

1 **Group size and visitor numbers predict faecal glucocorticoid**
2 **concentrations in zoo meerkats**

3
4

5 Short title: Hormonal stress in zoo meerkats

6 Authors: Katy Scott¹, Michael Heistermann², Michael A. Cant¹ & Emma I. K. Vitikainen¹

7 1. Centre for Ecology and Conservation, University of Exeter, Penryn, Cornwall TR10 9FE,
8 UK

9 2. German Primate Centre, Leibniz Institute for Primate Research, Göttingen, Germany

10 *Corresponding author: emma.vitikainen@gmail.com

11
12

13 **Abstract**

14 Measures of physiological stress in zoo animals can give important insights into how they
15 are affected by aspects of their captive environment. We analysed the factors influencing
16 variation in glucocorticoid metabolites in faeces (fGCs) from zoo meerkats as a proxy for
17 blood cortisol concentration, high levels of which are associated with a stress response.
18 Levels of fGCs in captive meerkats declined with increasing group size. Compared to data
19 from wild meerkats, this contrasts with the patterns seen in large stable groups but
20 matches the pattern seen in dispersing coalitions. In the wild, very small groups of meerkats
21 are at a higher risk of predation, while in larger groups there is increased competition for
22 resources. Indeed, group sizes in captivity tend to be closer to those seen in unstable
23 coalitions in the wild, which may represent a stressful condition to meerkats in captivity and

24 predispose them to chronic stress, even in absence of natural predators. Individuals in large
25 enclosures showed lower levels of stress, but meerkat density had no effect on the stress
26 measures. In contrast to data from wild meerkats, neither sex, age, nor dominance status
27 predicted physiological stress levels in captivity, which may reflect less food stress owing to
28 more equal access to resources in captivity versus wild. Median number of visitors at the
29 enclosure was positively correlated with fGC concentrations on the following day, with
30 variation in the visitor numbers having the opposite effect. Our results are consistent with
31 the hypothesis that there is an optimum group size which minimises physiological stress in
32 meerkats, and that zoo meerkats at most risk of physiological stress are those kept in small
33 groups and small enclosures and are exposed to consistently high numbers of visitors.

34

35 **Keywords:**

36 Fecal glucocorticoids; group size; animal welfare; zoo visitors; meerkat; *Suricata suricatta*

37

38

39 Introduction

40 Measuring stress in zoo animals is important to improve welfare and monitor the effect of
41 captivity, but is difficult in practice. Variation between species and between individual
42 animals in their behavioural responses to a stressor make it difficult to define fixed, reliable
43 criteria for assessing animal welfare based on their rearing conditions [1,2]. Observational
44 measures, such as behavioural repertoire, or breeding success can provide useful
45 information [3]. For example, large numbers of visitors may be stressful to the animals and
46 linked with changes in their behaviour (e.g. [4]). Yet, they may also be difficult to interpret
47 and to causally link to a particular set of conditions the animals are experiencing, for
48 example because the behaviour of the animals could be the driver of variation in visitor
49 numbers, as well as the consequence of it (e.g. [5,6]). A useful method therefore is to
50 measure directly how variation in captive conditions and exposure to visitors impacts the
51 physiological response of the animals, by measuring components of the hormonal stress
52 response [7].

53 The main characteristic of the physiological stress response in vertebrates is the release of
54 glucocorticoids (cortisol, corticosterone) from the adrenal gland in response to a stressor.
55 Glucocorticoids are released into the blood stream from the adrenal gland, and play an
56 essential role in general homeostasis. Their presence at elevated concentrations can also
57 indicate a stress response, as one of their functions is to trigger the mobilisation of energy
58 stores to allow the animal to respond to the current threat [1,8,9]. Analysing the level of
59 glucocorticoids in the animal's bloodstream is a way to measure the level of the hormonal
60 stress response at a given time. However, this requires catching the animal and extracting a
61 blood sample: this is impractical in zoos, and capture itself constitutes a stressor which will
62 compromise future samples for as long as the stress response lasts, and possibly even
63 longer if a stressor results in longer term changes in an animal's state [7,9]. An alternative
64 method is to analyse the level of glucocorticoid metabolites in excreta, e.g a faecal sample
65 from the animal (faecal glucocorticoids, or fGCs). The amount of fGCs provides an estimate
66 for glucocorticoid production over the preceding hours or days, depending on rate of
67 metabolism and volume of through-put [7]. For the same animal, or conspecifics on similar
68 diets, this provides a relative measure of hormonal stress response at different times or in

69 different situations. Using faecal sampling to monitor glucocorticoid levels in both captive-
70 housed animals as well as those living in the wild has become a widespread technique over
71 recent years (e.g. [7,10]).

72 The key question when studying the glucocorticoid response of zoo animals is how
73 characteristics of the captive environment, social group, and those of the individual itself
74 affect its stress response. Quantifying stress is not straightforward however, as there are no
75 clear guidelines of what constitutes an 'elevated' response, and as aspects of captivity may
76 in several ways be masking the stress response of the animals. Comparisons to wild
77 conspecifics are limited in that scarcity of food is a major source of stress for animals living
78 in the wild, yet often completely absent in animals in captivity that are typically fed to
79 requirement. Furthermore, chronic stress in captive animals may lead to downregulation or
80 suppression of the stress response (HPA axis; see e.g. [11]), leading to highly stressed
81 animals scoring misleadingly low in measures of physiological stress. Therefore, individual
82 variation in stress response measured against a range of conditions, if not the absolute
83 levels of the stress hormones, may better indicate how aspects of the environment affect
84 the animals' experience. Keeping these limitations in mind, comparisons to wild conspecifics
85 may be a useful method to understand how the conditions experienced by captive animals
86 are affecting their cortisol levels, and to guide decisions on how to best minimise stress
87 levels in captivity. In the current study we used meerkats (*Suricata suricatta*, Schreber 1776)
88 as a model to examine the effect of captivity on the stress-response of a highly social and
89 cooperatively-breeding species. Meerkats have been extensively studied in the wild, and the
90 effects of natural variation in cortisol levels in response to individual and group
91 characteristics are well documented [12-14]. Meerkats are also common in zoos, allowing
92 the study of a relatively large sample size of varying individuals in different social and
93 environmental conditions.

94 Meerkats are an obligate cooperatively-breeding species of mongoose which live in groups
95 of two to 50 animals in dry regions of southern Africa [15]. A social group consists of a
96 dominant female and a male, which are the parents of the majority of pups born in the
97 group [16] and both juvenile and adult subordinate helpers of both sexes, which
98 participate in cooperative behaviours such as vigilance, babysitting and feeding the pups

99 [16,17]. Their diet consists primarily of invertebrates and small vertebrates, which are
100 extracted from the ground in intensive bouts of digging in sand [17]. Glucocorticoids have
101 been linked in wild meerkats to behaviours which are important to a social species,
102 including babysitting, pup feeding, dispersal away from the group by males, and repression
103 of reproduction in subordinate females, vigilance, and response to alarm call playbacks
104 [8,12-14,18,19].

105 An important consideration for the welfare of social animals is group size. Is there an
106 optimum group size to minimise physiological stress? In the wild, meerkats which are on
107 their own, such as evicted females or roving males, have a much higher level of faecal
108 glucocorticoid metabolites (fGCs) than those within a group, probably because they are
109 vulnerable to predators [12,13]. In larger groups, increasing group size brings increased
110 protection from predators, but may also lead to increased conflict over resources and
111 reproduction. Young [20] found fGCs to decrease with group size in relatively small (1-10,
112 median group size = 3) dispersing coalitions of same-sex individuals, which may reflect the
113 antipredator benefits of grouping. On the other hand, Santema [21] found that in stable
114 groups, that also tend to be larger (2-32, median = 15), fGC concentrations increase with
115 increasing group size, suggestive of competitive costs of large group size. These results
116 suggest that group size may have complex relationship with measures of physiological
117 stress, depending on the social context (dispersing versus resident groups), as well as the
118 range of group sizes under investigation.

119 In this study we investigate patterns of physiological stress in captive meerkats using non-
120 invasive faecal sampling of 10 zoo groups. Specifically, we test (1) whether there are
121 consistent differences in fGC between dominant and subordinate individuals, and between
122 sexes; (2) how features of the captive environment, such as group size, enclosure size,
123 season, and population density, affect fGC levels; (3) what is the relationship between
124 physiological stress and number of visitors. We compare the patterns of fGC in captive
125 meerkats with those observed in the wild, and discuss the factors that may affect fGC in
126 these environments.

127

128

129 **Materials and Methods**

130 **Faecal sample collection**

131 We collected 140 faecal samples from meerkats living in 10 different social groups at eight
132 zoos in England between May 2011 and January 2013 (see Table 1). Forty-eight of these
133 samples, mostly of unknown origin, were collected daily from 4 zoo groups in summer
134 2011, with a further 21 samples collected from the same groups the following winter. In
135 addition, we collected 71 samples (40 in summer 2012; 31 in winter 2012) from known
136 individuals in 6 social groups using a glitter-feeding technique described in [22]. Briefly, a
137 small quantity of food taken from the animal's daily diet was coated in very fine embossing
138 glitter. Each piece of food was sprinkled with glitter of a particular colour, and given to a
139 different meerkat, identified either visually or from its microchip. We observed the
140 meerkats to check that the target individual consumed the food, and if not either removed
141 the food item or identified the individual that ate it. Based on stress hormone releasing
142 ACTH challenge tests and water injections carried out in individual meerkats [22], the lag
143 time for faecal GC excretion as measured by our corticosterone assay (CCST) ranged
144 between 3 and 33 hours with an average (i.e. median) time lag of 22 hours; faecal samples
145 were collected during the following 36 hours and the presence and colour of the glitter
146 they contained identified on site before freezing. Time from deposition to sample
147 collection was not recorded accurately, but it varied from a few minutes to a maximum of
148 three hours, with most samples being collected within half an hour from deposition.
149 Samples were stored at -70°C for between five and 87 weeks before being transferred,
150 frozen, to the Endocrinology Laboratory at the German Primate Centre in Göttingen for
151 hormone analysis. We were not able to test for storage effects in this dataset, but it has
152 been demonstrated that storing neat faecal samples at -20°C stabilizes faecal
153 glucocorticoid concentrations for up to 2 years in elephants and grizzly bears [23],
154 indicating that simple freezing reliably preserves faecal glucocorticoids long-term in these
155 and presumably also in other vertebrate species.

156 [Table 1]

157 Faecal glucocorticoid metabolite (fGC) concentrations were analysed using a corticosterone
158 enzyme immunoassay (CCST EIA) which used the same antibody that has been used in the
159 CCST radioimmunoassay (RIA) system validated for monitoring physiological stress
160 responses in wild meerkats by Young et al. [13]. Similar to the CCST RIA, the EIA has been
161 proven to be valid for assessing adrenocortical activity in meerkats in response to both
162 physiological and biological stimuli [22]. Extraction was performed following previously
163 described methodologies [24]. Samples were freeze dried at -20°C, then pulverised and
164 sieved to remove coarse material. At this stage, sample quality was estimated by noting
165 obvious physical qualities of the samples, such as the presence of large quantities of fur or
166 feathers in the faeces (which was thought to result from the animals having been fed
167 chicks the previous day), or substantial amounts of sand coating the sample, due to the
168 substrate from which the faeces were collected. As much extraneous sand was removed as
169 possible. Between 0.0900g and 0.1100g of each sample was weighed out and the weight
170 recorded to four decimal places. 3ml of 80% methanol was added to each sample, then
171 they were shaken for 10min in a vortex and centrifuged at 3000rpm for 10min. 2ml of
172 supernatant from each sample was decanted into an Eppendorf tube and stored at -20°C
173 until measured for glucocorticoid concentration.

174 Prior to assay, faecal extracts were diluted 1:10 (except 3 samples that were diluted 1:3
175 and 1 sample that was diluted 1:100) in assay buffer (0.04M PBS, pH 7.2) and duplicate
176 50µl aliquots were measured on microtiterplates along with 50µl aliquots of reference
177 standard in doubling dilutions over the range of 1.9-125pg as described elsewhere [25].
178 Sensitivity of the assay was 1.9pg. Specificity data (cross-reactivities) of the assay are
179 reported in [25]. Intra-assay coefficients of variation for low and high value quality controls
180 were 5.9% (n=16) and 7.9% (n=16), respectively. Respective figures for inter-assay CV
181 values were 8.1% (n=10) and 11.4%. (n=10). All fGC levels reported are expressed as ng/g
182 dry faecal mass.

183

184 **Visitor numbers**

185 Data on visitors were recorded on the day the samples were collected as well as the day
186 before. The number of people within 1m of the meerkat enclosure was counted every 2
187 minutes during each 20-minute observation session, and there were on average 7.7
188 observation sessions (1-11) each day, amounting to 2.6 hours of observations per
189 enclosure per collection day on average. This distance was chosen to distinguish visitors
190 that had their attention on the meerkats from visitors that were just passing by. The
191 median and standard deviation of the number of people observed on the day were
192 calculated, as well as on the day before, to be included as predictors in the statistical
193 analyses (see below).

194

195 **Statistical analysis**

196 Data were analysed using Generalized Linear Mixed Models (GLMM) with REML estimation
197 of random effects, as implemented in the package lme4 [26] in R 3.0.1 [27]. Although in
198 most cases all meerkats in a zoo belonged to the same group, two zoos had multiple
199 groups of meerkats that were housed in separate enclosures, see Table 1. Therefore, we
200 used meerkat group ID rather than zoo ID as a random factor in the analyses, to account
201 for multiple sampling among group members as well as for other possible differences
202 between groups in their genetic composition, and in their feeding and housing regimes.
203 Individual ID was also included as a random factor, to account for multiple sampling.
204 Sample condition as determined above (4-level factor: whether the sample contained large
205 amounts of sand, feathers or fur, both, or neither) was also included as a fixed effect in all
206 analyses, to account for its the potential effect on determination of fGC's. The fGC data
207 were ln-transformed prior to analysis to normalise errors and meet the assumptions of
208 parametric tests. As available data varied across samples and zoos, analyses were
209 conducted separately for individual and group level factors, to maximise the sample size in
210 each analysis as detailed below.

211 First, for the 59 samples for which individual identity was known, we investigated the
212 effect of sex, dominance status (dominant or subordinate), age (in years), reproductive
213 status of the group (pups present in the group or not) and sample condition on faecal

214 glucocorticoid metabolites, including individual and group as random factors in a mixed-
215 effects model (GLMM). Dominance was determined from zoo records of breeding patterns
216 (only dominant meerkats breed) and confirmed by behavioural observation. A known
217 problem in analysing fGC's is that sex differences in the absolute detected levels of fGC's
218 can arise because of differences in the proportion of GC metabolites excreted via the faecal
219 and urinary route, as well owing to differences in the actual metabolites that are formed
220 from cortisol/corticosterone (e.g. [28-30]). We can exclude the possibility that our CCST
221 assay picks up different metabolites in males versus females, based on an earlier study on
222 meerkats (see [22]), and to allow for different baseline of fGC between the sexes owing to
223 differences in excretion routes, we included all two-way interactions between sex and the
224 other variables in the initial analysis. To account for the possibility that status of the
225 individual affects its response to the presence of pups, a two-way interaction between
226 dominance and presence of pups was also included in the model.

227 Second, we pooled the data from 140 samples from known and unknown individuals to
228 investigate the influence of group level factors on fGC's. Specifically, we tested the effect of
229 group size, the total available outdoor space (m^2), indoor space (m^2), density (group size
230 divided by the enclosure size, in m^2), the season (summer or winter), reproductive status of
231 the group (pups present or not), and the two-way interactions between group size and
232 enclosure characteristics (see table 3). Sample condition was controlled for and individual
233 ID, or a running ID code for the samples of unknown origin, as well as the group ID, were
234 included as a random effects in the analysis.

235 Third, the number of members of the public visiting an enclosure could directly have an
236 effect either on the animals' glucocorticoid levels, or it could be correlated with the size of
237 the meerkat social group, and so be driving group-size effects. We used the median and
238 standard deviation of visitor numbers at the meerkat enclosure, both on the day of sample
239 collection as well as the previous day, as predictors in a GLMM on 94 samples where visitor
240 data was available. Sample quality was also controlled for, and group and individual
241 identity fitted as random factors.

242 In all analyses, we first fitted all main effects and the two-way interactions where
243 considered biologically relevant (see above). Nonsignificant ($p > 0.05$) interactions were

244 removed, to allow significance testing of main terms included in the interaction, but the
245 models were not simplified further in order to avoid problems with model selection. We
246 report significance of terms in the main text, and the full analysis results including
247 parameter estimates for all terms included in the models in Tables 2-4.

248

249 **RESULTS**

250 Seventeen of the 140 samples were found to contain glucocorticoid levels below the assay
251 sensitivity threshold, and these were assigned the maximum level possible (the assay
252 detection limit; 2.28ng/g faeces for a sample weight of 0.1g and a dilution of 3). Note that
253 this is a very conservative approach to our data, as it leads to overestimation of levels in
254 samples with undetectably low concentrations of fGCs, thereby potentially reducing
255 variation in our data set. The fGC levels in the remaining 123 samples varied between 7.34
256 and 2299.80ng/g faeces, with a mean of 100.34ng/g faeces and a median of 58.37ng/g
257 faeces; this difference in averages was due to a single outlier which was five times greater
258 than the next highest value (marked in red on Figure 1).

259

260 **1. Do individual characteristics predict fGCs?**

261 The only significant predictor of fGCs was sample condition (GLMM: $\chi^2_3 = 8.33$, $p = 0.04$)
262 with samples containing neither fur or feathers, nor sand, showing the highest fGC
263 contents (Table 2). Neither the sex of the animal, its age, its dominance status, nor the
264 two-way interactions between these variables had statistically significant effects on fGC
265 levels.

266 [Table 2]

267

268 **2. Do group-level factors and characteristics of the enclosure predict fGCs?**

269 Meerkats in larger groups had lower fGC levels (GLMM: $\beta \pm SE = -0.06 \pm 0.03$, $\chi^2_1 = 4.68$, $p =$
270 0.030 ; Figure 1). Meerkat fGC levels also decreased with increasing size of both the indoors
271 and outdoors enclosure (GLMM: $\beta \pm SE = -0.50 \pm 0.11$, $\chi^2_1 = 16.6$, $p < 0.001$, and $-0.30 \pm$
272 0.12 , $\chi^2_1 = 6.71$, $p = 0.010$, respectively). Again, samples with no large amounts of sand, fur
273 nor feathers, showed highest fGC levels, and other tested factors had no effect. (Figure 1;
274 Table 3). Results were qualitatively the same if the outlier (marked in red on Figure 1) was
275 excluded from the analysis: group size, size of both the outdoor and indoor enclosures, and
276 sample quality were all negatively related to fGCs while other factors had no effect
277 (GLMM: group size: $\chi^2_1 = 4.29$, $p = 0.038$; outdoor space: $\chi^2_1 = 7.24$, $p = 0.007$; indoor space:
278 $\chi^2_1 = 16.7$, $p < 0.001$; condition of sample: $\chi^2_3 = 8.19$, $p = 0.042$; all other $p > 0.24$).

279 [Table 3]

280

281 **3. What is the relationship between fGC levels and visitor numbers?**

282 fGC levels increased with increasing median number of visitors observed on the previous
283 day ($\beta \pm SE = 0.22 \pm 0.08$, $\chi^2_1 = 11.0$, $p < 0.001$), and decreased with increasing variation
284 (SD) in the visitor numbers of the previous day ($\beta \pm SE = -0.34 \pm 0.11$, $\chi^2_1 = 12.1$, $p < 0.001$;
285 Figure 2). Group size was again negatively related to fGCs ($\beta \pm SE = -0.10 \pm 0.05$, $\chi^2_1 = 7.31$,
286 $p = 0.007$). Other factors had no significant effects (Table 4). Taken together, these effects
287 show that the highest levels of physiological stress were measured in animals that were
288 exposed to consistently high visitor numbers on the previous day, and lowest levels in
289 animals with low median numbers of visitors. Visitor numbers on the day on which the
290 sample was collected had no effect on fGC levels (Table 4).

291 [Table 4]

292 [Figure 2]

293

294 **Discussion**

295 We found that three main factors predicted levels of faecal glucocorticoids in zoo
296 meerkats: the size of their social group, size of the enclosure, and the number of visitors
297 that the animals were exposed to, on the day before the sample collection. Levels of fGCs
298 were higher in smaller groups, in groups with smaller enclosures, and in groups with
299 consistently high median number of visitors. For those samples where we could match
300 faecal samples to specific individuals, we found no effect of age, sex or dominance class on
301 levels of fGC, which contrasts with studies done in the wild, but matches the findings of
302 Braga Goncalves et al. for a captive population [22].

303 The observed negative relationship between group size and physiological stress in this
304 study matches the findings of Young [20] working on dispersing coalitions of wild meerkats,
305 but is contrary to the pattern found in stable, mixed sex groups by Santema [21]. In the
306 wild, there is likely to be an optimum group size that minimises physiological stress, as
307 large groups are likely to experience higher within-group competition for food, whereas
308 smaller groups face increased predation pressure [21]. Indeed, the median group size in
309 zoos included in this study was 7 (SD = 4.5), which would be exceptionally small for wild
310 meerkats in stable groups, and much closer to that seen in the dispersing coalitions in the
311 wild. In captivity, group size has little correlation with food provision, as larger groups are
312 fed proportionally more food, often by scatter-feeding, which reduces the ability of
313 dominant animals to monopolise a food source [31]. This supports the idea that food
314 limitation is likely to play a larger role in determining the levels of fGC's in the wild,
315 whereas its role in determining variation in stress levels in captivity is negligible.

316 Individuals living in smaller groups in the wild may exhibit greater physiological stress
317 because they are forced into less productive areas or subjected to greater predation, lower
318 food intake or a trade-off between vigilance and foraging [32-34]. In captivity, many of
319 these factors are not present, but the same pattern still emerges. It may be that, while in
320 zoos these actual threats are not present, there is an innate hormonal stress response to
321 being in a small group, which prepares individuals to counter these potential risks. Since in
322 captivity food provisions is generally as high or higher per animal in large groups, both
323 lower through-put and food-stress can be ruled out as causes of the group-size effect in

324 captive meerkats [31,35]. The perceived threat of attack from either conspecifics or
325 predators, however, may still affect zoo animals. In wild meerkats, high blood cortisol
326 levels have been linked to an increasing likelihood of performing sentry duty [36]. If a fear
327 of attack is greater when in a small group, it would be expected that each animal should
328 perform sentry duty more often, and that is what is observed [37]. This suggests that
329 potential lack of control associated with uncertainty about the risk of predation and/or
330 attack from other meerkats may be a driving force for the higher fGC observed in small
331 groups both in captivity and in the wild.

332 Unsurprisingly, meerkats in larger enclosures had lower levels of fGC's, irrespective of the
333 relative density of animals in the enclosure. In the wild, meerkat groups defend territories
334 which can be up to several square kilometres in size, whereas enclosure sizes in zoos
335 included in this study ranged from 34 to 300m². Meerkats need a large territory in the wild
336 to secure sufficient food for the group, need for which is reduced under captive feeding
337 regimes. Nevertheless, additional enclosure space may facilitate natural foraging
338 behaviours that reduce stress, and it may also help individuals avoid or alleviate conflict,
339 for example by allowing subordinate individuals to physically escape aggression from
340 dominants (e.g. [13]). Other aspects of the housing environment, such as habitat
341 enrichment or the animals' ability to hide, often correlate with enclosure size, and
342 experimental approach would be needed in order to conclude whether these, rather than
343 the additional space per se, account for the lower physiological stress levels of meerkats in
344 larger enclosures. Complex and interacting effects of housing conditions in captivity have
345 been found in other species. For instance, pileated Gibbons (*Hylobates pileatus*) kept in
346 larger enclosures and less exposed to visitors had lower levels of fGC's [38], whereas
347 captive orangutans in groups that followed a natural fission-fusion dynamic were less
348 affected by increases in visitor numbers, than animals kept in unnaturally large, stable
349 groups [39].

350 Visitor numbers also predicted fGC's in meerkats, and median number of visitors and the
351 standard deviation had opposite effects. The lowest fGC levels were seen in animals that
352 had been exposed to a low number of visitors, while the highest fGC occurred when
353 meerkats had consistently high numbers of visitors throughout the day. It is not surprising

354 that the presence of fewer people most of the time results in a lower glucocorticoid level,
355 as a stressful effect of visitors is seen in other species [5,39,40]. However, this does
356 contradict a previous finding [37] that zoo meerkats exhibit lower levels of vigilance
357 behaviour when there are more people present. As the highest peaks in visitor numbers
358 often co-occur with feeding, this could lessen the impact on meerkats by drawing the
359 attention of the animals away from the crowd at the enclosure. Unfortunately, data on
360 behaviour of the meerkats was not available for this study, so we are unable to confirm
361 whether changes in visitor numbers were associated with behavioural changes. However,
362 our results suggest that experiencing high constant numbers of visitors would be most
363 stressful to meerkats, whereas occasional peaks may matter less; detailed investigation of
364 behavioural patterns associated with these changes would be useful in order to determine
365 causality and to draw inferences on how to best minimise the impact on meerkat welfare.

366
367 In conclusion, the size of social group, size of the enclosure, and the presence of visitors
368 appear to be the most important factors in determining the physiological stress levels in
369 captive meerkats. Meerkats in large groups had lower levels of faecal glucocorticoids (fGC)
370 probably due to a higher level of perceived predation and inter-group conflict risk inherent
371 to small groups in the wild. Unlike in studies done on wild meerkats, the age, sex and
372 dominance status of animals did not predict fGC concentrations, which may reflect
373 differences in determinants of physiological stress in captive versus wild animals. In
374 captivity, individuals are likely to experience stable nutritional status irrespective of their
375 reproductive or dominance status, which is unlikely to be true in the wild, where animals
376 particularly in larger groups face intense competition over food. The results reported here
377 suggest that the meerkats most at risk of unusually high and potentially detrimental levels
378 of stress hormones are those kept in small social groups and small enclosures, with
379 constantly high median numbers of visitors. From a husbandry policy viewpoint, although it
380 is often not possible to control the number of visitors, zoos should be aiming to keep
381 meerkats in larger groups and enclosures if they intend to minimise levels of physiological
382 stress.

383

384
385

Ethics statement

386 Glitter-feeding and faecal sample collection were approved in advance by the ethics
387 committee of each zoo before research began. The research was passed by the Research
388 Committee of the British and Irish Association of Zoos and Aquaria. No further ethical
389 approval was required as this was not an invasive procedure and caused no suffering or
390 harm, either permanent or temporary, to any animal.

391

Data accessibility

393 Supporting data are available on Figshare, DOI: [10.6084/m9.figshare.4665094](https://doi.org/10.6084/m9.figshare.4665094)
394

395

Competing interests

397 Authors have no competing interests.
398

Authors' contributions

400

401 MC and KS conceived the study; KS carried out sample collection; KS and EV analysed the
402 data; MH carried out the fGC analyses; EV, KS and MC drafted the manuscript with input
403 from MH. All authors gave final approval for publication.

404

Acknowledgments

406 The zoos from which samples were collected for this study were Blackpool Zoo, Bristol Zoo,
407 Cotswold Wildlife Park, Dartmoor Zoo, Longleat Safari Park, Newquay Zoo, Paignton Zoo
408 and Shaldon Wildlife Trust, and we are very grateful for their support and cooperation. We
409 would like to thank Andrea Heistermann and Tanja Wolf for their friendliness, support and
410 patient teaching during the visit of Katy Scott to the Heistermann lab. Marta Manser and
411 Ines Braga Gonçalves were always very helpful in our communications about glitter-feeding

412 methodologies. Peter Santema and Andrew Young have both been generous in their
413 discussion of the differences between their measurements of fGC concentrations in wild
414 meerkats and our captive results. We are grateful to Steffen Foerster and two anonymous
415 reviewers for their insightful comments, which greatly improved this manuscript. Finally,
416 many thanks to the Society for Endocrinology for providing the funding which made this
417 work possible.

418 419 **Funding**

420 Funding was provided by a European Social Fund studentship and a Society for
421 Endocrinology Grant awarded to KS, and by NERC Grant awarded to MC (NE/J010278/1).

422 423 **References**

- 424
- 425 1. Creel, S. 2001 Social dominance and stress hormones. *Trends in Ecology & Evolution* **16**, 491–
426 497. (doi:10.1016/S0169-5347(01)02227-3)
 - 427 2. Busch, D. S. & Hayward, L. S. 2009 Stress in a conservation context: A discussion of
428 glucocorticoid actions and how levels change with conservation-relevant variables. *Biological*
429 *Conservation* **142**, 2844–2853. (doi:10.1016/j.biocon.2009.08.013)
 - 430 3. Melfi, V. A. 2009 There are big gaps in our knowledge, and thus approach, to zoo animal
431 welfare: a case for evidence-based zoo animal management. *Zoo Biol.* **28**, 574–588.
432 (doi:10.1002/zoo.20288)
 - 433 4. Stevens, J., Thyssen, A., Laevens, H. & Vervaecke, H. 2013 The influence of zoo visitor
434 numbers on the behaviour of harbour seals (*Phoca vitulina*). *Journal of Zoo and Aquarium*
435 *Research* **1**, 31–34. (doi:10.19227/jzar.v1i1.20)
 - 436 5. Davey, G. 2007 Visitors' Effects on the Welfare of Animals in the Zoo: A Review. *Journal of*
437 *Applied Animal Welfare Science* **10**, 169–183. (doi:10.1080/10888700701313595)
 - 438 6. Luebke, J. F., Watters, J. V., Packer, J., Miller, L. J. & Powell, D. M. 2016 Zoo Visitors' Affective
439 Responses to Observing Animal Behaviors. *Visitor Studies* **19**, 60–76.
440 (doi:10.1080/10645578.2016.1144028)
 - 441 7. Schwarzenberger, F. 2007 The many uses of non-invasive faecal steroid monitoring in zoo and
442 wildlife species. *International Zoo Yearbook* **41**, 52–74. (doi:10.1111/j.1748-
443 1090.2007.00017.x)
 - 444 8. Santema, P., Teitel, Z., Manser, M., Bennett, N. & Clutton-Brock, T. 2013 Effects of cortisol
445 administration on cooperative behavior in meerkat helpers. *Behavioral Ecology* **24**, art039–

- 446 1127. (doi:10.1093/beheco/art039)
- 447 9. Young, K. M., Walker, S. L., Lanthier, C., Waddell, W. T., Monfort, S. L. & Brown, J. L. 2004
448 Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid
449 analyses. *General and Comparative Endocrinology* **137**, 148–165.
450 (doi:10.1016/j.ygcen.2004.02.016)
- 451 10. Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R. & Boonstra, R. 2011 Measuring stress in
452 wildlife: techniques for quantifying glucocorticoids. *Oecologia* **166**, 869–887.
453 (doi:10.1007/s00442-011-1943-y)
- 454 11. Novak, M. A., Hamel, A. F., Kelly, B. J., Dettmer, A. M. & Meyer, J. S. 2013 Stress, the HPA axis,
455 and nonhuman primate well-being: A review. *Appl. Anim. Behav. Sci.* **143**, 135–149.
456 (doi:10.1016/j.applanim.2012.10.012)
- 457 12. Young, A. J. & Monfort, S. L. 2009 Stress and the costs of extra-territorial movement in a
458 social carnivore. *Biology Letters* **5**, 439–441. (doi:10.1098/rsbl.2009.0032)
- 459 13. Young, A. J., Carlson, A. A., Monfort, S. L., Russell, A. F., Bennett, N. C. & Clutton-Brock, T.
460 2006 Stress and the suppression of subordinate reproduction in cooperatively breeding
461 meerkats. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12005–12010. (doi:10.1073/pnas.0510038103)
- 462 14. Carlson, A. A., Manser, M. B., Young, A. J., Russell, A. F., Jordan, N. R., McNeilly, A. S. &
463 Clutton-Brock, T. 2006 Cortisol levels are positively associated with pup-feeding rates in male
464 meerkats. *Proceedings of the Royal Society B: Biological Sciences* **273**, 571–577.
465 (doi:10.1098/rspb.2005.3087)
- 466 15. Clutton-Brock, T. H., Hodge, S. J. & Flower, T. P. 2008 Group size and the suppression of
467 subordinate reproduction in Kalahari meerkats. *Animal Behaviour* **76**, 689–700.
468 (doi:10.1016/j.anbehav.2008.03.015)
- 469 16. Hodge, S. J., Manica, A., Flower, T. P. & Clutton-Brock, T. H. 2008 Determinants of
470 reproductive success in dominant female meerkats. *Journal of Animal Ecology* **77**, 92–102.
471 (doi:10.1111/j.1365-2656.2007.01318.x)
- 472 17. Brotherton, P. N. M., Clutton-Brock, T. H., O'Riain, M. J., Gaynor, D., Sharpe, L., Kansky, R. &
473 McIlrath, G. M. 2001 Offspring food allocation by parents and helpers in a cooperative
474 mammal. *Behavioral Ecology* **12**, 590–599. (doi:10.1093/beheco/12.5.590)
- 475 18. Voellmy, I. K., Goncalves, I. B., Barrette, M.-F., Monfort, S. L. & Manser, M. B. 2014 Mean
476 fecal glucocorticoid metabolites are associated with vigilance, whereas immediate cortisol
477 levels better reflect acute anti-predator responses in meerkats. *Hormones and Behavior* **66**,
478 759–765. (doi:10.1016/j.yhbeh.2014.08.008)
- 479 19. Carlson, A. A., Russell, A. F., Young, A. J., Jordan, N. R., McNeilly, A. S., Parlow, A. F. & Clutton-
480 Brock, T. 2006 Elevated prolactin levels immediately precede decisions to babysit by male
481 meerkat helpers. *Hormones and Behavior* **50**, 94–100. (doi:10.1016/j.yhbeh.2006.01.009)
- 482 20. Young, A. J. 2004 Subordinate tactics in cooperative meerkats: helping, breeding and
483 dispersal. PhD Thesis, University of Cambridge.
- 484 21. Santema, P. 2013 Conflict, cooperation and cortisol in meerkats. PhD Thesis, University of
485 Cambridge.

486

- 487 22. Braga Goncalves, I., Heistermann, M., Santema, P., Dantzer, B., Mausbach, J., Ganswindt, A. &
488 Manser, M. B. 2016 Validation of a Fecal Glucocorticoid Assay to Assess Adrenocortical
489 Activity in Meerkats Using Physiological and Biological Stimuli. *PLoS One* **11**, e0153161.
490 (doi:10.1371/journal.pone.0153161)
- 491 23. Hunt, K. E. & Wasser, S. K. 2003 Effect of Long-Term Preservation Methods on Fecal
492 Glucocorticoid Concentrations of Grizzly Bear and African Elephant. *Physiol Biochem Zool* **76**,
493 918–928. (doi:10.1086/380209)
- 494 24. Heistermann, M., Ademmer, C. & Kaumanns, W. 2004 Ovarian Cycle and Effect of Social
495 Changes on Adrenal and Ovarian Function in *Pygathrix nemaeus*. *International Journal of*
496 *Primatology* **25**, 689–708. (doi:10.1023/B:IJOP.0000023581.17889.0f)
- 497 25. Heistermann, M., Palme, R. & Ganswindt, A. 2006 Comparison of different
498 enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal
499 analysis. *Am J Primatol* **68**, 257–273. (doi:10.1002/ajp.20222)
- 500 26. Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015 Fitting Linear Mixed-Effects Models Using
501 lme4. *J. Stat. Soft.* **67**. (doi:10.18637/jss.v067.i01)
- 502 27. R Core Team 2014 R: A language and environment for statistical computing. *R Foundation for*
503 *Statistical Computing, Vienna, Austria*, www.R-project.org.
- 504 28. Goymann W. 2012 On the use of non-invasive hormone research in uncontrolled, natural
505 environments: the problem with sex, diet, metabolic rate and the individual. *Methods Ecol.*
506 *Evol.* **3**: 757–765. (doi:10.1111/j.2041-210X.2012.00203.x)
- 507 29. Sipari S., Ylonen H. & Palme R. 2016 Excretion and measurement of corticosterone and
508 testosterone metabolites in bank voles (*Myodes glareolus*). *General and Comparative*
509 *Endocrinology*. **243**: 39–50. (doi: 10.1016/j.ygcen.2016.10.015)
- 510 30. Touma C., Sachser N., Mostl E. & Palme R. 2003 Effects of sex and time of day on metabolism
511 and excretion of corticosterone in urine and feces of mice. *General and Comparative*
512 *Endocrinology* **130**: 267–78. (doi: 10.1016/S0016-6480(02)00620-2)
- 513 31. Gutzmann, L. D., Hill, H. K. & Koutsos, E. A. 2009 Biochemical and physiological observations
514 in meerkats (*Suricata suricatta*) at two zoos during a dietary transition to a diet designed for
515 insectivores. *Zoo Biol.* **28**, 307–318. (doi:10.1002/zoo.20230)
- 516 32. Clutton-Brock, T. H. et al. 1998 Costs of cooperative behaviour in suricates (*Suricata*
517 *suricatta*). *Proceedings of the Royal Society of London B: Biological Sciences* **265**, 185–190.
518 (doi:10.1098/rspb.1998.0281)
- 519 33. Clutton-Brock, T. H., Gaynor, D., McIlrath, G. M., Maccoll, A. D. C., Kansky, R., Chadwick, P.,
520 Manser, M., Skinner, J. D. & Brotherton, P. N. M. 1999 Predation, group size and mortality in
521 a cooperative mongoose, *Suricata suricatta*. **68**, 672–683. (doi:10.1046/j.1365-
522 2656.1999.00317.x)
- 523 34. Clutton-Brock, T. H., O'Riain, M. J., Brotherton, P. N., Gaynor, D., Kansky, R., Griffin, A. S. &
524 Manser, M. 1999 Selfish sentinels in cooperative mammals. *Science* **284**, 1640–1644.
525 (doi:10.1126/science.284.5420.1640)

- 526 35. Kimble, K. 2003 Meerkat Studbook (*Suricata suricatta*) North American Region. *San Diego Zoo*
527 *Global Library*
- 528 36. Tatalovic, M. 2008 *Meerkat (Suricata suricatta) sentinel behaviour: variation in height and*
529 *contribution*. Master of Philosophy dissertation: University of Cambridge.
- 530 37. Scott, K. 2014 Behaviour and Endocrinology of Meerkats in Zoos. PhD Thesis, University of
531 Exeter.
- 532 38. Pirovino, M., Heistermann, M., Zimmermann, N., Zingg, R., Clauss, M., Codron, D., Kaup, F.-J.
533 & Steinmetz, H. W. 2011 Fecal Glucocorticoid Measurements and Their Relation to Rearing,
534 Behavior, and Environmental Factors in the Population of Pileated Gibbons (*Hylobates*
535 *pileatus*) Held in European Zoos. *International Journal of Primatology* **32**, 1161–1178.
536 (doi:10.1007/s10764-011-9532-9)
- 537 39. Amrein, M., Heistermann, M. & Weingrill, T. 2014 The Effect of Fission–Fusion Zoo Housing
538 on Hormonal and Behavioral Indicators of Stress in Bornean Orangutans (*Pongo pygmaeus*).
539 **35**, 509–528. (doi:10.1007/s10764-014-9765-5)
- 540 40. Hosey, G. R. 2000 Zoo animals and their human audiences: what is the visitor effect? *Animal*
541 *Welfare* **9**, 343–357.
- 542

543 Table captions:

544

545 Table 1. Sample sizes from the 8 zoos included in this study. For group size a range is shown
546 where the number of individuals within the group varied during the study; actual group size
547 for each sampling event is shown in Fig. 1.

548

549 Table 2. Full results from a GLMM analysis of fGCs in individually identified meerkat faeces.
550 Significant terms are denoted with an asterisk. For categorical variables, the parameter
551 estimate is given relative to the value in [brackets]. Non-significant interactions were
552 dropped from the model to allow significance testing of main terms included in the
553 interactions, but the model was not simplified further.

554

555 Table 3. Full results from a GLMM analysis of fGCs in all samples collected from both known
556 and unknown individuals, in relation to group-level variables. Significant terms are denoted
557 with an asterisk. For categorical variables, the parameter estimate is given relative to the
558 value in [brackets]. Non-significant interactions were dropped from the model to allow
559 significance testing of main terms included in the interactions, but the model was not
560 simplified further.

561

562 Table 4. Full results of a GLMM analysis of fGCs in all samples collected from both known
563 and unknown individuals, in relation to visitor numbers on previous and day of the sample
564 collection. Significant terms are denoted with an asterisk. For categorical variables, the
565 parameter estimate is given relative to the value in [brackets]. Non-significant interactions
566 were dropped from the model to allow significance testing of main terms included in the
567 interactions, but the model was not simplified further.

568

569

570 **Figure legends:**

571 Fig. 1. Faecal glucocorticoid levels decreased with increasing size of the social group. Dots
572 represent ln-transformed data and the line is the model prediction after correcting for
573 random effects of group and individual. The results were qualitatively the same when
574 omitting the outlier (in red). Sample size: N = 140 samples from 52 individuals.

575 Fig. 2. Faecal glucocorticoids increased with increasing median number of visitors at the
576 meerkat enclosure the previous day, and levels were higher when variation in visitor
577 numbers was lower. The line represents the model predictions for the effect of median
578 visitor number on fGC levels, with standard deviation of the visitor number held constant
579 (average SD = 4.73). For the purpose of illustration, observations with standard deviation
580 lower than the average are marked in red, and above this are marked in blue. Sample size:
581 N = 94 samples from 31 individuals.

582

583 Table 1.
584
585

Zoo / group	group size	number of females	no. sampled individuals	total N samples
Blackpool	2	1	2	5
Bristol	17	2 (7 of unknown sex)	9	13
Cotswold /1	10	4	9	15
Cotswold /2	3	0	2	4
Dartmoor	2-4	1	2	2
Longleat	14	6	7	10
Newquay	9-11	5	7	14
Paignton /1	2-4	2	5	35
Paignton /2	1-2	1	2	13
Shaldon	6-7	4	7	31
			52	140

586

587

588 Table 2.

589

Covariate	Parameter estimate ± SE	χ^2	d.f.	P
Sex:Age	0.002 ± 0.167	0.003	1	0.956
Sex:Dominance	0.956 ± 1.804	0.595	1	0.440
Sex:Pups	-1.644 ± 1.041	3.019	1	0.082
Sex:Sample condition [Both]	(0)	5.402	3	0.145
Dominance: Pups	0.187 ± 0.989	0.100	1	0.751
Sex [M]	0.456 ± 0.441	1.510	1	0.219
Pups [Yes]	-0.224 ± 0.600	0.076	1	0.783
Age	-0.040 ± 0.152	0.177	1	0.673
Dominance	-0.239 ± 0.749	0.263	1	0.608
Sample condition [Both]	(0)	8.331	3	0.040*
Fur / Feathers	-0.439 ± 1.169			
Sand	1.144 ± 1.053			
Neither	1.499 ± 0.945			
Random effects	Variance	SD	N	
Group	0.450	0.671	31	
Individual	0.098	0.312	10	10
Residual	1.654	1.286	59	59

590

591 Table 3.

592

Covariate	Parameter estimate ± SE	χ^2	d.f.	p
Group Size: Outdoor Space	0.100 ± 0.085	0.100	1	0.340
Group Size : Indoor Space	-0.089 ± 0.186	0.723	1	0.395
Group Size : Density	0.170 ± 2.678	0.203	1	0.653
Group Size	-0.056 ± 0.027	4.683	1	0.030*
Outdoor space	-0.304 ± 0.118	6.706	1	0.010*
Indoor Space	-0.502 ± 0.112	16.60	1	<0.001*
Density	0.001 ± 0.001	1.011	1	0.315
Season [Winter]	-0.079 ± 0.211	0.167	1	0.682
Pups [Yes]	-0.095 ± 0.222	0.149		0.699
Condition of Sample [Both]¶	(0)	7.752	3	0.051*
Feathers / fur	-0.465 ± 0.694			
sand	0.349 ± 0.692			
none	0.495 ± 0.663			
Random effects	Variance	SD	N levels	
ID	0.107	0.328	52	
Group	0.000	0.000	10	
Residual	1.040	1.020	140	

593

594 Table 4.

Factor / covariate	Parameter estimate ± SE	χ^2	d.f.	p
Group Size : Median Visitors Yesterday	0.028 ± 0.021	2.050	1	0.152
Group Size : SD Visitors Yesterday	-0.042 ± 0.033	1.734	1	0.187
Group Size Median Visitors Yesterday	-0.101 ± 0.049	7.312	1	0.007*
SD Visitors Yesterday	0.215 ± 0.078	11.04	1	<0.001*
Median Visitors Today	-0.343 ± 0.106	12.12	1	<0.001*
SD Visitors Today	-0.027 ± 0.067	0.046	1	0.830
Condition of Sample [Both]	0.069 ± 0.045	2.651	1	0.103
	(0)	0.237	3	0.971
Fur / feathers	0.059 ± 0.758	-		
Sand	0.377 ± 0.872	-		
Neither	0.102 ± 0.696	-		
Random effects	Variance	SD	N levels	
ID	0.096	0.309	31	
Group	0.182	0.427	10	
Residual	1.058	1.029	94	

595

596

597