20°C for biochemical analyses. The remaining blood is transferred to a tube
167 containing ethylenediaminetetraacetic acid (EDTA) for further determination of immune parameters
168 (see below). For each individual, faeces are collected rectally and stored at -20°C until extraction.

169 **2.3 Measurement of FCMs**

170 Faecal cortisol metabolites were extracted following the protocol developed by Palme and
171 colleagues (2013). Briefly, for each individual, 0.5 g of faeces were suspended in 5 mL of 80%
172 methanol, vortexed for 30 minutes and centrifuged for 15 min at 2500 g. The supernatants were
173 diluted 1:10 with assay buffer before FCM concentrations were determined with a group-specific
174 11-oxoetiocholanolone enzyme immunoassay as previously described (Möstl et al., 2002) and
175 validated for roe deer (Zbyryt et al., 2017). Measurements were carried out in duplicate. Intra- and
176 inter-assay coefficients of high and low concentration pool samples were less than 10% and 15%,
177 respectively. Results are expressed as nanograms per gram of wet faeces (ng/g).

178 **2.4 Measurement of immune parameters**

179 **2.4.1 Innate cellular immunity**

180 White blood cells (WBC) count was carried out by impedance technology (ABC Vet automaton,
181 Horiba Medical) and the proportions of each WBC type (neutrophils, monocytes, lymphocytes,
182 eosinophils and basophils) were quantified under microscope (x1000) by counting the first 100
183 WBC on blood smears, previously stained with a May-Grünwald and Giemsa solution (see
184 Houwen, 2001 for more details). The concentrations of each type of leukocyte were then
185 determined as (WBC*parameter cells count/100).

186 **2.4.2 Innate humoral immunity**

187 Innate humoral immunity was assessed by measuring circulating levels of natural antibodies
188 (NAbs) and complement activity of serum. The concentration of NAbs was measured by the
189 hemagglutination test (HA) that measures NAbs ability to agglutinate exogenous cells (Matson et
190 al., 2005). The complement is a group of proteins that acts in a chain reaction and causes lysis of
191 exogenous cells in presence of an antigen-antibody complex. It is revealed by the ability of proteins

192 to induce hemolysis (HL) (Matson et al., 2005). The HA/HL protocol developed by Matson et al.,
193 (2005) for birds has been previously adapted for roe deer (Gilot-Fromont et al., 2012).

194 **2.4.3 inflammatory markers**

195 Inflammatory status was evaluated using levels of alpha-1, alpha-2, and beta globulins. The total
196 concentrations of these proteins (in g/L) were quantified by a refractometer followed by an
197 electrophoresis on agarose gel, using an automaton (HYDRASYS). Haptoglobin concentration (in
198 mg/mL), which belongs to the alpha-2-globulin fraction and reflect infection or chronic
199 inflammation, was also measured. Analyses were performed with a Konelab 30i PLC (Fisher
200 Thermo Scientific) which operates on the principle of spectrophotometry. Unlike other immune
201 parameters, haptoglobin was measured only on the Trois-Fontaines and Chizé samples.

202 **2.4.4 Adaptive cellular immunity**

203 Adaptive cellular immunity was assessed using lymphocyte concentration, determined by the
204 leukocyte count described above, which includes both B and T lymphocytes.

205 **2.4.5 Adaptive humoral immunity**

206 The adaptive humoral immunity component was assessed using concentration of gamma globulins,
207 obtained by the protein analysis described above for other globulins. Gamma globulin concentration
208 has been used as an estimator of total antibodies, since they are essentially composed of circulating
209 antibodies (Stockham and Scott, 2008).

210 **2.5 Statistical analyses**

211 Due to conservation issues of faeces, the available data encompassed different year ranges
212 according to the population. Data were available for the years 2010 to 2019 in Trois-Fontaines,
213 while only the years 2013, 2014 and 2016 to 2019 were available in Chizé. For the Aurignac
214 population, neutrophils, eosinophils, basophils, monocytes and lymphocytes concentrations were

215 available for the years 2012 to 2017. For hemagglutination and hemolysis data were available from
216 the years 2013 to 2017, while for gamma-, alpha-1-, alpha-2- and beta-globulin, data were available
217 for the years 2014 to 2017. Consequently, the number of observations differed according to the
218 population and immune marker considered. In Aurignac, analyses were carried out on 144 to 188
219 observations, while they were conducted on 325 to 414 in Chizé, and 276 to 303 in Trois-Fontaines.
220 Overall, we implemented 35 series of models, including 12 series for the populations of Trois-
221 Fontaines and Chizé and 11 series for Aurignac (due to the absence of haptoglobin assays).

222 For each of the 12 immune parameters, we ran separate analyses for each of the three populations,
223 using linear mixed effect models (LMMs). Each immune trait was taken as a response variable and
224 some of them (concentrations of eosinophils, basophils, monocytes, lymphocytes, haptoglobin,
225 alpha-1, alpha-2 and beta globulins) were transformed as $\log(x+1)$ to ensure normality of model
226 residuals. Model selection was carried out by adjusting a reference model (see below) that included
227 all biologically relevant variables and interactions to test our hypotheses. We then compared this
228 model with all its sub-models. Each continuous explanatory variable (except for age) was centred
229 by population to obtain estimates corresponding to average values of the parameters in the
230 corresponding population. The reference model included:

231 i) Individual variables: baseline cortisol level (FCMs), log-transformed and centred around the
232 mean (mean value of the variable that is subtracted from every value), sex, body weight (in kg,
233 centred), and age. In Aurignac, age was considered with two classes (juveniles and adults). In Chizé
234 and Trois-Fontaines, age in years was considered to have either a linear effect, a quadratic function,
235 or a threshold effect) based on a previous study carried out on these two populations (Cheynel et al.,
236 2017).

237 ii) Environmental variables: in Chizé and Aurignac, we considered the sector of capture as a marker
238 of local resources quality and quantity. To account for temporal variations of resources among

239 years, we used a centred year quality index. The quality of a given year was indexed using the
240 average weight (in kg, centred) of juveniles caught the following winter (Pettorelli et al., 2003).

241 iii) Methodological variables: the time between capture and blood sampling is known to influence
242 the level of certain immune parameters such as neutrophils and lymphocytes (Carillet et al., 2019)
243 and was thus taken into account (thereafter, delay, in minutes, centred). In addition, we included the
244 Julian date of capture in our models to control for potential among-individual differences in
245 immune parameters, body weight and FCMs due to the timing of sampling.

246 Several plausible interactions based on our hypotheses were also included in our reference model.
247 First, the interaction between FCMs and age, to investigate a possible modification of the
248 relationship with advancing age (prediction 3). Second, we considered interactions between FCMs
249 and sector, and between FCMs and quality of the year, to account for a potential modulation of the
250 relationship under poorer environments or years of poorer quality (prediction 2). The interaction
251 between FCMs and sex was also included to control for a possible modification of the relationship
252 according to sex, as females generally allocate much more to immunity than males in the wild
253 (Metcalf et al., 2019). We also included an interaction between FCMs and body weight to control
254 for a possible modification of the relationship in individuals with the poorest physical condition
255 (Hau et al., 2016). Finally, an interaction between sex and age was also included in neutrophil
256 models for the Trois-Fontaines and Chizé populations because a previous study on roe deer from
257 these populations showed that neutrophil profile was affected by age in a sex-specific manner
258 (Cheynel et al., 2017). This interaction was also included in the Aurignac population for all immune
259 parameters, as no previous data was available on the sex-specific effect of age on other parameters
260 in this population. Individual's identity and year of capture were included as random effects to
261 avoid pseudo-replication problems (Hurlbert, 1984) and to control for unexplained variance due to
262 among-individual differences and inter-annual variation. For the Chizé and Trois-Fontaines
263 populations, the birth cohort is known and was also included as a random effect to account for

264 potential differences between individuals in the environmental conditions they experienced early in
265 life, which may have persistent effects on their phenotype (Douhard et al., 2014).

266 As a result, our (general) reference models read as follow:

267 Immune parameter = f(FCMs + year quality + sector + sex + age + weight + delay + julian date +
268 FCMs*sector + FCMs*year quality + FCMs*sex + FCMs*age (either linear, quadratic or threshold)
269 + FCMs*weight + 1|individual + 1|year of capture + 1|birth cohort).

270 To select the best models describing variation of each immune parameter, each reference model was
271 compared to all its sub-models (N=358 models overall) using a model selection method based on
272 the second-order Akaike's information criteria (AICc, Burnham and Anderson, 2003). Models with
273 a difference in AICc ($\Delta AICc$) > 2 units from the best model were considered to have less support,
274 following Burnham and Anderson (2003). In addition, we removed models within two AICc units
275 of the top model that differed from a higher-ranking model by the addition of one or more
276 parameters. These were rejected as uninformative, as recommended by Arnold (2010) and Richards
277 (2008). Using the selected models, we then applied a conditional model averaging procedure to
278 estimate parameters. We calculated AICc weights (AICcw) to measure the relative likelihood that a
279 given model was the best among the set of fitted models and goodness-of-fit was assessed by
280 calculating marginal (R²_m) and conditional (R²_c) variance using the `r.squaredGLMM` function of
281 the `MuMIn` package (Barton, 2016). When interactions between FCMs and age, year quality,
282 sectors, body weight, or sex were significant, we used F-tests in order to test prediction 1 of a
283 relationship between FCMs and immunity. For each parameter in each population, the normality of
284 residuals was tested (Shapiro-Wilk test) and visually assessed. All analyses were performed using R
285 version 3.5.1 (R Development Core Team 2018) and using the `lmer` function (`lme4` package, Bates
286 et al., 2014) and the `dredge` function (`MuMIn` package, Barton, 2016).

287 **3. Results**

288 The text below describes the covariations between FCMs and immune parameters, and their
289 variations according to the availability and quantity of food resources, age, sex, and body weight of
290 roe deer (Table 1, 2 & 3). The full results of the model selection procedure are given in Tables S1,
291 S2 and S3. A summary of these results is provided in Table S4. Correlation matrix between each
292 immune parameters for each population are provided in Table S5.

293 **3.1 Innate cellular immunity**

294 As expected from prediction 1, negative covariations between FCMs and cellular concentrations
295 were observed for neutrophils in Aurignac (slope of -0.51; CI = [-0.94; -0.08]; Fig. 1A) with a
296 similar trend in Chizé (-0.26; CI = [-0.54; 0.03]). A trend for a negative covariation between FCMs
297 and monocytes was also observed in Chizé ($F = 2.92$; $p = 0.09$; slope of -0.04; CI = [-0.07 - -0.007]
298 for average quality food resources) and in Trois-Fontaines (slope of -0.06; CI = [-0.13; 0.009]).
299 However, in Chizé only, the negative covariation was buffered in years offering better quality food
300 resources (Table 2; Fig. 2A), as expected from prediction 2. In Chizé, whereas basophil
301 concentration was negatively related to FCMs levels in general ($F = 6.71$; $p = 0.01$), this
302 particularly applied to areas of intermediate (sector 2) and poor quality (sector 3), while the
303 covariation was close to 0 in the area of best quality (Table 2; Fig. 3A; slope of -0.002; CI = [-0.01
304 - 0.01]). We found no significant association between eosinophil concentrations and FCMs in any
305 of the populations, and no interaction between FCMs and age (prediction 3) on any of the innate
306 immune parameters measured (Table 1, 2, 3).

307 **3.2 Innate humoral immunity**

308 In Aurignac, the overall covariation between FCMs and hemagglutination titers was not significant
309 ($F = 1.69$; $p = 0.20$; slope of -0.15; CI = [-0.37 - 0.08] for average quality resources) but a negative
310 covariation appeared when annual food quality resources decreased, as expected from prediction 2

311 (Table 1; Fig 2B). In Chizé, while hemagglutination titers and FCMs were overall positively related
312 ($F = 5.80$; $p = 0.02$; slope of 2.57; $CI = [0.37 - 4.77]$ for animals aged less than 8 years old), the
313 covariation became negative as age of roe deer increased (-0.33 per year; $CI = [-0.59; -0.06]$; Table
314 2; Fig. 4A). In addition, in Aurignac, FCMs were retained in the model selection to explain
315 hemolysis titers, with a negative trend (-0.19 ; $CI = [-0.43; 0.05]$; Table 1).

316 **3.3. Inflammatory markers**

317 In Aurignac, while alpha-1 globulin concentration was not related to FCM levels overall ($F = 1.45$;
318 $p = 0.23$), a trend for a negative covariation appeared in areas of intermediate (sector 2) and
319 especially poor quality (sector 3; Table 1), as expected from prediction 2. In addition, there was also
320 a trend for a negative covariation between alpha-1 globulins and FCMs in males compared to
321 females (slope of -0.05 ; $CI = [-0.11; 0.01]$), and in heavier individuals (slope of -0.006 ; $CI = [-0.01$;
322 $0.002]$), while beta-globulins seemed slightly positively related to FCMs (slope of 0.03; $CI = [-$
323 $0.009; 0.08]$). In Chizé, alpha-1 globulins were negatively related to FCMs (slope of -0.03 ; $CI = [-$
324 $0.05; -0.007]$; Table 2; Fig. 1B), as expected from prediction 1. Still in Chizé, the covariation
325 between FCMs and alpha-2 globulins was overall negative ($F = 11.9$; $p < 0.01$), as expected from
326 prediction 1. However, this negative covariation only held in the areas of intermediate (sector 2)
327 and poor (sector 3) quality, while no covariation was observed in the area of best quality (Table2;
328 Fig. 3B). In addition, and unexpectedly, the covariation between FCMs and alpha2-globulins was
329 more pronounced when the annual quality of food resources decreased, which is in contradiction
330 with prediction 2 (Table 2; Fig. 2C). In Chizé again, prediction 2 was not supported by results on
331 beta-globulin concentrations, as a positive covariation with FCMs was found in area of poor quality
332 (sector 3), with a similar trend in the area of intermediate quality (sector 2), while no covariation
333 was detected in the area of best quality (sectors 1, Table 2; Fig. 3C). In Trois-Fontaines, a slightly
334 negative covariation between haptoglobin and FCM levels was observed in youngest animals but
335 was exacerbated in old ones, in accordance with prediction 3 (slope of -0.08 per year; $CI = [-0.17; -$
336 $0.01]$; Table 2; Fig 4B).

337 **3.4 Adaptive cellular immunity**

338 Consistent with prediction 1, we observed no covariation between lymphocyte concentrations and
339 FCM levels in any of the 3 populations. However, in Chizé, and contrarily to prediction 2, a
340 positive covariation between FCMs and lymphocytes appeared during the worse quality years
341 (Table 2; Fig. 2D). In Trois-Fontaines, a negative covariation between FCMs and lymphocytes
342 appeared only in older roe deer (Table 3; Fig. 4C), in accordance with prediction 3.

343 **3.5 Adaptive humoral immunity**

344 Gamma-globulin concentrations were overall positively related to FCM levels in Aurignac ($F =$
345 2.62 ; $p < 0.01$), but the covariation differed among areas of different qualities, consistent with
346 prediction 2. Precisely, the positive covariation was only present in the area of best quality (sector
347 1), whereas negative covariations appeared in areas of intermediate (sector 2) and poor (sector 3)
348 quality (Table 1; Fig. 3D). Furthermore, in the Aurignac population, the covariation between
349 gamma-globulins and FCMs differed between females and males, with females showing a positive
350 link, while males had a negative covariation between FCMs and gamma-globulins (slope of -2.10 ;
351 $CI = [-3.62; -0.57]$). In Chizé, a similar overall slight positive covariation was observed between
352 gamma-globulins and FCMs ($F = 3.18$; $p < 0.01$), but the covariation became negative as age
353 increased (slope of -0.30 per year; $CI = [-0.56; -0.05]$; Table 2; Fig. 4D). As in Aurignac, the
354 positive covariation between gamma-globulin concentrations and FCMs also varied depending on
355 the sector. However, contrary to Aurignac and to prediction 2, in Chizé, the covariation was
356 positive only in the area of poor quality (Table 2; Fig. 3E). No significant correlation with FCMs
357 was observed in the Trois-Fontaines population.

358 **4. Discussion**

359 Overall, our results highlight clear associations between baseline glucocorticoid levels and immune
360 parameters and show that the strength and direction of these associations differ between the three

361 studied populations. Of the 12 measured immune parameters, only three (monocytes, lymphocytes
362 and haptoglobin) showed significant co-variations with FCMs in Trois-Fontaines, and six
363 (neutrophils, hemagglutination, hemolysis, alpha-1, beta and gamma-globulins) in Aurignac. The
364 co-variation between FCMs and immunological parameters was particularly marked in Chizé (nine
365 parameters), a site where the population faces harsh environmental conditions (Gaillard et al.,
366 1993). Taken together, our results constitute one of the rare pieces of evidence that adverse effects
367 on immune functions due to long-term elevation of glucocorticoid levels (Dhabhar and McEwen,
368 1997; Sapolsky et al., 2000; Dhabhar, 2009; Stier et al., 2009) also occur in the wild.

369 Furthermore, and as expected, we found that covariations between FCMs and immune parameters
370 differed between immunity components and that these covariations were affected by individual and
371 environmental factors. Our results support the hypothesis that immune system components are
372 differentially affected by glucocorticoid levels (Bourgeon and Raclot, 2006). As levels of FCMs
373 increased, we observed an overall decline in cellular (neutrophils, monocytes, basophils) and
374 humoral (hemolysis) innate immune functions, in accordance with prediction 1 and previous
375 reports. For instance, in the Belding's ground squirrel (*Urocitellus beldingi*), experimental chronic
376 elevation of cortisol levels reduced serum bactericidal competence, a component of the constitutive
377 innate immune response, compared to a control group (Brooks and Mateo, 2013). The most
378 plausible explanation for this negative covariation between FCMs and innate immune parameters
379 relies on the high energy cost of this immune system component. Indeed, the maintenance of
380 immune defences requires energy and nutrients (Lochmiller and Deerenberg, 2000), and the cost of
381 activation is higher for innate than for adaptive immunity (McDade, 2016). Consequently, long-
382 term elevation of glucocorticoid levels may selectively redirect energy away from the innate
383 immunity towards other energy demanding functions (Lee, 2006; Martin, 2009). This process could
384 be seen as a physiological adjustment for energy savings to maximize investment in reproduction
385 and long-term survival through mechanisms other than innate immunity.

386 Our results show that, of the four inflammatory markers measured, three (alpha-1, alpha-2 and
387 haptoglobin) were negatively associated with FCMs in line with our predictions. Indeed, negative
388 covariations between FCMs and inflammatory markers are consistent with the energy trade-off
389 hypothesis, and likely due to their particularly high activation costs (McDade, 2016). However, we
390 also found weak positive covariations between FGMs and beta-globulins. Why the relationship was
391 positive for one of the inflammatory markers while it was negative for the others remains
392 unanswered. First, beta-globulins, like alpha-1 and alpha-2 globulins, are a complex group of
393 proteins, that may each have distinct relationship with stress level. Previous studies also pointed out
394 that the relationship between glucocorticoids and immune parameters of the same arm may be
395 different, such that some antigen responses in chickens have been shown to be affected by stressors,
396 while others were not (El-Lethey et al., 2003). The proposed explanation for these differences was
397 an insensitivity to glucocorticoids for certain immune parameters, which could be a protective
398 mechanism. The discrepancy between markers of the same arm of immunity highlights an
399 important aspect of our work: the complexity of the relationship between immunity and
400 glucocorticoids calls for great caution in the choice of the immune markers and in their
401 interpretations.

402 Our results also partially supported our prediction of a moderate or absence of covariation between
403 FCMs and cellular (lymphocytes) or humoral (gamma-globulins) adaptive immune parameters,
404 which we observed in the three studied populations. Since the activation costs of adaptive immunity
405 are lower than those of innate immunity (McDade, 2016), adaptive immune functions are expected
406 to be less prone to energy trade-off. An alternative non-exclusive explanation to this lack of link, or
407 positive covariation (in the case of gamma-globulins in Aurignac in the high-quality sector),
408 between FCMs and adaptive immune parameters may be that individuals repeatedly or chronically
409 exposed to stressors may actually allocate more in adaptive immunity, especially if major stressors
410 are pathogens, in order to maximize long-term survival. This might be particularly true in long-
411 lived species such as roe deer, which repeatedly face the same pathogens in their environment, and

412 are expected to exhibit stronger allocation in adaptive, and particularly antibody-mediated
413 immunity, compared to innate immunity (Lee, 2006).

414 Besides the difficulty of making general assumptions about the immunity-glucocorticoid
415 relationship due to the above-mentioned differences between components, we found that other
416 factors may modulate the observed relationships. In particular, the age of individuals as well as the
417 spatial and annual heterogeneity of food resources influenced most of the covariations between
418 FCMs and innate, inflammatory, and adaptive markers of immunity in the three studied
419 populations. As predicted in 2, we found that during years providing less and/or lower-quality food
420 resources a negative covariation between innate immunity (monocytes and hemagglutination) and
421 baseline glucocorticoid levels appeared, contrary to better years. The same observation was made
422 for the spatial heterogeneity in food resources. In the Chizé and Aurignac populations, roe deer
423 from areas with low quality food resources showed negative covariations between some immune
424 markers (basophils, alpha-1, alpha-2 and gamma globulins) and FCM levels, while no or positive
425 covariations were observed in areas of high quality. High levels of food resources have been shown
426 to be associated with reduced glucocorticoid levels (Fokidis et al., 2012; Carbillet et al., 2020) but
427 may also provide sufficient energy and nutrients to sustain the cost of innate immune response, as
428 previously suggested in by Strandin and his colleagues (2018). In their review, these authors
429 showed that food provisioning in field studies tended to increase both innate and adaptive
430 immunity, whereas food restriction frequently impaired immunity. However, in contrast to those
431 results, our data also suggested that negative covariations may, in some cases, appear between
432 immunity and baseline glucocorticoid levels in areas and years of high food resources. Alpha-2
433 globulins and lymphocytes were positively associated with FCM levels when annual food resources
434 were scarce, and there were positive covariations with FCMs for beta-globulins and gamma
435 globulins in poor food resources areas, while no association was observed in areas providing better
436 food resources. At first glance, these results might appear counter-intuitive. However, these
437 observations were all made in the population of Chizé, which has the lowest availability in food

438 resources compared to the two other populations, even in the best sector (Gaillard et al., 1993).
439 These results thus seem to match with an alternative theoretical framework proposed by Davis and
440 Maney (2018), who suggested that in poor quality habitats, glucocorticoid secretion should be
441 downregulated to prevent impairment of immune functions. Therefore, in Chizé, during the worst
442 years, or in the worst sector, energy allocation to immune function could be prioritised and
443 independent of glucocorticoid levels, which should be minimised with low among-individual
444 variations. Consistent with this hypothesis, variance in FCMs appeared to be lower during years of
445 low food availability than during years of high food availability in Chizé (Fig.2). However, this
446 hypothesis is not supported for all immune parameters studied, suggesting that not all immune
447 parameters would be independent of glucocorticoid levels in poor quality habitats within the
448 framework proposed by Davis and Maney (2018). In addition, pathogen load, and thus immune
449 challenge, may covary with food resources availability across time. In particular, variations in
450 population abundance may determine both resource availability and pathogen exposure: high
451 population density leading to both limited resources and high exposure to directly or indirectly
452 transmitted pathogens. In this case, high pathogen pressure could act as a stressor on roe deer and
453 lead to an elevation of FCMs, while stimulating immune defences to cope with the threat.

454 Finally, age influenced the relationship between immunity and baseline glucocorticoid levels. In
455 accordance with our predictions 3, negative relationships between FCMs and immune parameters
456 (hemagglutination, haptoglobin, gamma globulins and lymphocytes) only appeared, or were
457 steeper, in older individuals. In roe deer, the ingestion capacity is known to be less efficient in old
458 individuals (Gaillard et al., 1993), leading to a diminution in available energy and nutrients, and
459 individuals suffer from a loss of condition, as documented through a senescence in body mass
460 (Douhard et al. 2017) and various biological markers of ageing (Cheynel et al. 2017, Wilbourn et al.
461 2017; Lemaître et al. 2022). Consequently, older individuals exhibiting high levels of baseline
462 glucocorticoids might have poorer abilities to increase their allocation toward the immune
463 functions. Moreover, in line with previous evidence that stress hormones might accelerate the aging

464 process in roe deer (Lemaître et al. 2021), our results suggest that they might also accelerate the
465 previously documented immunosenescence (Cheynel et al. 2017). Long-term or repeated exposure
466 to stressors may therefore constitute a major selective pressure, especially on old individuals that
467 may not be able to maintain an overall efficient immune response and would therefore be exposed
468 to higher risks of diseases, contributing to the reduction in survival and reproductive success.

469 To conclude, our results show that the immunity of wild ungulates is strongly shaped by baseline
470 glucocorticoid levels, but that this influence differ between innate, adaptive and inflammatory
471 markers of immunity. While glucocorticoids are overall negatively correlated with innate and
472 inflammatory immunity, they appear to be less or even positively linked to adaptive immunity. In
473 addition, spatial and temporal availability in food resources appear to shape the relationship
474 between glucocorticoids and immunity with non-linear patterns, while negative covariations
475 between FCMs and immunity is strongest in old individuals. Our work highlights the need to
476 consider a multi-marker approach for future studies investigating the effect of stress hormones on
477 immune functions in wild animals. Such an approach, combined with consideration of the
478 environmental context and individual phenotype, could help improve our understanding of the
479 mechanisms underlying among-individual differences in immunity and susceptibility to diseases in
480 the wild.

481

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487

488 **Authors' contributions**

489 JC, EGF and HV conceived and designed the study. JC, EGF, HV, BR, MP, JD, SP, FD, JM, JFL
490 performed fieldwork. EGF, AG and CR performed immunological analyses. RP and JC ran FCM
491 assays. JC and MH performed the statistical analysis, wrote the first draft of the paper, and then
492 received input from other co-authors. All authors approved the final version of the manuscript and
493 agree to be held accountable for the content therein.

494

495 **Data accessibility**

496 Data used in this study are available at <https://github.com/JeffreyCarbillet/CarbilletHollainEtal2022>

497

498 **Competing interests**

499 The authors declare that they have no conflict of interest.

500

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505

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Immune trait	Parameter	Estimate	CI
Innate immunity			
Neutrophils (n = 188) R ² _m = 0.07 ; R ² _c = 0.71	Intercept	6.07	5.75 to 6.39
	Delay	0.006	0.002 to 0.01
	FCMs	-0.51	-0.94 to -0.08
Eosinophils (n = 185) R ² _m = 0.12 ; R ² _c = 0.46	Intercept	0.05	0.04 to 0.07
	Age (juvenile)	-0.03	-0.06 to -0.0001
	Julian date	0.001	0.0003 to 0.002
	Delay	-0.0002	-0.0003 to -0.00002
	Weight	-0.005	-0.009 to -0.0008
	Year quality	-0.05	-0.08 to -0.01
	Sex (male)	-0.02	-0.04 to 0.003
Basophils (n = 188) R ² _m = 0.04 ; R ² _c = 0.32	Intercept	0.05	0.03 to 0.07
	Delay	-0.0001	-0.003 to 0.00001
	Sector (2)	0.01	-0.01 to 0.04
	Sector (3)	0.03	0.003 to 0.06
Monocytes (n = 187) R ² _m = 0.01 ; R ² _c = 0.01	Intercept	0.11	0.10 to 0.12
	Year quality	-0.04	-0.09 to 0.007
Hemagglutination (n = 168) R ² _m = 0.12 ; R ² _c = 0.74	Intercept	3.64	3.15 to 4.16
	FCMs	-0.15	-0.37 to 0.08
	Year quality	-1.54	-3.49 to 0.39

	FCMs*Year quality	0.96	0.07 to 1.86
Hemolysis (n = 170) R ² _m = 0.31 ; R ² _c = 0.66	Intercept	2.89	2.20 to 3.58
	FCMs	-0.19	-0.43 to 0.05
	Year Quality	-3.12	-5.84 to -0.41
	Inflammatory markers		
Alpha-1 globulins (n = 146) R ² _m = 0.35 ; R ² _c = 0.38	Intercept	1.40	1.36 to 1.44
	Julian date	0.002	0.0003 to 0.003
	FCMs	0.03	-0.01 to 0.07
	Sector (2)	-0.03	-0.08 to 0.01
	Sector (3)	-0.07	-0.12 to -0.02
	Weight	-0.02	-0.03 to -0.01
	Sex (Male)	0.07	0.03 to 0.11
	FCMs*Sector (2)	0.06	-0.01 to 0.13
	FCMs*Sector (3)	-0.03	-0.11 to 0.04
	FCMs*Weight	-0.006	-0.01 to 0.002
Alpha-2 globulins (n = 144) R ² _m = 0.35 ; R ² _c = 0.38	Intercept	1.67	1.64 to 1.70
	Julian date	0.0008	-0.0002 to 0.002
Beta globulins (n = 147) R ² _m = 0.10 ; R ² _c = 0.13	Intercept	1.95	1.91 to 1.99
	Age (juvenile)	-0.16	-0.25 to -0.07
	FCMs	0.03	-0.009 to 0.08
	Weight	-0.02	-0.03 to -0.004
Adaptive immunity			
Gamma globulins (n = 147) R ² _m = 0.24 ; R ² _c = 0.72	Intercept	13.16	12.05 to 14.28
	Delay	-0.006	-0.01 to 0.0003
	Age (juvenile)	-3.47	-5.12 to -1.81
	FCMs	1.58	0.40 to 2.76
	Sector (2)	-1.13	-2.31 to 0.05
	Sector (3)	-0.52	-1.88 to 0.85
	Weight	-0.33	-0.56 to -0.10
	Year quality	1.75	0.20 to 3.30
	Sex (male)	0.47	-0.62 to 1.55
	FCMs*Sector (2)	-2.68	-4.38 to -0.98
	FCMs*Sector (3)	-2.11	-3.87 to -0.35
FCMs*Sex (male)	-2.10	-3.62 to -0.57	
Lymphocytes (n = 188) R ² _m = 0.24 ; R ² _c = 0.72	Intercept	1.28	1.21 to 1.35
	Weight	-0.02	-0.03 to -0.01
	Sex	-0.07	-0.14 to -0.002

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735 **Table 1** – Parameters of linear mixed-effect models selected for each immune parameters of the

736 Aurignac population. R²_m and R²_c correspond respectively to the marginal and conditional

737 variance explained by the model, CI corresponds to the upper and lower limits of the 95%

738 confidence interval, and n represents the number of observations per analysis. See the material and

739 methods section for a full definition of model sets and explanation regarding the difference in the

740 number of observations.

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Immune trait	Parameter	Estimate	CI
Innate immunity			
Neutrophils (n = 390) R ² _m = 0.10 ; R ² _c = 0.44	Intercept	4.49	3.95 to 5.03
	Delay	0.003	0.0001 to 0.005
	Age (linear)	0.13	0.06 to 0.20
	I(Age ²)	0.008	0.001 to 0.01
	Julian date	0.01	0.000002 to 0.02
	FCMs	-0.26	-0.54 to 0.03
	Year quality	-0.32	-0.67 to 0.04
	Weight	0.06	0.004 to 0.11
Eosinophils (n = 390) R ² _m = 0.14 ; R ² _c = 0.19	Intercept	0.10	0.06 to 0.14
	Delay	-0.0003	-0.0004 to -0.0002
	Age (linear)	-0.01	-0.03 to 0.004
	I(Age ²)	0.0007	-0.0006 to 0.002
	Sector (2)	0.02	-0.007 to 0.05
	Sector (3)	-0.03	-0.06 to 0.005
	Weight	0.005	0.0006 to 0.008
	Year quality	-0.02	-0.04 to -0.005
	I(Age ²)*Sex (male)	-0.001	-0.002 to -0.0001
Basophils (n = 390) R ² _m = 0.04 ; R ² _c = 0.32	Intercept	0.04	0.01 to 0.07
	FCMs	-0.002	-0.01 to 0.01
	Age (linear)	0.002	0.0004 to 0.004
	Sector (2)	0.01	-0.004 to 0.02
	Sector (3)	0.004	-0.01 to 0.02
	Weight	0.002	0.0003 to 0.003
	FCMs*Sector (2)	-0.02	-0.04 to -0.0002
	FCMs*Sector (3)	-0.03	-0.06 to -0.004
Monocytes (n = 389) R ² _m = 0.07 ; R ² _c = 0.55	Intercept	0.67	0.54 to 0.81
	Age (linear)	-0.06	-0.08 to -0.05
	FCMs	-0.04	-0.07 to -0.007
	Year quality	-0.06	-0.09 to -0.03
	Sex (male)	-0.05	-0.09 to -0.009
	FCMs*Year quality	0.06	0.003 to 0.11
Hemagglutination (n = 325) R ² _m = 0.07 ; R ² _c = 0.59	Intercept	4.72	2.77 to 6.66
	Age (threshold: 8 years)	-0.08	-0.29 to 0.13
	Julian date	-0.007	-0.01 to 0.0009
	FCMs	2.57	0.37 to 4.77
	Weight	-0.07	-0.11 to -0.04
	FCMs*Age (threshold)	-0.33	-0.59 to -0.06
	Julian date	-0.01	-0.02 to -0.004
Hemolysis (n = 325) R ² _m = 0.02 ; R ² _c = 0.65	Intercept	3.88	-0.05 to 7.82
	Age (threshold: 10 years)	-0.30	-0.61 to 0.02
	Julian date	-0.01	-0.02 to -0.004
Inflammatory markers			
Alpha-1 globulins (n = 414) R ² _m = 0.22 ; R ² _c = 0.42	Intercept	1.43	1.39 to 1.47
	Delay	0.0002	0.00003 to 0.0003
	FCMs	-0.03	-0.05 to -0.007
	Weight	-0.01	-0.02 to -0.01
	Sex (male)	0.04	0.02 to 0.07
	Alpha-2 globulins (n = 414) R ² _m = 0.14 ; R ² _c = 0.24	Intercept	1.93
Julian date		-0.001	-0.002 to -0.00006
Delay		0.0002	-0.00002 to 0.0003
FCMs		-0.004	-0.03 to 0.04
Weight		-0.004	-0.008 to 0.0002
Year quality		0.06	0.03 to 0.09
Sector (2)		-0.01	-0.05 to 0.03
Sector (3)		-0.05	-0.01 to 0.005
FCMs*Year quality		-0.06	-0.01 to -0.005
FCMs*Sector (2)	-0.11	-0.17 to -0.05	

	FCMs*Sector (3)	-0.06	-0.13 to 0.007
Beta globulins (n = 412) R ² m = 0.24 ; R ² c = 0.51	Intercept	1.88	1.81 to 1.95
	Age (linear)	0.07	0.05 to 0.10
	I(Age^2)	-0.004	-0.006 to -0.002
	Julian date	0.001	0.0004 to 0.002
	FCMs	-0.02	-0.05 to 0.01
	Weight	-0.007	-0.01 to -0.0008
	Sector (2)	0.0001	-0.04 to 0.04
	Sector (3)	-0.02	-0.07 to 0.03
	Sex (male)	0.05	0.02 to 0.09
	FCMs*Sector (2)	0.03	-0.02 to 0.09
	FCMs*Sector (3)	0.11	0.04 to 0.17
	Haptoglobin (n = 406) R ² m = 0.13 ; R ² c = 0.28	Intercept	0.25
Julian date		0.002	0.0001 to 0.004
Year quality		-0.15	-0.23 to -0.08
Sex (male)		0.11	0.04 to 0.19
Adaptive immunity			
Gamma globulins (n = 414) R ² m = 0.07 ; R ² c = 0.67	Intercept	18.59	16.37 to 20.81
	Age (threshold: 4 years)	0.43	0.18 to 0.69
	Delay	0.004	-0.0001 to 0.008
	FCMs	1.13	-0.57 to 2.83
	Sector (2)	0.15	-0.94 to 1.25
	Sector (3)	-1.45	-2.82 to -0.08
	FCMs*Age (threshold)	-0.30	-0.56 to -0.05
	FCMs*Sector (2)	-0.78	-2.12 to 0.55
	FCMs*Sector (3)	1.77	0.04 to 3.51
Lymphocytes (n = 390) R ² m = 0.24 ; R ² c = 0.72	Intercept	1.14	1.01 to 1.27
	Age (linear)	-0.06	-0.09 to -0.03
	I(Age^2)	0.003	0.0007 to 0.006
	Delay	-0.0004	-0.0007 to -0.0002
	FCMs	-0.00005	-0.04 to 0.04
	Year quality	0.006	-0.13 to 0.14
	Sex (male)	-0.02	-0.09 to 0.05
	I(Age^2)*Sex (male)	-0.003	-0.005 to -0.0006
	FCMs*Year quality	-0.09	-0.17 to -0.02

742

743 **Table 2** – Parameters of linear mixed-effect models selected for each immune parameters of the
744 Chizé population. R²m and R²c correspond respectively to the marginal and conditional variance
745 explained by the model, CI corresponds to the upper and lower limits of the 95% confidence
746 interval, and n represents the number of observations per analysis. See the material and methods
747 section for a full definition of model sets.

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Immune trait	Parameter	Estimate	CI
Innate immunity			
Neutrophils (n = 293) R ² _m = 0.06 ; R ² _c = 0.42	Intercept	5.83	5.28 to 6.38
	Age (I ²)	0.02	0.007 to 0.03
	Delay	0.003	0.00007 to 0.005
	Sex (male)	-0.06	-0.66 to 0.54
	Age (I ²)*Sex (male)	-0.02	-0.03 to -0.003
Eosinophils (n = 292) R ² _m = 0.09 ; R ² _c = 0.34	Intercept	0.07	0.06 to 0.09
	Delay	-0.0003	-0.0004 to -0.0002
	Weight	0.004	0.001 to 0.006
Basophils (n = 291) R ² _m = 0.01 ; R ² _c = 0.37	Intercept	0.04	0.01 to 0.07
	Delay	-0.00005	-0.0001 to 0.00002
	Weight	0.001	0.00001 to 0.003
Monocytes (n = 293) R ² _m = 0.05 ; R ² _c = 0.46	Intercept	0.27	0.12 to 0.42
	Age (linear)	-0.01	-0.02 to 0.0005
	Julian date	-0.003	-0.005 to -0.0007
	Delay	-0.0004	-0.0007 to -0.00005
	FCMs	-0.06	-0.13 to 0.009
	Weight	-0.005	-0.01 to 0.001
Hemagglutination (n = 290) R ² _m = 0.02 ; R ² _c = 0.26	Intercept	1.54	1.40 to 1.68
	Julian date	-0.003	-0.005 to -0.0003
Hemolysis (n = 276) R ² _m = 0.00 ; R ² _c = 0.29	Intercept	2.32	1.72 to 2.90
Inflammatory markers			
Alpha-1 globulins (n = 288) R ² _m = 0.19 ; R ² _c = 0.69	Intercept	1.47	1.36 to 1.58
	Age (linear)	-0.03	-0.05 to -0.004
	Age (I ²)	0.002	0.0006 to 0.004
	Weight	-0.007	-0.01 to -0.002
	Julian date	-0.003	-0.004 to -0.002
Alpha-2 globulins (n = 287) R ² _m = 0.02 ; R ² _c = 0.28	Intercept	1.92	1.81 to 2.03
	Delay	0.0002	-0.0004 to 0.0005
	Sex (male)	-0.05	-0.10 to 0.004
Beta globulins (n = 288) R ² _m = 0.22 ; R ² _c = 0.56	Intercept	1.82	1.71 to 1.93
	Age (linear)	0.03	0.02 to 0.03
	Julian date	-0.004	-0.005 to -0.002
Haptoglobin (n = 303) R ² _m = 0.15 ; R ² _c = 0.60	Intercept	-0.38	-0.74 to -0.009
	Age (threshold: 9 years)	0.06	0.02 to 0.10
	FCMs	0.50	-0.42 to 1.42
	Year quality	-0.08	-0.16 to 0.005
	Sex (male)	0.02	-0.005 to 0.05
	FCMs*Age (threshold)	-0.08	-0.17 to 0.01
Adaptive immunity			
Gamma globulins (n = 288) R ² _m = 0.12 ; R ² _c = 0.56	Intercept	11.60	10.03 to 13.44
	Age (threshold: 4 years)	0.46	0.29 to 0.65
	Julian date	-0.04	-0.07 to -0.02
Lymphocytes (n = 293) R ² _m = 0.08 ; R ² _c = 0.60	Intercept	1.18	1.10 to 1.25
	Age (linear)	0.007	-0.008 to 0.02
	Julian date	-0.002	-0.003 to 0.0004
	Delay	-0.0003	-0.0006 to 0.00006
	FCMs	0.07	-0.04 to 0.17
	Weight	-0.02	-0.02 to -0.007
	FCMs*Age (linear)	-0.03	-0.06 to -0.007

749

750 **Table 3** – Parameters of linear mixed-effect models selected for each immune parameters of the

751 Trois-Fontaines population. R²_m and R²_c correspond respectively to the marginal and conditional

752 variance explained by the model, CI corresponds to the upper and lower limits of the 95%

753 confidence interval, and n represents the number of observations per analysis. See the material and
754 methods section for a full definition of model sets.

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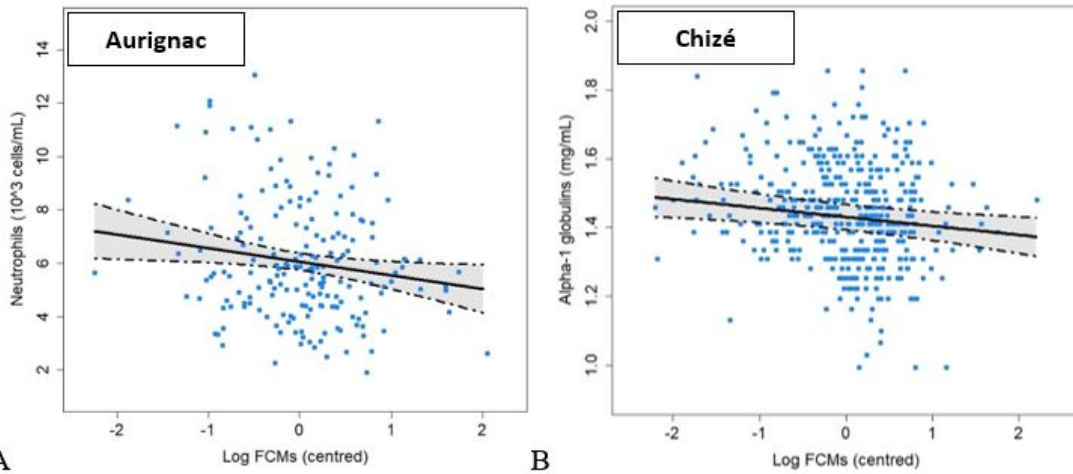
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Fig 1. Relationship between faecal cortisol metabolites (FCMs) and immune parameters in roe deer

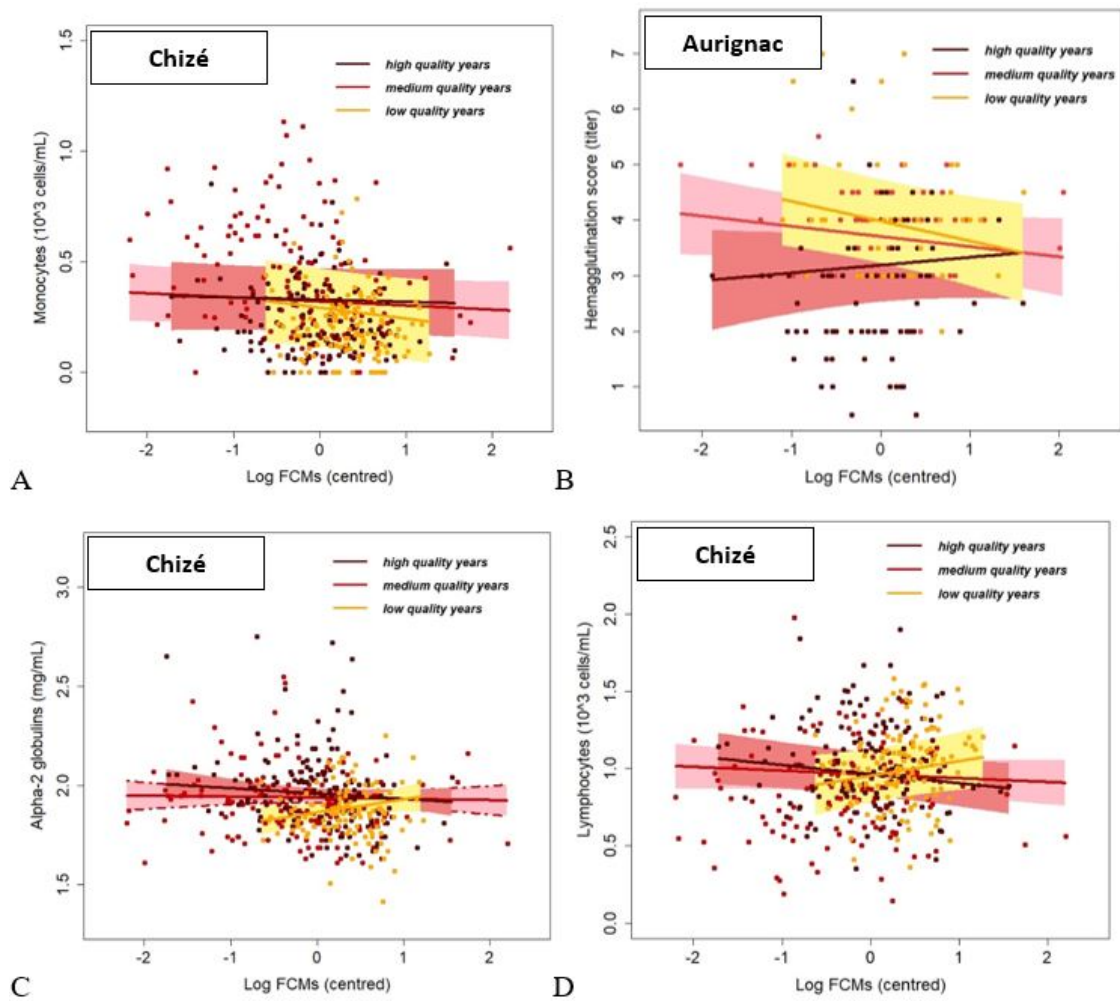
780 populations (prediction (i)): A) neutrophils in Aurignac; B) alpha-1 globulins in Chizé. Points

781 represent observed values. Solid black lines represent model predictions, dashed lines and shaded

782 area represent 95% confidence intervals. FCMs values are centred around the mean (mean value of

783 the variable that is subtracted from every value).

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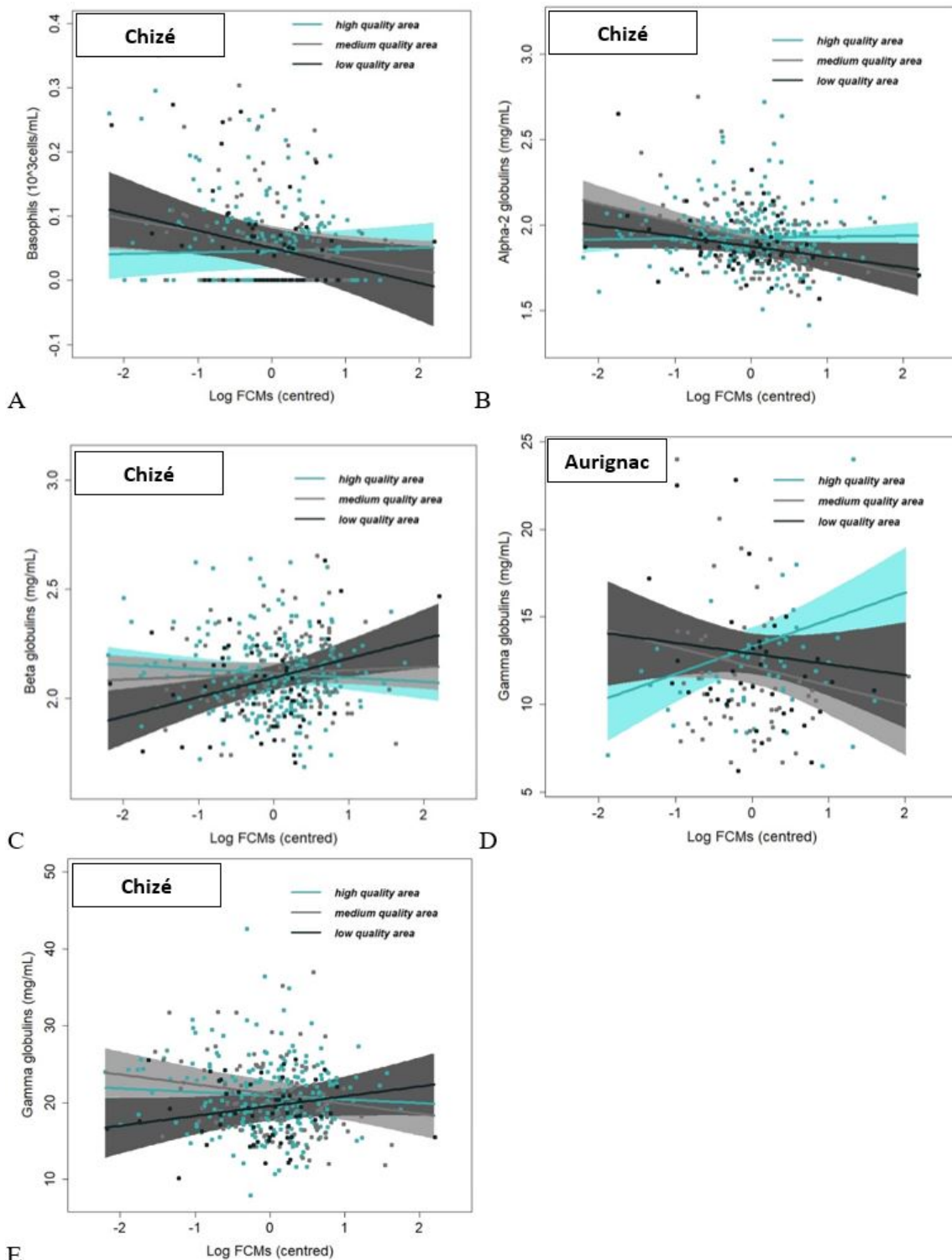
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Fig 2. Relationship between faecal cortisol metabolites (FCMs) and immune parameters in roe deer populations at varying year quality (prediction (ii)). Year quality was indexed using the population average body mass of juveniles (in kg) captured during the following winter: A) monocytes in Chizé; B) hemagglutination score in Aurignac; C) alpha-2 globulins in Chizé; D) lymphocytes in Chizé. Points represent observed values. Solid lines represent model predictions and shaded areas represent the 95% confidence interval. FCMs values are centred around the mean (mean value of the variable that is subtracted from every value).



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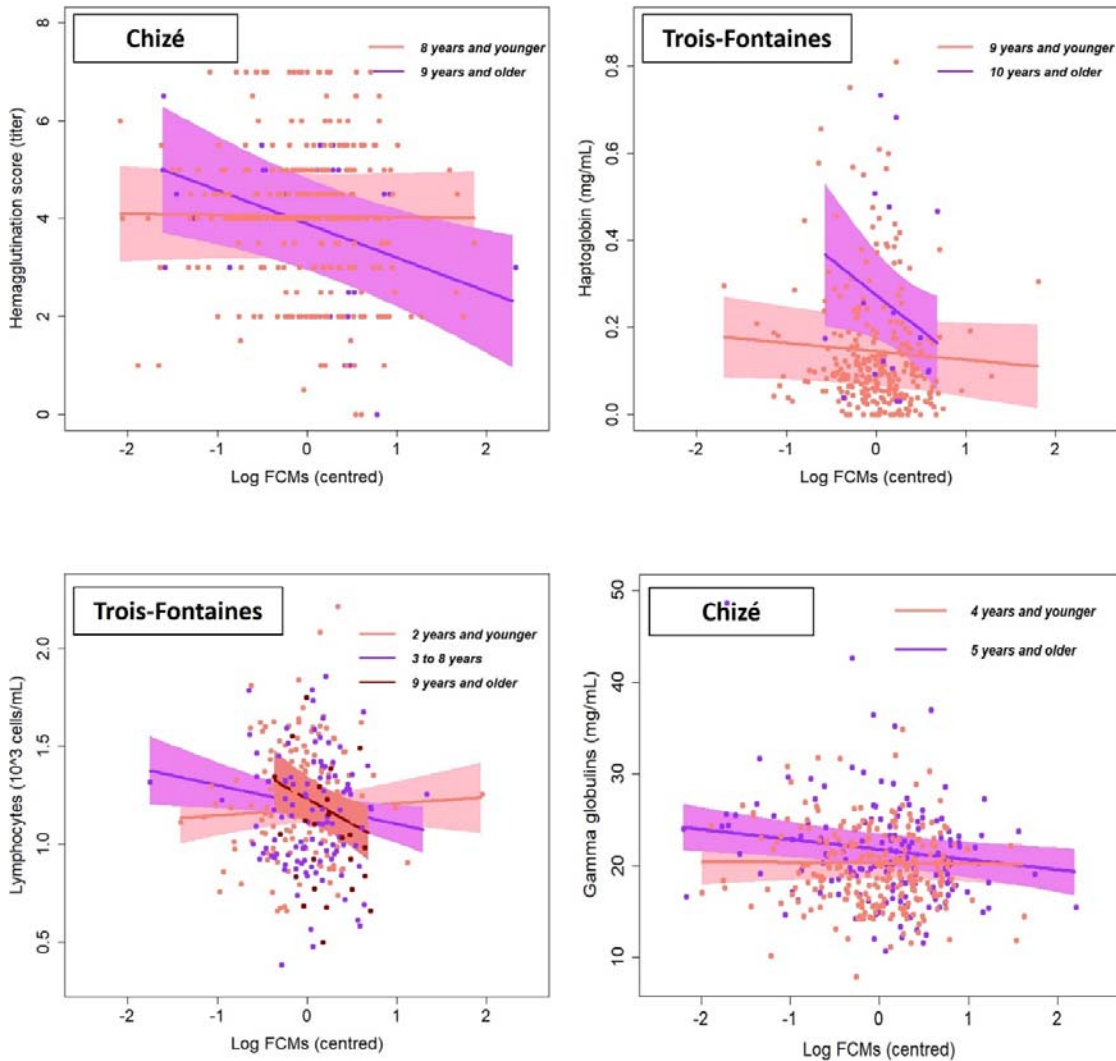
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Fig 3. Relationship between faecal cortisol metabolites (FCMs) and immune parameters in roe deer populations, at varying area quality (prediction (ii)). Areas quality differed among the three sectors described in the material and methods section: A) basophil concentrations in Chizé; B) alpha-2 globulins in Chizé; C) beta-globulins in Chizé; D) gamma-globulins in Aurignac; E) gamma-

800 globulins in Chizé. Points represent observed values. Solid lines represent model predictions and
801 shaded areas represent the 95% confidence interval. FCMs values are centred around the mean
802 (mean value of the variable that is subtracted from every value).
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805 **Fig 4.** Relationship between faecal cortisol metabolites (FCMs) and immune parameters at varying
806 ages in roe deer populations (prediction (iii)). Age was considered either as linear or with a
807 threshold function (determined from a previous study, see main text for details), in which case the
808 graphical representation compares before/after threshold age: A) hemagglutination in Chizé
809 (threshold: 8 years old), B) haptoglobin in Trois-Fontaines (threshold: 9 years old); C) lymphocytes
810 in Trois Fontaines (linear, but for the graphical display, three age classes were constituted on the
811 basis of sample size: up to 2 years, 3-8 years, 9 years and older); D) gamma-globulins in Chizé
812 (threshold: 4 years old). Points represent observed values. Solid lines represent model predictions
813 and shaded areas represent the 95% confidence interval. FCMs values are centred around the mean
814 (mean value of the variable that is subtracted from every value).