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Effects of culling on vigilance behaviour and endogenous stress response of female fallow deer

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Abstract

Context. Human activities can induce behavioural and stress responses in wild animals. Information is scarce on the effects of culling on anti-predator behaviour and endogenous stress response of wild ungulates.

Aims. In a Mediterranean area, we evaluated the effects of culling on vigilance, foraging and endogenous stress response of female fallow deer (*Dama dama*).

Methods. Effects of culling were evaluated through behavioural observations and hormone analyses of faecal samples.

Key results. In an area where culling occurred (C), individuals showed significantly greater vigilance rates and foraged closer to wood than in an area with no culling (NC). In C, 24 h after culling, faecal cortisol concentrations were greater than those recorded in NC, but they decreased significantly to values comparable to (48 h post-shot) and lower than (72 h post-shot) those observed in NC.

Conclusions. Most likely, culling determined behavioural responses in female fallow deer, but did not trigger long-term physiological effects.

Implications. Increased anti-predator behaviour may complicate the implementation of long-term culling programs.

Additional keywords: alertness, anti-predator behaviour, faecal cortisol, group size effect, ungulates.

Introduction

Animals may adopt trade-offs between reduction of predation risk and intake of high-quality food (e.g. Crosmary *et al*. 2012). To reduce predation risk, animals can divert part of their time to safety-related activities (e.g. Lima 1998*a*; Lung and Childress 2007; Benhaiem *et al*. 2008) and/or select feeding sites with a lower quality of food resources but greater safety (e.g. Creel *et al*. 2005; Hernándèz and Laundré 2005; Ciuti *et al*. 2006). Predation risk can change with habitat structure (Brown *et al*. 1999) and may increase with distance from escape terrains (Altendorf *et al*. 2001; Hochman and Kotler 2007) or when habitat features prevent visual detection of predators (Underwood 1982; Hopewell *et al*. 2005; Marino and Baldi 2008).

Vigilance has traditionally been considered as an anti-predator behaviour (Pulliam 1973) and may reduce food intake (Underwood 1982; Illius and Fitzgibbon 1994), which, in turn, may trigger long-term costs such as reduced fecundity and survival (Lima and Dill 1990; Lima 1998*b*). Group-living can provide indirect antipredatory benefits, because it usually allows individuals to reduce their time spent in vigilance ('group-size effect'; e.g. Elgar 1989; Childress and Lung 2003; Shorrocks and Cokayne 2005).

Human activities may induce behavioural and stress responses in wild animals (e.g. Frid and Dill 2002; Manor and Saltz 2003; Banks *et al*. 2007; Jayakody *et al*. 2008; Stankowich 2008; Zwijacz-Kozica *et al*. 2013). Hunting may have indirect non-lethal effects on the behaviour of animals (Brown *et al*. 1999; Benhaiem *et al*. 2008), with physiological, genetic and demographic implications (Bateson and Bradshaw 1997; Coltman *et al*. 2003; Cromsigt *et al*. 2013; see also Sforzi and Lovari 2000 for a review).

The activation of the hypothalamic–pituitary–adrenal axis (HPAA) and the subsequent release of glucocorticoids (cortisol or corticosterone; Sapolsky *et al*. 2000; Romero 2004) are normally associated with physiological stress (Sapolsky 1992; cf. Wikelski and Cooke 2006; Zwijacz-Kozica *et al*. 2013, for wild ungulates). Even though this adaptive mechanism may help animals cope with adverse conditions, prolonged exposure to high social and/or environmental stressor may lead to chronically elevated glucocorticoid concentrations which, in turn, may have detrimental effects on body conditions, reproduction or immune defence (Sapolsky *et al*. 2000; Romero 2004; Sheriff *et al*. 2009). For the quantification of stress-sensitive molecules in secreted or excreted material (e.g. urine, faeces, saliva, hair; Möstl and Palme 2002), non-invasive sampling methods are important in objective assessment of animal welfare. Particularly analysis of cortisol and cortisol metabolites in faecal samples has been proven to accurately reflect the concentrations of these hormones in the plasma (Sheriff *et al*. 2010), and has been repeatedly used in wild ungulates (Schwarzenberger *et al*. 1998; Huber *et al*. 2003; Arias *et al*. 2013; Zwijacz-Kozica *et al*. 2013).

Deer populations at high densities can impose heavy impacts on ecosystems as well as on human activities (e.g. Côté *et al*. 2004; Gordon *et al*. 2004; Gill and Morgan 2010; Reimoser and Putman 2011, for reviews). In turn, control of deer populations can be required. In ungulates, hunting can determine an increase of physiological stress (e.g. Bateson and Bradshaw 1997). However, deer can increase anti-predator behaviour such as vigilance (e.g. Benhaiem *et al*. 2008), or avoidance of areas where the risk of being shot is higher (e.g. Jeppesen 1987; Lloyd 1990; Vercauteren and Hygnstrom 1998; Grignolio *et al*. 2011). An increase of anti-predator behaviour may limit the effectiveness of population-control operations. Information on the effects of culling on behaviour of ungulates may help improve culling programs.

To our knowledge, information coupling the effects of culling on vigilance and anti-predator behaviour and those on endogenous stress response in wild ungulates is lacking. To fill this gap, we evaluated the effects of culling on vigilance and endogenous stress response in foraging female fallow deer. We compared vigilance and foraging rates, distance from escape terrain, as well as endogenous stress response between two areas, one where culling (C) was effected and one without culling (NC). On the basis of the scientific references, we would expect female fallow deer to show (1) greater alertness rate, (2) a lower proportion of time spent foraging and a greater proportion of time spent in vigilance, (3) lower distances from wood and (4) higher concentrations of faecal cortisol metabolites, in C than in NC animals.

Materials and methods

Study area

Our study was conducted in two meadows of the Maremma Regional Park (MRP, ~9 000 ha, central Italy, 42 39^0N, 11 05^0E) between November and mid-March 2006–08 and 2012/13. The local climate is Mediterranean, with dry summer and wet autumn/winter (mean annual rainfall: 670 mm; mean annual temperature: 13.2–15.5 C; RDM 2002). The two study areas were located ~6 km apart from each other, in a straight line. Local information on seasonal home-range size of female fallow deer suggested that movements of female fallow deer from an area to the other one were unlikely to occur throughout our study (mean home-range sizes: 187.9 ha, s.d. = 100.9 ha, in autumn; 213.9 ha, s.d. = 139.9 ha, in winter; *n* = 9 individuals; home ranges estimated through the 95% minimum convex polygon method; (Niglio 1995). The area where culling operations were effected (C area: 91.7 ha) included shrub and grassland (82%) and herbaceous crops (18%, wheat and sunflower, which are ploughed in summer and grow since the following late winter/ summer). The area where culling operations were not effected (NC area: 167.2 ha) included only shrub/grassland (100%; Ferretti *et al*. 2011*b*). In Area C, we did not collect data when agricultural works were occurring. In Area C, fallow-deer culling was conducted by authorised operators (from October to March) and Park Wardens (throughout the year), from fixed locations (~1 or 2 operations/week). Both areas are bordered by Mediterranean scrubwood, with prevalence of holm oak (*Quercus ilex*. Wild boar, *Sus scrofa*, and roe deer, *Capreolus capreolus*, were also present, and cattle and horses irregularly dwelled over part of the Area C. We did not collect data on individuals foraging in close proximity of cattle or horses (i.e. at a distance smaller than 200 m). Among large predators, wolf–dog hybrids, *Canis lupus* (Caniglia *et al*. 2013), were present; fallow deer represent their main prey (Manghi and Boitani 2012).

Summer densities of fallow deer were high (estimated through pellet group counts: 18.0–22.9 deer per 100 ha in C; 36.0–42.1 deer per 100 ha in NC; Fattorini *et al*. 2011; Ferretti *et al*. 2011*a*; Sforzi *et al*. 2014). Densities did not differ significantly between periods (2006–2008 vs 2012–2013, Wilcoxon test; C: $V = 11.5$, $P = 0.916$, $n = 7$ sampling plots; NC: $V = 6$, $P = 0.784$, $n = 6$).

Behavioural observations

Behavioural observation were conducted from vantage points, through 10 42 binoculars and 20 60 Leica spotting scopes, by one observer. Data were collected between November and mid-March 2006–08 and 2012/13, over sessions of 3 h each, at dawn and dusk (2006–08: 2 sessions day^{-1} $week^{-1}$; 2012/13: 5 sessions $site^{-1}$ $week^{-1}$). Activities of female fallow deer (1-year old) were recorded using a tape recorder, through focal animal sampling (Lehner 1996), in 10-min bouts per individual. We recorded feeding (the animal grazes or browses, still or in movement; San José *et al*. 1996) and vigilance (the animal lifts its head above the body-axis, intently looking at or around and orienting the ears towards the source of disturbance, if any; San José *et al*. 1996) behaviour. Before starting the observation, we recorded also the group size. A group was defined as the number of individuals at a mutual distance lower than 40 m (Bruno and Lovari 1989). In 2012/13, in each site and for each 10-min observation bout, we also estimated the distance between the focal individual and forest borders. Distances were estimated by eye, using the deer body length as a reference (Frid 1997). We used two distances, namely the minimum distance and the average of distances along the four cardinal points (north, east, south, west), as an estimate of the visibility around the focal individual; distances were categorised as follows: 1 (0–25 m), 2 (26–50 m), 3 (51–100 m), 4 (101– 200 m), 5 (201–500 m), 6 (501 m). Directions were estimated through a compass, and distances were estimated using the deer body length as a reference (Frid 1997). To minimise pseudoreplication, we collected data on individuals that we could temporarily distinguish by their location (Frid 1997). When possible, we used colour patterns and scars to identify

individuals (Marino 2010). Whenever a focal animal went out of sight, observations continued but no-sighting time was excluded from the analyses (San José *et al*. 1996). For analyses, we considered only intervals more than 5 min long (Shi *et al*. 2011).

Collection of faecal samples and hormonal assay

To detect the effects of culling on endogenous stress response, we assessed the concentration of faecal glucocorticoids, through enzyme immunoassays (EIA) that measure concentrations of cortisol and immunoreactive metabolites of cortisol (e.g. 11,17 dioxoandrostanes metabolites; cf. Palme and Möstl 1997; Palme *et al*. 2000; Morrow *et al*. 2002, for domestic ungulates; Dehnhard *et al*. 2001; Arias *et al*. 2013; Zwijacz-Kozica *et al*. 2013, for wild ungulates).

In both C and NC, between mid-January and early March 2013, we collected fresh faecal samples of female fallow deer, after observing defecations (~4 or 5 faecal samples per week). In NC, we collected 30 faecal samples as control groups. In C, we collected 30, 28 and 30 faecal samples, 24, 48 and 72 h after culling operations, respectively. Samples were labelled and promptly stored at 20 C, until laboratory analyses.

Faecal sample analyses

To extract steroids from non-liquid matrices (such as dried solids), faeces were subjected to an organic phase extraction using ethanol; the use of ethanol is recommended as a means to completely solubilise the dried steroid, because certain steroids have limited aqueous solubility.

Faeces were kiln dried at 55 C for 24 h, thoroughly crushed, and five aliquots of pulverised faeces (0.20 g each) were put into extraction tubes, which were then sealed with a Teflon cap. Next, 1 mL of ethanol (Sigma Aldrich, St Louis, MO, USA) for every 0.1 g of solid was added to each tube, and the mixture was shaken vigorously for 30 min. Samples were centrifuged at 3300*g* for 15 min, and the supernatant was recovered in a clean tube for evaporation to dryness in a SpeedVac (Thermo Fisher Scientific, Waltham, MA, USA). Extracts were stored at 80 C. Extracted samples were dissolved into 100 mL of ethanol, followed by at least 400 mL of kit assay buffer (Arbor Assays, AnnArbor, MI, USA); then, they were vortexed and rested for 5 min twice to ensure complete steroid solubility.

The faecal cortisol and immunoreactive cortisol metabolites (FCICM) were determined using a pan-specific cortisol enzyme immunoassay kit (K003; Arbor Assays) validated for dried faecal extracts. It is uncertain to which extent native molecules and immunoreactive metabolites of cortisol were quantified in the kit used. Consequently, we prefer to use the terminology 'cortisol and immunoreactive cortisol metabolites'.

All analyses were repeated twice. Inter- and intra-assay coefficients of variation were less than 10%. The test sensitivity was determined by measuring the least amount of hormone standard consistently distinguishable from the zero concentration standard and was calculated to be 17.3 ng mL^{-1}.

All faecal samples were analysed at multiple dilutions (1 : 4, 1 : 8, 1 : 16 and 1 : 32) and were found to be parallel to the standard curve ($P < 0.05$). The mean recovery rate of cortisol added to dried faeces was 96.7%. According to the manufacturer, the cortisol kit presents the following cross-reactivity: 100% with cortisol, 18.8% with dexamethasone, 7.8% with prednisolone, 1.2% with corticosterone and 1.2% with cortisone.

Statistical analyses

Differences in proportion of time feeding (PTF), proportion of time in vigilance (PTV) and alertness rate (AR) between the study areas were evaluated with generalised linear models with zero-inflated b distributions (PTF and PTV) and zero-inflated gamma distributions (AR). In global models, PTF, PTV or AR were the response variables; site, group size and their two-way interaction were the predictors. Then,

we compared the distance of focal female fallow deer from the forest borders (i.e. the minimum distance and the average distance of those of 4 cardinal points) between the two areas, through generalised linear models with negative binomial errors (minimum distance) and general linear models (mean distance). For analyses of distances, we used data collected in 2012/13 (*n* = 195 observation bouts). For all analyses, minimum adequate models were estimated by removing the least significant term at each step, starting from interactions, until the elimination of terms caused an increase of AIC values of >2 (Crawley 2007).

We assessed differences in concentrations of faecal cortisol (ng g^{-1}) among four treatments (no culling area, and culling area 24 h, 48 h and 72 h after culling operations) through a general linear model (Crawley 2007). In the general linear model, concentrations of faecal cortisol metabolites per faecal sample were the response variable, and the treatment was the predictor. Analyses were conducted with the R3.0.2 software (R Development Core Team 2013).

Results

Behavioural response and distance from wood

We collected 350 sampling bouts on adult females (C area: *n* = 198; NC area: *n* = 152). Increasing group size had a positive effect on the PTF and a negative effect on both the PTV and AR (Table 1, Figs 1, 2). Female fallow deer showed a lower PTF and a greater PTV in C than in NC. AR tended also to be greater in C than in NC, especially in large groups (Table 1, Figs 1, 2).

Both the minimum and the mean distances from wood, along the four cardinal points, were significantly greater in individuals foraging in NC than in individuals foraging in C (Table 1, Fig. 3).

Endogenous stress response

Endogenous stress response differed between NC and C, depending on the time after culling operations (Fig. 4, Table 2). Concentrations of FCICM were significantly lower in NC than in C, 24 h after culling (Fig. 4, Table 2). In C, from 24 h to 48–72 h after culling operations, endogenous stress response decreased significantly to FCICM concentrations comparable (48 h) and lower (72 h) than those of NC (Fig. 4, Table 2).

Discussion

Our results suggested that culling could determine behavioural responses (increase of vigilance; foraging in sites with a wide visibility), but not long-term physiological effects (increase in concentrations of faecal cortisol) in female fallow deer. We conducted our study out of the period when most agricultural crops are active (i.e. late spring/summer). Furthermore, in Area C, we did not collect data when agricultural works occurred. It is likely that our results in Area C were not affected by anthropogenic disturbance related to agricultural activity. As to natural predators, one pack of wolf–dog hybrids has been reported in our study area (Manghi and Boitani 2012). The territory size of wolves is generally greater than 100 km^2 (Fuller *et al*. 2003); therefore, it is likely that the predators used both our study areas, and fallow deer of both study areas should have experienced predation risk by natural predators. Deer density was about two times greater in NC than in C. Although different deer densities between our study areas may have influenced our results, we compared behavioural rates between individuals foraging in similar-sized groups, which may have allowed to control for the effect of local density on foraging and vigilance.

A negative correlation between group size and vigilance has been reported in many taxa (e.g. Roberts 1996; Hunter and Skinner 1998; Dias 2006). Larger groups may detect a predator more easily than do smaller ones, because of the greater number of individuals; this would allow animals to decrease their own vigilance and benefit from that of other group members ('*many-eyes*' or '*detection effect*' hypothesis; Pulliam 1973). The risk of predation would be reduced in larger groups ('*safety in numbers*'

or '*dilution effect*' hypothesis; Foster and Treherne 1981). Alternatively, increasing group size may determine an increase of intra-specific competition for food, resulting in an increase of individual foraging time, so as to maintain a high food intake, and a reduction of vigilance ('*scramble competition*' hypothesis; Beauchamp 1998, 2008; Lima *et al*. 1999; Blumstein *et al*. 2001).

Glucocorticoid makes glucose immediately available for escape behaviour (Michelena *et al*. 2012). In ruminants, the highest concentration of faecal cortisol metabolites have been reported 8–24 h after injection of adrenocorticotropic hormone (ACTH; Wasser *et al*. 2000; Dehnhard *et al*. 2001; Ashley *et al*. 2011). In red deer, *Cervus elaphus*, the concentration of cortisol in blood samples was much higher in hunted individuals than in non-hunted ones (Bateson and Bradshaw 1997). Conversely, in our culling area, concentrations of cortisol metabolites of female fallow deer peaked only 1 day after operations, which, most likely, was triggered by the fear induced by the shot. Nevertheless, concentrations of cortisol metabolites consistently decreased over the 3 days after shooting, to values comparable (48 h post-shot) or lower (72 h) than those observed in the no-culling site, which suggests that an adaptive short-term endogenous response to stress occurred. As vigilance was greater in the culling site than in the no-culling site, presumably, the perception of predation risk was greater in the former than in the latter. Vigilance allows animals to scan and control the surrounding environment, which could not always reflect an endocrine response to stress. Most likely, culling determined an increase of anti-predator behaviour, and only an acute, short-term, rather than chronic, stress response, in fallow deer.

Vercauteren and Hygnstrom (1998) observed that resident white-tailed deer, *Odocoileus virginianus*, females were occasionally flushed by agricultural activities, mushroom hunters or hunting, but they typically returned to their home ranges by the next morning. Jeppesen (1987) reported that red deer moved from their familiar areas in response to intensive hunting, but they came back after 2–4 days. However, red deer tended to move continuously, over wide areas (Jeppesen 1987), suggesting nervousness. Our results suggested that fallow deer may have learned periodicity and areas where culling takes place, and got used to them (Lloyd 1990).

In the area where culling occurred, 72 h post-shot, concentrations of faecal cortisol metabolites were lower than those found in samples collected in the area where culling did not occur. When culling operations did not occur, endogenous response to stress could have been comparatively greater in the area without culling than in the area with culling, although different basal concentrations of faecal cortisol metabolites between our two study sites cannot be ruled out. In the area without culling, the density of fallow deer was about two times greater than that in the area with culling. Culling may lead animals to concentrate in culling-free, refuge areas, resulting in great population densities (Braza 1975, for fallow deer; Jeppesen 1987, for red deer and roe deer). High population density can determine intraspecific competition for food, which may result in aggression and/or stress (Li *et al*. 2007). However, no evidence for an increase of fallow deer densities in the area without culling has been reported in the past decade (see Materials and methods). Furthermore, fallow deer herds have been repeatedly observed grazing at a close proximity of culling locations, when culling operations did not occur (F. Ferretti, pers. obs.). Most likely, culling has not induced changes in population distribution of these deer.

Our results suggested that fallow deer did not show a long-term physiological response to culling and increased their vigilance behaviour.

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Table 1. Effects of site (culling (C) vs no culling (NC)) and group size on grazing female fallow deer: proportion of time foraging (PTF), proportion of time in vigilance (PTV) and number of head-lifts min^{-1} (AR) (*n* = 350 observation bouts, collected in November–March 2006–08, and 2012–2013), as well as minimum and mean distances from wood, along the four cardinal points (*n* = 195 observation bouts, collected in November–March 2012/13)

Response variable	Predictor	*B*	s.e.	*P*
Proportion of time feeding	Intercept	1.589	0.095	<0.001
	Site (NC)	0.260	0.114	0.023
	Group size	0.011	0.004	0.008
Proportion of time in vigilance	Intercept	–2.163	0.094	<0.001
	Site (NC)	–0.223	0.112	0.048
	Group size	–0.014	0.004	<0.001
Number of head-lifts per min	Intercept	–0.358	0.096	<0.001
	Site (NC)	0.229	0.167	0.172
	Group size	–0.016	0.005	0.004
	Site (NC) group size	–0.022	0.008	0.006
Minimum distance from wood	Intercept	0.249	0.089	0.005
	Site (NC)	0.379	0.116	0.001
Mean distance from wood	Intercept	3.033	0.079	<0.001
	Site (NC)	0.996	0.112	<0.001

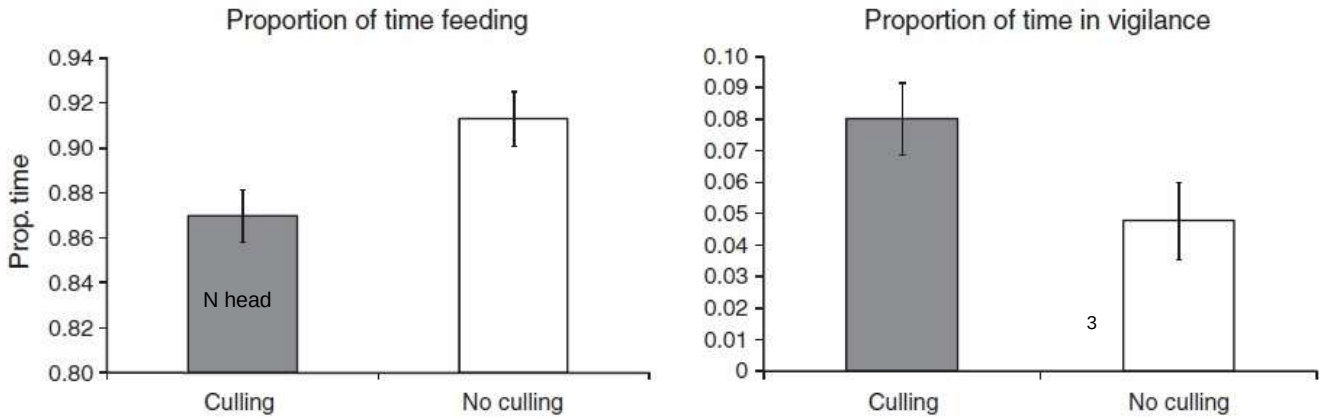

Fig. 1. Feeding and vigilance behaviour of female fallow deer (mean standard errors) grazing in sites with or without culling, in the Maremma Regional Park (*n* = 350 observation bouts).

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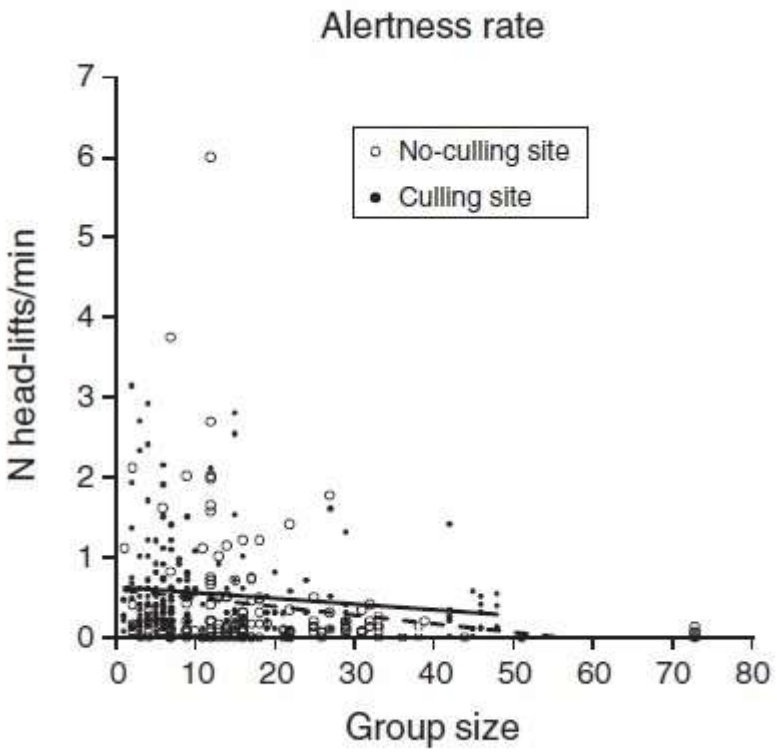

Fig. 2. Alertness rate in relation to group size of female fallow deer grazing in sites with or without culling, in the Maremma Regional Park (*n* = 350 observation bouts). Site with culling (solid line); site with no culling (dotted line).

Table 2. Effects of culling operations on levels of faecal cortisol metabolites in female fallow deer, estimated through general linear models

Treatment	*B*	s.e.	*P*
Culling area – 24 h after operations	87.350	16.840	<0.001
Culling area – 48 h after operations	–19.270	17.140	0.263
Culling area – 72 h after operations	–61.320	16.840	<0.001
Intercept	278.170	11.910	<0.001

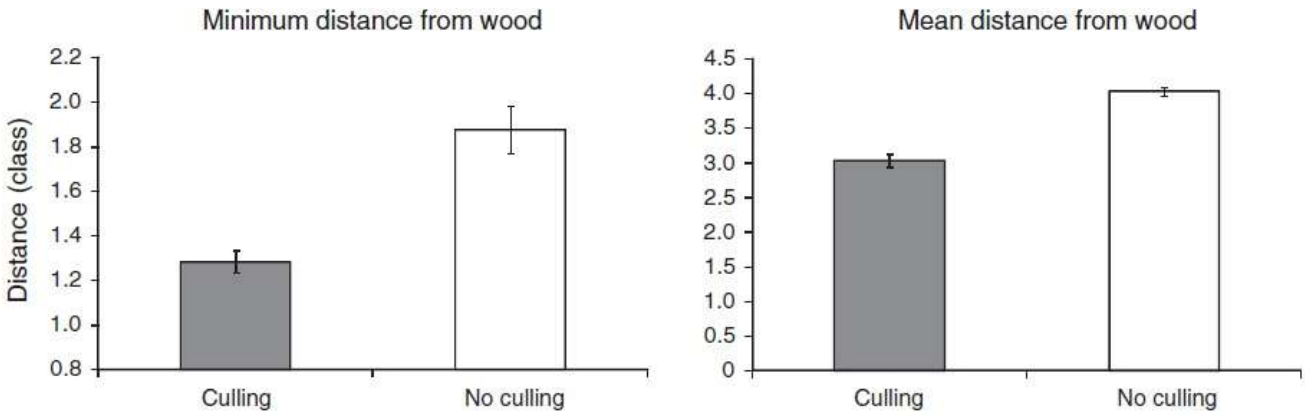

Fig. 3. Distance from wood (in classes: minimum distance and mean distance along the four cardinal points; mean standard errors) of female fallow deer grazing in sites with or without culling, in the Maremma Regional Park (*n* = 195 observation bouts).

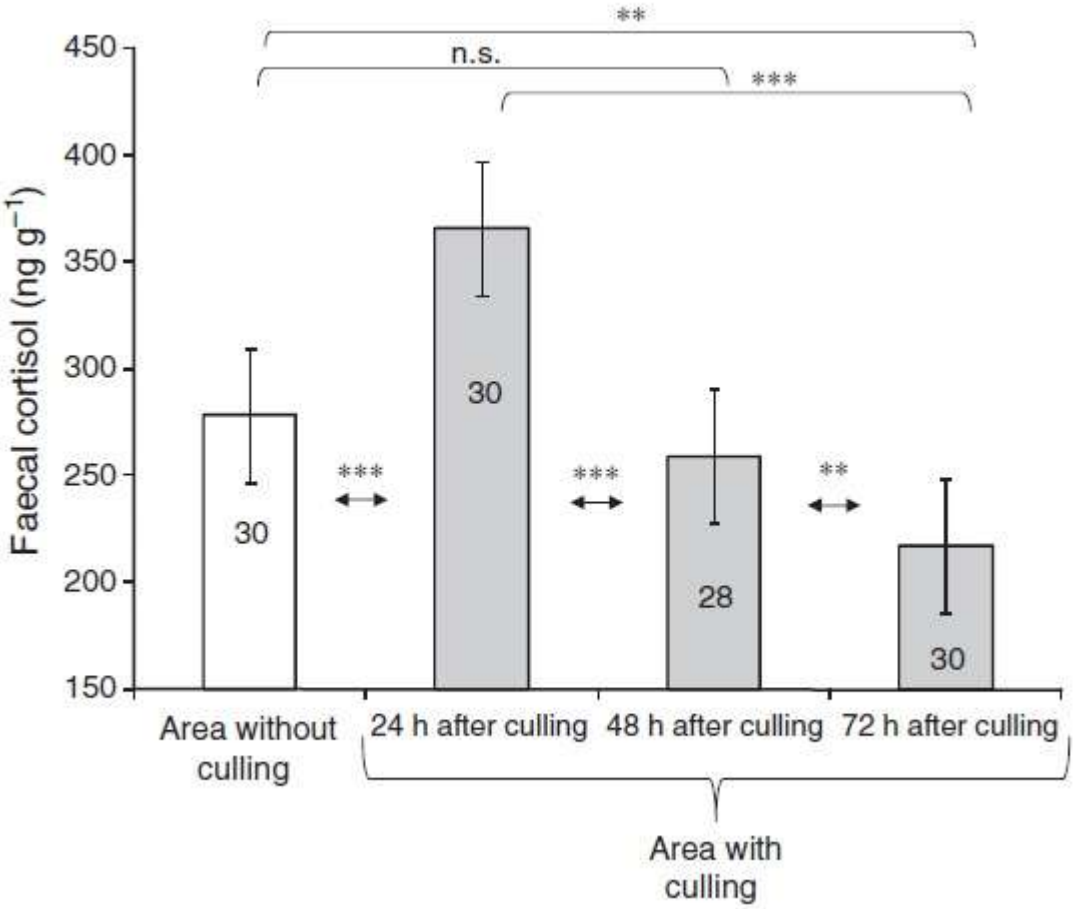

Fig. 4. Concentrations (mean s.e., ng g^{-1}) of faecal cortisol metabolites of female fallow deer in an area without culling and in an area where culling occurred, 24 h, 48 h and 72 h after culling operations. $^{**}P < 0.01$; $^{***}P < 0.001$, n.s., not significant (general linear models).