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RESEARCH

Stay calm and keep grooming: exploring the link between vigilance and self-grooming in wild fallow deer under wolf predation risk

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Abstract

Predation is a key ecological interaction shaping prey populations through both consumptive and non-consumptive pathways, influencing prey behavior and eliciting antipredator responses. Vigilance is a fundamental antipredator strategy that may induce stress and motivational conflict in prey, potentially giving rise to displacement activities. Among these activities, self-grooming is a behavior widely recognised as an anxiety and stress-ameliorating response across many *taxa*. However, its role in wild ungulates remains poorly understood. We investigated the relationship between self-grooming, vigilance, and perceived predation risk in a wild, gregarious ungulate, the fallow deer, in an area recently recolonised by wolves. Using an extensive camera-trapping dataset, we quantified the relationship between vigilance and three indices of self-grooming (probability, frequency, and proportion), while testing the effects of age, sex, season, wolf and human visitation rates, habitat openness, and group size. Our results revealed a strong, direct relationship between vigilance rate and all three self-grooming indices. In contrast, group size, wolf detection rate, and season showed weak or inconsistent effects. Habitat openness was associated with reduced self-grooming in some models for males, although only one of these models was weakly significant. Age and sex also weakly influenced self-grooming patterns: younger males groomed less frequently, while younger females groomed more frequently than adults. Overall, our findings provide the first evidence that self-grooming in fallow deer is closely linked to vigilance, supporting its interpretation as a vigilance-linked self-directed behavior. These results highlight the potential of self-grooming as a non-invasive behavioral indicator of perceived risk in wild ungulates, with important implications in predator-recolonised landscapes.

Significance statement

Predators shape prey populations not only through killing but also through non-consumptive effects on behavior and physiology, a cost that is hard to quantify in free-ranging animals. This study identifies self-grooming, a widespread comfort behavior, as a vigilance-linked displacement activity in fallow deer living in an area recently recolonised by wolves. Using camera-trapping, we show that self-grooming closely tracks vigilance rate in both sexes, consistent with its role as a de-arousal response following a stressful, motivational conflict between feeding and scanning for danger. Notably, this relationship held regardless of group size or wolf detection rate, suggesting perceived predation risk acts on self-directed behavior primarily at the individual level rather than through group-mediated antipredator strategies. These findings support self-grooming as a practical, non-invasive behavioral indicator of perceived risk, with direct relevance for monitoring wildlife welfare in areas undergoing large carnivore recolonisation.

Keywords Displacement activity · Comfort behavior · Self-directed behavior · Antipredator behavior · Cervids

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Introduction

Among the different types of interspecific interactions, predation plays a crucial role in ecosystems, both due to its ubiquity and its potential to substantially influence a system's properties and functions (Bump et al. 2009; Hawlena and Schmitz 2010; Schmitz et al. 2010). Apex predators exert a strong influence on ecological communities by directly and indirectly affecting the abundance and dynamics of their competitors, prey, and other species in lower trophic levels (Beschta and Ripple 2009; Schmitz et al. 2010). Most studies on predator-prey interactions have focused on the consumptive effects, where the predators affect population densities by killing and consuming their prey (Hebblewhite et al. 2005b). However, besides lethal effects, the presence of predators in the area can trigger non-consumptive effects, such as changes in the physiology and behavior of the prey species (Gerber et al. 2024). Interest in prey behavioral tactics to reduce predation risk (Kuijper et al. 2013), including spatial (e.g., Thaker et al. 2011) and temporal avoidance (e.g., Tambling et al. 2015), or increased individual vigilance (Sirot and Touzalin 2009) and group size (Lima and Dill 1990) has increased in recent decades (Say-Sallaz et al. 2019), also due to the recovery of large carnivores in western ecosystems (Gerber et al. 2024). Antipredator strategies are not ubiquitous, as different species can modify their antipredator tactics based on the perceived level of threat (Hebblewhite et al. 2005a), on the predator's hunting method (e.g., ambush vs. cursorial; Makin et al. 2017), and on the structural complexity of their habitat (Hebblewhite et al. 2005a). Additionally, they can adopt different strategies in response to the same predator (Valeix et al. 2009). Unfortunately, studies focused on antipredator strategies remain scarce for areas recently recolonised by predators and where prey have lived for long time in the absence of major predation pressure (e.g., Atwood et al. ur2007; Månsson et al. 2017; Dellinger et al. 2019; Sarmento and Berger 2020).

The impact of predation-risk-induced stress on the behavior of the prey is an interesting, yet traditionally overlooked, aspect of prey-predator behavior (Lima 1998). In vertebrates, exposure to a stressor activates the hypothalamic–pituitary–adrenal axis, resulting in increased glucocorticoid production (Adkins-Regan 2005). This response characterizes proper stress (*sensu* Koolhaas et al. 2011) when environmental demands exceed the natural regulatory capacity of the organism, and particularly in the absence of an anticipatory response and reduced recovery.

The detrimental effects of long-term exposure to stress on captive animals are well-documented (Möstl and Palme 2002). However, the outcomes of similar experiments in wild populations may differ significantly (Calisi and Bentley 2009; Karaer et al. 2023). While studying stress in the wild offers valuable insights, it also presents challenges, particularly due to the need to capture, handle, and potentially recapture elusive, free-ranging species in dense or inaccessible environments (e.g., Monestier et al. 2016). Additionally, high logistical demands and costs (e.g., Hunt et al. 2013), coupled with the meticulous planning necessary when using less-invasive sampling methods (Palme 2019), further restrict research on stress physiology in wild populations.

Interestingly, when glucocorticoid concentrations rise, organisms enter an "emergency state", reflected in observable behavioral changes (Wingfield et al. 1998; Blas et al. 2007) aimed at reducing the anxiety connected to the stressful situation (Klenowski et al. 2023). As some behaviors have been traditionally linked to de-arousal (Spruijt et al. 1992; Estanislau et al. 2013) and relaxation (Song et al. 2016) after exposure to a stressor, their observation can be used as an indicator of the emotional states of the animal expressing them. For example, the occurrence of comfort behaviors (i.e., actions that contribute to enhancing an individual's overall physical well-being and comfort; Allaby 2014) outside the range of self-maintenance (i.e., as displacement activity) shows remarkably similar progressions independently of the species (Kyzar et al. 2011; Kalueff et al. 2016). Thus, the analysis of these behaviors represents a good starting point to investigate stress-amelioration across *taxa*.

Displacement activities (i.e., behavioral patterns exhibited by an animal that are "apparently irrelevant" to its ongoing activity; Tinbergen 1952; Zeigler 1964) are thought to occur in situations of motivational conflict (Schino et al. 1988) and are considered a reliable ethological indicator of increased emotional and physiological arousal throughout the phylogenetic scale (Troisi et al. 2000). Among these activities, self-grooming, also known as autogrooming, is an important and evolutionarily ancient behavior that is observed across many animal *taxa* (Denmark et al. 2010). Defined as a cleaning behavior where an individual licks, nibbles, or scratches itself to maintain hygiene, remove parasites, or regulate body condition (Sachs 1988), grooming is enhanced during stressful situations that do not involve dirtying one's body (Kalueff and Tuohimaa 2005). It has therefore been suggested as a reliable indicator of dearousal after exposure to a stressor (Spruijt et al. 1992; Estanislau et al. 2013). Evidence in support of the use of self-grooming as stress-ameliorating displacement activity has been found in primates (Castles et al. 1999; Elder and Menzel 2001; Kutsukake and Castles 2001; Wells 2005; Fraser 2008; Higham et al. 2009; Kaplan et al. 2012; Wallace et al. 2013; Amrein et al. 2014; Galvao-Coelho et al. 2017), rodents (Ferron 1976; Patenaude and Bovet 1984; Sachs

1988; Spruijt et al. 1992; Eads et al. 2017; Klyuchnikova 2024), lagomorphs (Song et al. 2016), and other species (e.g., bats; Fejtek et al. 1995; dogs; Beerda et al. 1999), in both natural and experimental conditions.

The situation is, however, quite different for gregarious foragers living in the wild, such as many ungulate species. Thus, while self-grooming is a widely accepted indicator of welfare in farm animals (e.g., cattle :Mattiello et al. 2002; Herskin et al. 2004; Lyu et al. 2023), sheep and goats :Bojkovski et al. 2014), the literature on wild species is extremely scarce, counting only one study on Tibetan antelopes (*Pantholops hodgsonii*), and Tibetan gazelle (*Procapra picticaudata;* Luo et al. 2023), and a study on African elephants (*Loxodonta africana*; Manning et al. 2022). Luo et al. (2023) showed a positive association between self-grooming and vigilance in both species (being more frequent after vigilance than before) and suggested that grooming could be a reliable indicator of vigilance-related stress in ungulates. Instead, Manning et al. (2022) showed that self-directed behaviors may be correlated with a state of anxiety and overall lower-level stress, acting as a coping style in anxiety-inducing situations.

Here, we focused on a gregarious forager, i.e., the fallow deer **(***Dama dama)*, and tested for the occurrence of a relationship between self-grooming and vigilance, group size, indirect clues of potential predation risk, as well as habitat, in a protected area recently recolonised by natural predators (i.e., the wolf, *Canis lupus*). Previous studies have identified antipredator behavioral responses of fallow deer to wolves based on temporal avoidance and increased vigilance in sites with higher predator activity (e.g., Rossa et al. 2021; Esattore et al. 2023; Lazzeri et al. 2024b). Building on previous results (Esattore et al. 2023), in this study we considered self-grooming as a vigilance-induced self-directed behavior. Being a major prey of the wolf in the area (Ferretti et al. 2019; Lazzeri et al. 2024a), fallow deer can also modify the antipredator effort according to the time of the year and consequent perceived level of risk (Esattore et al. 2023). Falling within the framework of an extensive camera-trapping effort, we aimed to provide the first record of self-grooming in wild, free-ranging fallow deer in connection with vigilance and perceived predation risk by wolf.

Although defining stress *sensu* Koolhaas et al. (2011) would require physiological measures to assess the occurrence of a detrimental condition for the organism, we suggest both unpredictability and reduced recovery apply to our study subject. Therefore, following Luo et al. (2023), we assume that the occurrence of self-grooming after a vigilance bout may serve as a behavioral indicator of de-arousal after a potentially stressful experience. Based on previous work (e.g., Esattore et al. 2023; Lazzeri et al. 2024a), we predicted that the occurrence of fallow deer self-grooming Pi) will be higher after an individual experienced a higher vigilance rate or after allocating more time in vigilance without chewing activity, and Pii) will decrease with increasing group size. We also predict the occurrence of self-grooming to be Piii) higher in sites with high wolf activity and Piv) at times of the day when the wolf is more active, and Pv) higher in closer and denser environments, due to increased perceived predation risk in these contexts.

Materials and methods

Study area

Our study was conducted in a protected coastal area in central Italy (Maremma Regional Park; approximately 90 km²; 42.626371° N, 11.099303° E; Fig. 1). The local climate is Mediterranean, characterized by hot, dry summers, and seasonal temperatures range from 4 to 14 °C in winter to 17–29 °C in summer. Vegetation is primarily composed of Mediterranean shrubland (40%), including oakwood and scrubland/garigue. This is dominated by holm oak (*Quercus ilex*), strawberry tree (*Arbutus unedo*), juniper (*Juniperus* spp.), heather (*Erica* spp.), myrten (*Myrtus communis*), and *Phillyrea* spp. Other habitat types include pinewood (9%, mainly domestic pine, *Pinus pinea*), and wetlands (5%), but the landscape is also covered by crops (30%), abandoned olive groves and pastures (13%), and human settlements (2%) (Calosi et al. 2026).

Wolves occurred in our study area with three packs during the study period (Ferretti et al. 2023b). Between 2017 and 2022, wolf numbers estimated through long-term monitoring ranged from 15 (spring) to 25 (autumn), with a mean autumn pack size of 7.8 (SD = 1.8), 12.8 (SD = 3.2), and 4.8 (SD = 2.4) for the three packs, respectively (Belardi et al. 2026). Three wild ungulate species, fallow deer, wild boar (*Sus scrofa*), and roe deer (*Capreolus capreolus*), are present. Ungulate densities in the study year were medium-high for wild boar (~ 10.5 individuals/km²) and fallow deer (~ 8.3 individuals/km²), and low for roe deer (~ 3.1 individuals/km²), as estimated by concurrent research (Ferretti et al. 2023a). In our study area, wolves are predominantly nocturnal (Rossa et al. 2021; Lazzeri et al. 2024b) and their diet is made up of wild ungulates (~ 85%), including fallow deer, which represents the second most important prey after wild boar, accounting for ~ 25% of the predator diet (Ferretti et al. 2023b; Lazzeri et al. 2024a). Previous studies conducted in the area have shown that fallow deer increased diurnality after wolf recolonisation as a major antipredatory

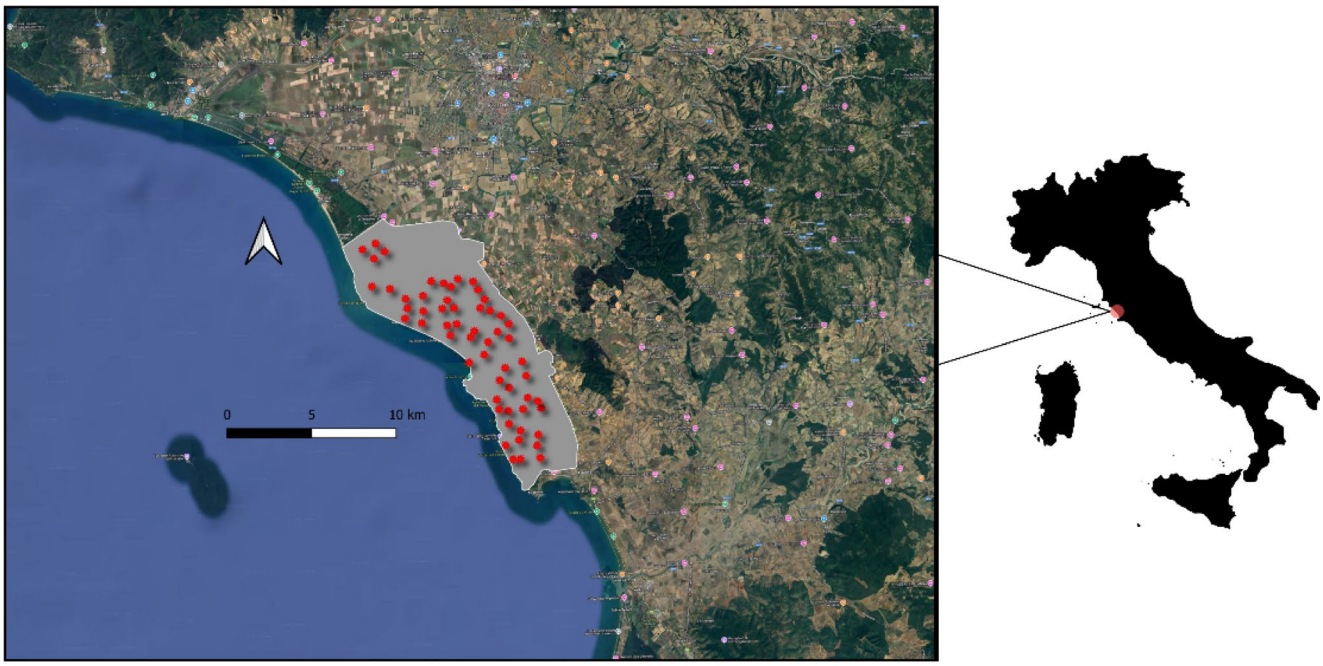

Fig. 1 Map of the study area (grey) with the locations of camera traps (red stars)

strategy (e.g., Rossa et al. 2021; Esattore et al. 2023; Lazzeri et al. 2024b), and that the increase of predators throughout 2015–2019 has been followed by a numerical decrease of fallow deer density in the following years (Fattorini et al. 2026). Livestock density is approximately 20 heads/km² (Ferretti et al. 2019), consisting of free-ranging cattle and horses that graze mainly in pinewoods and abandoned olive groves and pastures, as well as two sheep herds in localized sectors of the Park. However, wolf predation on livestock is scanty (< 5% of wolf diet; Lazzeri et al. 2024a). Population control of wild boar and fallow deer is carried out by the Park Agency through culling (both species) and trapping and removal (wild boar), aiming to reduce their negative impacts on habitats and species of conservation relevance as well as on agriculture. During our study period, only 31 fallow deer were culled in April-August, and 24 in September-early October (i.e., out of the observation period; Maremma Regional Park Archive data), and culling locations were concentrated mainly in and around the agricultural areas (Pecorella et al. 2016), where data on self-grooming were not collected. Previous work showed that the endogenous response of fallow deer to culling activities was short-term (Pecorella et al. 2016). Human density in the Park is relatively low (~ 3.8 inhabitants/km^2, on average; data from Schiavina et al. 2022), although tourist visitation occurs on trails and roads, mainly for hiking and cycling, especially in summer. Previous studies showed that fallow deer vigilance was not affected by human visitation rates (Esattore et al. 2023).

Data collection

We conducted our study in April 2019 - March 2020 within the framework of a long-term study on wolf-prey and wolf-mesocarnivore interactions (e.g., Rossa et al. 2021; Esattore et al. 2023; Ferretti et al. 2023b; Lazzeri et al. 2024b). The sampling design has been repeatedly described in previous work (Esattore et al. 2023; Lazzeri et al. 2024b): a total of 57 camera-trap locations (Fig. 1) were selected using a 1 × 1 km sampling grid superimposed on the non-agricultural part of the study area in a Geographic Information System (GIS), with one camera per suitable grid cell. Each location was monitored according to a rotational scheme, for approximately 30 days per season (spring: April–June; summer: July–September; autumn: October–December; winter: January–March). Cameras (IR-Plus HD-2, Scout-Guard) were installed generally 30–150 cm above the ground along animal trails, trails, or forest roads suitable for detecting both carnivores and ungulates (Li et al. 2010, 2012; Bu et al. 2016; Torretta et al. 2017). They operated continuously (24 h/day), recording 30-s videos with no delay between consecutive recordings, and had a trigger time ≤ 1 s. Each camera was equipped with a 16-GB SD card and an external 6-V battery, and was checked approximately every 15–30 days to download data and replace cards or batteries. All videos were reviewed and classified, and relevant information was transcribed into Microsoft Excel. Daylight-saving time was not applied to maintain time consistency relative to solar position (Foster et al. 2013). At each camera location, we visually recorded the major habitat type by

discriminating between open habitats (i.e., ecotones such as pastures, glades, and transitional habitats with scattered trees) and covered habitats (i.e., oakwood, pinewood, and dense shrubland). For each location, we also calculated the yearly detection rate for both wolf and humans, using the yearly number of 'independent' wolf and human detections (*sensu* Esattore et al 2023), respectively, divided by the overall number of camera working days in the same year. We used these rates as proxies for the long-term site use by wolves and humans at each location, thus risk perception by fallow deer. Indeed, previous work showed that fallow deer vigilance were higher in sites with greater wolf detection rates (Esattore et al. 2023).

Behavioral observations

For behavioral observations on fallow deer, we used a conservative approach and excluded videos collected during the rut (September 2019 – November 2019) as the increased frequency of short, non–vigilance-related self-directed behaviors, including grooming, would have complicated and confounded behavioral scoring (Buschhaus et al. 1990). For analyses, we categorized the period into warmer (April 2019-August 2019) and colder (December 2019-March 2020) months. We collected behavioral data on different sex and age classes of fallow deer. We estimated age classes considering overall morphology, body size and, for males, size and shape of antlers (Apollonio 2003). We discarded all individuals whose age class assessment was uncertain. Initially, we considered young females (<2 years old), adult females (≥2 years old), young males (<2 years old), subadult males (2–4 years old), and adult males (>4 years old). For analyses, we grouped young and subadult males against adults. This was decided based on the low sample size of the former category, the expected behavioral similarities in non-socially mature males (McElligott et al. 1999; Bateman-Neubert et al. 2023), as well as significant differences in vigilance between adults and subadult fallow deer shown in previous studies in the same area (Pecorella et al. 2019).

Following a procedure from our previous work (Esattore et al. 2023), we collected data on all individuals whose head was visible in the video and that could be assigned to a certain sex and age class. Then, for these analyses, we considered all the videos for which the head of at least one individual fallow deer could be observed for at least 10 s. We chose this threshold, i.e., one third of the overall video length, to maximise sample size while avoiding inferring behavioral frequencies from individuals observed for a few seconds only. We defined a "head lift" as a vigilance posture where the animal lifted its head above the body axis, intently looking at/around and orienting the ears towards the source of disturbance, if any (San José et al. 1996; Ferretti et al. 2008; Sönnichsen et al. 2013). Thus, for each observed individual, we recorded the duration (in seconds) of the interval where its head was visible and recorded both the number and the duration of head lifts. For each vigilance event, we recorded the occurrence and duration of self-grooming afterwards, focusing only on cases when self-grooming occurred after a vigilance bout. Chewing activity during vigilance behavior has been considered as a proxy for vigilance intensity (Favreau et al. 2015). Thus, we classified the head lift in three categories (with chewing activity, without chewing activity, and undetermined), assuming vigilance without chewing to be more attentive and focused than with chewing (Blanchard and Fritz 2007), and recorded the occurrence and duration of self-grooming following each head lift. We also recorded the total group size, defined as the minimum number of distinct individuals belonging to the same group observed across consecutive videos within 30 min from the first video of the series. Hence, for group size, videos of fallow deer recorded within a 30-minute intervals were considered a single detection event, with only the first video retained for analysis to minimise autocorrelation (Rowcliffe et al. 2008; Tobler et al. 2008; Lucherini et al. 2009; Torretta et al. 2016; Esattore et al. 2023). Considering the impossibility of identifying individual animals beyond the 30-minute bout and being our study's aim to define within-bout behavioral associations between self-grooming and vigilance, rather than attempting to define a baseline rate for the behavior itself, we chose not to focus on self-grooming occurring outside the vigilance context. For each video, we used the solar time (hh:mm) and the R package suncalc (Thieurmel et al. 2019) to assess the occurrence of darkness by discriminating between videos recorded under dark conditions (nighttime; after civil twilight ends, in the evening, and before civil twilight begins the next morning; i.e., reference angle of the sun below the horizon = 6°) with those where fallow deer were under daylight (daytime or crepuscular periods). Eventually, each video was ascribed to the relevant seasonal period by distinguishing between videos collected in warmer months (April 2019 - September 2019; mean ± SD temperature: 20.4 ± 6.1 °C; n = 17,566 15-min readings; data from Alberese weather station: https://sir.toscana.it) and those in relatively colder months (December 2019 - March 2020; mean ± SD temperature: 10.2 ± 4.4 °C; n = 11,711 15-min readings).

In each focal bout, we calculated self-grooming and vigilance rate (number of events/second) as the number

of self-grooming events and head lifts, respectively, divided by the time (seconds) the focal individual had the head visible. We also calculated the proportion of time spent in self-grooming and vigilance as the duration of time (seconds) allocated to self-grooming and vigilance, respectively, divided by the time (seconds) the focal individual had the head visible. Finally, for focal observation bouts with head lifts where chewing activity (chewing or non-chewing) could be identified, we calculated the proportion of non-chewing vigilance as the duration of time (seconds) allocated to non-chewing vigilance divided by the overall time (seconds) allocated to vigilance. As our study involved observation of focal animals in the field, it was not possible to record data blind.

Statistical analyses

We investigated the effect of vigilance on self-grooming using generalised linear mixed models (GLMMs; Zuur and Ieno 2016). We conducted separate GLMMs for males and females. We modelled three indices of self-grooming as response variables, separately. Specifically, we modelled (i) the occurrence probability of self-grooming using Bernoulli errors (link function: cloglog), as recommended for imbalanced binary (presence/absence) data (Alves et al. 2023), (ii) the frequency of self-grooming with Poisson errors (link function: log) combined to an offset term for measurement time, as suggested for rates from count data (Hinde et al. 2024), and (iii) the proportion of time spent in self-grooming using Beta errors (link function: logit) and the ordered beta parametrization, as recommended when modelling continuous proportion in the 0–1 range (Kubinec 2023).

In a first step, we considered all focal observation bouts (males: $n=341$ bouts; females: $n=946$ bouts). We built a model considering several focal predictor variables used to test our predictions (Pi-Pv). We included (1) vigilance rate (continuous, as number of head lifts/second) to investigate the effect of vigilance on self-grooming (Pi). Because the vigilance rate of fallow deer in any focal bout positively correlated with the proportion of time spent in vigilance in the same bout ($r=0.64$, $p<0.0001$, for both males and females), we only used the former for analyses. Besides the predictor (1), we tested the other predictions (Pii-Pv) by considering the variables related to predator risk perception such as wolf visitation rate, habitat cover, light availability, and gregariousness, namely (2) yearly wolf detection rate (continuous, as number of wolf detections/camera working day); (3) habitat type (categorical; reference category: covered habitats); (4) occurrence of darkness (categorical; reference category: daylight); (5) group size (integer, as number of individuals in group with the focal individual). We also considered confounding variables intimately linked to external pressures as well as social dynamics such as age class and season, which may shape fallow deer vigilance (Pecorella et al. 2019; in our study area) and in turn self-grooming, namely (6) yearly human detection rate (continuous; as number of human detections/camera working day); (7) age class (categorical; reference levels: adult, for both males and females); (8) season (categorical; reference category: colder months). In the model evaluating the probability of self-grooming, we also considered as a confounder (9) the time an individual had the head visible for observation (continuous, as seconds), to account for greater probability of observing self-grooming with increasing observation time, while in the model evaluating the frequency of self-grooming, we log-transformed the same variable (as per the link function) and set it as an offset term to standardise the number of self-grooming events by observation time. As camera-trap videos did not allow us to reliably determine whether a female is accompanied by a fawn, nor whether a particular fawn can be attributed to a specific female, we did not consider fawn presence among the predictors. In all models, we treated the camera location and the identity of the analysed video as random intercepts to account for repeated focal observation bouts conducted at the same camera location and within the same time slot, respectively.

In a second step, we focused on the effect of vigilance with chewing versus without chewing. Thus, we repeated the above analyses by considering only focal observation bouts with vigilance and, within these, only those where we could discriminate between head lifts with and without chewing activity. We fitted models using the same fixed and random structures, except that we added the (10) time spent in non-chewing vigilance out of the total vigilance time (continuous, as a proportion) as a further focal predictor.

We found neither collinearity amongst numeric covariates ($r < |0.52|$) nor multicollinearity amongst predictors (VIFs<2 in all models). We scaled and centered numeric covariates before modelling to improve model convergence. Since we aimed at investigating the effects of focal predictors while accounting for the effects of the other variables, we based our inference on the entire model (forced entry-method). We fitted models in R 4.3.0 through the function glmmTMB::glmmTMB (Brooks et al. 2017) and validated them through visual inspection of DHARMa residuals (Hartig 2022). For modelling of the proportion of time spent in self-grooming by males using the full dataset, we used the BFGS optimizer as a glmmTMB control parameter to allow model convergence. For each predictor, we estimated the coefficient and the 95% confidence interval (CIs), assessing whether the latter overlapped with '0' to identify informative predictors.

Results

After data filtering (see Methods), we obtained 341 bouts for males and 946 bouts for females, from a total of 34 locations. Both male and female fallow deer exhibited self-grooming in 8.8% of focal observation bouts. We analysed two versions of the dataset: one including all observations (full dataset, results summarized in Table 1) and one restricted to videos in which chewing activity could be reliably observed and scored (restricted dataset, results summarized in Table 2). Across camera locations, yearly detection rates ranged from 0.010 to 0.956 for wolves and from 0.037 to 6.407 for humans, with mean ± SD values of 0.167 ± 0.183 and 0.944 ± 1.488 detections/day, respectively.

Full Dataset

Males

Males showed a mean ± SD self-grooming rate of 0.264 ± 0.942 events/minute and spent on average 1.5% ± 6.3% of their time self-grooming. The probability of self-grooming was strongly and directly associated with the vigilance rate (Fig. 2a), and the duration for which the head was visible also had a positive effect, indicating that longer observation periods improved detection of self-grooming events. A weak negative association was detected between probability of self-grooming and wolf detection rate, while no significant association was found between probability of self-grooming and human detection rate. Substantial variation occurred among videos, whereas variation among locations was minimal. The frequency of self-grooming was positively associated with vigilance rate (Fig. 2c) and was lower in open habitats than in closed ones, with a weak tendency suggesting higher frequency of self-grooming in warmer months. Random-effect variances were very low among locations. Last, the proportion of time spent self-grooming increased with vigilance rate (Fig. 2e). Younger males spent less time self-grooming than older individuals, and random-effect variances were negligible across models. The proportion of time spent self-grooming also decreased in open habitats, even though this effect was weak.

Females

Females had a mean ± SD self-grooming rate of 0.258 ± 0.924 events/minute and spent 2.1 ± 8.6% of their time self-grooming. Both vigilance rate (Fig. 2b) and head visibility time were positively associated with the probability of self-grooming. Other predictors did not show significant contributions. Random effects indicated moderate variation among locations and smaller variation among videos. For the frequency of self-grooming, vigilance rate (Fig. 2d) was the main significant predictor, and variance was minimal among videos but higher among locations. Finally, the proportion of time spent self-grooming increased with vigilance rate (Fig. 2f) and decreased with age, as younger females spent more time self-grooming than adult ones.

Restricted dataset

Males

When analyses were restricted to bouts in which chewing activity could be scored, there was no support for an association between the proportion of non-chewing vigilance and self-grooming in males ($n = 198$ bouts). The probability of self-grooming was positively associated with increasing vigilance rate and time when the head was visible. Wolf detection rate showed a weakly supported positive association with the probability of self-grooming, while the effects of all the other predictors were not supported. Random-effect variation at both video and location levels was negligible. The frequency of self-grooming was positively related to vigilance rate and to group size. A weak trend suggested higher frequency of self-grooming during warmer months; habitat type, age class, and wolf detection rate had no clear effects on the frequency of self-grooming. Random-effect variation was minimal among videos and negligible among locations. For the proportion of time spent self-grooming, age class was the only significant predictor, with younger males spending less time self-grooming than adult ones. Vigilance rate and all other predictors showed no meaningful effects, and variation was moderate among locations but negligible among videos.

Females

In females ($n = 523$ bouts), vigilance rate and head visibility time showed strong positive associations with the probability of self-grooming, while other predictors remained non-significant. Random effects indicated moderate variation among both locations and videos. The frequency of self-grooming was again primarily associated with vigilance rate, and younger females exhibited higher frequencies of self-grooming. Other predictors were not significant. Random-effect variances were moderate across locations and negligible among videos. For the proportion of time spent self-grooming, younger females spent more time self-grooming than adult ones, and vigilance rate only showed a weak positive effect. Variation was higher for locations than for videos.

Table 1 Parameters estimated from the GLMMs predicting indices of self-grooming in fallow deer (*Dama dama*): variance of random intercepts (σ^2), predictors' coefficient estimates (β) and their 95% confidence intervals (CIs). An asterisk marks p-values < 0.05. All covariates are scaled and centered. The effect of vigilance rate is bolded

Sex	Response variable	Predictor	β coefficient	95% CI	*p*
# obs = 341, # locations = 24, # ID video = 244					
Males	Probability of self-grooming	Intercept	-4.087	-6.210; -1.965	*<0.001
	σ^2 Location	Light conditions (dark)	-0.615	-2.425; 1.195	0.506
	$=2.17\times10^{-9}$	Season (warmer months)	0.907	-0.445; 2.258	0.189
	σ^2Video ID	Habitat type (open habitats)	-1.150	-2.854; 0.553	0.186
	=3.93	Age class (young)	-0.992	-2.407; 0.423	0.170
		Group size	0.348	-0.221; 0.917	0.230
		Vigilance rate	**1.415**	**0.694; 2.135**	***<0.001**
		Wolf detection rate	-0.930	-1.852; -0.008	*0.048
		Human detection rate	-0.411	-1.524; 0.702	0.469
		Time with head visible	0.851	0.174; 1.529	*0.014
	Frequency of self-grooming	Intercept	-5.909	-7.084; -4.734	*<0.001
	σ^2 Location	Light conditions (dark)	-0.216	-1.286; 0.853	0.692
	$=2.19\times10^{-9}$	Season (warmer months)	0.936	-0.033; 1.905	0.058
	σ^2Video ID	Habitat type (open habitats)	-1.329	-2.689; 0.032	0.056
	=0.197	Age class (young)	-0.591	-1.479; 0.297	0.192
		Group size	0.274	−0.075; 0.622	0.124
		Vigilance rate	**0.945**	**0.581; 1.310**	***<0.001**
		Wolf detection rate	-0.373	-0.878; 0.132	0.148
		Human detection rate	-0.257	-0.963; 0.449	0.475
	Proportion of time spent in self-grooming	Intercept	-1.781	-2.434; -1.127	*<0.001
	σ^2 Location	Light conditions (dark)	-0.320	-0.992; 0.351	0.350
	$=2.68\times10^{-9}$	Season (warmer months)	0.479	-0.109; 1.067	0.111
	σ^2Video ID	Habitat type (open habitats)	-0.651	-1.477; 0.175	0.123
	$=1.26\times10^{-8}$	Age class (young)	-0.863	-1.410; -0.317	*0.002
		Group size	-0.024	-0.347; 0.298	0.883
		Vigilance rate	**0.555**	**0.258; 0.852**	***<0.001**
		Wolf detection rate	-0.242	-0.550; 0.066	0.123
		Human detection rate	-0.371	-0.920; 0.179	0.186
# obs = 946, # locations = 32, # ID video = 550					
Females	Probability of self-grooming	Intercept	-3.532	-4.553; -2.510	*<0.001
	σ^2 Location	Light conditions (dark)	0.168	-0.495; 0.830	0.620
	=1.075	Season (warmer months)	-0.298	-0.923; 0.328	0.350
	σ^2Video ID	Habitat type (open habitats)	-0.022	-1.410; 1.366	0.980
	=0.271	Age class (young)	0.336	-0.256; 0.927	0.270
		Group size	0.186	-0.078; 0.450	0.170
		Vigilance rate	**1.202**	**0.886; 1.518**	***<0.001**
		Wolf detection rate	-0.075	-0.636; 0.485	0.790
		Human detection rate	-0.102	-0.764; 0.561	0.760
		Time with head visible	0.846	0.5119; 1.178	***<0.001**
	Frequency of self-grooming	Intercept	-6.201	-7.033; -5.369	*<0.001
	σ^2 Location	Light conditions (dark)	0.260	-0.306; 0.827	0.370
	=0.863	Season (warmer months)	-0.396	-0.928; 0.136	0.140
	σ^2Video ID	Habitat type (open habitats)	0.078	-1.176; 1.331	0.900
	$=1.38\times^{10-8}$	Age class (young)	0.402	-0.091; 0.895	0.110
		Group size	0.108	-0.121 0.338	0.350
		Vigilance rate	**0.945**	**0.751; 1.138**	***<0.001**
		Wolf detection rate	-0.076	-0.576; 0.425	0.770
		Human detection rate	-0.074	-0.671; 0.524	0.810
	Proportion of time spent in self-grooming	Intercept	-2.077	-2.719; -1.433	*<0.001
	σ^2 Location	Light conditions (dark)	0.157	-0.258; 0.572	0.458
	=0.268	Season (warmer months)	-0.186	-0.590; 0.218	0.366
	σ^2Video ID	Habitat type (open habitats)	0.084	-0.715; 0.883	0.836
	=0.07	Age class (young)	0.403	0.031; 0.774	*0.034
		Group size	-0.025	-0.212; 0.162	0.793
		Vigilance rate	**0.497**	**0.316; 0.677**	***<0.001**
		Wolf detection rate	-0.123	-0.438; 0.192	0.444
		Human detection rate	-0.135	-0.508; 0.238	0.479

Table 2 Parameters estimated from the GLMMs predicting indices of self-grooming in fallow deer (*Dama dama)*, when we considered only focal observation bouts with head lifts where chewing activity could be determined: variance of random intercepts (σ^2), predictors' coefficient estimates (β), and their 95% confidence intervals (CIs). An asterisk marks p-values < 0.05. All covariates are scaled and centered. The effect of the proportion of non-chewing (NC) vigilance is bolded

Sex	Response variable	Predictor	β coefficient	95% CI	*p*
# obs = 198, # locations = 22, # ID video = 156					
Males	Probability of self-grooming	Intercept	-2.131	-3.268; -0.994	*<0.001
	σ^2 Location	Light conditions (dark)	0.303	-0.826; 1.431	0.599
	$=3.9\times10^{-16}$	Season (warmer months)	0.808	-0.172; 1.789	0.106
	σ^2Video ID	Habitat type (open habitats)	-0.678	-2.007 0.651	0.317
	$=5.1\times10^{-9}$	Age class (young)	-0.646	-1.561; 0.268	0.166
		Group size	0.214	-0.058; 0.486	0.124
		Vigilance rate	0.507	0.078; 0.935	*0.021
		Proportion NC vigilance	**0.056**	**-0.363; 0.476**	**0.792**
		Wolf detection rate	-0.559	-1.129; 0.011	0.054
		Human detection rate	-0.196	-0.810; 0.418	0.531
		Time with head visible	0.543	0.045; 1.040	*0.033
	Frequency of self-grooming	Intercept	-5.214	-6.386; -4.041	*<0.001
	σ^2 Location	Light conditions (dark)	0.297	-0.736; 1.330	0.573
	$=3.19\times10^{-8}$	Season (warmer months)	0.947	-0.061; 1.955	0.066
	σ^2 Video ID	Habitat type (open habitats)	-0.803	-2.141; 0.535	0.240
	$=3.29\times10^{-2}$	Age class (young)	-0.653	-1.520; 0.213	0.140
		Group size	0.275	0.018; 0.532	*0.036
		Vigilance rate	0.473	0.128; 0.818	*0.007
		Proportion NC vigilance	**0.093**	**-0.327; 0.513**	**0.664**
		Wolf detection rate	-0.293	-0.826; 0.240	0.281
		Human detection rate	-0.232	-0.838; 0.374	0.453
	Proportion of time spent in self-grooming	Intercept	-1.667	-2.391; -0.942	*<0.001
	σ^2 Location	Light conditions (dark)	0.146	-0.788; 0.497	0.657
	$=0.368$	Season (warmer months)	0.550	-0.099; 1.198	0.097
	σ^2 Video ID	Habitat type (open habitats)	-0.621	-1.634; 0.392	0.230
	$=7.86\times10^{-11}$	Age class (young)	-0.954	-1.500; -0.408	*0.001
		Group size	-0.023	-0.273; 0.319	0.880
		Vigilance rate	0.111	-0.181; 0.404	0.455
		Proportion NC vigilance	**-0.066**	**-0.372; 0.239**	**0.671**
		Wolf detection rate	-0.267	-0.875; 0.341	0.390
		Human detection rate	-0.706	-1.605; 0.193	0.124
# obs = 523, # locations = 30, # ID video = 359					
Females	Probability of self-grooming	Intercept	-2.633	-3.493; -1.772	*<0.001
	σ^2 Location				
	$=0.433$				
	σ^2 Video ID				
	$=0.365$				
		Light conditions (dark)	-0.336	-1.208; 0.535	0.450
		Season (warmer months)	-0.100	-0.781; 0.582	0.774
		Habitat type (open habitats)	0.296	-0.796; 1.388	0.560
		Age class (young)	0.511	-0.133; 1.154	0.120
		Group size	0.235	-0.041; 0.511	0.096
		Vigilance rate	0.648	0.339; 0.958	*<0.001
		Proportion NC vigilance	**0.064**	**-0.220; 0.347**	**0.660**
		Wolf detection rate	-0.238	-0.721; 0.245	0.334
		Human detection rate	-0.122	-0.614; 0.371	0.629
		Time with head visible	0.652	0.303; 1.000	*<0.001
	Frequency of self-grooming	Intercept	-5.555	-6.233; -4.877	*<0.001
	σ^2 Location				
	$=0.315$				
	σ^2 Video ID				
	$=1.94\times10^{-8}$				
		Light conditions (dark)	-0203	-0.961; 0.555	0.600
		Season (warmer months)	-0.112	-0.703; 0.479	0.710
		Habitat type (open habitats)	0.348	-0.619; 1.314	0.481
		Age class (young)	0.628	0.094; 1.161	*0.021
		Group size	0.160	-0.070; 0.389	0.173

Table 2 (continued)

Sex	Response variable	Predictor	β coefficient	95% CI	*p*
		Vigilance rate	0.459	0.235; 0.683	*<0.001
		Proportion NC vigilance	**0.104**	**-0.159; 0.367**	**0.440**
		Wolf detection rate	-0.235	-0.667; 0.197	0.286
		Human detection rate	-0.084	-0.508; 0.341	0.699
	Proportion of time spent in self-grooming σ^2 Location =0.102 σ^2 Video ID =0.06	Intercept	-1.585	-2.124; -1.047	*<0.001
		Light conditions (dark)	-0.008	-0.550; 0.534	0.977
		Season (warmer months)	-0.044	-0.481; 0.392	0.842
		Habitat type (open habitats)	0.147	-0.532; 0.826	0.672
		Age class (young)	0.482	0.063; 0.900	*0.024
		Group size	0.049	-0.145; 0.243	0.619
		Vigilance rate	0.144	-0.010; 0.298	0.067
		Proportion NC vigilance	**0.014**	**-0.170; 0.199**	**0.879**
		Wolf detection rate	-0.188	-0.475; 0.100	0.200
		Human detection rate	-0.147	-0.428; 0.135	0.307

Discussion and conclusions

We documented a close and consistent relationship between self-grooming and vigilance in a free-ranging ungulate species exposed to predation risk. The observed association with vigilance behavior suggests that self-grooming may function as a displacement activity linked to a stressful situation in a wild, gregarious herbivore. Overall, our results align with the literature showing that self-directed behaviors may serve multiple functions beyond simple hygienic maintenance (Sachs 1988), including relieving tension deriving from stress or anxiety response (van den Bos 1998; Beerda et al. 1999; Castles et al. 1999; Densing et al. 2018; Manning et al. 2022; Schino et al. 2026). Our findings also contribute to the development of methodological and conceptual frameworks for the interpretation of self-directed behaviors in camera-based studies. We observed that self-grooming was closely linked to vigilance and occurred alongside, or even as part of, an alert state. Furthermore, individuals exhibiting higher rates of head lifts were consistently more likely to engage in self-grooming for longer periods. This pattern was observed in both males and females and was not overly influenced by habitat type or other environmental variables. These findings support our first prediction (Pi) that links self-grooming occurrence and the rate of the alert state. Accordingly, this behavior may provide a brief, low-risk outlet during periods of high vigilance, acting as a coping or displacement behavior, as self-directed behaviors may represent a balance between feeding or grazing and maintaining alertness. Displacement activities are also common in situations of motivational conflict in which inclinations to perform more than one activity are simultaneously expressed (Schino et al. 1988; Troisi 2002; Ferkin 2005). Similarly, some authors have documented them in view of an imminent change of behavior (Peignot et al. 2004). In fallow deer, self-grooming was expressed after vigilance bouts during which the individual had to stop exploring the surroundings or foraging to engage in the scanning of its surroundings. In turn, it may express the result of a motivational conflict between keeping feeding and scanning the surroundings, or between keeping feeding and fleeing, as well as an interlude to a complete switch to a different behavior (e.g., feeding to fleeing).

Our second prediction, expecting self-grooming to decrease with increasing group size (Pii), did not find strong support, as group size was associated only with the frequency of allogrooming in males. Some authors highlighted the importance of self-grooming as a way of social signalling, even though this seems to be mostly limited to reproductively active, opposite-sex conspecifics (e.g., in voles; Ferkin and Leonard 2010; Zhang et al. 2022). We excluded the rutting period from the analyses to avoid potential misinterpretation. Thus, during this period, self-grooming may be used to spread odoriferous substances on the body, thereby increasing their volatility (Bossert and Wilson 1963), rather than functioning as a self-relief response following prolonged vigilance. Similarly, vigilance itself may be directed toward scanning for potential mates and rivals, rather than assessing the danger of the situation. For the remaining time of the year, our results suggest that, even in a highly social species, the link between vigilance and self-directed behaviors may be primarily individual-mediated rather than group-mediated.

Age and sex seemed to have a strong role, with young males grooming less frequently than adults, and younger females grooming more frequently than adult females. These divergent patterns may reflect sex-specific strategies relating to risk management, as well as differences related to

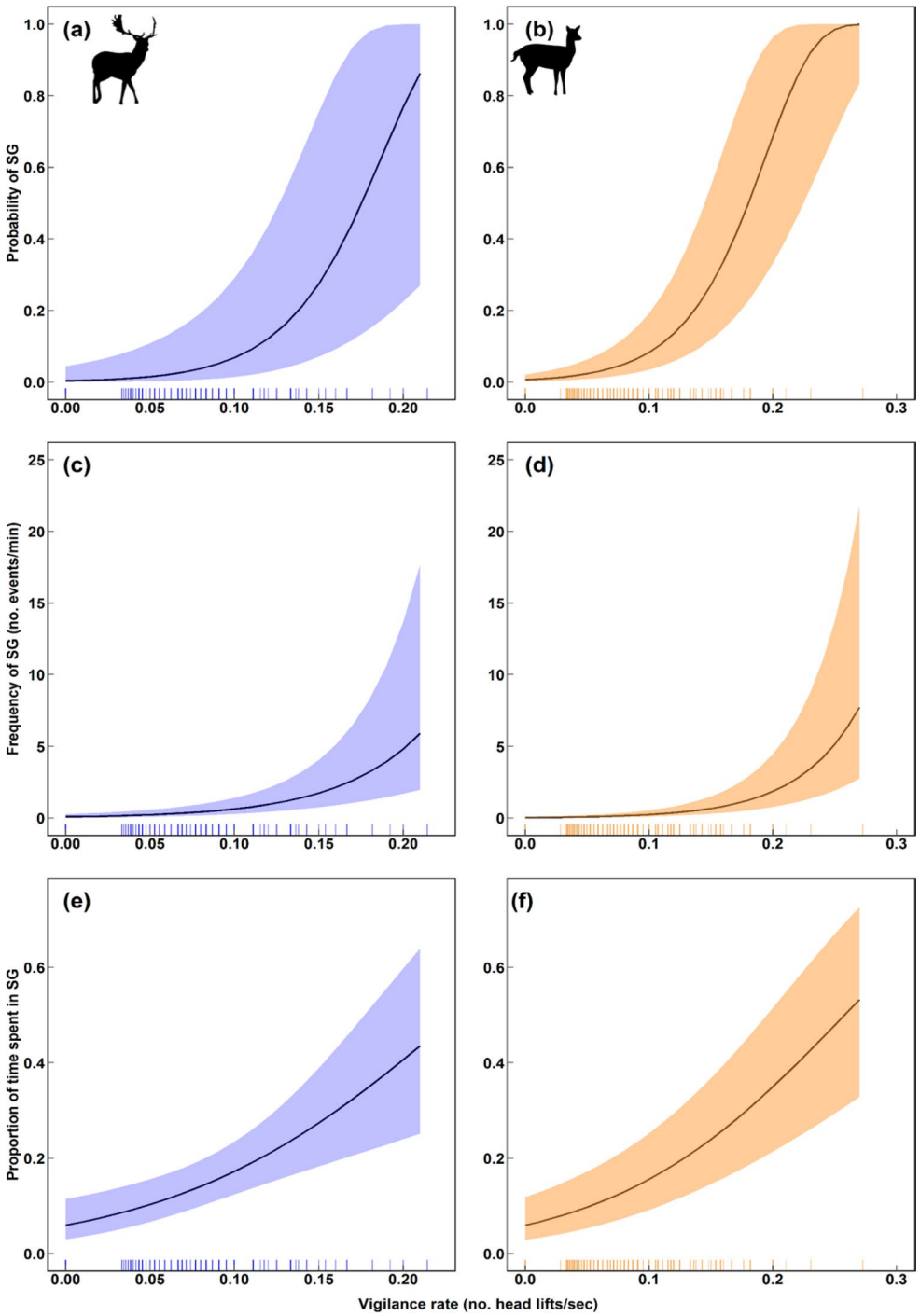

Fig. 2 The effect of vigilance rate on (**a-b**) the probability of exhibiting self-grooming (SG), (**c-d**) the frequency of self-grooming, and (**e-f**) the proportion of time spent in self-grooming, in male (left panels) and female (right panels) fallow deer (*Dama dama*). Lines depict predicted values while keeping constant the other confounding effects, and bands show 95% confidence intervals. Marks along the x-axis show the distribution of observed values. For frequency of self-grooming, predicted values are expressed as number of events/minute to facilitate interpretation

physical or social maturity. This is consistent with Pecorella et al. (2019), who showed that female fallow deer spent less time foraging and were more alert than males, with vigilance decreasing with group size in females but varying across male age classes. These findings are also in line with our previous study (Esattore et al. 2023), where a positive relationship was observed between vigilance indices of female fallow deer and the spatial variation of wolf detection rates. Increased self-grooming in females could have been a way to release tension after increased vigilance, which was not confirmed by our results. Similarly, no group-size effect on self-grooming was supported, although it is associated with vigilance (lower vigilance in larger groups: see Esattore et al. 2023 for our study area).

Obstructive cover can elicit vigilance (Frid 1997), and thick and bushy vegetation has been suggested to make it harder to spot an approaching predator (Torretta et al. 2018). Accordingly, the lower occurrence of self-grooming in open

habitats would be a direct consequence of lower vigilance in those environments (Esattore et al. 2023). In line with prediction v), habitat openness was associated with reduced self-grooming in some models for males, although only the model on frequency of self-grooming showed a weak significance ($p = 0.056$). These findings may suggest a negligibly lower perceived risk in open habitats than in forested ones.

Predictions iii) and iv) were only weakly supported, as wolf activity and time of day had limited or inconsistent effects on self-grooming. During our study, three wolf packs were reported, which indicates a very high predator density in our 90 km^2 study area (Mech and Boitani 2003). In 2017–2018, at an initial stage of wolf recovery, fallow deer were reported to show diurnal activity peaks only in sites with higher wolf detection rates, which suggests the ability to spatially modulate temporal avoidance (Rossa et al. 2021). In 2019–2020 (our study period), fallow deer diurnal activity was spread all over the study area, suggesting a widespread perception of risk associated with longer-term predator presence (Esattore et al. 2023). However, a positive relationship between spatial variation of wolf detection rates and fallow deer vigilance (Esattore et al. 2023) suggests that predators only affected vigilance responses, without directly impacting self-grooming, which is then affected only by increasing vigilance. Wolves are flexible predators (Peterson and Ciucci 2003), able to operate different hunting tactics (Escobedo et al. 2014; Nichols 2015; Gable et al. 2016, 2018; Mech et al. 2021), taking advantage of dense vegetation. Our results further support the idea that risk perception varies throughout the landscape, requiring continuous and fine-tuned adjustment of antipredator strategies to different habitat features (Muro et al. 2011).

Future directions

Our study shows the possibility to quantify fine-scale vigilance-related self-directed behavior by means of camera traps. This method represents a useful non-invasive method, possible to implement in contexts where physiological measurements are challenging (Kuijper et al. 2014). Moreover, our results suggest that self-grooming may serve as a useful behavioral indicator of individual welfare or behavioral state when interpreted in light of species-specific behavioral repertoires, in both wild and captive environments (Maestripieri et al. 1992; Beerda et al. 1999; Manson and Perry 2000; Thompson et al. 2019; Manning et al. 2022). Monitoring behavioral indicators is pivotal and increasingly used for management and conservation purposes (Kotler et al. 2016). Quantifying occurrence, frequency, and duration of self-grooming, particularly when coupled with vigilance, may provide insights into attentional allocation within individuals or groups in wild mammals. Expanding on the use of this methodology, especially through automatic tools such as motion-sensitive cameras, may be particularly relevant to monitor such behavioral states in species dwelling in areas characterized by dense vegetation, for which our ability to capture behavioral variation and information on prey-predator interactions is still limited (Kuijper et al. 2014; Esattore et al. 2023).

Future research could build on these findings by comparing areas with and without predator presence, or areas showing recent significant changes in predator density, to help clarify the role of predation risk in driving self-grooming. Furthermore, comparisons with mammal species that do not form large social groups, or non-obligate gregarious foragers, could help determine whether social context, group vigilance dynamics, or species-specific behavioral strategies influence the observed patterns.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-026-03785-7.

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Author contributions BE conceptualized the work, developed the methodology, coded the data, wrote the original draft, and reviewed and edited the final version of the manuscript. NF developed the methodology, conducted the analyses, took care of data visualization, and wrote and reviewed the final version of the manuscript. FF developed the methodology, acquired the data, wrote and reviewed the final version of the manuscript, and supervised its development. LS coded the data, wrote the first draft, and wrote and reviewed the final version of the manuscript.

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Data availability All data generated or analyzed during this study are included as supplementary material.

Declarations

Ethical note We collected behavioral data through a non-invasive method (camera trapping), avoiding direct contact and manipulation of the study animals. Camera installation, checking, and removal generally required < 10 min of attendance by the operator at each location, thereby minimising disturbance to wildlife. The study was conducted according to existing laws and was authorised by the responsible institution (Ente Parco Regionale della Maremma).

Competing interests The authors declare no competing interests.

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