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1 **Glucocorticoids of European bison in relation to their status: age, dominance, social centrality and**
2 **leadership.**

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5

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11

12 **Abstract:** Stress is the body's response to cope with the environment and better survive.

13 While some studies suggest that it would be more stressful to be the dominant of the group, others
14 support the opposite hypothesis. Several variables can actually affect this relationship, or even cancel
15 it. This study therefore aims to make the link between social status and the basal level of stress of 14
16 wild European bison (*Bison bonasus*) living together. We collected faeces and measured the faecal
17 glucocorticoid metabolites (FGM), a proxy of stress. We showed that FGM is linked to different
18 variables of social status of European bison, specifically age, dominance rank, eigenvector centrality
19 but also to several variable interactions. Hence, glucocorticoids seem to be a mediator of social life of
20 bison. Preferential leaders in bison, *i.e.* the older and more dominant individuals which are also the
21 more central ones, are less stressed compared to other group members. Measurement of such
22 variables could thus be a valuable tool to follow and improve the conservation of species.

23

24

25 **Keywords:** *Bison bonasus*, stress, sociality, collective decisions, ruminants, conservation

26 1. Introduction

27 Stress is the body's response to cope with the environment and better survive (Boonstra,
28 2013; Crespi et al., 2013). For many social species, an individual's baseline stress level is generally
29 associated with their dominance status (Creel, 2001). However, while some studies suggest that it
30 would be more stressful to be the dominant of the group (Kotrschal et al., 1998; Mooring et al., 2006;
31 Sands and Creel, 2004), others support the opposite hypothesis (Abbott et al., 2003; McLeod et al.,
32 1996). Several variables can actually affect this relationship, or even cancel it, such as the social
33 system of the species, the seasonality of reproduction, domestication, personality, age or even the
34 context of the study, *i.e.* in captivity or in the wild (Creel, 2001; Engh et al., 2005; Sapolsky, 2005;
35 York and Schulte, 2014).

36 The basal level of stress, measured via the secretion of glucocorticoid hormones, can also be
37 intrinsically linked to the propensity to be a leader during group movements, *i.e.* to initiate the
38 departure and be followed (Ramos et al., 2021). In equines, for example, the stallion usually moves
39 behind the progression; this position allows it to maintain social cohesion by bringing its females
40 together, *i.e.* herding behaviour (Berger, 1977; Feist and McCullough, 1976). However, a study in
41 Przewalski's horse species, *Equus ferus przewalskii*, showed that a change in the structure of the
42 enclosure caused a significant increase in the secretion of glucocorticoids in the male. As a result, the
43 latter then more often took the lead of collective movements (Wolter et al., 2014).

44 A growing number of studies attempt to explain the phenomenon of leadership by focusing
45 on individual characteristics (*e.g.* age, sex, physiological status, temperament, *etc.*) and on the social
46 relationships that individuals maintain with each other (*e.g.* dominance, social centrality...). However,
47 the majority only focus on the interaction between two or three factors (often age and dominance or
48 dominance and sex) whilst these factors are often entangled and dependent on internal processes
49 such as stress.

50 This study therefore aims to make the link between leadership, age, dominance, social
51 centrality and the basal level of stress of individuals. Individual animals were identified and the stress
52 level was measured by the concentration of faecal glucocorticoid metabolites (FGM, ng.g⁻¹). We
53 focused our study on semi free-roaming European bison (*Bison bonasus*), a wild ungulate living in
54 groups with an average of 20 individuals (Kraśniński, 1967; Ramos et al., 2019). The only study
55 reporting the interaction between glucocorticoid levels, individual characteristics and social structure
56 in European bison was performed on captive herds and showed that cortisol levels were not
57 influenced by sex or age (Klich et al., 2021a). However, the study site and the interaction between
58 study site and visitor pressure were statistically significant. On the basis of behavioural observations
59 made on leadership and sociality in this species (Ramos et al., 2018, 2016a, 2016b, 2015) and the
60 literature on the subject, we made the following hypothesis: the oldest females in the herd, which

61 are the preferential leaders and the more dominant and central individuals, should have a higher
 62 average rate of FGM compared to the other group members.

63

64 2. Material and methods

65 2.1. Study area

66 Our study was conducted in the Kraansvlak area, a coastal dune area that it is part of the
 67 Zuid-Kennemerland National Park (3800 ha), the Netherlands (52° 23' 16.1" N, 4° 34' 41.3" E). This
 68 area was 330 ha large and consisted of six main habitats: sand dunes, grasslands, deciduous forest,
 69 pine forest, shrubbery areas and scattered water points. No natural predator of bison, *i.e.* grey wolf
 70 (*Canis lupus*) and brown bear (*Ursus arctos*), was present in the Kraansvlak area.

71 Kraansvlak is a nature core area within the National Parks where access to visitors is limited
 72 to guided excursions year round and a hiking trail accessible for the public during the months of
 73 September to March. The rest of the year the free-access walking trail remains closed. Further on
 74 only researchers and park rangers track the animals to monitor or study them.

75 2.2. Study Subjects

76 During the study period, the herd of European bison consisted of 14 individuals. Bison were
 77 identified at individual level (Table 1). The herd included eight adult females aged 4 to 15 years, two
 78 subadult females of 2 and 3 years old, two juvenile males both 10 months old and two juvenile
 79 females of 9 and 8 months old. The kin relationships between all individuals were known (Fig. 1).
 80 Unlike numerous European bison populations living in natural reserves, the herd of Kraansvlak is not
 81 supplemented with food during winter or any other time during the year. Therefore, this herd shows
 82 the most natural foraging behaviour throughout the different habitats all year round. More details
 83 are given in Ramos et al. (2018) and Ramos et al. (2019).

84

85 Table 1: Individual measures for the total observation period

<i>Individual</i>	Age (months)	Eigenvector (A.U.)	Dominance rank	Leadership (relative ratio)	FGM mean (ng.g-1)
<i>Kareta (Kr)</i>	180	0.29	2	0.23880597	272±89
<i>Kaga (Kg)</i>	132	0.27	5	0.097014925	297±78
<i>Katarina (Kt)</i>	132	0.32	3	0.253731343	210±52
<i>Katona (Ko)</i>	102	0.32	1	0.02238806	187±47
<i>Ina (In)</i>	78	0.17	6	0.104477612	177±70
<i>Moesja (Mo)</i>	78	0.19	8	0.171641791	264±73
<i>Wisha (Wi)</i>	78	0.22	4	0.052238806	227±47

<i>Frida (Fr)</i>	54	0.21	7	0.014925373	200±44
<i>Kristy (Ky)</i>	42	0.22	9	0.029850746	216±93
<i>Neréna (Nr)</i>	30	0.29	10	0.014925373	216±49
<i>Nelson (Nl)</i>	10	0.32	12	0	309±80
<i>Némar (Nm)</i>	10	0.2	11	0	283±101
<i>Nejen (Nj)</i>	9	0.34	13	0	275±74
<i>Névita (Nv)</i>	8	0.3	14	0	199±42

86

87

88 2.3. Data Collection

89 Throughout the data acquisition period (from 5 April 2016 to 22 June 2016), we observed the
90 bison herd from 09:30 to 16:30, for an average of 4 h per day depending on the climatic conditions as
91 well as the time required to locate the group in the area. The total observation time for the entire
92 study period is 156 h. We observed the bison at sufficient distance, minimum distance of 50 metres,
93 which sometimes decreased slightly due to terrain geography or herd movement direction, to avoid
94 setting off stress behaviours and ensure that our presence did not interfere with natural activity
95 patterns and the welfare of the herd.

96 We recorded social relationships of individuals, dominance interactions, movement initiations
97 (leadership) and collected faeces in order to obtain measure stress via glucocorticoids. Social and
98 dominance data were collected using CyberTracker 3.0 Software (Steventon et al., 2011), installed on
99 a Samsung Galaxy Tab3 Lite. Leadership data was recorded with a HDR-CX320E Sony camera
100 recorder.

101 Social relationships: Each 5 minutes, the identity of the nearest neighbour for each individual was
102 recorded by the *instantaneous sampling method* (Altmann, 1974). When it was not possible for the
103 observers to distinguish only one nearest neighbour (focal individual placed at the same distance of
104 several individuals), the identities of these different neighbours were recorded. We used the
105 eigenvector centrality as a measure of social centrality. This index was calculated using the nearest-
106 neighbour matrix implemented in SocProg 2.7 (Whitehead, 2009). More details are given in Ramos et
107 al. (2019, 2018).

108 Dominance interactions: All unidirectional agonistic interactions were recorded using the *continuous*
109 *sampling method* (Altmann, 1974). For each interaction (see Ramos et al. 2019) for details), the
110 identity of the emitter and the receiver were noted. Agonistic interactions were carried over an
111 asymmetrical matrix to calculate dominance scores of individuals. This was analysed using SocProg
112 2.7 Software to measure the linearity of the hierarchy ($h' = 0.949$, $P < 0.0001$, 10,000 permutations;

113 de Vries et al. 2006) and to obtain a dominance index for each animal, the Modified David's Score
114 (MDS). From these MDS, a dominance score, ranging from 1 (most dominant) to 14 (least dominant),
115 was attributed to each bison. The dominance never changed during the observation period.
116 Movement initiations: We recorded the initiation phase of spontaneous collective movements with
117 the *continuous sampling method* (Whitehead, 2009) by triggering the camera as soon as an individual
118 moved away from the herd. The initiation of a movement was defined as the departure of the first
119 individual (*i.e.* the initiator) out of the group diameter, for at least 20 steps in a constant direction (\pm
120 45°), with its head raised (Ramos et al., 2018, 2015). This head position has been described to be a
121 good cue of individual motivation to change location (Bourjade et al., 2009; Ramseyer et al., 2009).
122 An initiation was finally considered to be a success when the initiator was followed by at least one
123 congener (Ramos et al., 2018, 2015). If no bison followed the movement within the 15 min time
124 window, the initiation was considered as a failure. The leadership score was then calculated by
125 dividing the number of successful initiations per individual per the total of successful initiations (see
126 Ramos et al. 2018 for the complete method).

127 **2.4. Measures of faecal glucocorticoid metabolites (FGM)**

128 The faeces samples were collected during the observations. For this, sterile 5 ml Eppendorf
129 tubes were used, as well as disposable sampling spatulas. At least, one sample per day was taken,
130 and all samples were identified on an individual level. The tubes thus filled were identified, dated and
131 then immediately stored in a coolbag (containing an ice pack) in the field for a maximum period of 3
132 hours, then they were stored at -20°C until laboratory analyses took place. At the time of sampling,
133 we noted the environmental conditions (ambient temperature, sunshine or cloudiness, presence or
134 absence of wind, rainfall level) and the time elapsed between defecation and sampling in order to
135 determine whether fluctuations in environmental conditions and field constraints would have had a
136 significant effect on the concentrations of FGM. To avoid contamination and deterioration, samples
137 were not taken on rainy days, when defecation took place in water or when individuals had
138 diarrhoea. Indeed, after defecation, several factors, such as temperature, humidity, and other
139 environmental conditions, may influence concentrations of FGM in the sample (Millspaugh and
140 Washburn, 2004; Möstl et al., 2002; Washburn and Millspaugh, 2002). Body condition might also
141 confuse interpretation of FGM measures (Heath and Dufty, 1998; Smith et al., 1994). Because
142 bacterial enzymes are reported to increase or decrease levels of FGM if samples are not frozen
143 shortly after sampling, only faeces dating from a maximum of 2 hours after defecation, were taken
144 (Millspaugh and Washburn, 2004; Möstl et al., 2002; Washburn and Millspaugh, 2002). Finally, only
145 the faeces sufficiently isolated from each other and therefore reliably identified were collected. A
146 total of 199 faecal samples were thus obtained and 103 were selected for analyses. The choice of
147 samples to analyse was made in such a way as to have an equivalent quantity of samples per

148 individual (N = 7±1), while making sure to have collection dates for each animal covering the entire
149 period.

150 In collaboration with Professor Rupert Palme, the analysis of the level of FGM was carried out by the
151 Physiology, Physiopathology and Experimental Endocrinology Unit of the University of Vienna
152 (Austria). The protocol described in (Palme et al., 2013) and (Möstl et al., 2002) was used for the
153 extraction and measurement of FGM using the EIA (Enzyme Immunoassays) technique. We obtained
154 a value of FGM in ng/g-1 per sample (Table 1). This protocol was checked and approved covering
155 many species including ungulates (Christensen et al., 2021; Kovács et al., 2021; Lemaître et al., 2021;
156 Touma and Palme, 2005; Veissier et al., 2018; Wagner et al., 2012). We performed the measures
157 twice, on wet and dry faeces and the values were correlated at 98.18 % ($r = 0.9818$, $p < 0.0001$). We
158 chose to use FGM values on wet faeces as there is less protocol steps for measurement. The detailed
159 protocol is given in the supplementary material.

160 **2.5. Statistical Analyses**

161 We used eigenvector centrality, leadership score and FGM per week during the observation
162 period. Age and dominance rank were stable. Therefore, we could not implement individual
163 identities as random factor in the next analyses as it was collinear with age and dominance rank (VIF
164 > 4). Indeed, the potential collinearity between our predictor variables was tested by the calculation
165 of variance inflation factor (VIF) from the R package *car*. In our different models (except Model 2, see
166 below), age and dominance rank always showed a VIF > 4 , meaning that they were highly correlated
167 ($\text{adj } r^2 = 0.79$, $F = 388$, $p < 0.0001$, Figure 1a). Therefore, we decided to remove dominance rank as
168 variable in the models 1, 3 and 4 as it is dependent on age, which is also shown in previous studies
169 (Bouissou et al., 2001; Ramos et al., 2019; Sosa et al., 2019; Sueur et al., 2017). Subsequently,
170 multifactorial linear models (LM) were applied as follows:

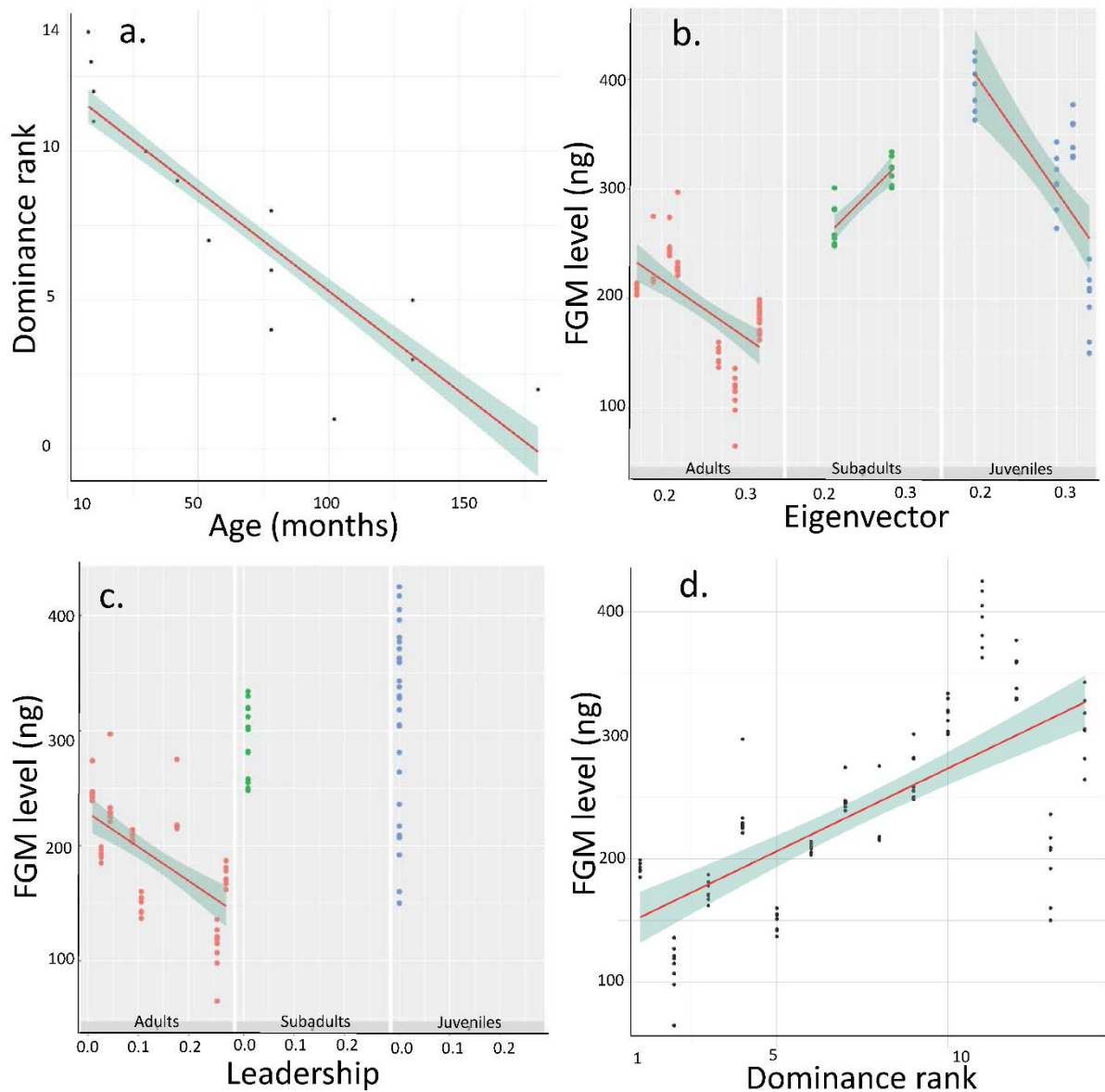
171 - $\text{FGM} \sim \text{Age} * \text{Leadership} + \text{Age} * \text{Eigenvector} + \text{Leadership} * \text{Eigenvector}$ (Model 1)

172 - $\text{Rank} \sim \text{Age} * \text{FGM}$ (Model 2)

173 - $\text{Eigenvector} \sim \text{Age} * \text{FGM}$ (Model 3)

174 - $\text{Leadership} \sim \text{Age} * \text{FGM} + \text{Eigenvector} * \text{FGM}$ (Model 4)

175 We tested leadership as influenced by the eigenvector centrality and not the converse, as
176 usually done in studies on leadership (Fischhoff et al., 2007; Jacobs et al., 2008; Van Belle et al.,
177 2013), by using the function *lm()* from the R package *car* (Fox et al., 2007). *P*-values for LM were
178 calculated by *Monte Carlo sampling* with 10 000 permutations, using the function *PermTest()* of the R
179 package *pgirmess* (Giraudoux et al., 2018). Permutation tests for LM are well adjusted for moderate
180 sample size and do not require normal distribution of model residuals (Good, 2013). We repeated
181 three times the tests to check for the stability of the statistics ($\alpha = 0.05$). All statistical analyses were
182 performed with R, version 4.0.3 (R Development Core Team, 2009).



184

185 Figure 1: Main effects obtained between Faecal Glucocorticoid Metabolites (FGM, $\text{ng}\cdot\text{g}^{-1}$) and social
 186 status in European bison. a. Dominance in function of Age, b. FGM level in function of Eigenvector
 187 (arbitrary unit) and Age (months), c. FGM level in function of Leadership (relative frequency) score
 188 and Age, d. FGM level in function of Dominance rank.

189

190

191 3. Results

192 Results of Model 1 ($\text{adj } r^2 = 0.79$, $F = 67.29$, $p < 0.0001$) show that the FGM is influenced by age ($p <$
 193 0.0001 , Figure 1b, c), eigenvector centrality ($p = 0.002$, Figure 1b), interaction Age*Eigenvector ($p <$
 194 0.0001 , Figure 1b) and interaction Age*Leadership ($p = 0.0044$, Figure 1c). Leadership and
 195 Leadership*Eigenvector did not influence the FGM (respectively $p = 0.341$ and $p = 0.172$).

196 Results of Model 2 (adj $r^2 = 0.81$, $F = 150.2$, $p < 0.0001$) indicate that the dominance rank is
197 influenced by age of individuals ($p < 0.0001$, Figure 1a), by FGM ($p = 0.006$, Figure 1d) and interaction
198 Age*FGM ($p = 0.026$).

199 Results of Model 3 (adj $r^2 = 0.18$, $F = 8.75$, $p < 0.0001$) show that the eigenvector centrality is
200 influenced by FGM ($p = 0.006$), interaction Age*FGM ($p < 0.0001$) but not by age alone ($p=0.408$).
201 Finally, results of Model 4 (adj $r^2 = 0.69$, $F = 45.94$, $p < 0.0001$) indicate that the leadership score is
202 influenced by age ($p < 0.0001$) but not by other factors or interactions ($p > 0.353$).

203

204 **4. Discussion**

205 In this study, we found that the FGM, a proxy of stress (Chinnadurai et al., 2009; Möstl and
206 Palme, 2002), is linked to different variables of social status of European bison, specifically age,
207 dominance rank, eigenvector centrality but also to several variable interactions. Hence,
208 glucocorticoids seem to be a mediator of social life of bison.

209 We had to remove dominance rank from three of our models, because this factor is highly
210 determined by age in European bison (Ramos et al., 2019) but also in other bovines (Bouissou et al.,
211 2001; Sosa et al., 2019; Sueur et al., 2017). However, as shown in many studies, dominance rank is
212 linked to glucocorticoid levels. While some studies suggest that it would be more stressful to be the
213 dominant of the group (Kotrschal et al., 1998; Mooring et al., 2006; Sands and Creel, 2004), others
214 support the opposite hypothesis (Abbott et al., 2003; McLeod et al., 1996). For instance, in captive
215 animals, there are more aggression from the dominant towards the dominated for whom the
216 inability to escape results in an increase of the glucocorticoid hormone secretions (Creel, 2001;
217 Kotrschal et al., 1998). Under natural conditions, a single study, carried out on olive baboons (*Papio*
218 *anubis*), showed that the basal level of glucocorticoids was also higher in subordinate individuals
219 (Sapolsky, 1983), while five other studies, grouping together several taxa, demonstrated a higher
220 level of stress in dominant individuals (Arnold and Dittami, 1997; Cavigelli, 1999; Creel et al., 1997,
221 1996; Schoech et al., 1991). Thus, according to the literature, even if there is no absolute consensus
222 on the question (Sapolsky, 2005), a high secretion of glucocorticoids is more generally observed in
223 the dominant than in the subordinate individuals, in particular for species living in stable social
224 groups (Sands and Creel, 2004). According to some authors, the high level of glucocorticoids in the
225 dominant individual is not directly due to hierarchical status, but to agonistic behaviours expressed
226 by the individual that allow him to access and maintain such a position (de Villiers et al., 1997;
227 Sapolsky, 1992). In our study, we found that subordinate individuals have higher FGM levels than
228 dominant ones but this is strongly mediated by age as subordinate individuals are younger
229 individuals (Ramos et al., 2019). It is thus difficult to conclude about whether age or dominance is the

230 prior factor affecting glucocorticoids, whilst studies have shown that, in same age groups of
231 individuals, a correlation between dominance and FGM levels still existed (Hasegawa et al., 1997;
232 Hubbard et al., 2021). Such group composition experiments would be interesting to test on bison,
233 but this species being wild and vulnerable, it's not manageable like domestic groups.

234 In addition, we found a link between the FGM levels and the eigenvector centrality. More
235 central individuals are less stressed. This social centrality is based on spatial proximity and thus is
236 more a spatial centrality. Animals being more spatially central in the group can feel more protected
237 (Lopes et al., 2016; McCann et al., 2016). This correlation is also found with species showing
238 grooming behaviour as central individuals are more groomed and this grooming decreases
239 glucocorticoids level (Brent et al., 2011). Socially isolated individuals also show more stress (Sueur et
240 al., 2021) as well in cattle (Rushen et al., 1999; Sec et al., 2013) as in other species (Mumtaz et al.,
241 2018; Sapolsky et al., 1997) including humans (Cacioppo and Cacioppo, 2014). The relationship
242 FGM*Eigenvector centrality seems to be mediated by the age of individuals. In fact, Ramos et al.
243 (2019, 2018) showed that juveniles have similar centralities than adults because of their dependency
244 but subadults have lower ones. The interaction here shows that FGM level decreases with centrality
245 in juveniles and adults but not in subadults. The subadults are often meeting higher stress because
246 they are less protected by their mother, especially if the mother has a new calf, need to find a place
247 in the group and are subordinates.

248 A similar influence is found on the FGM level with the interaction Leadership*Age meaning
249 that individuals who initiate more successful movements are less stress compared to other ones. This
250 result can be explained by the greater experience and knowledge of older individuals which cope
251 better with the uncertainty of changing location and resource availability (Ramos et al., 2021). The
252 other explanation could be that individuals initiating movements do it because they feel the need to
253 do so (Fischhoff et al., 2007; Ramos et al., 2018; Sueur et al., 2013) and by initiating these
254 movements, they thus fill their needs and are less stressed. One may think that leaders are more
255 stressed because of their position (Harms et al., 2017; Smith and Cooper, 1994), but in humans, a
256 study showed that leaders have lower levels of glucocorticoids probably due to a sense of control
257 (Sherman et al., 2012).

258 Finally, and contrary to our initial hypothesis, we found that preferential leaders in bison, *i.e.*
259 the older and more dominant individuals which are also the more central ones (Ramos et al., 2019,
260 2018), are less stressed compared to other group members. Other factors with a potential influence
261 such as reproductive status, the transmission of social rank by previous generations, season (and
262 consequently resource availability), or even parasitic infections should be studied further.

263 **6. Conclusion**

264 All these results are not only interesting for a better understanding of links between
265 physiological and behavioural mechanisms in animal societies, but also for European bison
266 management itself. Indeed, the species is listed on the IUCN Red List of Threatened Species and in
267 need of receiving especially high attention in conservation and rewilding proceedings (Klich et al.,
268 2021b; Pucek, 2004; Pucek et al., 2004; Ramos et al., 2021). Measurement of faecal cortisol
269 metabolites, associated with other parameters, could thus be a valuable tool to follow and improve
270 the adaptation and the welfare of herds in the semi-wild and in captivity (Klich et al., 2021a). This
271 new knowledge could, for instance, allow us to take into account the importance of older females as
272 group members as being stable and reassuring figures during captures, veterinary interventions or
273 new reintroductions (Ramos et al., 2021, 2019). This could have a direct positive impact on survival of
274 the herd.

275

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283

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