

1  
2  
3  
4 **1 Is social dispersal stressful? A study in male crested macaques (*Macaca nigra*)**  
5  
6  
7  
8

9 4 Pascal R. Marty<sup>1,2,3</sup> \*, Keith Hodges<sup>2</sup>, Michael Heistermann<sup>4</sup>, Muhammad Agil<sup>5</sup>, Antje Engelhardt<sup>2,6</sup>  
10  
11  
12  
13  
14  
15  
16  
17

18 9 <sup>1</sup>Department of Population Health and Reproduction, University of California Davis, USA  
19

20 10 <sup>2</sup> Junior Research Group Primate Sexual Selection, German Primate Center, Göttingen, Germany  
21

22 11 <sup>3</sup> Reproductive Biology Unit, German Primate Center, Göttingen, Germany  
23

24 12 <sup>4</sup> Endocrinology Laboratory, German Primate Center, Göttingen, Germany  
25

26 13 <sup>5</sup> Faculty of Veterinary Medicine, Bogor Agriculture University, Indonesia  
27

28 14 <sup>6</sup> Faculty of Science, School of Natural Sciences and Psychology, Liverpool John Moores University, UK  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39

40 20 \* *Corresponding author:*

41 21 *Pascal R. Marty*

42 22 *University of California, Davis*

43 23 *1089 Veterinary Medicine Dr.*

44 24 *95161 Davis*

45 25 *USA*

46 26 *E-Mail: [pmarty@ucdavis.edu](mailto:pmarty@ucdavis.edu)*  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56

57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112

27 **Summary**

28 In gregarious species, dispersal events represent one of the most dramatic changes in social life and  
29 environment an animal will experience during life due to increased predation risk, aggression from  
30 unfamiliar conspecifics and the lack of social support. However, little is known about how individuals  
31 respond physiologically to dispersal and whether this process is stressful for the individuals involved. We  
32 therefore studied the physiological stress response during dispersal in the crested macaque, a primate  
33 species in which males often change groups. Over a period of 14 months and 14 dispersal events in 4  
34 groups, we determined faecal glucocorticoid metabolite (FGCM) levels during the process of immigration  
35 into a new group and examined a variety of factors (e.g. male age, rank achieved, number of males in the  
36 group) potentially affecting FGCM levels during this process. We found that FGCM levels were  
37 significantly elevated in the first few days upon immigration, after which levels returned quickly to  
38 baseline. FGCM response levels upon immigration were significantly and positively influenced by the  
39 number of males in the group. The rank a male achieved upon immigration, aggression received, as well  
40 as the proximity to other males did not significantly influence FGCM levels. Our data confirm previous  
41 findings on other species demonstrating that in crested macaques immigration into a new social group is  
42 associated with an acute endocrine stress response. However, given that stress hormone levels remained  
43 elevated only for a short period of time, we do not expect males to experience high physiological costs  
44 during immigration. Given our limited knowledge on the physiological responses to dispersal in animals,  
45 this study contributes to our understanding of dispersal more generally, and particularly inter-individual  
46 differences in the stress response and the potential physiological costs associated with these.

## 60 Introduction

61 In gregarious animals, certain individuals leave their natal group as a consequence of selective pressures  
62 from within-group competition and inbreeding avoidance (Clutton-Brock and Lukas, 2012; Crnokrak and  
63 Roff, 1999; Henzi and Lucas, 1980; Pusey, 1987). The dispersal of an individual from one social unit to  
64 another is likely to represent one of the most fundamental shifts in social life and environment in an  
65 animal's life (Smale et al., 1997). Dispersal is often accompanied by substantial benefits such as access to  
66 unrelated females but may also come with a number of costs to the individual involved. For instance, in  
67 comparison to philopatric individuals, conspecifics transferring from one group or territory to another  
68 often face increased predation risk and restricted access to known food resources (e.g. Alberts and  
69 Altmann, 1995; Pärt, 1995; Ridley et al., 2008). Furthermore, upon arrival in a new social group,  
70 dispersing individuals may face an elevation in aggression (Teichroeb et al., 2011; Ydenberg et al., 1988),  
71 the need to establish a permanent residency and dominance rank, and unfamiliar competitors – which  
72 require them to adapt their behaviour accordingly (Smale et al., 1997). Dispersal from a social group and  
73 immigration into a new group represent therefore potentially highly stressful and costly life-phases for the  
74 dispersing individual. To date, however, information on the physiological response and potential costs of  
75 such dispersal events and, in particular, how stressful the different phases (i.e. transition, immigration,  
76 membership in new group) associated with dispersal events are, is scarce. Such information, however,  
77 would be important to better understand the implications of this event on the health, survival and  
78 consequently the fitness of individuals.

79         Generally, as an adaptation to cope with the new environment, increased predation risk, and  
80 social challenges during the transition period and upon immigration, dispersing individuals can be  
81 expected to show a physiological stress response, i.e. an activation of the hypothalmo-pituitary-adrenal  
82 (HPA) axis and the secretion of increased levels of stress hormone (i.e. glucocorticoid; GC) (e.g.  
83 Sapolsky, 2002). This stress response enables the mobilization of energy for immediate use (e.g. 'flight or  
84 fight')(Sapolsky, 2002). Enhanced cognition, analgesia, and sensory function, as well as decreased pain  
85 perception are further adaptive consequences of an acute physiological stress response increasing the  
86 chances to overcome stressful and life-threatening situations (Nelson, 2005; Sapolsky, 1992). A direct  
87 link between a physiological stress response and such situations (e.g. increased predation risk, food  
88 scarcity) has been shown in diverse taxa (e.g. amphibians: Narayan et al., 2013; mammals: Sheriff et al.,  
89 2009). Studies investigating the direct link between time spent outside a social group and its effect on GC  
90 levels are, however, scarce. To our knowledge, the only study to examine this relationship, showed that  
91 subordinate male meerkats (*Suricata suricatta*) show increased stress hormone levels when conducting  
92 extra-territorial forays (Young and Monfort, 2009). Elevated GC levels upon immigration into a new

169  
170  
171 93 social group, the second important phase individuals face during dispersal, has been reported for a  
172  
173 94 number of primate species (long-tailed macaques (*Macaca fascicularis*): van Schaik et al., 1991; yellow  
174 95 baboons (*Papio cynocephalus*): Alberts et al., 1992; chacma baboons (*Papio ursinus*): Bergman et al.,  
175 96 2005; gray-cheeked mangabeys (*Lophocebus albigena*): Arlet et al., 2009; but see black howler monkeys  
177 97 (*Alouatta pigra*): van Belle et al., 2009) and thus seems to be a more common pattern, at least in this  
178  
179 98 taxon.

180 99 However, the duration of these elevations is mostly unknown despite its potential implication on  
181  
182 100 an individual's health and fitness. Whereas a short term (i.e. acute) increase in GCs is adaptive and allows  
183 101 an individual to better cope with a stressful situation, long-term or frequent activation of the HPA axis  
184 102 may lead to chronically elevated GC levels that can lead to physiological costs and thus compromise  
185 103 fitness due to its diverse negative effects on health (e.g. immunosuppression, decreased growth, impaired  
187 104 reproduction), potentially even leading to death (Sapolsky, 1992; Balm, 1999; Nelson, 2005). As such,  
189 105 individuals that are better able to downregulate their physiological stress response experience diminished  
190 106 health and fitness impairments. If a stress response is purely adaptive, a rise in GC levels is expected to be  
191  
192 107 followed by a quick return to baseline levels.

193 108 Although current data on the stress response following dispersal is extremely limited due to the  
194  
195 109 difficulty to predict the timing of dispersal and immigration, we expect that both intrinsic factors (e.g.  
196 110 fighting ability/rank achieved) and extrinsic factors (e.g. number of males in the new group, aggression  
197 111 received) will predict inter-individual stress responses for the dispersing males. In primates, males are the  
199 112 dispersing sex in the majority of species, and newly-arriving individuals of high fighting ability often  
200 113 challenge resident males to achieve a high rank in the new group, often at the expense of high risks such  
201 114 as severe injuries (Marty et al., 2016; Drews 1995). Immigrants with comparably lower fighting ability  
202 115 often only achieve a lower rank in the new group and develop strategies with which to circumvent  
203 116 contest, in this way reducing the potential costs of immigration (Clarke et al., 2008; Marty et al., 2016).  
206 117 Given these differences in immigration costs, males of different fighting ability can be expected to also  
208 118 differ in their stress hormone responses upon immigration.

209 119 Once arrived in a new group, a successful competition for mates does not only depend on  
210 120 intrinsic factors such as fighting ability but also on the number of competitors. We assume that the  
212 121 number of competitors and therefore the degree of male-male competition may have an influence on the  
213 122 males FGCM levels. FGCM levels are expected to be positively associated with the number of  
215 123 competitors in the group. Alternatively, males may circumvent competition by avoiding proximity to  
216 124 other males and becoming peripheral (Harcourt 1987).

218 125 To investigate individual stress levels during dispersal and immigration, we studied crested  
219 126 macaques (*Macaca nigra*). Crested macaques are an excellent model species as males are known to

225  
226  
227 127 disperse from one group to another several times in their life (Marty et al., 2016). This species has a high  
228  
229 128 reproductive skew leading to high male-male competition and a steep linear hierarchy (Neumann et al.,  
230 129 2011; Reed et al., 1997)(Engelhardt et al. under review). Reproduction in crested macaques is moderately  
231  
232 130 seasonal whereby females can give birth year round. Crested macaques are endemic to the island of  
233 131 Sulawesi (Indonesia) and show a social system typical for cercopithecines with groups consisting of  
234  
235 132 several males and females. As in most primates, females are philopatric whereas males disperse after  
236 133 reaching their physical prime (Marty et al., in press). Predation risk during the transition between groups  
237  
238 134 seems to be low (Marty et al., 2016). Immigrations into a new group are non-random and many males are  
239 135 observed to immigrate into a group around the same time as other males. Males who achieve a high rank  
240 136 upon immigration (i.e. high fighting ability) are mainly young adult males dispersing from their natal  
241  
242 137 group for the first time, immigrate independent of other males, are more likely to get injured, and can  
243  
244 138 expect high future reproductive success. Males who achieve a low rank upon immigration (low fighting  
245 139 ability) align their immigration to a recent change in the alpha male position (exclusively conducted from  
246 140 new immigrants) (Marty et al., in press).

248 141 The overall aim of this study was to investigate the pattern of stress hormone output shown by  
249 142 dispersing males of a gregarious primate, and, by doing so, to examine whether dispersal events are  
250  
251 143 associated with long-term physiological costs to these animals. We also investigated the effect of a variety  
252 144 of factors potentially influencing stress hormone output during immigration. Generally, we posed the  
253  
254 145 following predictions: for solitary living males in the transition between two social groups (transient  
255 146 males), we predict that (1) they will show higher FGCM levels than individuals living in a group (resident  
256  
257 147 males). Directly upon immigration, we predict (2) elevated FGCM levels in the immigrants. However,  
258 148 due to differences in a males' physical condition and the target group constellation, inter-individual  
259  
260 149 differences in the magnitude of the physiological stress response are expected. Specifically, we therefore  
261 150 predict (3) high rank achievers who usually challenge the top rank position upon immigration (Marty et  
262  
263 151 al, 2016.) to initially show higher FGCM levels than males who achieve a low rank. Regarding male-male  
264 152 competition, we expect (4) males immigrating into groups with comparably more competitors, and males  
265 153 that spend more time in the vicinity of such males upon immigration (5) to experience higher FGCM  
266  
267 154 levels.

268 155

## 270 156 **Methods**

272 157

### 273 158 **Study subjects and study site**

274  
275 159 Four groups of wild crested macaques were studied from November 2011 until January 2013 (15  
276 160 months) in the Tangkoko Reserve in North Sulawesi, Indonesia (1°33'N, 125°10'E) as part of an on-  
277  
278  
279  
280

161 going long term project (Macaca Nigra Project, [www.macaca-nigra.org](http://www.macaca-nigra.org)). The reserve ranges from sea  
 162 level to 1350m and comprises 8867 ha of lowland rainforest (Collins et al.1991; Rosenbaum et al.1998).  
 163 The groups live in a mixture of regenerating former gardens, secondary forest, and undisturbed primary  
 164 forest. Two of the observed groups (R1 and R2) have been periodically studied during the last ten years  
 165 (O'Brien and Kinnaird, 1997; Reed et al., 1997) and continuously since 2006 (e.g. Neumann et al., 2010),  
 166 whereas the two other groups (PB and R3) were habituated in 2008 and 2010, respectively (for more  
 167 details see Marty et al. 2016). All individuals were fully habituated to the presence of human observers  
 168 and individually known by the observers. All dispersing males were fully adult and none of the males  
 169 included in this study were considered to be of old age (i.e. worn down canines, skinny body and slower  
 170 movement).

### 172 Behaviour data collection

173 Upon the arrival of a new male in a group, 12 new immigrating males were followed all day,  
 174 from one sleeping tree to the next sleeping tree. Two of these males immigrated twice into different  
 175 groups (Table 1). Whenever possible, these males were followed on a daily basis for the first 14 days.  
 176 Focal data were collected using all occurrence event sampling of all agonistic and affiliative behaviours  
 177 with group members, as well as scan sampling every 5 minutes recording position, nearest neighbours,  
 178 and activity (Altmann, 1974). The number of adult males in proximity was measured using the data on  
 179 males within 10m which was collected every 5 min during a focal observation. A daily average of adult  
 180 males within 10m was used for further analyses. All interactions between the focal animal and other  
 181 group members were entered into handheld computers (Psion Workabout Pro G2) using spread-sheet  
 182 software (PTab Spreadsheet v.3.0; Z4Soft). In addition, data on four solitarily roaming males was  
 183 collected while following them for a total of 111 focal hours. Overall, behavioural data was collected  
 184 during more than 2300 focal hours.

186 Table 1: Study male ID, number of observed immigrations, ID of the group the males immigrated into,  
 187 rank achieved upon immigration, and number of samples within the first 14 days following immigration.  
 188

ID	Group	Rank	no of samples
BN	R1	0.07	10
OL	PB	1.00	15
QL	PB	1.00	4
UL	PB	0.78	19
AN	R1	0.00	5
JL	PB	0.00	16

JM	PB	1.00	21
LL	R1	0.63	18
PL	R3	0.33	29
CN	R3	0.00	26
NL *	PB	1.00	2
NL**	R3	1.00	17
FL *	PB	0.11	21
FL**	R2	0.00	14

\* = first observed dispersal, \*\* = second observed dispersal  
 Rank: Standardized rank between zero and one, 0.00 represents the highest rank, 1.00 the lowest

### Determination of the dominance hierarchy

All displacements (approach/leave interactions) and agonistic dyadic aggressive interactions between males with a clear winner/loser outcome were considered in order to quantify dominance hierarchies. Depending on the available data, interactions within the first three to six months (depending on the group tenure of the males) after the immigration were included into analysis in order to obtain an accurate rank for the newly immigrated male. Dominance rank was assessed using corrected normalized David's score (de Vries et al., 2006), using the package "Steepness" (Leiva and de Vries, 2011) in R (R Development Core Team, 2009) based on a matrix of proportions of wins calculated for each dyad. All ranks were standardized between 0 and 1 with the lower number representing a higher rank (see also Marty et al. 2016).

### Sample collection

Urine-uncontaminated faecal samples were collected from transient males, newly immigrated males, and resident males. Samples from newly immigrated males were collected continuously for the first 14 days upon immigration into the new group. If possible, one sample was collected each in the morning, noon, and late afternoon. The freshly defecated faeces were homogenized before an aliquot of 2-3g was placed in a polypropylene tube (Hodges and Heistermann, 2011). Samples were directly stored in a cool box filled with ice until they were placed in a freezer (-18°C) after return to camp. Overall, 217 faecal samples from immigrating males within the first 14 days upon immigration were collected. An additional 187 faecal samples were collected from the immigrating males after the initial 14 days. Furthermore, 130 samples from eight non-natal resident males were collected during the study period for comparison. Eight samples from four transient males were also collected. The first fecal samples for these transient males were collected two days after these males were detected to account for the 1-2 day lag

393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448

217 time in fecal glucocorticoid metabolite excretion, thus ensuring the hormone levels represent days the  
218 male was not in a group.

219  
220 **Hormone analyses**

221 For analysis, all faecal samples were freeze-dried and pulverized and an aliquot of 0.05 – 0.08g of  
222 the faecal powder was extracted with 3ml of 80% methanol in water (Palme et al., 2013) as described in  
223 detail by Heistermann et al. (1995). Faecal extracts were analysed for immunoreactive 11 $\beta$ -  
224 hydroxyetiocholanolone, a major metabolite of cortisol in the feces of primates (e.g. Heistermann et al.,  
225 2006; Marty et al., 2015) by using enzyme immunoassay (Ganswindt et al., 2003). The assay, carried out  
226 as described in Heistermann et al. (2004), has been validated for monitoring adrenocortical activity in  
227 numerous primate species of all major taxa, including several species of macaques (Fichtel et al., 2007;  
228 Heistermann et al., 2006; Ostner et al., 2008; Shutt et al., 2012; Weingrill et al., 2011). The assay was  
229 recently also validated for use in crested macaques by confirming the presence of high amounts of 11 $\beta$ -  
230 hydroxyetiocholanolone in the feces of the species, and demonstrating a significant increase in levels in  
231 response to external stressors, such as injury, caught in a poachers trap or severe harassment by  
232 conspecifics (Gholib, 2011). Prior to each assay, extracts were diluted 100 to 3000 times (depending on  
233 concentration) with assay buffer to bring hormone concentrations into the working range of the assay.  
234 Sensitivity of the assays at 90% binding was 1.0 pg. Inter- and intra-assay coefficients of variation,  
235 determined by replicate measurements of high- and low-value quality controls, were 10.9% and 5.2%  
236 (high) and 14.7% and 8.1% (low), respectively. We ran each sample in duplicate and calculated mass  
237 steroid metabolite per mass fecal dried weight in ng/g.

238  
239 **Statistical analyses**

240 Samples collected at least one month after the immigration were used to calculate baseline FGCM  
241 levels. In order to compare the mean FGCM levels between the four transient and the eight resident  
242 males, a Mann-Whitney-U Test was conducted in R (2.15.2)(R Development Core Team 2009) using  
243 mean FGCM levels per individual. Baseline values of an additional six males who immigrated during the  
244 study period were added as resident males to increase the sample size. To investigate factors determining  
245 FGCM levels upon immigration, a generalized linear mixed model (GLMM) with Gaussian error  
246 distribution was carried out. For this, FGCM levels were all ln-transformed to meet assumptions of a  
247 normal distribution. Hormone values in the immigrating males were adjusted (i.e. shifted) for two days to  
248 account for the time lag in FGCM excretion (Gholib, 2011). Each sample entered the analysis as the  
249 response variable (N=217). The predictor variables were: 1. number of days after immigration, 2. number  
250 of males in proximity (10m), 3. rank achieved upon immigration, 4. number of males in the group, 5.



449  
450  
451 251 daily aggression rate received from other males. Rank upon immigration entered the model as a control  
452 variable. Two males changed group twice within the study period and were accordingly given a number  
453 252 for the immigration event (1 for the first immigration, 2 for the second immigration). This immigration  
454 253 number, as well as the group identity entered the model as random factors. ID was treated as a nested  
455 254 random effect within day to control for multiple samples per day and individual. The model was  
456 255 conducted using the package “lmer” in R.  
457 256

460 257 We checked various diagnostics of model validity and stability (Cook's distance, DFBetas,  
461 258 DFFits, and Variance Inflation Factors; distribution of residuals, residuals plotted against fitted values),  
462 259 and none of these indicated obvious influential cases, nor obvious deviations from the assumptions of  
463 260 normality and homogeneity of residuals (Field, 2005; Quinn and Keough, 2002). To obtain reliable p-  
464 261 values, a likelihood ratio test was used to compare the full model with respective reduced models using  
465 262 the function ‘drop1’ in the package ‘car’ (Chambers, 1992).

469 263 To compare FGCM levels of immigrating males with their respective baseline levels after the first  
470 264 four weeks following immigration, FGCM levels of each individual were averaged for the first 14 days  
471 265 following immigration and compared to the mean FGCM value recorded during the period 2-6 months  
472 266 after immigration using the Wilcoxon paired rank test was used. All significance levels were set at two-  
473 267 tailed p-values < 0.05.  
474 268

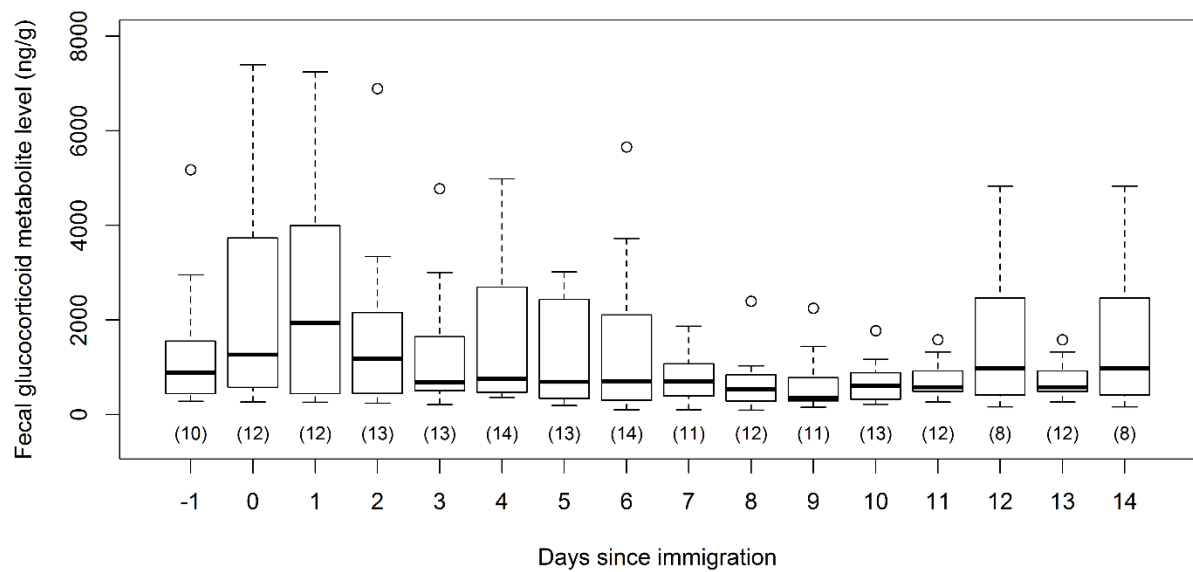
## 477 269 **Results**

480 270  
481 271 Although the transition period after departure from a group was not associated with elevated  
482 272 FGCMs for dispersing males, entry into a new group was. During the transition period, FGCM levels of  
483 273 the four dispersing males sampled did not significantly differ from those recorded for resident males  
484 274 (N=14) (Mann-Whitney-Test, U=33, p=0.32, n=18). Upon immigration, the daily mean FGCM levels of  
485 275 immigrating males showed a high variation within the first seven days, but did not differ from baseline  
486 276 values (assessed several weeks later, see above) in the second week after immigration (Wilcoxon rank  
487 277 sum test, W=51, P=0.24, N=24) (Figure 1).

491 278 In the GLMM investigating the factors that influence FGCM levels in response to immigration,  
492 279 the null model was significantly different from the full model ( $\chi^2=51.19$ ,  $df=12$ ,  $P<0.001$ , effect size  
493 280  $R^2=0.76$ ). The best predictors of FGCMs in immigrant males were time since arrival and number of other  
494 281 males in the group. Day after immigration was a significant predictor for FGCM levels in immigrating  
495 282 males (Table 2) with highest FGCM levels being recorded in the two days directly following immigration  
496 283 (Figure 1) and markedly declining levels thereafter. Mean FGCM levels were clearly less variable and  
497 284 consistently low following day 7. In addition, the number of males in the target group had a significant

505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560

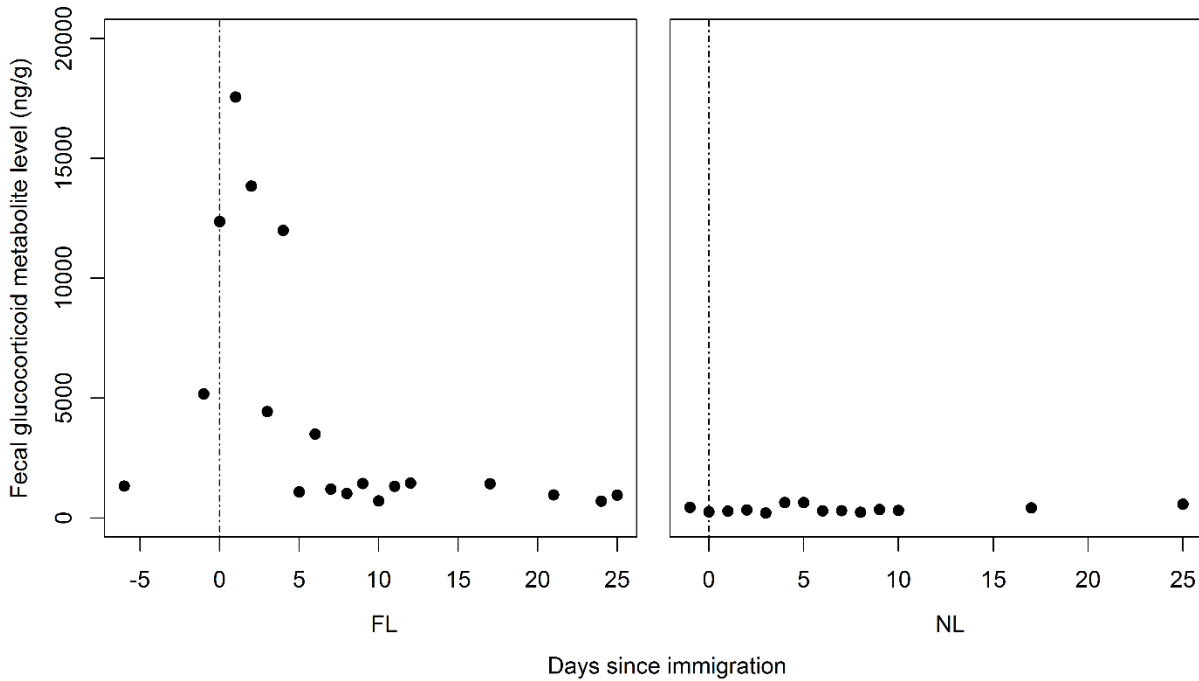
285 influence on the FGCM levels of the immigrating males. The higher the number of males in the target  
286 group, the higher were the immigrating males' FGCM levels (Table 2). The rank achieved upon  
287 immigration, as well as the proximity to other males did not significantly influence FGCM levels (Table  
288 2).



290  
291 Figure 1: Boxplot of FGCM levels of immigrating males within the first 14 days upon immigration (the  
292 lines represent the minimum/maximum whereas the rectangle represents data from the lower quartile to  
293 the upper quartile, outliers above 6000 ng/g were are not shown). Numbers in brackets represent the  
294 number of males' sampled on the given day. All hormone data have already been adjusted for the FGCM  
295 excretion time lag of two days.  
296

561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616

297



298

299 Figure 2: Visualization of the development of two males' (FL, NL) FGCM levels during and after the  
300 immigration process. Each point represents the mean daily FGCM. The line represents the time of  
301 immigration.

302

303

304 Table 2: Results of the GLMM testing the influence of the day after immigration, the aggression received  
305 from males, the rank achieved, and proximity to other males on the FGCM levels of immigrating males.

306

Model	Estimate	SE	t	P
Intercept	5.39	0.51	10.61	< 0.001
Day after immigration	-0.09	0.22	-4.00	<b>0.002</b>
Proximity to other males	-0.05	0.10	-0.52	0.604
Rank achieved	0.40	0.20	2.00	0.07
Number of males	0.20	0.04	5.62	< <b>0.001</b>
Aggression received	-0.01	1.53	0.00	0.996

307

308

309

## 310 Discussion

311

617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672

312 In the present study, we investigated in wild crested macaques the extent to which the process of  
313 male dispersal and immigration into a new group elicits a physiological stress response (i.e. increased  
314 HPA axis activity as measured by faecal glucocorticoid (FGCM) levels) in the dispersing individuals. We  
315 also, more specifically, examined the relative importance of several factors on a male's glucocorticoid  
316 excretion upon immigration. Opposite to what we expected, there was no indication that males show  
317 elevated FGCM levels during the transition between groups. Males demonstrated, however, elevated  
318 FGCM levels during the first few days after immigration into the new group, indicating that this critical  
319 phase of the dispersal process elicits a physiological stress response. However, the period of elevated  
320 FGCM levels was relatively short even in newly immigrating males, with FGCM levels dropping to  
321 baseline already in the second week after immigration. Inter-individual variation of FGCM levels upon  
322 immigration was mainly modulated by the number of potential competitors in the new group. A high  
323 number of males in the target group was associated with comparably higher FGCM levels, suggesting a  
324 more marked stress response in the immigrating individuals under such conditions.

325 The combination of the absence of social partners, an increased risk of being attacked by  
326 conspecifics and predators, and life in an unknown environment is usually considered to represent a  
327 stressful situation for a dispersing individual (Smale et al., 1997; Young and Monfort, 2009).  
328 Interestingly, in our study species, dispersing individuals during the transition phase of their dispersal did  
329 not show increased FGCM levels compared to resident males. The lack of elevated FGCM levels in our  
330 roaming males may be ascribed to the small samples size that limits statistical power; thus the results  
331 need to be treated with caution. Alternatively, however, the relatively low FGCM levels in our roaming  
332 males may be related to the low predation pressure that macaques face on Sulawesi. The risk of predation  
333 in crested macaques may in principle be low for both group living animals as well as for those roaming  
334 alone. If so, our limited results suggest that the primary stressor for male crested macaques in this  
335 population may be social in nature rather than ecological (i.e., predation). In addition, aggression from  
336 conspecifics towards strangers is common also in dispersing male crested macaques and severe injuries  
337 inflicted by attacks on solitary males have been observed (personal observation). The four roaming males  
338 followed in this study, however, did not face such potentially stressful and harmful attacks during the  
339 observation time, which may add to the reasons for why FGCM levels were not elevated in these  
340 individuals. Whether the lack of encounters with other potentially threatening males was due to active  
341 avoidance strategies of the dispersing males, or just a matter of chance, remains unclear. In the few cases  
342 where we observed attacks towards solitary males, these occurred only when transient males approached  
343 a group for immigration. These attacks may therefore be part of the immigration process rather than of the  
344 transition period. However, as already mentioned, our sample size here is inherently small and results thus  
345 need to be confirmed, ideally by studying dispersing individuals prior to and during the dispersal process.

673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728

346 This will be an extremely challenging task though as the timing of dispersal is not predictable and  
347 following roaming males for extended periods of time is inherently difficult to do.

348 As expected, and corroborating findings for other primate species (Alberts et al., 1992; Arlet et  
349 al., 2009; Bergman et al., 2005), FGCM levels of male crested macaques were significantly elevated upon  
350 immigration indicating that this critical step of dispersal represents a stressful event also for our study  
351 species. Excreting stress hormones during a challenging period is clearly adaptive as it enables the  
352 mobilization of energy for immediate use (e.g., the ‘flight or fight’ response) (Sapolsky, 2002),  
353 presumably to cope with a new uncertain environment with potential challengers/competitors.  
354 Competition is often associated with elevated levels of physiological stress, whereby individuals do not  
355 only show elevated stress levels during, but already prior to the competitive event (e.g. *Macaca*  
356 *fascicularis*: Girard-Buttez et al. 2009; humans: Aubets and Segura, 1995). However, our results also  
357 show that the elevation in stress hormone output associated with immigration is not maladaptive as the  
358 increase only lasted a few days at most, helping the individual to cope with the energetic challenges  
359 associated with this process but not leading it into allostatic overload (McEwen 1998). Thus, detrimental  
360 and long-lasting effects on an individual’s health as seen under chronic conditions of stress (e.g.  
361 Sapolsky, 2002) are unlikely to occur in male crested macaques as a consequence of dispersal, at least not  
362 if immigrations are successful as they all were in our study. In recent years, it has been debated whether  
363 or not animals suffer from chronic stress in a wild environment at all or if our knowledge is too much  
364 influenced by biomedical research on captive animals (e.g. Boonstra, 2013; Wingfield and Ramenofsky,  
365 2011). Research on fitness consequences of prolonged or chronic physiological stress in a natural setting  
366 is very limited in comparison to the overwhelming literature in biomedical research (Boonstra 2013). It  
367 has, however, been shown that the risk of extended periods of elevated stress hormone levels might affect  
368 an individual’s behavioural strategy leading it to withdraw from a challenge (Girard-Buttoz et al. 2014).  
369 Our finding that dispersing between groups does not pose a long-term stressor to male crested macaques  
370 does not only show the absence of a maladaptive stress response but might also facilitate male dispersals  
371 in this species and thus explain the many immigration events we observed even for males entering at the  
372 lower end of the hierarchy.

373 On an individual level, our results show that differences in the magnitude of the stress response  
374 are linked to the number of potential challengers/competitors in the target group. Males who immigrated  
375 into groups with a higher number of adult males showed comparably higher FGCM levels than males  
376 who immigrated into groups with fewer potential competitors. Interestingly, not males who are actually at  
377 the highest risk of getting injured (high rank achievers) but males with the highest potential for being  
378 attacked (number of competitors) show higher FGCM levels. Even though immigration into a group with  
379 a comparable high number of males elicits a stronger physiological stress response, male crested

729  
730  
731 380 macaques do not preferentially immigrate into groups with a lower number of competitors (Marty et al.,  
732 381 2016). This supports our assumption that the stress response upon immigration is purely adaptive with no  
733 382 negative consequences on the males' fitness. The stress response probably simply prepares the individual  
734 383 for upcoming potential or real challenges. The initial strong stress response may come along with rank  
735 384 uncertainties upon immigration and may return to homeostasis as soon as the potential of challenges  
736 385 declines with an increase in rank certainty. The higher the number of competitors in a group is, the longer  
737 386 it may take to consolidate the rank a male achieved.

741 387       Even though dispersal is often accompanied by cost in terms of injuries, predation, or starvation  
742 388 (e.g. Alberts and Altmann, 1995; Cheney and Seyfarth, 1983; Pärt, 1995) and represents one of the most  
743 389 stressful periods in a male's life (Smale et al., 1997), physiological costs might be overestimated. Both on  
744 390 an individual and a population level, the stress responses we observed to immigration followed the pattern  
745 391 expected for an adaptive response to an acute stressor. Our results do not provide any evidence of chronic  
746 392 stress or a prolonged stress response. Individuals in the wild may have adapted to stressful situations such  
747 393 as dispersal and immigration over time by using strategies to reduce costs (Marty et al., 2016). Our results  
748 394 suggest that inter-individual differences in the stress response are likely to be adaptive due to varying  
749 395 external conditions during and upon immigration.

750 396 Overall, our study demonstrates that the physiological stress response to migration shown by male crested  
751 397 macaques is adaptive and does most likely not carry any physiological costs. Interestingly, we did not  
752 398 find any statistically significant individual differences in the males' stress response suggesting that  
753 399 immigration is similarly stressful in this species whether males try to achieve a high or a low rank. This  
754 400 might explain why dispersal in crested macaques is highly dynamic with males migrating even frequently  
755 401 when the prospect for reproductive benefits achieved in the new group is bad. Similar studies on other  
756 402 species are now needed to better understand in how far duration of and inter-individual differences in the  
757 403 physiological stress response influence a species' migration dynamic.

758 404

759 405

## 760 406 **ACKNOWLEDGEMENTS**

761 407

762 408       We thank the Indonesian State Ministry for Research and Technology (RISTEK), the Director  
763 409 General Department Kehutanan (PHKA), the Department Dalam Negeri, the local Government in North  
764 410 Sulawesi and BKSDA Manado for giving us the permission to conduct research in Indonesia. We are  
765 411 thankful to all assistants, in particular to Agung, Arief, Caitlin Hannah and Maura Tyrrell for their help in  
766 412 collecting data. We thank Ugiek, Stephan Lentey, Maria Panggur and Edith Sabara for administrative  
767 413 support in Indonesia. For helpful comments on the manuscript, we would like to thank the two

729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784

785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840

414 anonymous reviewers For her help in the laboratory we thank Andrea Heistermann. We thank the  
415 Leibnitz Association and the German Research Council (grant no. EN 719/2) for financial support.

416  
417

## 418 **References**

419

420 Adamo, S.A., Kovalko, I., Mosher, B., 2013. The behavioural effects of predator-induced stress responses  
421 in the cricket (*Gryllus texensis*): the upside of the stress response. *J. Exp. Biol.* 216, 4608–14.

422 Alberts, S.C., Altmann, J., 1995. Balancing costs and opportunities: dispersal in male baboons. *Am. Nat.*  
423 145, 279–306.

424 Alberts, S.C., Sapolsky, R.M., Altmann, J., 1992. Behavioral, endocrine, and immunological correlates of  
425 immigration by an aggressive male into a natural primate group. *Horm. Behav.* 26, 167–78.

426 Altmann, J., 1974. Observational study of behavior: sampling methods. *Behaviour* 49, 227–267.

427 Arlet, M., Grote, M., Molleman, F., 2009. Reproductive tactics influence cortisol levels in individual male  
428 gray-cheeked mangabeys (*Lophocebus albigena*). *Horm. Behav.*

429 Aubets, J., Segura, J., 1995. Salivary cortisol as a marker of competition related stress. *Sci. Sports* 10,  
430 149–154.

431 Balm, P.H., 1999. *Stress Physiology in Animals*. Sheffield Academic Press, Sheffield.

432 Bergman, T., Beehner, J., Cheney, D., Seyfarth, R., Whitten, P., 2005. Correlates of stress in free-ranging  
433 male chacma baboons, *Papio hamadryas ursinus*. *Anim. Behav.* 70, 703–713.

434 Boonstra, R., 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in  
435 nature. *Funct. Ecol.* 27, 11–23.

436 Chambers, J.M., 1992. Linear models, in: Chambers, J.M., Hastie, T.J. (Eds.), *Statistical Models in S*.  
437 Wadsworth & Brooks.

438 Cheney, D.L., Seyfarth, R.M., 1983. Nonrandom Dispersal in Free-Ranging Vervet Monkeys: Social and  
439 Genetic Consequences. *Am. Nat.* 122, 392.

440 Clarke, P.M.R., Henzi, S.P., Barrett, L., Rendall, D., 2008. On the road again: competitive effects and  
441 condition-dependent dispersal in male baboons. *Anim. Behav.* 76, 55–63.

442 Clutton-Brock, T., Lukas, D., 2012. The evolution of social philopatry and dispersal in female mammals.  
443 *Mol. Ecol.* 472–492.

444 Crnokrak, P., Roff, D.A., 1999. Inbreeding depression in the wild. *Heredity (Edinb)*. 83, 260–270.

445 de Vries, H., Stevens, J.M.G., Vervaecke, H., 2006. Measuring and testing the steepness of dominance  
446 hierarchies. *Anim. Behav.* 71, 585–592.

447 Drews, C., 1995. Context and patterns of injuries in free-ranging male baboons (*Papio cynocephalus*).  
448 *Behaviour* 133, 443–474.

449 Fichtel, C., Kraus, C., Ganswindt, A., Heistermann, M., 2007. Influence of reproductive season and rank  
450 on fecal glucocorticoid levels in free-ranging male Verreaux's sifakas (*Propithecus verreauxi*). *Horm.*  
451 *Behav.* 51, 640–8.

452 Field, A., 2005. *Discovering statistics using SPSS*. Sage Publications, London.

453 Ganswindt, A., Palme, R., Heistermann, M., Borragan, S., Hodges, J., 2003. Non-invasive assessment of  
454 adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to  
455 musth. *Gen. Comp. Endocrinol.* 134, 156–166.

456 Gholib, 2011. Non-invasive hormone monitoring: Faecal androgen and glucocorticoid in male crested  
457 macaques (*Macaca nigra*) in relation to seasonal and social factors. Master thesis, Bogor  
458 Agricultural University.

459 Girard-Buttoz C, Heistermann M, Krummel S, Engelhardt A. 2009. Seasonal and social influences on fecal

841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896

460 androgen and glucocorticoid excretion in wild male long-tailed macaques (*Macaca fascicularis*)  
461 Physiology & Behavior, 98 :168-175

462 Girard-Buttoz C, Heistermann M, Rahmi E, Agil M, Ahmad Fauzan P, Engelhardt A. 2014. Costs of mate-  
463 guarding in wild male long-tailed macaques (*Macaca fascicularis*): Physiological stress and  
464 aggression. Hormones and Behavior, 66 :637-648

465 Heistermann, M., Ademmer, C., Kaumanns, W., 2004. Ovarian cycle and effect of social changes on  
466 adrenal and ovarian function in *Pygathrix nemaeus*. Int. J. Primatol. 25, 689–708.

467 Heistermann, M., Finke, M., Hodges, J.K., 1995. Assessment of female reproductive status in captive-  
468 housed Hanuman langurs (*Presbytis entellus*) by measurement of urinary and fecal steroid  
469 excretion patterns. Am. J. Primatol. 37, 275–284.

470 Heistermann, M., Palme, R., Ganswindt, A., 2006. Comparison of different enzymeimmunoassays for  
471 assessment of adrenocortical activity in primates based on fecal analysis. Am. J. Primatol. 68, 257–  
472 273.

473 Henzi, S.P., Lucas, J.W., 1980. Observations on the Inter-Troop Movement of Adult Vervet Monkeys  
474 (*Cercopithecus aethiops*). Folia Primatol. 33, 220–235.

475 Hodges, J., Heistermann, M., 2011. Field endocrinology: monitoring hormonal changes in free-ranging  
476 primates, in: Field and Laboratory Methods in Primatology, A Practical Guide. Cambridge University  
477 Press, Cambridge, pp. 353–370.

478 Leiva, D., de Vries, H., 2011. Testing Steepness of Dominance Hierarchies.

479 Marty, P.R., Hodges, K., Agil, M., Engelhardt, A., 2016. Determinants of immigration strategies in male  
480 crested macaques (*Macaca nigra*). Sci. Rep. 6, 32028.

481 Marty, P.R., Hodges, K., Agil, M., Engelhardt, A., 2015. Alpha male replacements and delayed dispersal in  
482 crested macaques (*Macaca nigra*). Am. J. Primatol. doi: 10.1002/ajp.22448.

483 Marty, P.R., van Noordwijk, M.A., Heistermann, M., Willems, E.P., Dunkel, L.P., Cadilek, M., Agil, M.,  
484 Weingrill, T., 2015. Endocrinological correlates of male bimaturism in wild Bornean orangutans.  
485 Am. J. Primatol. 77, 1170–1178.

486 McEwen BS., 1998. Stress, adaptation, and disease. Allostasis and allostatic load.  
487 Ann N Y Acad Sci. 1;840:33-44

488 Narayan, E.J., Cockrem, J.F., Hero, J.-M., 2013. Sight of a predator induces a corticosterone stress  
489 response and generates fear in an amphibian. PLoS One 8, e73564.

490 Nelson, R., 2005. An introduction to behaviorial endocrinology. Sinauer Associates, Inc., Sunderland,  
491 MA.

492 Neumann, C., Assahad, G., Hammerschmidt, K., Perwitasari-Farajallah, D., Engelhardt, A., 2010. Loud  
493 calls in male crested macaques, *Macaca nigra*: a signal of dominance in a tolerant species. Anim.  
494 Behav. 79, 187–193.

495 Neumann, C., Duboscq, J., Dubuc, C., Ginting, A., Irwan, A.M., Agil, M., Widdig, A., Engelhardt, A., 2011.  
496 Assessing dominance hierarchies: validation and advantages of progressive evaluation with Elo-  
497 rating. Anim. Behav. 82, 911–921.

498 O'Brien, T.G., Kinnaird, M., 1997. Behavior, diet, and movements of the Sulawesi crested black macaque  
499 (*Macaca nigra*). Int. J. Primatol. 18, 321–351.

500 Ostner, J., Kappeler, P., Heistermann, M., 2008. Androgen and glucocorticoid levels reflect seasonally  
501 occurring social challenges in male redfronted lemurs (*Eulemur fulvus rufus*). Behav. Ecol.  
502 Sociobiol. 62, 627–638.

503 Palme, R., Touma, C., Arias, N., Dominchin, M., Lepschy, M., 2013. Steroid extraction: Get the best out of  
504 faecal samples. Wiener tierärztliche Monatszeitschrift 100, 238–246.

505 Pärt, T., 1995. The importance of local familiarity and search costs for age- and sex-biased philopatry in  
506 the collared flycatcher. Anim. Behav. 49, 1029–1038.

507 Pusey, A.E., 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. Trends Ecol.



897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952

508           Evol. 2, 295–9.

509   Quinn, G.P., Keough, M.J., 2002. Experimental Designs and Data Analysis for Biologists. Cambridge  
510           university press, Cambridge.

511   R Development Core Team, 2009. R: A language and environment for statistical computing.

512   Reed, C., O'Brien, T.G., Kinnaird, M.F., 1997. Male social behavior and dominance hierarchy in the  
513           Sulawesi crested black macaque (*Macaca nigra*). Int. J. Primatol. 18, 247–260.

514   Ridley, A.R., Raihani, N.J., Nelson-Flower, M.J., 2008. The cost of being alone: the fate of floaters in a  
515           population of cooperatively breeding pied babblers *Turdoides bicolor*. J. Avian Biol. 39, 389–392.

516   Sapolsky, R.M., 2002. Endocrinology of the stress-response, in: Becker, J., Breedlove, S., Crews, D.,  
517           McCarthy, M. (Eds.), Behavior Endocrinology. The MIT Press, Cambridge, pp. 409–450.

518   Sapolsky, R.M., 1992. Stress, the Aging Brain, and the Mechanisms of Neuron Death. MIT Press,  
519           Cambridge.

520   Sheriff, M.J., Krebs, C.J., Boonstra, R., 2009. The sensitive hare: sublethal effects of predator stress on  
521           reproduction in snowshoe hares. J. Anim. Ecol. 78, 1249–58.

522   Shutt, K., Setchell, J.M., Heistermann, M., 2012. Non-invasive monitoring of physiological stress in the  
523           Western lowland gorilla (*Gorilla gorilla gorilla*): validation of a fecal glucocorticoid assay and  
524           methods for practical application in the field. Gen. Comp. Endocrinol. 179, 167–77.

525   Slos, S., Stoks, R., 2008. Predation risk induces stress proteins and reduces antioxidant defense. Funct.  
526           Ecol. 22, 637–642.

527   Smale, L., Nunes, S., Holekamp, K., 1997. Sexually dimorphic dispersal in mammals: patterns, causes, and  
528           consequences. Adv. Study Behav. 26, 181–250.

529   Teichroeb, J. a., Wikberg, E.C., Sicotte, P., 2011. Dispersal in male ursine colobus monkeys (*Colobus*  
530           *vellerosus*): influence of age, rank and contact with other groups on dispersal decisions. Behaviour  
531           148, 765–793.

532   van Belle, S., Estrada, A., Ziegler, T.E., Strier, K.B., 2009. Social and hormonal mechanisms underlying  
533           male reproductive strategies in black howler monkeys (*Alouatta pigra*). Horm. Behav. 56, 355–63.

534   van Schaik, C.P., Van Noordwijk, M.A., Vanbragt, T., Blankenstein, M.A., 1991. A Pilot-Study of the Social  
535           Correlates of Levels of Urinary Cortisol, Prolactin, and Testosterone in Wild Long-Tailed Macaques  
536           (*Macaca fascicularis*). Primates 32, 345–356.

537   Weingrill, T., Willems, E.P., Zimmermann, N., Steinmetz, H., Heistermann, M., 2011. Species-specific  
538           patterns in fecal glucocorticoid and androgen levels in zoo-living orangutans (*Pongo* spp.). Gen.  
539           Comp. Endocrinol. 172, 446–57.

540   Wingfield, J.C., Ramenofsky, M., 2011. Hormone-Behavior Interrelationships of Birds in Response to  
541           Weather, in: Brockmann, H.J., Roper, T., Naguib, M., Mitani, J.C., Simmons, L.W. (Eds.), ADVANCES  
542           IN THE STUDY OF BEHAVIOR, VOL. 43. Academic Press, Burlington, pp. 93–188.

543   Ydenberg, R., Giraldeau, L., Falls, J., 1988. Neighbours, strangers, and the asymmetric war of attrition.  
544           Anim. Behav. 36, 343–347.

545   Young, A.J., Monfort, S.L., 2009. Stress and the costs of extra-territorial movement in a social carnivore.  
546           Biol. Lett. 5, 439–441.