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Impact of capture and chemical immobilization on the spatial behaviour of red deer *Cervus elaphus* hinds

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Research and management programs of wild animal populations often require intensive field actions, which make direct interventions on animals such as capture, immobilization and manipulation, frequently necessary. Such events, however, could induce a stress response causing physiological and behavioural alterations in the individuals. Knowing the impact of these practices in the post-release phase is of crucial importance when studying the spatial behaviour of wild species. Our study aimed to investigate the potential effect of narcosis and application of GPS collars on 17 red deer *Cervus elaphus* hinds living in a mountainous area in northern Apennine, Italy. We conducted the analysis at a temporal scale of 45 days after release, in order to assess the response of animals in terms of changes in movement rates, distance from the barycentre of their positions and distance from the capture sites. We then compared habitat selection between the period of disturbance and the following days. This work represents the first study of the behavioural responses of wild red deer to capture and handling procedures. We showed that these operations significantly affect the spatial behaviour of hinds by inducing a short-term increase in movement rates and by keeping the animals away from the centre of their activity, for a period no longer than 10 days. The habitat selection analysis remarked a tendency to escape from human disturbance, by avoiding hunting sites and anthropized locations in favour of forest cover. Red deer hinds thus, seem to cope with stressful events and disturbance by adopting a flight behaviour, which includes moving away from the capture area and seeking refuges. The differences highlighted between the 'stressful' period and the following one, showed that such modifications persist for a limited time frame and suggested that this capture method is unlikely to lead to permanent alterations of behaviour.

Keywords: narcosis, disturbance, generalised additive mixed models, habitat selection, ungulates, GPS telemetry

Research and management programs involving wild animal populations require intensive field actions, and direct interventions on animals are frequently necessary to ensure their monitoring and conservation. Interventions such as translocations, tagging, sampling, insertion of implants and fitting of radio-collars have different levels of invasiveness, nevertheless they require the animals to be captured, immobilised and manipulated. Such unpredictable events induce immediate physiological and behavioural adjustments in order to cope with the perturbation (Wingfield 2005) and may result in an intense stress response. In this context, response can be attributed to either the restraint during capture operations or to the pharmacological treatment. Capture stress may induce physical (Del Giudice et al. 2001, Haulton et al. 2001, Arnemo et al. 2006), physiological (Kock et al. 1987, Marco

and Lavin 1999, Del Giudice et al. 2001, Montané et al. 2002, West et al. 2014) as well as behavioural consequences, such as injuries, alteration of normal patterns of behaviour or even mortality (Rachlow et al. 2014). Physiological and behavioural reactions to stress are under hormonal control: animals exposed to noxious stimuli respond by releasing glucocorticoids, i.e. cortisol in most mammals (Romero 2004), which are responsible for recovery mechanisms. Such responses can include the stimulation of the immune system, escape or avoidance behaviours, suppression of feeding as well as of reproduction and serve to focus the animal's attention to behaviours necessary to deal with the stressor (Reeder and Kramer 2005). In free-ranging animals post-capture stress can result in a modification of spatial behaviour, such as moving away from the capture site (Arnemo et al. 2006) or increasing displacement, as well as reducing or increasing movement rates (Cattet et al. 2008, Neumann et al. 2011, Quinn et al. 2012, Northrup et al. 2014). Common modifications of behaviour involve variations in activity levels or rhythms, e.g. reduced activity rates (Morellet et al. 2009, Brivio et al. 2015, Graf et al. 2016), or alteration of habitat

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selection e.g. refuge seeking (Argenti et al. 2015) and positive selection of wooded areas (Morellet et al. 2009) were frequently observed in wild ungulates. Moreover, changes in behavioural patterns and habits are supposed to affect the animal's life-history traits, for instance by negatively influencing fighting ability and consequently mating success (Pelletier et al. 2004) or inducing the female to separate from offspring (Arnemo et al. 2006, Del Giudice et al. 2018).

Rigorous monitoring and handling protocols have been developed to minimize capture related injuries and mortality. However, short, medium and long term effects on behaviour in sensitive species can hardly be prevented. In addition, a certain amount of time could be required for animals to be accustomed to wear monitoring devices such as GPS or VHF collars. Thus, a better understanding of the influence of capture, manipulation and monitoring devices on post-capture behaviour is mandatory to be able to work with reliable data representing the usual animal's habits.

Although the effects of capture and immobilization on movement and activity were studied for diverse wild ungulate species (e.g. *Capreolus capreolus*, *Alces alces*, *Capra ibex*, *Rupicapra rupicapra*), to our knowledge no investigations were conducted on the modifications of behaviour in red deer *Cervus elaphus*. The aim of our study was to investigate the short-term effects of narcosis, handling and application of GPS collars on red deer spatial behaviour: we hypothesized that post-capture stress would result in displacement from the capture site as well as an alteration of movement patterns and habitat selection. We also expected that such

modifications would be prominent in the first hours immediately following the operations and that, subsequently, the animal would return to a baseline situation. In order to test these hypotheses, we conducted the analysis in two stages: 1) assessing the response of animals to capture-related operations and the duration of the eventual period of disturbance; 2) comparing habitat selection between the period of disturbance and the following period, when the animal returns to its usual activities.

Material and methods

Study area

The study was conducted in a mountainous area stretching over several municipalities in the Provinces of Prato, Pistoia and Bologna, central Italy. The territory extends for approximately 22 000 ha (43°92'–44°07'N, 10°99'–11°15'E) over the two sides of the northern Apennine mountain chain (Fig. 1). The topography of the study site encompasses mountains, with peaks reaching 1250 m above sea level, hills, plains and valleys. The climate is oceanic, precipitation peaks during autumn and winter, while temperatures vary from a minimum of –1.1°C in February to a maximum of 24.3°C in August (average of the values recorded in the last 30 years).

The study area is mainly covered by woodlands: chestnut trees *Castanea sativa*, oaks *Quercus cerris* and mixed

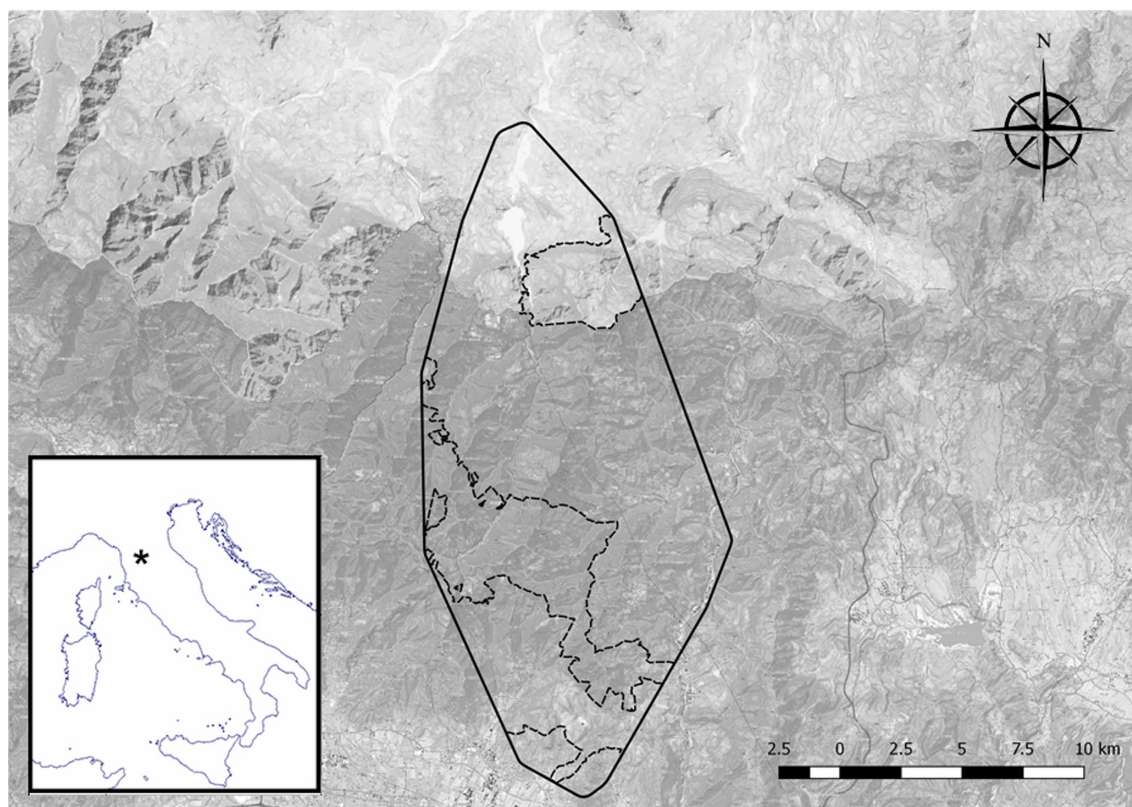


Figure 1. Study area (black solid line of the polygon in the map). The area delimitates the outer boundaries of the territories frequented by the 17 hinds. The edge separating the darkest and the lightest part of the map corresponds to the northern Apennine crest, Italy. The dotted lines represent the boundaries of the protected areas.

hardwood formations, mainly *Ostrya carpinifolia*, dominate forests at lowest altitudes while, in the highest parts, mountain beech *Fagus sylvatica*, in association with maples *Acer* spp., prevails. The two sides of the Apennine chain, however, are characterized by different landscape structures as well as by a diverse mixture of natural and cultivated plant communities. At the southern side (Prato and Pistoia, Tuscany), mountain agriculture and pastoralism were abandoned during the last decades, resulting in a rather homogenous landscape dominated by woodlands, while agricultural patches are fragmented and less extended (about 8% of the area). Conversely, at the northern side (Bologna, Emilia Romagna) crops and natural pastures are more common (16%) and interspersed with woodlands, thus generating a mixed landscape structure. Ungulates (*C. capreolus*, *C. elaphus*, *D. dama*, *S. scrofa*) are well represented in the area and they are hunted on a regular basis in the whole territory, with the exception of protected areas, which represent 27% of the study site (Fig. 1). Here, the red deer population underwent a rapid growth during the last decades, with a parallel increase of the occupied territories. During the past 10 years, the population stabilised with an estimated density of 1.92 deer/100 ha.

Chemical immobilization and data collection

Between 2008 and 2016, 17 red deer hinds were captured (Supplementary material Appendix 1 Table A1) as part of two projects aimed to study the spatial behaviour of red deer population in northern Apennine. The capture sessions were conducted during nighttime by two teams with substantial experience in the immobilization of ungulates, composed by at least two wildlife technicians and a veterinarian. The animals were chemically immobilized from a car using a dart gun, with a 1:1 mixture of Zoletil and Xylazine. After the shot, the teams observed the animals and followed them for up to 15–20 min and, when they lied down, the veterinarian checked their health condition. We collected biometric data as well as the coordinates of the capture site, and then applied Vectronic Aerospace GPS/GSM collars (ProLight4, weight ~ 980 g); finally, the veterinarian administered Antisedan in order to reverse the effects of the sedatives. All capture and handling procedures were approved by Istituto Superiore per la Protezione e Ricerca Ambientale (ISPRA) and by local authorities. We scheduled the GPS/GSM collars to collect one position every hour, and they were equipped with a timer-controlled drop-off, programmed to release the device after 2.5 years. In case we were able to successfully retrieve the collars, we downloaded the data directly from the devices; otherwise, we used the positions received by GSM using a Ground Station and the software GPS Plus Collar Manager (Vectronic Aerospace). We retained only positions obtained using at least four satellites (i.e. 3D fix) and with a DOP < 10 (96.9% of the original data), in order to exclude the ones with poor accuracy (Adrados et al. 2002). For every hind, we obtained on average 23.1 ± 1.2 positions day⁻¹, homogeneously during the entire study period. We derived sun position at the time of every animal position by ephemeris tables taken from US Naval Observatory (<www.usno.navy.mil/USNO/>), and we defined twilight the interval between nautical twilight and sunrise or sunset.

Statistical analysis

Assessing duration of disturbance period

To test the hypotheses that capture and handling operations would impact the hinds displacement from capture site and modify their movement patterns, we first selected three metrics summarising their ranging behaviour: 1) movement rate, i.e. Euclidean distance between consecutive positions at hourly intervals (meters/hour), as an index of the animal's intensity of movement. 2) Distance of each position from the barycentre of all GPS fixes (meters), as a measure of the animal's displacement from the centre of its activity. 3) Distance of each position from the capture site (meters). We conducted the analysis at a temporal scale of 45 days, starting from the moment of the capture. We tailored this time span for our study case and it proved to be adequately long to detect short-term alterations of behaviour due to capture stress in ungulates, as already found by Morellet et al. (2009) and Brivio et al. (2015), but restricted enough not to encompass changes in spatial behaviour of our hinds, such as seasonal migration which occurred later. For each individual, we calculated the three metrics in GIS environment (QGIS ver. 2.18.20). Under the hypothesis that capture-related stress would influence the animal's activity and site selection, we expected that the relationship between each of these measurements and time since release would be significant and vary in a non-linear manner. Accordingly, we utilised generalised additive mixed models (GAMM) (Wood 2006) and examined these relationships with a smoothing spline (R ver. 3.4.1 <www.r-project.org>, 'mgcv' package). We fitted three models using movement rate (MRate), distance from the barycentre of GPS fixes (Db) and distance from the capture site (Dc) as independent variables, and applied logarithmic transformation in order to improve normality of the residuals and to limit the skew of the distribution. Time since capture was expressed in hours. Apennine side (northern, southern) was included in the GAMM due to the different availability of key habitat and consequently of food resources for the animals. Apennine side, time of the day (day, twilight, night) and month of capture were included in the analysis as fixed factors. Finally, we considered the individual hinds as random effect. We evaluated the goodness of fit of the models by inspecting the residuals. Using values predicted by the three models, for each hind we plotted behavioural metrics against hours from capture and, in every case, a steady decrease in predicted values was evident along the first days following release. Thus, we considered the first minimum point of the predicted smoothing spline as the threshold to define the individual phase of post-capture disturbance. We then defined a population-level threshold by averaging the individual phases (i.e. hours after capture), to discern an overall 'disturbance period' (hereafter 'period A') from the following days (hereafter 'period B'). In order to verify and quantify the differences between the two periods, we repeated the GAMM analysis including this categorical variable in the model as fixed effect.

Habitat selection

Once we ascertained the occurrence of alterations in spatial behaviour, we aimed to investigate whether capture related 'stress' could have influenced resource selection in hinds: in

Table 1. Movement rate during the 45 days post-capture period. Coefficients estimates, standard error and significance for the generalised additive mixed model fitted to the logarithm of MRate.

Predictors	Coefficient estimate	Standard error	Probability
Intercept	3.779	0.124	< 0.001
Period:			
B	-0.044	0.080	0.582
Apennine side:			
southern	-0.342	0.124	0.006
Sun position:			
day	-0.103	0.022	< 0.001
twilight	0.257	0.037	< 0.001
Capture period:			
October	-0.139	0.218	0.525
November	0.371	0.144	0.01
January	-0.080	0.123	0.514
February	-0.076	0.213	0.723
March	0.007	0.213	0.973

particular, we predicted that the animals would seek undisturbed sites and avoid anthropized areas. For this purpose, we selected four environmental variables as indicators: distance to the nearest protected area (DistP), type of land cover (i.e. woodland or open areas), distance to the nearest road (DistR) and distance to the nearest anthropized area (DistA). By means of resource selection functions (RSF) (Johnson et al. 2006), we aimed to study habitat selection during the ‘disturbance period’ and the following days. For period A, based on the results of our previous analyses, we set a duration of 10 days after release, while in period B we analysed the remaining 35 days. We conducted the analysis at the individual level by fitting two logistic regression models (one for each period) for every hind.

The restricted time frame chosen for the selection study and the individuality of the analysis were selected to meet the assumptions behind resource selection analysis. Being the selection process affected, for instance, by season, age class and effective availability of resources, these assumptions suggest to avoid pooling information across variable contexts in order to prevent erroneous inference (Manly et al. 2002). Prior fitting resource selection functions, we tested for correlation between the selected independent habitat

variables: considering a threshold cut-off of $r=0.6$ (Hosmer and Lemeshow 2000), there was a low level of multicollinearity in our dataset, thus we retained all the four candidate variables in the models. We defined as ‘available’ the resources within each animal’s minimum convex polygon (MCP) built using 100% of their positions during the 45 days period. MCP does not offer information on the variance of space use within the delimited area, however it is considered suitable for characterizing the available habitat inside a home range (Roffler et al. 2018). We sampled available resources by generating a grid of 5000 points within each MCP, whereas to define ‘utilised’ habitat we used all positions collected on the hinds.

Results

The results of the GAMM analysis of MRate indicated that the hinds inhabiting the two sides of the Apennine Mountain displayed significant differences in the extent of movement rate during the investigated period (Table 1). In particular, the animals of the southern (Tuscany) side showed an overall lower mobility. Different levels of movement were also observed in various phases of the day: the maximum was detected during twilight and the least during daylight. Moreover, the hinds captured in November displayed higher levels of mobility. Overall, in the hours immediately after the release, the movement rates were substantially high, however they rapidly decreased during the first week and then remained relatively constant for the rest of the period (Fig. 2). When considering values predicted from the model at the individual level, this same initial trend appeared for all the hinds; on average the decline ended at 8.42 ± 1.13 days after release, when the splines reached their first minimum point. The estimated coefficients of the GAMM indicate that movement rate slightly decreased in period B (Table 1), yet not significantly.

When observing the behaviour of the animals in relation to the distance from the barycentre of their positions (Table 2), the results appeared similar to those of MRate, showing in general lower displacement in hinds from the

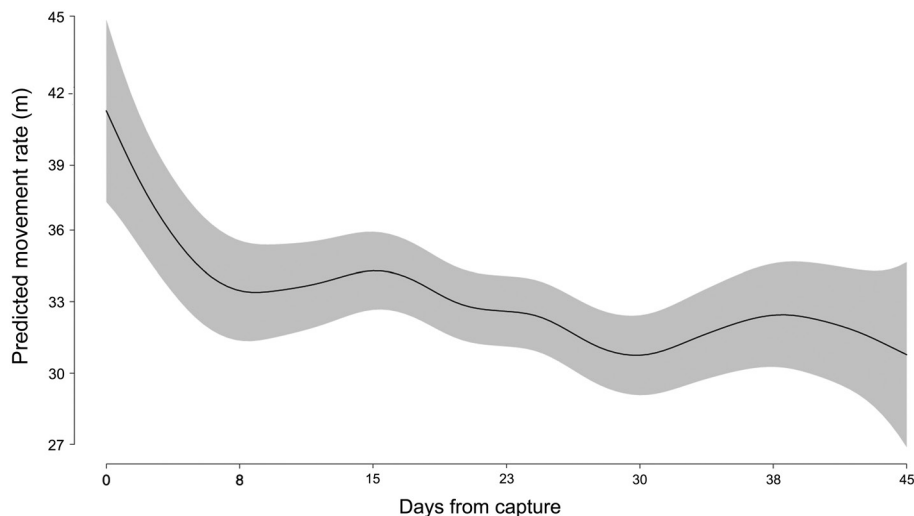


Figure 2. Smoothing spline of the predicted movement rate (MRate) from the generalised additive mixed model for the 17 red deer hinds.

Table 2. Distance from the barycentre of the hinds positions during the 45 days post-capture period. Coefficients estimates, standard error and significance for the generalised additive mixed model fitted to the logarithm of Db.

Predictors	Coefficient estimate	Standard error	Probability
Intercept	6.414	0.269	< 0.001
Period:			
B	-0.121	0.053	0.022
Apennine side:			
southern	-0.630	0.316	0.046
Sun position:			
day	-0.020	0.011	0.067
twilight	-0.047	0.018	0.011
Capture period:			
October	-0.467	0.560	0.405
November	1.188	0.368	0.001
January	-0.114	0.316	0.717
February	-0.227	0.547	0.678
March	0.072	0.547	0.896

southern Apennine side and higher values for the ones captured in the month of November. The overall model displays a general tendency for the hinds to move farther from the centre of their activities soon after the release phase (Fig. 3). The examination of individual predicted values revealed a similar trend for all the animals in the first days post-capture, followed by a rapid return to their habitual areas with restricted, although regular, fluctuations. On average, the duration of the period of altered behaviour was 5.93 ± 3.25 days since release, after which the hinds moved significantly closer to the centre of their home range (Table 2).

None of the factors evaluated in this analysis affected the behaviour of the hinds in relation to the site of capture, with the exception of the time of the day (Table 3): on average, the animals appeared to move farther from those sites in presence of sunlight. The spline obtained from the overall model displayed no clear pattern during the first hours post-release: Dc peaked after approximately 70 h, however the trend appeared rather fluctuating during the study period (Fig. 4). The inspection of the individual

patterns of predicted values (Supplementary material Appendix 1 Fig. A1) revealed that, for the majority of the hinds, Dc tended to decrease over time after reaching the highest peak after around 2.70 ± 0.19 days. However, three animals (F1, F2, F6; Supplementary material Appendix 1 Table A1) progressively increased their displacement from the capture site during the 45 days, while only in one hind no clear trend was evident. F1 was a migrating hind, which progressively expanded its home range, but started to shift two months after the capture. F2 and F6 were resident hinds which started expanding their home range. Overall, distance from the capture site slightly decreased in period B (Table 3), although this variation was not significant. Globally, narcosis, handling and application of GPS collars appeared to have an effect on the spatial behaviour of red deer hinds for a period no longer than 10 days.

During period A, a significant selectivity towards protected areas was detected in the majority of hinds (16 out of 17). Among these, 11 tended to prefer sites near or within areas where hunting is forbidden; conversely the other five selected sites located far from protected areas.

Subsequently, after the end of the capture-induced disturbance period, 15 out of 17 hinds significantly selected their habitat in relation to distance from protected areas, but only six animals selected areas nearest to protected sites. When analysing habitat selection in relation to distance from anthropized areas and from roads, most hinds (15 and 14, respectively) displayed a significant selectivity during both periods. In period A, selection was addressed mostly for areas located farther from urban sites and roads, while during period B an opposite behaviour emerged. Finally, by examining the two different land cover types, a significant selectivity emerged in period A only for six hinds: among these, four preferred the woodland habitat category and two the open areas. Instead, a greater tendency to select land cover types emerged during period B, when the hinds' choice was equally divided between the two habitat categories, with seven animals preferring open areas and seven selecting woodlands.

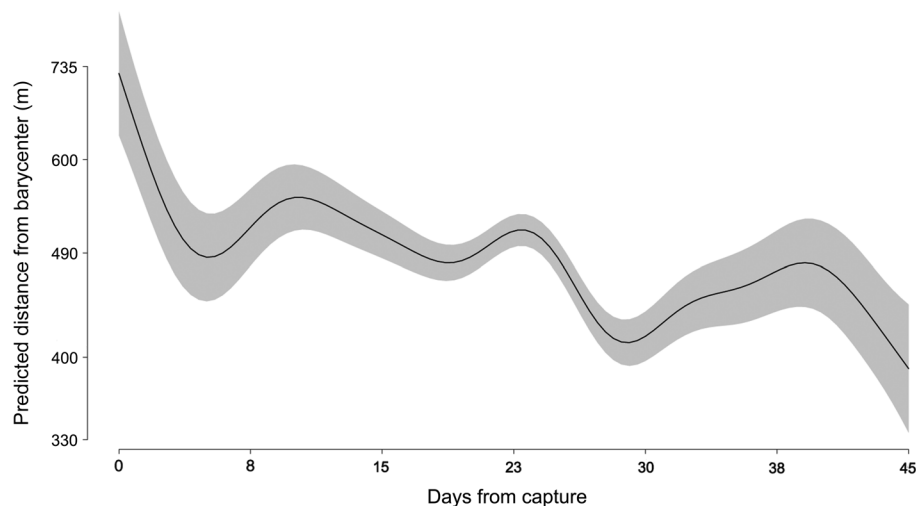


Figure 3. Smoothing spline of the predicted distance from the barycentre (Db) from the generalised additive mixed model for the 17 red deer hinds.

Table 3. Distance from capture site of the hinds during the 45 days post-capture period. Coefficients estimates, standard error and significance for the generalised additive mixed model fitted to the logarithm of Dc.

Predictors	Coefficient estimate	Standard error	Probability
Intercept	6.484	0.373	< 0.001
Period:			
B	-0.009	0.055	0.872
Apennine side:			
southern	-0.351	0.418	0.402
Sun position:			
day	0.154	0.010	< 0.001
twilight	0.078	0.018	< 0.001
Capture period:			
October	0.695	0.742	0.349
November	0.881	0.487	0.071
January	0.125	0.418	0.766
February	0.231	0.724	0.749
March	-0.485	0.724	0.503

Discussion

This work represents the first study of the behavioural responses of wild red deer to capture and handling procedures. Blanc and Brelurut (1997) studied the effects of wearing a satellite-tracking collar on the social relationships and food activity in captive red deer. The research, however, was conducted under different conditions (the animals were kept in pens and the collars were heavier), thus their results probably do not reflect the responses of wild deer fitted with modern devices.

Our research showed that capture and handling operations significantly affect the spatial behaviour of hinds, by inducing a short-term increase in movement rates and by displacing the animals farther from the centre of their activity. Movement is crucial not solely for individuals but also for population dynamics, and it is the result of the interaction between intrinsic (i.e. the animal's internal state) and environmental (i.e. external factors) cues (Nathan et al. 2008). By analysing the extent of movements and of displacement in a short-medium temporal frame, we accounted

for differences in spatial behaviour related to phases of the day, period of the year as well as to environmental variables. Red deer are known to forage mainly at night, with peaks of activity around sunset and sunrise (Georgii 1981, Georgii and Schroder 1983, Ensing et al. 2014), as for most ungulate species living in temperate regions (Signer et al. 2011, Pagon et al. 2013). Our data showed that movement rate follows this pattern and that hinds tend to move farther from the centre of their activity during night. The spatial behaviour varied also in relation to the month of capture, showing a significant increase of mobility in hinds captured and collared in November. The increase in movement rates occurring in this period could be partly owed to changes in ranging behaviour due to the seasonal decrease in food availability. However, it has to be considered that, in this month, the wild boar hunting season opens in the study area. This culling technique involves the presence of hunter teams composed by several groups of people and dogs, thus causing disturbance and alarm not only in the target species but also in other wildlife (Scillitani et al. 2010). Differences in post-capture behaviour related to the extent of mobility and displacement, seem to arise between animals inhabiting areas with diverse landscapes and habitats, as in this case. In particular, the study area located in Southern side of the Apennine chain, where the animals showed a more "sedentary" behaviour, is characterised by a larger occurrence of woodlands, offering a habitat rich in shelters and thus less subjected to anthropogenic disturbance. This is consistent with behaviour patterns detected by Allen et al. (2014), who found that red deer moved greater distances in landscapes dominated by arable areas and pastures than in those mostly covered by forests. Moreover, red deer display a preference for closed canopy with high density of trees (Bobek et al. 2016).

The amount of time necessary for the hinds to return to their baseline behaviour, no longer than 10 days in our study, is consistent with the results of analogous studies on wild ungulates, which remarked several effects of capture-related operations. Such effects comprise: alterations in animal displacement for few days post release, as found in

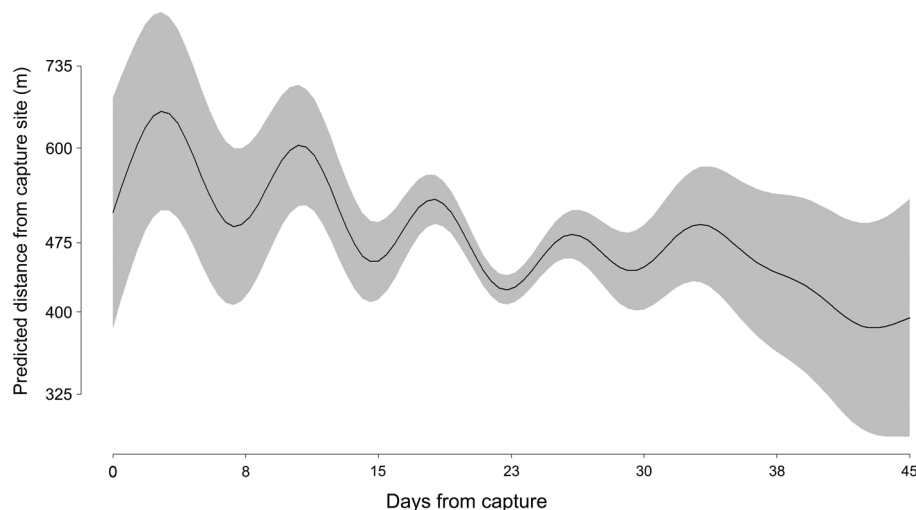


Figure 4. Smoothing spline of the predicted distance from the capture site (Dc) from the generalised additive mixed model for the 17 red deer hinds.

moose *Alces alces* (Neumann et al. 2011) and in mule deer *Odocoileus hemionus* (Northrup et al. 2014); an increase in movement rates for a period up to 10 days, as recorded in roe deer *Capreolus capreolus* (Morellet et al. 2009) and in bighorn sheep *Ovis canadensis* (Clapp et al. 2014); a short-term reduction of activity rates, as in the case of alpine ibex *Capra ibex* (Brivio et al. 2015).

Red deer thus, as other medium and large sized ungulates, seems to cope with stressful events and disturbance by adopting a flight behaviour, which includes moving away from the capture area and seeking for refuges. The habitat selection analysis remarked a tendency to escape from human disturbance, by avoiding areas where hunting is permitted and anthropized locations in favour of forest cover.

The result agrees with the typical response recorded in ungulate species when they are subjected to stressful events such as predation or hunting pressure (Creel et al. 2005, Sullivan et al. 2018). However, the pronounced differences in habitat selection between the 'stressful' period and the following one showed that such modifications persist for a limited time frame, suggesting that this capture method is unlikely to lead to permanent alterations of behaviour.

GPS collars with high sampling frequency allow for not only investigating long-term changes in animal movement, but also represent valuable instruments to evaluate the impact of narcosis and handling procedures which are often necessary to deploy such devices. Assessing the impact of these operations should become a necessary step, in order to improve the reliability of the results when studying fine scale animal behaviour, and to help developing low-impact field capture protocols.

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Supplementary material (available online as Appendix wlb-00499 at <www.wildlifebiology.org/appendix/wlb-00499>). Appendix 1.