biology letters

Biol. Lett. (2012) **8**, 537–539
doi:10.1098/rsbl.2012.0197
Published online 11 April 2012

Animal behaviour

Group size elicits specific physiological response in herbivores

Pablo Michelena[1,*], Marie-Hélène Pillot[2,3], Carole Henrion[2,3], Sylvain Toulet[2,3], Alain Boissy[4] and Richard Bon[2,3,*]

[1]*Unit of Social Ecology, CP 231, Université libre de Bruxelles (ULB), Brussels, Belgium*
[2]*UPS, Centre de Recherches sur la Cognition Animale, Université de Toulouse, Toulouse, France*
[3]*CNRS, Centre de Recherches sur la Cognition Animale, Toulouse, France*
[4]*INRA, UR1213 Herbivores, 63122 Saint-Genès Champanelle, France*
**Authors for correspondence (pablo.michelena@ulb.ac.be; rbon@cict.fr).*

With increasing group size, individuals commonly spend less time standing head-up (scanning) and more time feeding. In small groups, a higher predation risk is likely to increase stress, which will be reflected by behavioural and endocrine responses. However, without any predator cues, we ask how the predation risk is actually processed by animals as group size decreases. We hypothesize that group size on its own acts as a stressor. We studied undisturbed groups of sheep under controlled pasture conditions, and measured *in situ* the cortisol and vigilance responses of identified individuals in groups ranging from 2 to 100 sheep. Both vigilance and average cortisol concentration decreased as group size increased. However, the cortisol response varied markedly among individuals in small groups, resulting in a lack of correlation between cortisol and vigilance responses. Further experiments are required to explore the mechanisms that underlie both the decay and the convergence of individual stress in larger groups, and whether these mechanisms promote adaptive anti-predator responses.

Keywords: group size; stress; cortisol; vigilance; sheep

1. INTRODUCTION

Participating in large groups generally reduces the risk of predation for an individual [1]. This observation has generated an extended literature focused on the dilution of predation risks and enhanced predator detection as group size increases. Individuals in larger groups spend less time scanning their environment and more time feeding, while predator detection rate at the group level is maintained [2]. However, while predation risk is commonly assumed to drive scanning decisions, little is known about the mechanisms that regulate individual vigilance as group size varies.

There is considerable evidence that predation risk affects behavioural decisions [3]. For example, impalas (*Aepyceros melampus*) are more vigilant under high predation pressure than in low predation sites [4]. At a finer scale, impalas raise their head faster and chew less when experimentally exposed to recordings of lion roars compared with a control situation [5].

Physiological responses of wild animals to brief exposure to predation risk have also been documented [6]. Under life-challenging circumstances, a neuroendocrine response stimulated by a threatening event involves the sympathetic adrenal medullar system and the hypothalamo–pituitary–adrenal (HPA) axis. Glucocorticoids and other hormones in the HPA cascade initiate and orchestrate the emergency response, triggered within minutes to hours. Accordingly, the concentration of HPA axis hormones (i.e. cortisol) is used as an index of acute stress and any stimulus that causes such an increase is identified as a stressor [7]. However, a major question still remains: in the absence of predator cues, how is the risk of predation processed by animals as group size varies? We hypothesized that group size itself acts as a stress modulator. We therefore predicted that both physiological and behavioural stress responses should decrease with increasing group size, even in the absence of external stress stimuli. To test this hypothesis, we studied undisturbed groups of sheep under controlled pasture conditions, and measured *in situ* cortisol and vigilance responses of identified individuals within groups of differing size.

2. MATERIAL AND METHODS

Fieldwork was carried out in November–December 2008 at the Domaine du Merle (5.74° E, 48.50° N). From a flock of 1200 sheep, 200 unrelated 18-month-old Merino Arles females (mean body mass 41 ± 1 kg) were randomly selected and identified by a painted number. The experiments were conducted between 10.30 and 12.00 h in four 80 × 80 m adjacent pens, within a familiar native pasture. The pens were mutually visually isolated by a 1.2 m-high green polypropylene net.

Four groups of either 2, 3, 4, 8, 16 or 32 individuals were introduced into the pens, simultaneously selecting at random the four group sizes tested daily. Grazing pressure was quasi-balanced by a rotation of groups of different sizes in each plot. Because of adverse winter weather, not all planned replications were possible (nine with groups of 2 and 4, ten with groups of 8, 16 and 32 and eleven with groups of 3 sheep). Four replications were done with groups of 100 sheep prior the aforementioned ones.

The 12 sheep (cortisol-sheep) for which we extracted saliva were tested only once per group size. The behaviour of each cortisol-sheep was recorded for 1 h using four digital cameras (Canon EOS D50) from the top of an 8 m-high tower located at the centre of the set-up, taking one snapshot every second. In order to avoid recording atypical behaviour owing to the introduction event, only the last 30 min were analysed. The behaviour of sheep (grazing, walking, vigilant and others) was extracted every second using a custom software [8]. However, behaviour could not be determined in groups that were clumped, too far from the camera, or in groups of more than 16. We obtained 10 full sequences of behaviour in groups of 3 and 16, nine in groups of 2, eight in groups of 4 and six in groups of 8 sheep.

Within 10 min following the end of the tests, groups were moved back to a holding pen where saliva was collected using a swab (Hartmann). This 10-min period was short enough to extract cortisol secreted only during the test [7]. The saliva-moistened swabs were placed in a 10 ml polypropylene tube (Dominique Dutscher) and centrifuged (3000*g*, 4°C, 10 min). The extracted saliva samples ($n = 62$) were each placed in a 1.5 ml tube and stored at −20°C until radioimmunoassay [9].

Cortisol concentrations were log-transformed and the proportions of time devoted to grazing, walking and scanning by each cortisol-sheep were logit-transformed to meet normality assumptions. The effect of group size on both cortisol concentrations and activity budget was tested by running a mixed-effects linear model with group size as a fixed factor and sheep identity as a random factor.

3. RESULTS

Salivary cortisol concentrations decreased with increasing group size ($F_{1,48} = 6.28$, $p = 0.015$; figure 1*a*). Interestingly, this relationship results from the high sensitivity of some (10–40%) individuals tested in small groups (of up to eight sheep), whereas all individuals

Received 6 March 2012
Accepted 20 March 2012

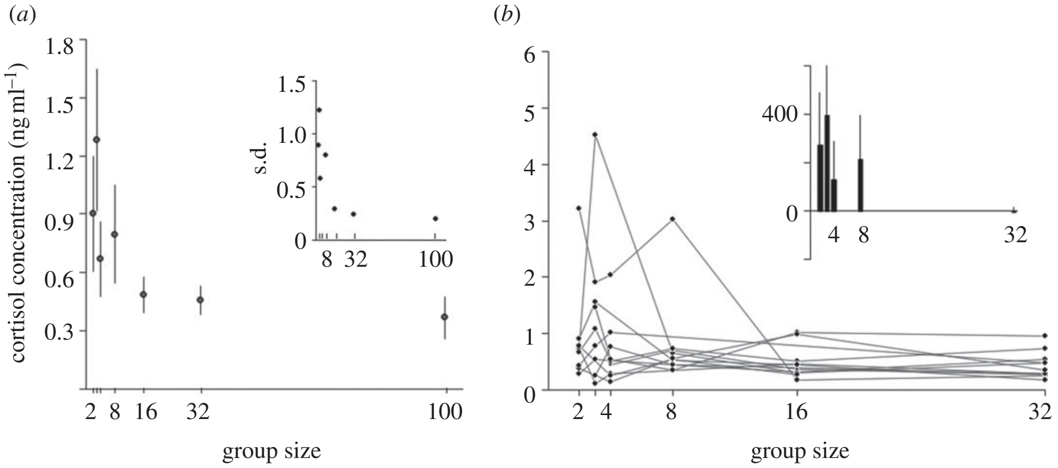

Figure 1. (*a*) Mean (±s.e.) saliva cortisol concentrations (ng ml^{-1}). The inset indicates the standard deviation of cortisol concentrations among individuals for each group size. (*b*) Individual sheep cortisol profiles. The lines link the concentrations of cortisol for each individual in each group size. The inset indicates the mean (+s.e.) per cent difference in cortisol concentrations for groups of 2, 3, 4, 8 and 32 sheep compared with groups of 16 used as a baseline.

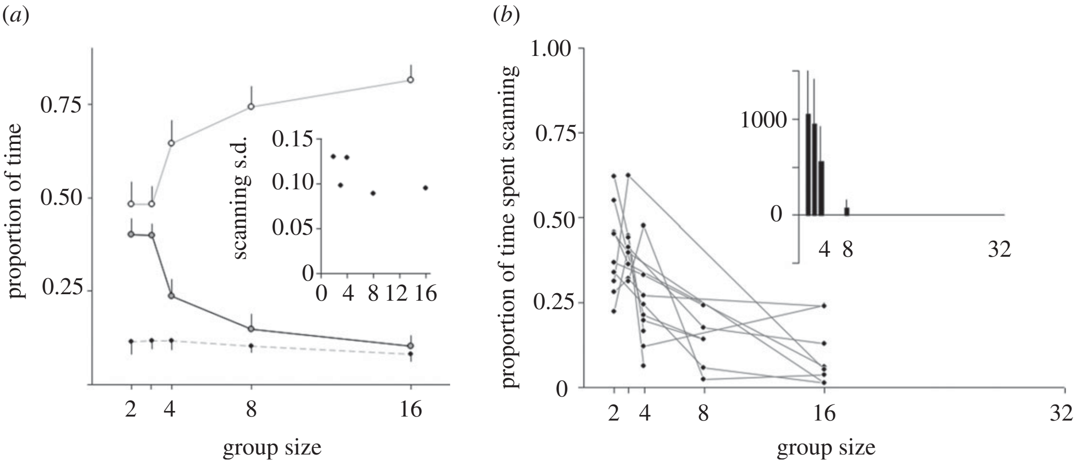

Figure 2. (*a*) Mean (±s.e.) proportion of time spent grazing, scanning and walking (top, middle and bottom traces, respectively) as a function of group size. The inset indicates the standard deviation among individuals of the proportion of time spent scanning for each group size. (*b*) Individual sheep scanning profiles. The lines link the proportion of time spent scanning for each individual tested in each group size. The inset indicates the mean (+s.e.) per cent difference in scanning activity for groups of 2, 3, 4 and 8 compared with groups of 16 sheep.

secreted less cortisol in larger groups (figure 1). Consequently, the variability of cortisol concentrations increases as group size decreases (Bartlett test: $K = 31.86$, d.f. $= 6$, $p < 0.001$; figure 1*a*). Figure 1*a* makes it possible to estimate a basal cortisol concentration per individual, using values obtained for group sizes of 16 or more. We found significantly higher levels of cortisol in groups of up to eight sheep when compared with groups of 16 sheep (Sign test: $p = 0.04$, $n = 22$; figure 1*b*).

Sheep spent more time grazing and less time scanning as group size increased ($F_{1,30} = 30.77$ and $F_{1,30} = 40.24$, respectively, both $p < 0.0001$), whereas the time spent walking remained constant ($F_{1,30} = 1.91$, $p = 0.18$; figure 2). The inter-individual variability of time spent scanning did not differ significantly among individuals for group sizes ranging from 2 to 16 (Bartlett's test: $K = 1.71$, d.f. $= 4$, $p = 0.79$; figure 2*b*).

In order to examine the links between behavioural and physiological responses, we used the individual responses for scanning rate and cortisol concentration measured in groups of 16 sheep as a baseline and then calculated the per cent difference for smaller groups, which provided a standardized assessment of departure from basal levels. We found no significant correlation in the magnitude of this departure between time spent scanning and cortisol concentration (Spearman test: $\rho = 0.4$, $p = 0.066$, $n = 22$).

4. DISCUSSION

Our experimental design allowed us to test whether individual behaviour and physiology respond to variation in group size in the absence of external stress stimuli. The observed group size-specific responses, despite the absence of any predation risk, indicate that the behavioural and the endocrine modifications reflect a marked influence of the social environment.

Clearly, individual vigilance decreases (while time spent foraging increases) with increasing group size, in agreement with the group size effect observed in birds and ungulates [2,10–11]. Secondly, cortisol was secreted in higher quantities and more variably among sheep in small than in larger groups. A marked modification occurred between groups of up to eight and larger groups, presumably reflecting lower stress in large groups.

Previous studies showed that the presence of conspecifics or social cues reduced stress responses in several species [12]. Our results show that stress attenuation also depends on the number of conspecifics [13]. One might assume that higher cortisol levels underlie higher levels of stress and arousal, and thus could facilitate both the acuity of the detection of predators and glucose available for escape behaviour in small groups [7]. Whether all individuals respond in the same way to group size remains unclear. On the one hand, higher inter-individual variability of cortisol levels in small groups suggests that some individuals are more sensitive to small group sizes than others [14], as already shown in kangaroo vigilance [15]. On the other hand, a collective amplification of stress and/or appeasement through interactions between group members is plausible.

Unexpectedly, group size-specific variation in cortisol concentration and vigilance levels did not correlate. Most individuals displayed moderate variation in cortisol concentrations when tested in different group sizes, whereas vigilance level was highly sensitive to varying group size. Further behavioural analyses are needed to improve our understanding of how individuals cope with stressful social situations, and to clarify the links between scanning and stress-physiology when controlling sheep identity and social factors.

This study provides evidence that decreasing group size itself probably acts as a stress modulator. Because of the higher predation risk when in small groups, this stress response might in turn facilitate adaptive responses such as arousal and preparative actions for escape behaviour in case of predator attacks [16]. However, the lack of correlation between individual cortisol concentrations and scanning suggests that the regulation of scanning as group size varies emerges instead from finer social interactions [17]. Furthermore, our results are consistent with the 'social buffering' effect on relief from stress and open a new avenue on how the presence of conspecifics modulates stress reactions, but also question the influence of idiosyncratic responses on stress at the group level. Additional experiments are required to explore the mechanisms that underlie the decline and convergence of individual stress levels in large groups and whether such effects impact anti-predator responses.

Animal care and experimental manipulations complied with the rules of the French committee of animal experimentation ethics. We thank P.M. Bouquet for experimental facilities, J.D. Guyonneau and P. Maingot for technical help and M. Hewison for English revision. S. Andanson and C. Ravel performed the physiological analyses. This research was supported by the PANURGE project (ANR-BLAN07-3_200418) and a grant from the GDR Ethologie 2822.

1 Krause, J. & Ruxton, G. D. 2002 *Living in groups*. Oxford, UK: Oxford University Press.
2 Roberts, G. 1996 Why individual vigilance declines as group size increases. *Anim. Behav.* **51**, 1077–1086. (doi:10.1006/anbe.1996.0109)
3 Lima, S. L. & Dill, L. M. 1990 Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619–640. (doi:10.1139/z90-092)
4 Hunter, L. T. B. & Skinner, J. D. 1998 Vigilance in African ungulates: the role of predation pressure. *Behaviour* **135**, 195–211. (doi:10.1163/156853998793066320)
5 Blanchard, P. & Fritz, H. 2007 Induced or routine vigilance while foraging. *Oikos* **116**, 1603–1608. (doi:10.1111/j.0030-1299.2007.15799.x)
6 Hawlena, D. & Schmitz, O. J. 2010 Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *Am. Nat.* **176**, 537–556. (doi:10.1086/656495)
7 Mormède, P. *et al.* 2007 Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. *Physiol. Behav.* **92**, 317–339. (doi:10.1016/j.physbeh.2006.12.003)
8 Michelena, P., Noël, S., Gautrais, J., Gerard, J.-F., Deneubourg, J.-L. & Bon, R. 2006 Sexual dimorphism, activity budget and synchrony in groups of sheep. *Oecologia* **148**, 170–180. (doi:10.1007/s00442-005-0347-2)
9 Boissy, A. & Bouissou, M. F. 1994 Effects of androgen treatment on behavioral and physiological responses of heifers to fear-eliciting situations. *Horm. Behav.* **28**, 66–83. (doi:10.1006/hbeh.1994.1006)
10 Risenhoover, K. L. & Bailey, K. A. 1985 Relationship between group size, feeding time, and agonistic behavior of mountain goats. *Can. J. Zool.* **63**, 2501–2506. (doi:10.1139/z85-370)
11 Beauchamp, G. 2008 What is the magnitude of the group-size effect on vigilance? *Behav. Ecol.* **19**, 1361–1368. (doi:10.1093/beheco/arn096)
12 Kikusui, T., Winslow, J. T. & Mori, Y. 2006 Social buffering: relief from stress and anxiety. *Phil. Trans. R. Soc. B* **361**, 2215–2228. (doi:10.1098/rstb.2006.1941)
13 Takeda, K., Sato, S. & Sugawara, K. 2003 Familiarity and group size affect emotional stress in Japanese Black heifers. *Appl. Anim. Behav. Sci.* **82**, 1–11. (doi:10.1016/S0168-1591(03)00039-X)
14 Michelena, P., Sibbald, A. M., Erhard, H. W. & McLeod, J. E. 2009 Effects of group size and personality on social foraging: the distribution of sheep across patches. *Behav. Ecol.* **20**, 145–152. (doi:10.1093/beheco/arn126)
15 Carter, A. J., Pays, O. & Goldizen, A. W. 2009 Individual variation in the relationship between vigilance and group size in eastern grey kangaroos. *Behav. Ecol. Sociobiol.* **64**, 237–245. (doi:10.1007/s00265-009-0840-4)
16 Sapolsky, R. M., Romero, L. M. & Munck, A. U. 2000 How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* **21**, 55–89. (doi:10.1210/er.21.1.55)
17 Michelena, P. & Deneubourg, J.-L. 2011 How group size affects vigilance dynamics and time allocation patterns: the key role of imitation and tempo. *PloS ONE* **6**, e18631. (doi:10.1371/journal.pone.0018631)