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Capture effects in wild boar: a multifaceted behavioural investigation

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Although the proliferation of the wild boar in Europe makes capturing and handling necessary for both management and research, the behavioural responses of this species to capture are still unknown. We evaluated how capture affects wild boar behaviour during the first 30 days after the release, focusing on the animals' total activity, mobility and activity rhythms and their variation in response to different drug mixtures used for sedation. Low levels of activity and mobility characterized the first 10 post-capture days. After this period, a gradual restoring of stable levels occurred. Wild boar captured by using different drug mixtures exhibited slightly different patterns of activity depression. We also showed capture to produce a partial effect on wild boar behavioural rhythmicity. Our findings highlight the case study variability of the capture effect and offer useful insights into several conservation and management implications.

Keywords: activity rhythms, chemical immobilisation, spatial behaviour, *Sus scrofa*

The capture of individuals is a key tool for pest species management, both as a direct management option and as a fundamental resource for research on their biology. In order to mitigate the impact on agriculture and ecosystems, it is often useful to remove individuals from the environment: capturing living animals allows for their displacement or confinement in areas where their presence is not in conflict with human activities. This practise is essential when culling is legally or ethically unfeasible, as in the case of many protected areas and in most urban or suburban contexts. Moreover, an efficient pest species management needs continuous updates of information on the species' biology, ecology and behaviour. While non-invasive procedures provide some useful research data (e.g. direct observation for behavioural studies and collection of faecal, hair, feather or carcass samples for molecular investigation), certain pieces of information can only be obtained by capturing and handling animals. This is the case of blood samples, repeated biometric measures, individual marking for identity recognition and the application of tools for biotelemetry studies.

In the last decades, wild boar *Sus scrofa* populations rapidly increased in Europe because of both human manipulation and environmental changes (Apollonio et al. 2010, Massei et al. 2015, Vetter et al. 2015). As this proliferation has caused conflicts with human activities (damages to crops, zoonoses transmission and vehicles collisions) and is a threat for local biodiversity conservation (Massei and Genov 2004), wild boar is considered a pest species in many European countries and the capture of individuals has become an increasingly common practice. Nonetheless, how and how long a capture event can affect wild boar behaviour remains yet unknown. This lack of information results in unpredictable potential disturb effects on behavioural research results when capture is involved.

Capture is probably one of the most stressful episodes which can occur in the life of large mammals (Koch et al. 2017) as it often overturns their behavioural patterns (Chi et al. 1998, Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014) and can even increase their mortality rate (Kock et al. 1987, Beringer et al. 1996, Arnemo et al. 2006, Jacques et al. 2009). Capture-related stress can affect animal behaviour in many ways. A general higher tendency to avoid humans after capture events was observed by

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Chi et al. (1998) in black bear *Ursus americanus*. Similarly, Morellet et al. (2009) found that captured roe deer *Capreolus capreolus* remained further from anthropic structures in the first 10 days after the capture event in comparison with the subsequent 40 days. A sharp reduction of activity and/or movements of the captured individual was also observed (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015). Activity gradually increases back to the normal base-line situation over a period that varies, depending on species and capture methods, from a maximum of 36 days reported for black bear movement rates (Cattet et al. 2008) to a minimum of two days for restoration of normal activity levels of Alpine ibex *Capra ibex* (Brivio et al. 2015). On the other hand, a weak, but still notable, inverse effect has been observed on moose *Alces alces* by Neumann et al. (2011), who found greater spatial displacements for up to 4.5 days after capture.

Only recently, researchers have devoted their attention to investigate the circadian rhythms of wild mammals through a chronobiological approach, one which must include analyses of the periodicity of locomotor activity (Brivio et al. 2016, 2017, Grignolio et al. 2018). This kind of analysis is rare in research on wild fauna, partly because it demands detailed information on wild animals' activity that can only be provided by highly sophisticated technology, such as GPS-collars equipped with accelerometers. On the other hand, this approach would provide the opportunity to examine the potential alterations of behavioural circadian rhythms related to capture-stress, which have never been evaluated in large mammals.

The relation between stress and circadian system, however, has been thoroughly investigated in laboratory rodents. Stress is able to affect the circadian clock and stress responsiveness varies during the day (Koch et al. 2017). For instance, the expression of *Period1* and *Period2*, two cardinal components of the molecular circadian clock network, were found to be affected by acute or chronic stress (Takahashi et al. 2012, Al-Safadi et al. 2015). Animals have evolved to adapt to stress at both a physiological and a behavioural level by the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of glucocorticoids. The HPA axis and its hormonal components are under the direct control of the circadian timekeeping system (Oster et al. 2006, Nader et al. 2010). Indeed, glucocorticoids display marked diurnal rhythms, with the highest levels during the active phase, and their response elements are present in the promoter of *Period* genes (Kalsbeek et al. 2012, Dickmeis et al. 2013). Capture is bound to cause acute stress in animals, potentially inducing these modifications in their circadian rhythms.

Capture induced stress is caused mainly by manipulation (i.e. trapping, handling, eventual translocation and releasing). Accordingly, the method implemented to capture wild animals can differently affect the animals' health conditions, their long-term survival probability and their behavioural responses to capture (Kock et al. 1987, Beringer et al. 1996, Brivio et al. 2015). Large mammals can be captured with different methods, such as leg snares, vertical and horizontal dropping nets, net-guns, traps and teleanaesthesia. Although avoiding the use of drugs during the capture prevents any drug side effect, it entails higher injury risk for both animals and operators and an even higher potential stress effect, due to the fact that animals are handled while awake. For example, although roe deer were captured without sedation, they showed depressed activity levels and shifted space and habitat use for up to 10 post-capture days (Morellet et al. 2009). Moreover, in case of larger or potentially aggressive species (such as adult wild boar) the animals' body mass and strength make sedation a necessary choice to prevent risks for operators during handling. On the other hand, anaesthesia may trigger several side effects, including hyperthermia, hypoxemia and heart rate variation (Fahlman et al. 2011). Different in vivo and in vitro investigations showed that anaesthesia also strongly affects the circadian clock by altering the expression of its molecular components and by phase-shifting or disrupting behavioural rhythmicity (reviewed by Poulsen et al. 2018). Interestingly, the impact of anaesthesia on circadian rhythms appears to be stronger when drugs are administered during the animals' active phases and when the selected drug mimics the mechanism involved in the adaptation to photoperiodic variations (Cheeseman et al. 2012, Ludin et al. 2016).

Our aim was to investigate how and how long the protocols generally implemented by managers to capture wild boar can affect its behaviour, focusing on its behavioural circadian rhythms, activity and movements rates. Based on previous research on other species, we predicted that wild boar would exhibit a depression of activity and movements for a period of *n* days after capture and that achievement of stable levels would follow a gradual increase. Secondly, we predicted that different drug mixtures would affect post-capture behavioural patterns differently.

Material and methods

Study area

The study was conducted in two different study areas located in the Casentino valley, in the Tuscan Apennine (Province of Arezzo, central Italy, 43°48′N, 11°49′E, Fig. 1). In both study areas, the climate is temperate-continental, with hot and dry summers and cold and wet winters. The highest mean temperatures are reached in July and the lowest in January. Snowfalls are occasional and usually start in October and may continue through April.

The Oasi Alpe di Catenaia study area (OAC) covers a surface of about 120 km^2 and includes a forested protected area of 27 km^2. Elevation ranges from 300 to 1414 m a.s.l. Seventy-six percent of OAC is composed of mixed deciduous woods, dominated by copses of oaks *Quercus* spp. and chestnuts *Castanea sativa* as well as beeches *Fagus sylvatica* used as high stand; 17% of it consists of open areas and bushes and the remaining 7% of conifer woods (mainly composed of black pine *Pinus nigra* and Douglas fir *Pseudotsuga menziesii*; see Merli et al. (2017) for more details about OAC). The wild boar and the roe deer are the most abundant ungulate species, but red deer *Cervus elaphus* and fallow deer *Dama dama* have also been observed. In the OAC study area, the wild boar is the main prey for wolves *Canis lupus*, while the

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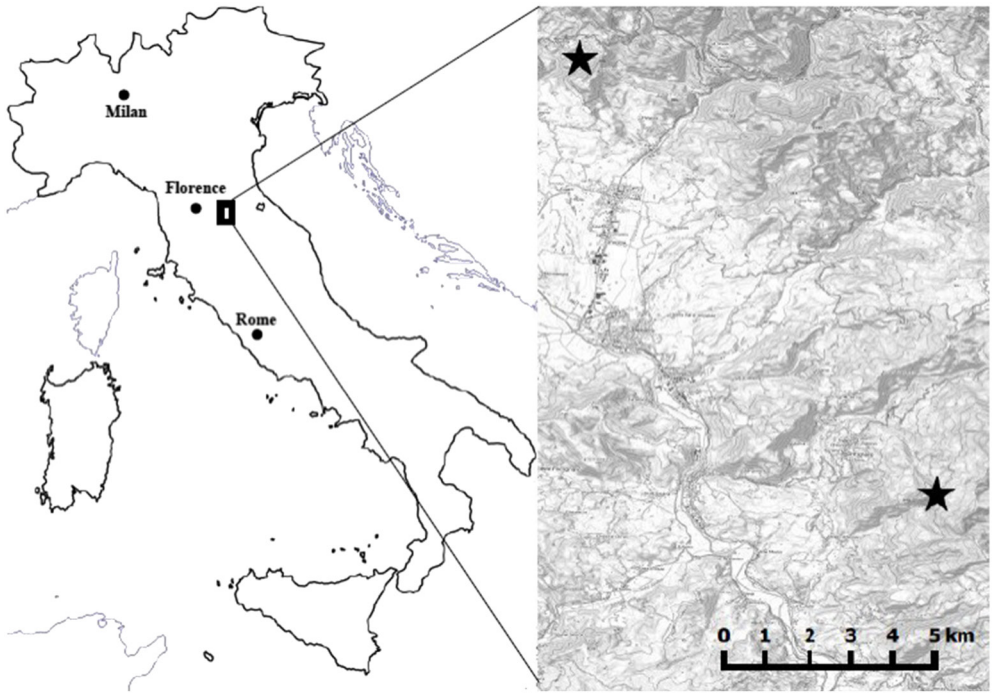


Figure 1. Map of Italy (left) showing the localisation of Casentino valley and an enlargement of the map with both study areas localisation (right). The southern and the northern stars represent the Oasi Alpe di Catenaia and the Foreste Casentinesi National Park study areas, respectively.

red fox *Vulpes vulpes* preys only on piglets (Bassi et al. 2012, Davis et al. 2012).

The second study area lies in the southern part of the Tuscan slope of Foreste Casentinesi National Park (FCNP). About 137 km^2 of its surface (150 km^2) is included in the protected area and elevation ranges from 500 to 1289 m a.s.l. About 85% of landscape is covered by woods (mainly composed by beech, oaks, chestnut, silver fir, *Abies alba*, Douglas fir and black pine), in large part used as high stands, while 15% is occupied by shrubs and pastures. The FCNP study area is inhabited by a rich ungulate community, with high densities of wild boar, red deer, fallow deer and roe deer. As in OAC, in FCNP the wolf preys mainly on wild boar (Mattioli et al. 2011).

In both protected areas any form of hunting is strictly forbidden, while outside wild boar hunting is permitted from about mid-September to the beginning of January (for more details see Grignolio et al. 2011).

Data collection

Wild boar were captured by means of traps baited with maize from June 2013 to January 2017 (Supplementary material Appendix 1 Table A1). Baited traps were set at night only, in order to minimize the physiological stress due to high temperatures during the hot season. Traps were activated at dusk and checked in the early morning to minimize the period of time any captured animal would spend in the trap. Each captured wild boar was first forced into a small cage that strongly limited its movements and then manually sedated. We sedated the captured animals in the early morning: thus, the temporal effect of drugs on their circadian system was minimized (Poulsen et al. 2018), as the wild boar resting period typically starts in the early morning (Brivio et al. 2017). Sedation was performed using a mixture of zolazepam and tiletamine (Zoletil 50 + 50 mg ml^{-1}), either alone or in combination with xylazine (Fournier et al. 1995, Casas-Díaz et al. 2015). At each capture, type and amount of the injected drugs and time of injection were recorded. The operators visually estimated the weight of the captured boar in order to define the dosages to inject. The actual mean of performed injections (i.e. drug dosage /animal body weight estimated by dynamometer) was: 4.00 ± 1.59 mg kg^{-1} of zolazepam–tiletamine mixture, when used alone, and 0.99 ± 0.18 mg kg^{-1} of zolazepam–tiletamine mixture when used together with xylazine (1.70 ± 0.47 mg kg^{-1}). Biometric measures (i.e. body weight, total length, neck and thorax circumference and age, estimated by teeth eruption and consumption) were taken for each individual. Finally, a GPS collar (GPS PRO Light collar) was applied. The handling of each captured animal took about 40 min. All collars were configured to record their GPS position every two hours. Moreover, collars were equipped with activity sensors (i.e. dual-axis accelerometers) so as to measure the acceleration experienced by the collar themselves (within the dynamic range –2G / +2G, with G = gravitational constant). Activity was measured four times/second as the acceleration variation between consecutive values on axis x (forward/backward direction) and y (sideward and rotary direction) independently. Activity data were averaged over a time interval of 4 min and recorded in the collar memory within the relative range between 0 (no activity) and 255 (–2G / +2G), with associated date and time. Only activity measured on x-axis has been analysed, as it was found to be highly correlated with y-axis activity (Heurich et al. 2014, Brivio et al. 2017).

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The Regional Hydrological Service provided weather data (mean air temperature, mean air humidity and total rain), hourly recorded in the weather station of Poppi (Arezzo province, 43°44′09″ N, 11°45′42″ E).

Data analysis

Actograms were drawn with Activity Pattern software (ver. 1.3.1, Vectronic Aerospace GmbH). In each actogram the presence of activity rhythm was determined by χ^2 periodogram analysis (ActogramJ 1.0; Schmid et al. 2011). Periodogram analyses were performed on 10-day intervals on the whole actogram. Furthermore, we calculated the daily acrophase (ActogramJ 1.0) and determined the average acrophase on 10-day intervals by using vector addition. We then performed a Rayleigh test to determine whether the acrophases deviated from uniform dispersion around the clock and whether they were concentrated at a given time of the day ($p < 0.05$). A Mardia–Watson–Wheeler test was performed to look for differences among average acrophases of different periods ($p < 0.05$).

Our multivariate analysis focused on two patterns of wild boar behaviour: total activity and mobility, expressed within two variables named activity rate (AR) and mobility rate (MR). To assess whether and how they are affected by environmental and capture-related factors, AR and MR were used as dependent variables in two sets of generalised additive mixed models (GAMMs). Wild boar identity was used as a random factor given the nested nature of data. For each individual, only data (activity and GPS positions) recorded during the first 30 days after their capture were included in the analysis. We ran all analyses in R software (ver. 3.2.2, <www.r-project.org>).

Activity values were first transformed by dividing them by the maximum value recorded by the activity sensor (255), obtaining values varying within the relative range 0–1. Depending on the time when they were recorded, all activity records were assigned to twelve 2-h intervals. Then, an AR value was calculated for each interval for each date, as the arithmetic mean of all activity values included. To improve the models' normality of residuals, AR was arcsine square root-transformed and used as dependent variable.

Only ascertained localisations, recorded with at least four satellites and with dilution of precision (DOP) smaller than 10, were used in our analysis. MR was obtained by dividing the straight-line distance between two consecutive positions (m) by their time interval (h). As collars can fail some positioning attempts, the time interval between consecutive localisations could be greater than 2 h. Nevertheless, we excluded from our analysis all MR records with time intervals greater than 6 h. Finally, MR was natural logarithm-transformed and used as dependent variable in the models.

Following the information-theoretic approach (Dochtermann and Jenkins 2011), we started by building a set of alternative hypotheses explaining the possible relations between dependent and explanatory variables, based on the effect of environmental conditions on wild boar activity assessed by Brivio et al. (2017) and on previous research investigating the effect of capture on other species (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015). Each of the four resulting hypotheses was transformed into a statistical model (Table 1). Each competing model was run and the best one selected following the minimum AIC criterion (Symonds and Moussalli 2011), for AR and MR, respectively. Models with $\Delta AIC<2$ were assumed to be as good as the minimum AIC model. When models had equivalent goodness of fit (Symonds and Moussalli 2011), the simplest one was selected.

In order to account also for not capture-related sources of variation in wild boar behavioural patterns, we used variables that were known to shape this species' behaviour. Considering wild boar activity variation patterns observed by Brivio et al. (2017) on both seasonal and daily scales, the Julian date and the time of day were included as continuous predictor variables in the models. In the same study, a significant relation between activity and weather conditions was observed. Thus, we added mean air temperature (°C), mean air humidity (%) and rain precipitation (mm) as continuous variables in the models (mean values of temperature and humidity were calculated for each activity and mobility value, averaging all records within the corresponding time interval, while total rain precipitation values were obtained from the sum of all records found in the same interval). To investigate any detectable effect that a capture event could have had on wild boar behaviour, we added the time elapsed since the capture event (hours) as predictor variable in the models. The kind of drug used to sedate each individual (zolazepam–tiletamine versus zolazepam–tiletamine–xylazine mixture) was included within the interaction term with the time after the capture, as any drug effect was supposed to be related to the time elapsed since the drug was injected. Finally, the study area was used as a categorical variable in order to detect possible behavioural

Table 1. Set of alternative hypotheses predicting the variation of activity rate and mobility rate of wild boar in the Alpe di Catenaia and Foreste Casentinesi National Park (Tuscany, Italy).

No.	Model	Hypothesis	Supporting evidence
1	Base	Wild boar behaviour was only affected by seasonal and daily cycles and by weather conditions as temperature and rain precipitation, without any capture-related effects.	Brivio et al. 2017
2	Capture effect	In addition to day of the year, time of day and weather, wild boar activity and movements were affected by the capture event.	Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015.
3	Capture and drug effect	Same as hypothesis no. 2, but with capture effect varying according to the kind of drug used to sedate the wild boars.	Cattet et al. 2003
4	Study area effect	Similar to hypothesis no. 1, but with wild boar behaviour markedly differing between individuals from different environmental conditions of the two study areas.	Brivio et al. 2017

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differences between wild boar captured in different locations (OAC and FCNP).

To avoid collinearity, we checked for possible correlations between continuous predictor variables, calculating Pearson correlation coefficient within all possible predictor variables pairs (Zuur et al. 2009). We found a not negligible correlation only between mean air temperature and mean air humidity ($r = -0.7$). A random forest calculation (R package 'randomForest') showed that mean air temperature was the best predictor of variation for both AR and MR, therefore mean air humidity was dropped from the predictor variables sets.

Results

We captured and monitored six wild boar (four females and two males) in OAC and 12 (six females and six males) in the FCNP (Supplementary material Appendix 1 Table A1). We excluded two males (no. 8319 and no. 8749) of FCNP from the activity rhythms analysis and one male (no. 12288.2c) of OAC from the movement analysis, because of data failure. We thus used the data related to 16 wild boar for daily activity rhythms analysis and AR analysis, and data on 17 wild boar for the models fitting MR. We recorded an average of 354.88 ± 16.79 AR/wild boar and of 284.06 ± 89.58 MR/wild boar.

Daily activity rhythms

Capture did not alter the daily activity rhythm of most of the wild boar investigated: 10 out of 16, five males and five females (Fig. 2a–b, Supplementary material Appendix 1 Fig. A1a–h). Both males and females of this unaffected group showed a unimodal and nocturnal activity pattern synchronised to the onset of civil dusk. The mean daily acrophase occurred between 21:16 and 23:12 (Fig. 2a–b, Supplementary material Appendix 1 Fig. A1a–h; Rayleigh test, $p < 0.0001$) and did not significantly change throughout the period investigated (Mardia–Watson–Wheeler test; $p > 0.05$).

In six out of 16 wild boar, capture had a marked effect on activity rhythms. After the release, two wild boar (females: no. 16597 and no. 16599; Fig. 2c, Supplementary material Appendix 1 Fig. A1i) showed a diurnal pattern with acrophase in the late afternoon (between 15:00 and 17:00). In contrast, a male (no. 16603, Fig. 2d) showed an inversion of activity pattern from crepuscular to diurnal and the mean acrophase changed from 19:20 during twilight to 05:30–07:00 during diurnal activities (Mardia–Watson–Wheeler test, $p < 0.00001$). The inversion of activity pattern was also found in a female (no. 12292c, Supplementary material Appendix 1 Fig. A1j), though for a short period of four days only. Capture had a marked effect on the daily activity rhythms of two wild boar (females: no. 12290c and no. 16602; Fig. 2e–f). In one individual (no. 12290c; Fig. 2e), we observed arrhythmia in daily activity for a period of about a week. Subsequently, this wild boar showed a daily rhythm with a clear nocturnal activity with acrophase about at 21:00. Another female (no. 16602, Fig. 2f) showed a similar response to capture: during the subsequent three days, her activity was considerably reduced and spread across the 24 h. After these initial alterations, all wild boar showed a nocturnal pattern of activity with a peak during the early hours of the night.

Activity rate

The best model explaining the variation of AR included Julian date, time of day, mean air temperature, total rain precipitation and the interaction term between time after capture and drug type (i.e. model no. 3; R^2 (adj) = 0.423; Table 2A). AR did not show a significant relation with Julian date, while its daily pattern highlighted the importance of the predictor variable time of day, clearly showing the preference of wild boar for nocturnal activity (Supplementary material Appendix 1 Fig. A2a). Both air temperature and rain precipitation affected wild boar activity (Supplementary material Appendix 1 Fig. A2b–c), the first with a non-linear and unclear pattern, the latter with a positive relation with AR reaching a plateau with values of about 5 mm of rain precipitation, but with wide confidence intervals (especially with high precipitation values, Supplementary material Appendix 1 Fig. A2c). Results showed that the interaction between the time elapsed from the capture and the drug mixture treatment significantly contributed to explain the activity pattern of the captured individuals. Wild boar exhibited lowest AR values immediately after their capture and gradually increased their activity until the reaching of a plateau, about 10 days after their capture, with both kinds of drug mixture (Fig. 3). Results suggested that this reduction of activity was slightly more pronounced in the wild boar sedated with a mixture of zolazepam–tiletamine and xylazine compared with individuals treated with zolazepam–tiletamine only. Nonetheless, the estimated activity patterns for both sets of individuals had either partly or completely overlapping confidence intervals. Finally, wild boar sedated with zolazepam–tiletamine–xylazine had a more irregular activity pattern. The weak effect of the drug mixture caused a relatively low difference of R^2-values between the first and the second ranked models (Table 2). Moreover, as the time elapsed from the capture only influenced wild boar's behavioural patterns during 10 days out of 30, there was little difference in R^2 between the best models and the alternative models including or excluding this variable.

Mobility rate

The best model explaining the variation of MR included Julian date, time of day, mean air temperature, total rain precipitation and time after capture (model no. 2; R^2 (adj) = 0.307, Table 2). Julian date affected wild boar movements with a weakly significant relation and a non-linear pattern; a higher MR was observed around the 270th day of the year (Supplementary material Appendix 1 Fig. A3a). The effect of the time of day was very similar to that for AR, with wild boar moving longer distances at night (Supplementary material Appendix 1 Fig. A3b). The relation between mean air temperature and MR was almost steady for temperatures below 25°C but became positive when temperatures exceeded this threshold (Supplementary

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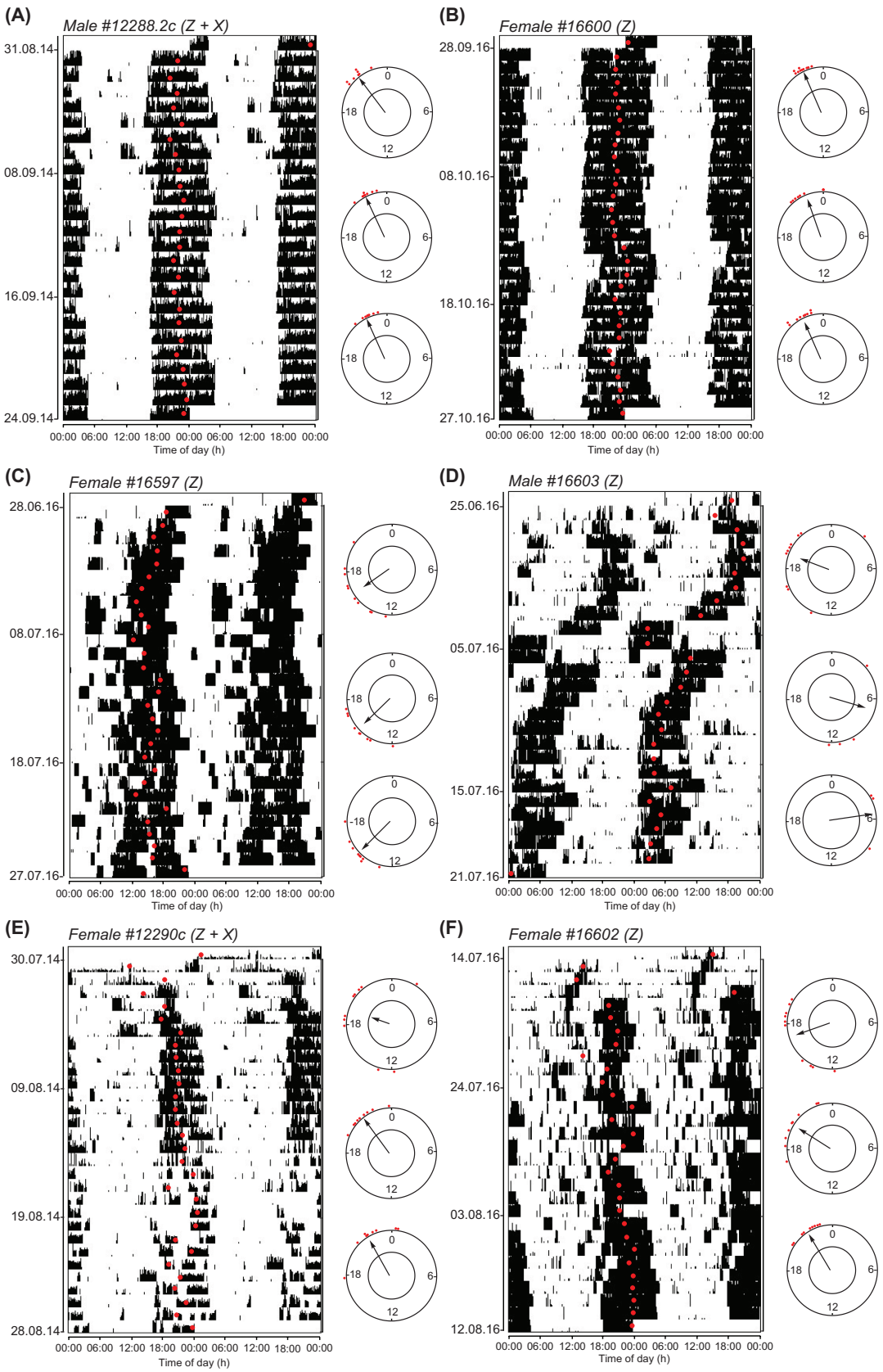


Figure 2. Representative actograms of daily activity of 6 radio-collared wild boar. Records are double plotted on a 48-h time scale to help the interpretation. Red dots on the actograms mark daily acrophases. On the right-hand of the actograms, circular diagrams showing acrophases for 10-day intervals are plotted. Dots represent daily acrophases and arrows indicate the average acrophases represented as vector. The circle inside each panel represents critical values of Rayleigh test ($p < 0.05$). Z: wild boar sedated with zolazepam–tiletamine; Z + X: wild boar sedated with zolazepam–tiletamine–xylazine.

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Table 2. Generalised additive mixed models predicting the activity (A) and movement (B) rates after capture in wild boar in the Oasi Alpe di Catenaia and Foreste Casentinesi National Park (Italy).

No. model	Model structure	AIC	Δ AIC	R^2
(A)				
3	AR~ Julian date + time of the day + temperature + precipitation + time since release × drug	−1426.2	0	0.423
2	AR~ Julian date + time of the day + temperature + precipitation + time since release	−1421.6	4.6	0.422
4	AR~ Julian date + time of the day + temperature + precipitation + study area	−1344.5	81.7	0.413
1	AR~ Julian date + time of the day + temperature + precipitation	−1344.5	81.7	0.413
(B)				
2	MR~ Julian date + time of the day + temperature + precipitation + time since release	18553.5	0	0.307
3	MR~ Julian date + time of the day + temperature + precipitation + time since release × drug	18559.0	5.5	0.307
4	MR~ Julian date + time of the day + temperature + precipitation + study area	18610.7	57.1	0.299
1	MR~ Julian date + time of the day + temperature + precipitation	18610.7	57.1	0.299

The best model was selected with the minimum AIC criterion [AIC = Akaike information criterion; ΔAIC = difference in AIC value between the AIC of a given model and the best model (with the lowest AIC); AR = activity rate; MR = mobility rate].

material Appendix 1 Fig. A3c). Wild boar movements increased together with total rain precipitation, showing a clear, although weak, positive pattern (Supplementary material Appendix 1 Fig. A3d). Finally, wild boar were found to cover short distances immediately after their capture and then they gradually increased their mobility until achieving a stable situation around 10 days after their capture, in accordance with the pattern found for AR (Fig. 4). The different mixture of drugs used for sedation was not included in the best model selected.

Discussion

Immediately after capture, wild boar showed low values of AR and MR. AR and MR highly increased during the first 10 post-capture days and then reached stable values. Capture was also found to produce a partial and variable effect on wild boar activity rhythms periodicity, affecting only some individuals of the study group.

More specifically, the analysis of daily activity rhythms showed a potential effect of capture and anaesthesia on wild boar periodicity: in six out of 16 wild boar, we observed

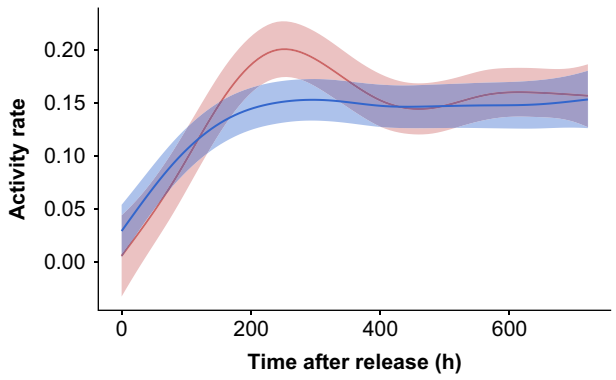


Figure 3. Effect of the interaction between time after release (hours) and the drug mixture on wild boar activity rate after capture. The values of activity rate reported were predicted by the best generalised additive mixed model (see the text for more details). Wild boar treated with zolazepam–tiletamine only and with the mixture of zolazepam–tiletamine and xylazine are represented by the blue and by the red line, respectively. Estimated standard errors are represented by the colour-shaded areas.

locomotor arrhythmicity or inversion of activity pattern from nocturnal to diurnal, considering a unimodal and nocturnal activity pattern as the standard baseline condition in our study area (Brivio et al. 2017). Different investigations in invertebrates and vertebrates, including humans, clearly demonstrate that general anaesthesia disrupts or alters behavioural circadian rhythms (Dijk and Lockley 2002, Chassard et al. 2007, Poulsen et al. 2018). In this respect, marked differences related to the time of drugs administration were found. For instance, general anaesthesia during the active phase highly altered daily activity rhythms (Mihara et al. 2012, Anzai et al. 2013). Both honeybees and rats treated with isoflurane or ketamine, two general anaesthetics commonly used, showed a phase-shifts in the locomotor activity if the treatments were applied during the daytime (Cheeseman et al. 2012, Ludin et al. 2016). Conversely, administration of anaesthesia during the resting period appeared to have minor effects on activity rhythms (Prudian et al. 1997, Mihara et al. 2012). It is worth noting that different anaesthetic drugs and different durations of the anaesthetic treatment may induce diverse species-specific reactions. Although the drug mixture was administered when wild boar typically start their resting period (i.e., in the early morning), the changes in the circadian behaviour observed in this study provide a piece of evidence in the complex puzzle of how anaesthetics can affect the circadian timekeeping system in large wild mammals.

An alternative explanation for the behavioural pattern observed after capture and anaesthesia is a direct effect of stress on the regulation of circadian clocks. At the best of our knowledge, this has been observed in rodents, under controlled laboratory conditions only (Koch et al. 2017). Ours is one of the first findings on how stress can affect the circadian clocks in free-ranging large mammals. Since the affected wild boar were few (n = 7), we were not able to detect any clear effect of age, sex, study area, drug mixture used or season of capture. Nevertheless, our results remark the strong potential stress effect of a capture event on animal behaviour, as it may affect both pattern (arrhythmia) and phase (inversion) of the activity rhythms, therefore influencing both internal and environmental-related aspects of activity rhythms. Cortisol concentration significantly increases in wild boar after stressful situations (Morton et al. 1995, Gentsch et al. 2018) and this endogenous signal could alter the circadian timekeeping system (Kalsbeek et al. 2012, Dickmeis et al.

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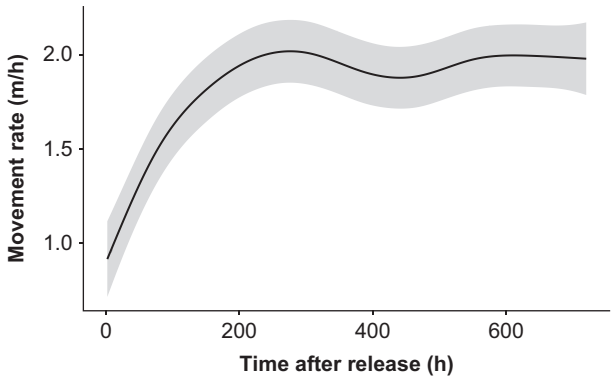


Figure 4. Effect of the time after release (hours) on wild boar movement rate. The values of movement rate reported were predicted by the best generalised additive mixed model (see the text for more details). Estimated standard errors are represented by the colour-shaded areas.

2013). Brivio and colleagues (2017) also observed an inversion of activity patterns in female wild boar in the same study area, occurring likely during the weaning period. Thus, we can conjecture that the wild boar may switch its activity periodicity when under stress.

Our results are in accordance with our first prediction: wild boar exhibited low activity and movement rates after the capture and the achievement of stable AR and MR values followed a gradually increasing pattern that lasted about 10 days, consistently with the range observed in other species (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015). In particular, the reduction of activity in wild boar lasted longer than in Alpine ibex (Brivio et al. 2015), but it was relatively short if compared with the 36 days of perturbation observed in black bears' movements (Cattet et al. 2008), hence confirming the high heterogeneity of results obtained by different studies on different species. So far, studies evaluating the capture effect on mammals are still few, making the comparison of results unfeasible. For the same reason, it is difficult to attribute the variability of results in the available studies to systematic, environmental or drug-related factors. Thus, our aim in this study was to understand wild boar reactions to capture in different study areas and to two different drug mixtures. Eventually, though, AR and MR best models did not include the study area, probably because of the fair similarity between OAC and FCNP. Anyhow, any slight difference in environmental factors characterising the two study areas would have had an attenuated effect, as we already took into account environmental factors variability by including some climatic variables (air temperature, humidity and precipitation) in our models.

As to the effect of sedation, we found a weakly significant different effect of the two drug mixtures only on wild boar activity, while the different drug mixtures used similarly affected MR. AR and MR reached the stable values after the same amount of post-capture hours with either one of the drug mixtures. The wild boar sedated with a mixture of zolazepam–tiletamine–xylazine showed a slightly more irregular AR pattern compared with individuals treated with zolazepam–tiletamine only. This weak effect can be due to the presence of xylazine in the mixture administered, and/or to the potential synergistic effect potentially triggered by its association with zolazepam–tiletamine. Nevertheless, the addiction of xylazine did not affect the total duration of the period required to restore stable activity levels. In the light of this, we can speculate that the length of the restoration period was likely driven by the overall stress caused by the capture and/or by the administration of zolazepam–tiletamine, as the latter was used for all individuals.

Nowadays, wildlife managers and researchers encounter a wide variety of circumstances in which the capture of animals is required. Whichever the purpose for the capture, the lightest and shortest capture effects would be desirable for ethical, conservationist and management reasons. Animals' welfare is a fundamental issue in wildlife research and management, but capture events can threaten it both directly and indirectly: capture can induce mortality (Kock et al. 1987, Beringer et al. 1996, Arnemo et al. 2006, Jacques et al. 2009) and cause a decrease in activity and mobility (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015, this study), thus increasing the risk to be predated or involved in collisions with vehicles. Moreover, stronger capture effects result in significant distortions of the data acquired within a research project. The comparison of our results with those of other studies shows a remarkable heterogeneity in capture effect duration, which can arise from systematic, environmental and method-related factors. Further accurate investigations on the role of the method used for capturing, handling and releasing wild animals could permit to establish standard field protocols with minimum stress effects. Further studies should therefore focus on methodological aspects such as capture method, time spent in the trap, time of total handling, number of operators and kind and dosage of the drugs administered.

In conclusion, any capture event that includes chemical immobilization is likely followed by behavioural alterations of not negligible duration and the most evident effects are exhibited in the first hours after the release. Here, we showed that in wild boar this alteration consists, at the least, in a partial periodicity modification and in a depression of activity and mobility rates for a long period. Since captured individuals are not fully alert when handling is concluded, they should be released in places that are free from risks. This surely includes high traffic roads, but also lakes, streams and gorges as well. Moreover, the presence of predators is likely to affect released wild boar survival rate. While dangerous human or geographical elements may be avoided by simply displacing the releasing site, though, the stable presence of a predator would be more difficult to elude. Finally, since the addition of xylazine to a tiletamine/zolazepam protocol did not affect the long-term behavioural alteration time, its use needs to be considered a strictly veterinary issue, not providing any clear biological advantage or disadvantage. Hence, carefully evaluating drug combination and dosages for sedation appears to be a useful strategy to minimize capture effects. In this context, as the individual's state of stress at the moment of the drug injection presumably affects its response to anaesthesia, capture-handling protocols should be designed to reduce stress even before the starting of

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handling (i.e. when animals are still awake). Reduced initial state of stress could thus permit lighter dosages with still adequate anaesthetization and safe manipulation, which, in turn, will likely produce lighter long-term stress effects as well.

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Conflict of interest – The authors declare that they have no conflict of interest.
Ethical standards/permits – This study complies with all national and regional laws dealing with ethics and animal welfare. Capture and manipulation protocols were approved by Tuscany Regional Administration (no. 103/5936/152 – 13/03/2002). The research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research.
Author contributions – SG and FB originally formulated the idea. RB, SL, NCa, EB, SG and MA implemented the animal captures. NCa and EB were the vets responsible in FCNP and OAC capture sections, respectively. MA and CP contributed materials/analysis tools. RB and SL conducted fieldwork. FB, SG and CB collaborated in imaging analysis. RB, FB, SG, CB and MB performed statistical analyses. RB, SG, CB and FB wrote the manuscript. MA and NCo provided editorial advices.

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Supplementary material (available online as Appendix wlb-00497 at <www.wildlifebiology.org/appendix/wlb-00497>). Appendix 1.

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