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Stress-hormone levels of wolves in relation to breeding season, pack size, human activity, and prey density

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Human disturbance is thought to be a major source of stress for animals but breeding status, social interactions and food availability are also potential sources. Long-lasting stress may adversely affect the fitness of animals and for that reason the evaluation of stressors is important for conservation of threatened species. The aim of our study was therefore to assess which factors cause stress in wolves (*Canis lupus*). We evaluated the stress levels of wolves from six packs by measuring the concentration of glucocorticoid metabolites in 59 faecal samples with a cortisol enzyme-immunoassay. During the breeding season, stress hormone concentration was higher than during the rest of the year, with two peaks around mating and begin of denning, respectively. Multiple regressions ranked by AIC showed that breeding had the highest impact on the wolves' stress levels, followed by human activity, pack size, and prey density. We conclude that human activity is only one of several factors contributing to stress in wolves and that intraspecific competition during breeding is likely to cause elevated levels of glucocorticoids.

Introduction

Centuries of wolf (*Canis lupus*) persecution caused wolves to avoid humans (e.g. Thurber *et al*. 1994, Theuerkauf *et al*. 2003a, 2003b), but human presence does not necessarily mean that it negatively impacts wolves. The long history of wolf persecution has evolutionarily favoured wolves that avoided humans but, at the same time, has forced them to adapt to live and breed in close proximity to them. This mixture of avoidance and habituation seems to be the basis of wolf-human coexistence in areas where wolves occupy habitats with relatively high human activity (Theuerkauf *et al*. 2007). In such situations, it is difficult to discriminate which kind of human activity actually reduces fitness of wolves and can as such be regarded as a disturbance.

An elevated level of glucocorticoids is a physiological reaction allowing animals to efficiently hunt or flee. Prolonged elevation of glucocorticoids, however, has serious negative effects and is defined as chronic stress (McEwen & Sapolsky 1995, Wingfield & Sapolsky 2003). It has a considerable impact on virtually all bodily functions and can disrupt reproduction, alter the animal's behaviour and cognition, and degrade the performance of the animal's immune system, resulting in reduced resistance to disease (McEwen & Sapolsky 1995, Wingfield & Sapolsky 2003). It is unknown which type of human activity within the wolf environment can cause chronic stress and adversely affect their fitness. Human recreational activity and snow sports were shown to increase faecal glucocorticoids in several mammalian species (Creel *et al*. 2002, Taylor & Knight 2003, Arlettaz *et al*. 2007). Such stress response suggests that human presence may cause prolonged stress in animals and hence adversely affect their fitness.

Wolves deal with human activity by temporarily avoiding areas used by humans at that particular moment (spatio-temporal segregation as defined in Theuerkauf *et al*. 2003b). Another possibility that allows wolves to live in the proximity of humans without experiencing the negative consequence of permanent stress may be habituation to human presence and subsequent reduced stress response. However, empirically little is known if these assumptions apply in the wild, because no study so far measured the actual levels of stress response by wolves in relation to varying intensities of human presence.

Glucocorticoids in scats were used as physiological indicators of stress in a variety of species (Touma & Palme 2005). As capture and handling of the animals is omitted, stress measurements from scats are unaffected by the observer and thus reflect the actual stress level of the animal more accurately (Kotrschal *et al*. 1998). Moreover, glucocorticoid metabolites in scats are pooled over a certain period, determined by gut passage time and dynamics of excretion (Scheiber *et al*. 2005). Thus, hormone concentrations in scats represent an assessment of chronic stress. For these reasons, we measured faecal glucocorticoid metabolite concentrations to assess stress levels of wolves noninvasively. We aimed at assessing the effect of breeding, pack size, prey abundance, and human activity within six wolf pack home ranges on the stress levels of wolves. We hypothesised that human activity would not be the major factor influencing stress in wolves.

Methods

This study was conducted in two distinct areas. One was situated in the southeast of Poland, in the Bieszczady Mountains (49°19´–49°50´N, 22°15´–22°45´E). We took faecal samples of five wolf packs that inhabited an area of about 1000 km^2 (Eggermann *et al*. 2009). The second region (250 km^2) was situated in the Holy Cross Forest (Puszcza Świętokrzyska) in central Poland (51°02´N, 20°44´E). Here, we took faecal samples of a wolf pack that was discovered only in 2006, which was the first record of wolves in this region since they were extirpated in 1953 (Gula 2008a, 2008b).

We collected faecal samples incidentally in 2004 and 2005 ($n = 4$), but carried out a systematic scat survey from March 2006 to March 2007 ($n = 55$). We searched for scats along randomly selected transects on roads or tracks throughout the study area. During summer and autumn, only fresh (still humid) scats were collected to avoid microbial fermentation (Khan *et al*. 2002). Samples were frozen within a few hours after collection and stored at –20 °C until analysis, as recommended by Hunt and Wasser (2003). As we did not assign faecal samples to individual wolves, we might have sampled some wolves more often than others.

We extracted metabolites from the scats following the protocol by Schatz and Palme (2001). They yielded the highest amount of metabolites (about 70%) with 80% methanol. For the measurement of glucocorticoid metabolite concentrations, we used a cortisol enzyme immunoassay developed by Palme and Möstl (1997), which was validated in the red wolf *Canis rufus* (Young *et al*. 2004) and the dog *Canis lupus familiaris* (Schatz & Palme 2001).

To detect potential seasonal variation, we grouped faecal samples in five periods and calculated mean glucocorticoid metabolite concentrations for each of them. During the breeding season we chose shorter intervals, to reveal potential peaks connected to breeding. Based on their sampling date, we classified faecal samples as collected during the breeding period (February–May) or the non-breeding period (June–December). We considered pack size as the maximum number of wolves seen or snow-tracked (Gula 2008c) during the same year we collected the scats. We calculated prey abundance within the home ranges of each pack as the sum of harvest densities of ungulates (red deer *Cervus elaphus*, roe deer *Capreolus capreolus* and wild boar *Sus scrofa*; data provided by local hunting authorities), which are the main prey of wolves in the study areas (Gula 2004). We calculated home ranges as MCP (minimum convex polygon) of multiannual radio telemetry locations or multiannual snow tracking data (Gula 2008c).

A magnetic traffic counter (NC-30 NU-metrics, Uniontown, Pennsylvania, USA) recorded the traffic on roads within the home ranges. We assigned roads in each MCP to four classes (1–500, 501–5000, 5001–10 000 and more than 10 000 vehicles per week) according to the recorded intensity of traffic. We then calculated the road density of each class (km km^{-2}) for every home range and multiplied it by the average traffic in each class. We calculated a traffic index for each pack by summing up the calculated traffic values for each road class (Table 1).

We used multiple linear regression models (SPSS ver. 19), with the level of stress hormones in individual wolf scats as a response variable and breeding, pack size, prey density, and traffic index as predictor variables. Breeding was entered as a nominal (0/1) variable whereas the other three parameters were metric. We then ranked the models by Akaike weights (w) (Burnham & Anderson 2002) to assess which of the four parameters influenced stress hormone levels the most. We log-transformed hormone concentrations for the regression analyses, because they were not normally distributed. All means are provided with 95% confidence intervals (CI).

Results

The mean glucocorticoid metabolite concentration of individual scats was 11.4 ± 2.8 (CI) ng g^{-1} of fresh faecal mass and ranged from 0.6 ng g^{-1} to 53.9 ng g^{-1}. Average hormone concentrations varied among wolf packs from 8.5 ng g^{-1} to 16.3 ng g^{-1} (Table 1). Glucocorticoid metabolite concentrations peaked during mating and at the beginning of denning (Fig. 1). Breeding was the highest-ranking factor (Table 2) influencing log-transformed levels of glucocorticoid metabolites (sum of Akaike weights: 0.60), followed by traffic index (0.53), pack size (0.40), and prey density (0.35).

Discussion

Despite the intensive traffic, high density of roads and a human density of 36 inhabitants per km (Theuerkauf *et al*. 2007) within the home ranges of the studied packs, the levels of glucocorticoids in wolf scats were most influenced by breeding. Breeding is a period of elevated aggression among members of a wolf pack (Rabb *et al*. 1967, Zimen 1976). During this period, sexual

Table 1. Number of faecal samples, average hormone concentration (ng g^{-1}), home range size (km^2), pack size, traffic index (vehicles week^{-1} km^{-2}) and prey density (indiv. km^{-2}) within the home ranges of the six wolf packs.

Wolf pack	Number of samples	Hormone concentration	Home range	Pack size	Traffic index	Prey density
Stebnik	10	8.51	126	4–7[a]	486	2.13
Holy Cross Forest	20	9.77	100	4	344	2.37
Andro	6	10.98	233	7–9[b]	810	2.49
Nika	11	13.49	94	6	345	2.15
Paniszczew	7	13.73	146	6	635	1.58
Lodyna	5	16.26	245	5	1958	2.35

[a] 7 in 2004, 5 in 2005 and 4 in 2006; [b] 7 in 2005 and 9 in 2006.

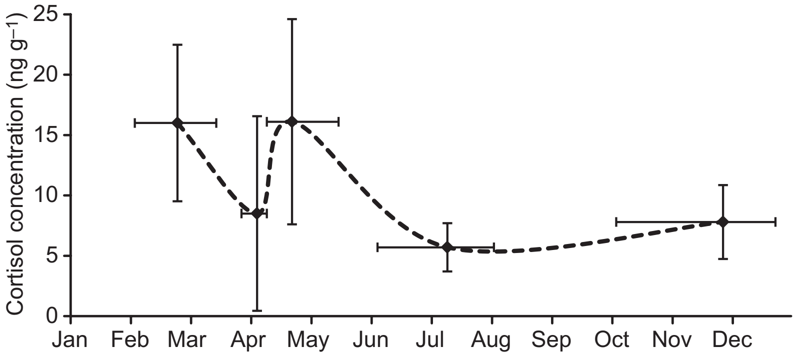

Fig. 1. Mean glucocorticoid metabolite concentrations in late winter (*n* = 13), early spring (*n* = 14), spring (*n* = 13), summer (*n* = 6) and autumn (*n* = 13). Horizontal bars indicate the time span of sample collection and vertical bars indicate 95% confidence intervals.

competition and the related aggression can even trigger dispersal of young, but sexually mature, wolves (Mech & Boitani 2003). Our data suggest that social interactions among wolves cause more stress than human presence in wolf habitat. Therefore, we argue that human activity is not the major factor inducing stress in wolves. These findings support earlier conclusions that wolves living in areas with higher human densities might habituate to human activity (Theuerkauf *et al.* 2003b, 2007). Habituation and a reduced stress response is another indication for the behavioural plasticity of wolves, such as high variation in their daily activity patterns (Eggermann *et al.* 2009) and spatio-temporal avoidance of humans (Theuerkauf *et al.* 2003b). We believe that behavioural plasticity is a decisive adaptation, which allowed wolves to survive in close proximity of people as long as they are not persecuted.

Although our sample size was too small to produce conclusive results, it seems that wolves experience the highest stress in two periods of the year: during the mating period in February and at the start of the denning period. While stress during the first period is likely caused by sexual competition and the resulting conflicts, it is more difficult to explain why the denning induces stress. An explanation might be that wolves need more effort than usual for hunting to provide enough food for nursing females, and later for the growing pups. Prey availability along with pack size and human activity was one of the factors that also contributed to the level of stress hormones. While the impact of pack size is likely to be explained by the intensity of social interactions, with higher stress levels in larger packs, the influence of prey availability is related to the level of difficulties in hunting and subse-

Table 2. Linear models of four parameters influencing wolf stress hormone levels ranked by ΔAIC_c.

Rank	Parameters in the model	*w*	ΔAIC_c	AIC_c
1	breeding	0.145	0.00	–109.82
2	traffic index	0.114	0.48	–109.34
3	breeding, traffic index	0.113	0.49	–109.33
4	breeding, pack size	0.098	0.78	–109.04
5	traffic index, prey density	0.064	1.64	–108.19
6	breeding, traffic index, pack size	0.061	1.71	–108.11
7	breeding, traffic index, prey density	0.061	1.71	–108.11
8	pack size	0.061	1.73	–108.09
9	traffic index, pack size	0.060	1.77	–108.06
10	breeding, prey density	0.058	1.83	–107.99
11	prey density	0.042	2.45	–107.37
12	breeding, prey density, pack size	0.038	2.68	–107.14
13	traffic index, prey density, pack size	0.031	3.06	–106.77
14	prey density, pack size	0.028	3.28	–106.54
15	breeding, traffic index, prey density, pack size	0.026	3.44	–106.38

quent food stress. Prey abundance is also known to determine the habitat selection by wolves (i.e. Eggermann *et al*. 2011) and their activity patterns (Theuerkauf 2009). However, under the study conditions, it does not seem that prey availability was an important factor for stress in wolves. We conclude that mostly intrinsic factors influence the level of stress in wolves and that human activity is less important.

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References

Arlettaz, R., Patthey, P., Baltic, M., Leu, T., Schaub, M., Palme, R. & Jenni-Eiermann, S. 2007: Spreading free-riding snow sports represent a novel serious threat for wildlife. — *Proceedings of the Royal Society B* 274: 1219–1224.

Burnham, K. P. & Anderson, D. R. 2002: *Model selection and multi-model inference: a practical information-theoretic approach*, 2nd ed. — Springer-Verlag, New York.

Creel, S., Fox, J. E., Hardy, A., Sands, J., Garrott, B. & Peterson, R. O. 2002: Snowmobile activity and glucocorticoid stress responses in wolves and elk. — *Conservation Biology* 16: 809–814.

Eggermann, J., da Costa, G. F., Guerra, A. M., Kirchner, W. H. & Petrucci-Fonseca, F. 2011: Presence of Iberian wolf (*Canis lupus signatus*) in relation to land cover, livestock and human influence in Portugal. — *Mammalian Biology* 76: 217–221.

Eggermann, J., Gula, R., Pirga, B., Theuerkauf, J., Tsunoda, H., Brzezowska, B., Rouys, S. & Radler, S. 2009: Daily and seasonal variation in wolf activity in the Bieszczady Mountains, SE Poland. — *Mammalian Biology* 74: 159–163.

Gula, R. 2004: Influence of snow cover on wolf *Canis lupus* predation patterns in Bieszczady Mountains, Poland. — *Wildlife Biology* 10: 17–23.

Gula, R. 2008a: Legal protection of wolves in Poland: implications for the status of the wolf population. — *European Journal of Wildlife Research* 54: 163–170.

Gula, R. 2008b: Wolves Return to Poland's Holy Cross Primeval Forest. — *International Wolf Magazine* Spring 2008: 17–21.

Gula, R. 2008c: Wolf depredation on domestic animals in the Polish Carpathian Mountains. — *Journal of Wildlife Management* 72: 283–289.

Hunt, K. E. & Wasser, S. K. 2003: Effect of long-term preservation methods on faecal glucocorticoid concentrations of grizzly bear and African elephant. — *Physiological and Biochemical Zoology* 76: 918–928.

Khan, M. Z., Altmann, J., Isani, S. S. & Yu, J. 2002: A matter of time: evaluating the storage of faecal samples for steroid analysis. — *General and Comparative Endocrinology* 128: 57–64.

Kotrschal, K., Hirschenhauser, K. & Möstl, E. 1998: The relationship between social stress and dominance is seasonal in greylag geese. — *Animal Behaviour* 55: 171–176.

McEwen, B. S. & Sapolsky, R. M. 1995: Stress and cognitive function. — *Current Opinion in Neurobiology* 5: 205–216.

Mech, L. D. & Boitani, L. 2003: Wolf social ecology. — In: Mech, L. D. & Boitani, L. (eds.), *Wolves: behaviour, ecology and conservation*: 1–34. University of Chicago Press, Chicago, IL.

Palme, R. & Möstl, E. 1997: Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. — *Zeitschrift für Säugetierkunde* 62: 192–197.

Rabb, G. B., Woolpy, J. H. & Ginsburg, B. E. 1967: Social relationships in a group of captive wolves. — *American Zoologist* 7: 305–311.

Schatz, S. & Palme, R. 2001: Measurement of faecal cortisol metabolites in cats and dogs: a non-invasive method for evaluating adrenocortical function. — *Veterinary Research Communications* 25: 271–287.

Scheiber, I. B. R., Kralj, S. & Kotrschal, K. 2005: Sampling effort/frequency necessary to infer individual acute stress responses from faecal analysis in greylag geese (*Anser anser*). — *Annals of the New York Academy of Sciences* 1046: 154–167.

Taylor, A. R. & Knight, R. L. 2003: Wildlife responses to recreation and associated visitor perceptions. — *Ecological Applications* 13: 951–963.

Theuerkauf, J. 2009: What drives wolves: fear or hunger? Humans, diet, climate and wolf activity patterns. — *Ethology* 115: 649–657.

Theuerkauf, J., Rouys, S. & Jędrzejewski, W. 2003a: Selection of den, rendezvous and resting sites by wolves in the Białowieża Forest, Poland. — *Canadian Journal of Zoology* 81: 163–167.

Theuerkauf, J., Jędrzejewski, W., Schmidt, K. & Gula, R. 2003b: Spatiotemporal segregation of wolves from man in the Białowieża Forest (Poland). — *Journal of Wildlife Management* 67: 706–716.

Theuerkauf, J., Gula, R., Pirga, B., Tsunoda, H., Eggermann, J., Brzezowska, B., Rouys, S. & Radler, S. 2007: Human impact on wolf activity in the Bieszczady Mountains, SE Poland. — *Annales Zoologici Fennici* 44: 225–231.

Thurber, J. M., Peterson, R. O., Drummer, T. D. & Thomasma, S. A. 1994: Gray wolf response to refuge boundaries and roads in Alaska. — *Wildlife Society Bulletin* 22: 61–68.

Touma, C. & Palme, R. 2005: Measuring faecal glucocorticoid metabolites in mammals and birds: the importance of validation. — *Annals of the New York Academy of Sciences* 1046: 54–74.

Wingfield, J. C. & Sapolsky, R. M. 2003: Reproduction and resistance to stress: when and how. — *Journal of Neuroendocrinology* 15: 711–724.

Young, K. M., Walker, S. L., Lanthier, C., Waddell, W. T., Monfort, S. L. & Brown, J. L. 2004: Noninvasive monitoring of adrenocortical activity in carnivores by faecal glucocorticoid analysis. — *General and Comparative Endocrinology* 137: 148–165.

Ziemen, E. 1976. On the regulation of pack size in wolves. — *Zeitschrift für Tierpsychologie* 40: 300–341.