UNIVERSITÀ
DEGLI STUDI
FIRENZE

DOTTORATO DI RICERCA IN
Scienze Agrarie e Ambientali

CICLO XXVII

ACTIVITY DATA FROM ACCELEROMETERS IN GPS COLLARS: CLASSIFICATION OF BEHAVIOUR AND ACTIVITY PATTERNS IN FREE-RANGING UNGULATES

Settore Scientifico Disciplinare AGR/19

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Anni 2012/2014

ACKNOWLEDGEMENTS

I would like to thank my tutor, Dr. Ponzetta, for making this research possible and for the support during the whole experimentation.

I would also like to thank all the persons I worked with during these years; a special thanks goes to Dr. Bocci, Dr. Cervasio, Dr. Trunfio, Dr. Parrini, who helped me during my work and during data collection.

My gratitude goes to the people that offered their work, time and structures for the experimentation; thanks to “Agriturismo Montaglioni” in San Marcello Pistoiese, “Azienda Valdastra” in Borgo San Lorenzo, “Agriturismo I Cancelli” in Marradi, “Azienda Agricola Brunelli” in Scandicci, “Parco Zoo della Fauna Europea di Poppi” in Poppi, “Centro assistenza fauna selvatica” in Ponte in Valtellina.

My greatest thanks are for all my family and friends. I thank my parents, Daniela and Valerio, for giving me their continuous love and support, and for encouraging me to follow my dreams. Thanks to Emanuele, for his love, help, support. Thank you to all my dear friends, for giving me advice, joy and love.

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ABSTRACT

The applications of GPS telemetry have become a widespread means for wildlife and livestock tracking; the reasons for this success lie both in their growing accuracy and reliability, as well as in the great potential they can express in animal monitoring, especially when compared to traditional tracking techniques. The study of animal behaviour with the traditional techniques, in particular for wild and elusive species, but also for free-ranging livestock, has required a remarkable amount of time and field work. Soon after their release, GPS collars have been equipped with activity sensors; their use allows to obtain continuous and high frequency activity data. The employment of these devices has an enormous potential for ecological and ethological research, since it permits to collect a remarkable amount of data, which can be utilized for the study of the entity and variations of animal activity at various time scales.

However, these data have rarely been analysed and their potential is not fully expressed; this is probably due to their difficult interpretation in a behavioural sense.

The aim of this research is thus to provide a method for the interpretation of activity data in term of behavioural recognition and activity pattern analysis, as well as giving some practical indications for future application in research; in particular we aimed at interpreting activity data recorded during the tracking with GPS collars of free-ranging red deer (*Cervus elaphus*) living in Northern Apennine (Tuscany); moreover, we intended to enrich the knowledge of some aspects of red deer activity patterns since, as far as we know, no studies have performed before an analysis of long-term activity data remotely recorded on this species. Finally, we applied the models for the classification of behaviour in order to predict the time budget of tracked animals.

We also aimed at applying these techniques and methods to beef cattle reared at confined pasture, belonging to two breeds: Chianina, a local breed, and Limousine.

By using two classification procedures, Discriminant Analysis applied to Gaussian mixture models and recursive partitioning, we were able to build classification models predicting 88.1% of active behaviours and 94.2% of resting behaviours for captive red deer. In cattle, 91.8% of active behaviours and 99% of resting behaviours were correctly predicted for Chianina cows, while in Limousine cows 91.8% and 96.1% of active and resting behaviours respectively, were correctly predicted.

The analysis of red deer activity patterns displayed that, despite slight individual differences, the general patterns are similar for all monitored individuals both at annual and at daily level; moreover, the patterns remain constant in consecutive years. Individual differences were detected, in particular periods of the year, in the distribution of activity levels between night and day. However, the daily patterns of activity displayed a bimodal trend, with peaks of activity in correspondence of dusk and dawn; in some periods of the year, a third peak of activity occurred soon before dawn. Individual differences emerged also in the daily activity budget; on average, monitored deer dedicated 50% of the time to active behaviours, i.e. feeding and moving. It is to notice that this value, despite consistent with previous studies on European red deer, likely underestimate the actual proportion of activity time.

The analysis of activity patterns of beef cattle showed a strong similarity of both seasonal and daily patterns between different individuals, but also among the two breeds. Despite the similarity of patterns, activity levels and activity budgets of Chianina cows differed from the ones of Limousine cows. Daily budget is dominated by passive behaviour: 64% of time is dedicated to resting and ruminating.

This research allowed to highlight different opportunities for the use and interpretation of activity data recoded by sensors on GPS collars, displaying their interesting potential but also some limitations; additionally, we described for the first time some features of activity patterns in Apennine red deer.

We believe that the potential of these information for wildlife and livestock monitoring may be enhanced both by introducing some modifications to the sensors in order to increase the interpretability of the data, and by using such data in synergy with as many environmental data as possible.

We wish that our work could represent a starting point for further and deepest investigations in this research field.

RIASSUNTO

Le applicazioni della telemetria GPS nell'ambito del monitoraggio a distanza di fauna selvatica e domestica, hanno avuto un sempre più largo impiego negli ultimi quindici anni, sia per la crescente affidabilità e precisione degli strumenti, che per le notevoli potenzialità che questi sono in grado di esprimere rispetto alle tradizionali tecniche di monitoraggio. Lo studio del comportamento spaziale delle specie selvatiche, per natura particolarmente elusive e difficilmente monitorabili, ha da sempre comportato un notevole impiego di tempo e di risorse umane; lo studio del comportamento di specie domestiche allevate allo stato brado o in condizioni di semi-libertà, tradizionalmente effettuato a mezzo di osservazioni dirette, presenta le medesime criticità.

A breve distanza dalla produzione dei primi radiocollari GPS, sono stati integrati in questi strumenti diversi modelli di sensori di attività. L'impiego di tali sensori consente di misurare, continuativamente e a brevi intervalli di tempo, l'intensità o la frequenza di movimento; tale strumento offre quindi delle enormi potenzialità in ambito di ricerca, consentendo di collezionare regolarmente un'enorme quantità di dati, che possono essere utilizzati per studiare l'entità e le variazioni dell'attività a varie scale temporali. Tali dati non sono stati tuttavia impiegati e valorizzati così frequentemente come nel caso dei dati di posizione, probabilmente a causa delle difficoltà nella gestione di una grande quantità di informazioni non direttamente interpretabili in termini comportamentali.

Questa ricerca è nata dalla necessità di interpretare i dati di attività raccolti durante il monitoraggio mediante collari GPS di esemplari di cervo rosso (*Cervus elaphus*) viventi allo stato libero nell' Appennino toscano.

In particolare, ci siamo proposti di individuare dei modelli per l'identificazione del comportamento, fornendo inoltre alcune

indicazioni pratiche per un futuro potenziamento del metodo. La presente sperimentazione inoltre, intende arricchire il panorama delle conoscenze sui ritmi di attività del cervo in ambiente appenninico, specie per la quale sono assenti studi a lungo termine condotti su dati rilevati con tale tipo di sensore; infine si sono voluti applicare i modelli sviluppati per l'identificazione del comportamento, per calcolare l'*activity budget* dei soggetti monitorati. Abbiamo inoltre voluto valutare l'applicabilità di questa metodologia di monitoraggio a bovini appartenenti a due razze da carne, Limousine e Chianina, allevati allo stato semi-brado.

Attraverso l'utilizzo di due tecniche di classificazione, ovvero l'analisi discriminante applicata a modelli a misture di Gaussiane e la *recursive partitioning*, è stato possibile identificare correttamente l'88,1% dei comportamenti attivi ed il 94,2% di quelli a riposo per il cervo; nei i bovini di razza Chianina è stato possibile classificare correttamente il 91,8% dei comportamenti attivi ed 99% di quelli a riposo, mentre per la razza Limousine il 91,8% dei comportamenti attivi ed il 96,1% di quelli a riposo.

L'analisi degli *activity pattern* sul cervo ha permesso di mettere in luce come, nonostante le differenze individuali, i modelli di attività siano comuni a tutti i soggetti monitorati sia a livello annuale che giornaliero, e che questi si mantengono stabili negli anni. Alcuni animali hanno mostrato, relativamente a determinati periodi dell'anno, una diversa distribuzione dell'attività tra notte e giorno. I soggetti monitorati hanno mostrato, in linea con quanto già conosciuto per la specie, un pattern di attività giornaliero tendenzialmente bimodale con due picchi in corrispondenza di alba e tramonto; in alcuni mesi, tuttavia, è emerso un ulteriore momento di elevata attività poche ore prima dell'alba: questo pattern trimodale non è mai stato precedentemente messo in luce per *Cervus elaphus* in territorio europeo.

Sono inoltre emerse delle differenze individuali anche nell'*activity budget* complessivo: mediamente gli animali hanno dedicato il 50%

del tempo, a livello giornaliero, ad alimentazione e movimento. Si ritiene opportuno sottolineare che tale valore, pur risultando coerente con quanto rilevato in precedenti studi, probabilmente sottostima la reale proporzione di tempo dedicato all'attività.

L'analisi degli *activity pattern* per le due razze bovine ha messo in luce una forte somiglianza nei modelli di attività a livello stagionale e giornaliero, sia tra individui che tra razze diverse. Pur condividendo un pattern comune, le due razze hanno mostrato differenze nei livelli di attività e nella quantità di ore giornalmente dedicate ad alimentazione e spostamenti, che sono risultate sempre maggiori nella razza Chianina. Il budget giornaliero è dominato dalle attività stazionarie: il 64% del tempo è impiegato infatti per riposo e ruminazione.

La presente ricerca ha permesso dunque di evidenziare diverse chiavi di utilizzo e interpretazione dei dati rilevati da sensori di attività, mettendo in luce potenzialità ma anche alcuni limiti di questa tecnica di monitoraggio. Inoltre il nostro studio, oltre a costituire uno spunto per affinare la ricerca effettuata con questi strumenti, ha consentito di studiare per la prima volta i pattern di attività del cervo in ambiente appenninico.

Riteniamo che il valore di queste informazioni per il monitoraggio di specie domestiche e selvatiche possa essere potenziato sia introducendo opportune modifiche nei sensori, volte a migliorare l'interpretabilità dei dati, sia utilizzando questi ultimi in sinergia con la maggior quantità possibile di informazioni a livello ambientale.

Auspichiamo pertanto che il nostro lavoro possa costituire un punto di partenza per perfezionare ed approfondire le iniziative in questo ambito di ricerca.

CHAPTER 1
INTRODUCTION

1.1 GPS RADIOTELEMETRY AND ACTIVITY SENSORS

GPS telemetry for wildlife

In the past 40 years new technological devices have been produced and employed for tracking and studying free-ranging animals; however, the use of GPS radiotelemetry probably represents the most revolutionary advance in obtaining animal locations. The application of this technology for wildlife tracking has become a widespread means for the study of animal ecology; since the use of the first collars in mid-1990s, this technique has gained major importance, both due to an increase in performances of positioning and to the progressive decline of cost per GPS unit.

Precision and accuracy improved greatly since the removal of selective availability (SA): starting from 1990, the performances of non-military receivers were intentionally degraded to approximately 100 m (Tomkiewicz et al. 2010); SA was suspended in 2000 and definitively decommissioned in 2007. Since then, horizontal error of non-differentially corrected positions reduced to 10 - 30 m (D'Eon et al. 2002; Frair et al. 2010). Contextually, the quick technical advances in receivers allowed to produce smaller GPS units and to minimize the time to acquire *fix* (i.e. location estimate), thus reducing power consumption. These improvements led to the possibility to adapt the devices to small terrestrial, marine and avian species (Tomkiewicz et al. 2010), as well as to extend tracking periods. The point of this innovation relies in the opportunity to collect systematic, highly accurate and relatively unbiased data, compared with traditional VHF data collection (Hebblewhite & Haydon 2010), even for highly evasive or migratory species. The benefits offered by GPS-based telemetry involve many aspects of animal ecology research;

the most immediate implication concerns the identification of important habitats as well as migratory paths for wild species and the possibility to improve resource selection modelling, providing tools for the conservation of endangered species and understanding the extent of human impacts on wild populations. The following step involves the study of combined variations and influences between animals and environmental resources. Changes in climate are known to affect in future the distribution of seasonal forage, both in time and in space, for many species; for example, for ungulates, changes in plant phenology could emphasize the mismatch between peaks of resources availability and demand by reproducing individuals, having thus potentially consequences on offspring production (Post et al. 2008), especially in habitats characterized by strong seasonality in forage production. Moreover, the effects of climate changes are likely to involve grasslands and grazing systems, thus potentially affecting welfare, behaviour and productivity of livestock. Thus, the potential applications of GPS technology in these study themes are considerable and partially not fully expressed.

However, this technique presents some disadvantages. Although GPS has limited labour costs and field work, compared to VHF technology, the cost of investment per unit is higher; the necessity of a trade-off between number and cost of GPS unit has led to a decreasing number of tracked animals, reducing thus sample sizes and causing poor population-level inference (Hebblewhite et al. 2010). In addition, despite the high rate of position acquisition and accuracy of data, in particular cases when obstructions occur between the signal from the satellites and the GPS receiver (e.g. dense canopy cover, topographic obstructions), the device may be incapable to obtain a fix or the accuracy of position may be reduced (D'Eon et al. 2002; Hebblewhite et al. 2007; Frair et al. 2010).

Moreover, automated remote tracking is not able to meet every necessity in studies on animal ecology and biology, since certain aspects can only be measured by means of field surveys.

Activity sensors

In regard to behaviour, advances in technology have progressively increased the opportunities for incorporating tracking and biotelemetry (Cooke et al. 2004; Rutz & Hays 2009). Formerly in VHF collars, changes in radio pulses were used to monitor wildlife activity patterns (e.g. on red deer by Carranza et al. 1991, Georgii et al. 1981); variation in quality of signal however may cause considerable bias in detection of active and inactive behaviours (Pépin et al. 2006 a), thus different types of motion sensors were subsequently developed and tested. Activity sensors for the monitoring of behaviour started to be built in GPS collars in late 1990s; the first prototypes integrated activity counters in collars, and their utility in interpretation of behaviour started to be tested (e.g. on moose by Moen et al. 1996). The need for continuous and remote measurements of animal behaviour has led to the parallel development of devices designed exclusively for behavioural monitoring, for example devices basing on tracking the position of animal's head, activity counters and accelerometers (e.g. ETHOSYS® developed by Scheibe et al. 1998). Such devices were employed to perform continuous recording of animal behaviour, activity pattern studies (*red deer* by Berger et al. 2002, Pépin et al. 2006 b; *Przewalskii horse* by Berger et al. 1999) as well as various attempts of behaviour identification (*red deer* by Pépin et al. 2006 a). In a similar manner, a new concept of activity sensor was included in GPS collars, suitable for monitoring intensity of activity at long-time scale and for an easier identification of behaviour.

The devices currently available in radiocollars differ in the mode they measure activity: they can provide alternatively the counts of movements events, the counts of “head down” events, acceleration measurements reported as row data (acceleration in G or m/s^2) or as scaled data (acceleration reported on a relative range of values, often 0-255). The question that naturally arises is: what information can we derive from long-term activity measurements of free-ranging animals? And how may these information be employed both for wildlife management and conservation and for studying interactions between livestock and their environment? The organization and distribution of activities in time, which are determined by behavioural and physiological rhythms, are crucial for all animals. Activity patterns have evolved specifically in each species in response to environmental influences as, for instance, food availability, abiotic factors and predators. It follows that the timing of alternation of resting and active bouts cannot be a random event, but it is markedly influenced by external changes: therefore the question of when to do what is subject to fitness maximization (Berger 2011). However, changes in living conditions and in external stimuli are likely to determine behavioural adaptations, as well as stressors and physiological alterations may influence intensity of activity and timing of rhythms. Continuous and uninterrupted measurements of activity allows to monitor changes in activity levels at different time scales, monitor their rhythmic component and possibly detect alterations, identifying active and inactive behaviours and locate these events at geographical scale. Therefore, studying activity patterns and their changes may improve for free-ranging wildlife the understanding of interactions with their habitat, and help interpreting the effects of anthropic activities on them; for livestock, the accurate description of the individual components of behaviour (e.g. foraging, bedding, ruminating, movements) may help understanding sustainable grazing systems, investigating the different responses of breeds to environmental criticalities and variations, as well as monitoring animal health.

Although intensity of activity, as recorded by collars, is directly usable for studying activity patterns, the identification of behaviour requires additional work. Classification of behaviour from activity data recorded by GPS collars or activity loggers has been attempted for different taxa, both wild and domestic: roe deer (Gottardi et al. 2010; Heurich et al. 2012), red deer (Adrados et al. 2003; Adrados et al. 2008; Löttker et al. 2009), bear (brown bear in Gervasi et al. 2006; black bear in Yamazaki et al. 2008), cheetah (Grünewälder et al. 2012), badger (McClune et al. 2014), crab-plover (Bom et al. 2014), penguin (Yoda et al. 1999), goats (Moreau et al. 2009) and cattle (Guo et al. 2009).

In general two types of validations have been performed in these studies: the first one discriminates active from inactive behaviours and the second type recognizes a major number of main activities: for example resting, feeding and walking. However, there is no standardization of the method used to validate the relationship between the values recorded by the activity sensors and the real activities (Gottardi et. al 2010); moreover, frequently the classification models have been built using only "pure" data, namely with only one behaviour expressed during the recorded lapse of time (Body et al. 2012). This procedure, neglecting data referring to mixtures of active and inactive behaviours, represents a simplification and provides a biased classification when applied to the whole activity dataset recorded on animals, which will be naturally composed both by pure and mixed data.

It has to be pointed out that, in order to perform such "calibration" of sensors in order to classify behaviour, direct observations on tracked animals are necessary; with regard to this point, only in a few cases (e.g. Pépin et al. 2006a) the possible effects of the season of survey has been mentioned or taken into account. Therefore, some methods and procedures remain still to be tested.

1.2 BIOLOGICAL RHYTHMS AND ACTIVITY PATTERNS

The recurrence of any event within a biological system at more or less regular intervals can be considered a biological rhythm (Aschoff 1981). Biological rhythms occur at different frequencies: one cycle can last from a millisecond to several years; moreover, they can exist at different levels in biological systems, such as in cells, tissues, organs, individuals or in populations.

Rhythms can be controlled externally (*exogenous*) or internally (*endogenous*). *Free-running rhythms* are endogenous rhythms, synchronized with environmental cycles, which can persist with a slightly longer or shorter period when isolated from the respective cycle (Aschoff 1967). Since the period of free-running rhythm doesn't match exactly the environmental periodicity, the prefix *circa* has been introduced.

Since biological rhythms are oscillations, they can be described by wave parameters.

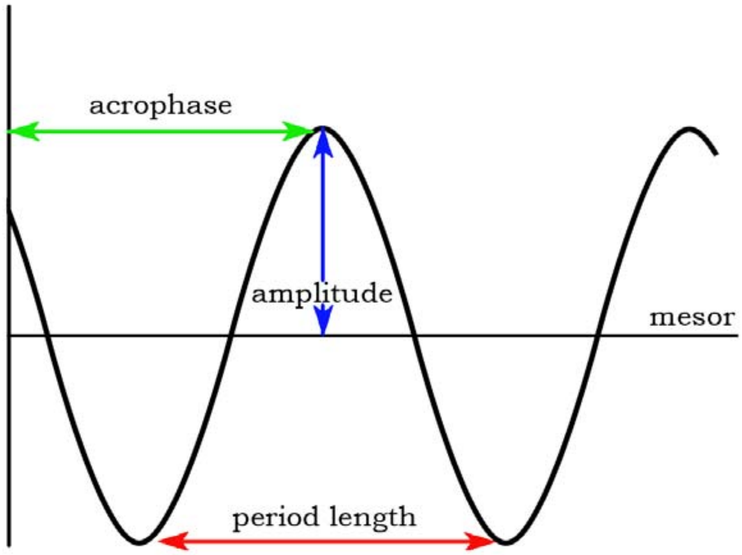

Fig. 1.1 Parameters of oscillations

Rhythms may be classified, according to period length, in different levels: ultradian rhythms, which have a period length of less than 24 hours; circadian rhythms, with a period length of about 24 hours; in circannual rhythms period length is about one year; period length in infradian rhythms is longer than 24 hours. Ultradian and infradian rhythms do not have an external equivalent cycle.

Rhythms involve many aspects of animal biology, from physiologic processes to reproductive timing. As example, concentration of several hormones (e.g. growth hormone in bulls, cortisol and corticosterone in pigs and mares) follows a ultradian or circadian periodicity; infradian periodicities are found in reproduction for example in cows and sow, which display heats every 21 days, and circannual rhythms are typical of species with seasonal reproduction (cervids, sheep, mares).

As most body parameters, also activity patterns are based on these periodicities, and have evolved in a specie-specific manner to prepare for predictable changes on daily or seasonal scale instead of a simply direct reaction on the current situation (Enright 1970, Berger 2011). Activity patterns provide insights in the relationship between species and environment, since they depend from the adaptation of a species to habitat, season and time of the day, thus reflecting the time-energy relationship (Pipia et al. 2008).

Circadian rhythms and patterns

The 24-hours rhythms are essentials, since they control animal life. Circadian rhythms displayed by most animals in field conditions are not only reactions to periodical changes in environmental stimuli (Aschoff 1966), but they are generally endogenously controlled; some 24-hours cycles depend exclusively by external factors, as artificial feeding, but they persist only in presence of the stimulus.

Most circadian rhythms are self-sustaining and have a period of 22-28 hours, but being entrained by cyclic and predictable environmental factors called zeitgebers (Aschoff 1981), they acquire an exact 24-h period; these entraining factors, also named synchronizers, are able to modify one or more parameters (e.g. period, phase) of a biological rhythm (Favreau et al. 2009).

The most powerful zeitgeber is the day-night cycle of illumination, but other factors as temperature, atmospheric pressure, food availability or even social cues (Favreau et al. 2009) can affect endogenous rhythms. The endogenous rhythm is influenced indirectly by environmental stimuli, being registered by receptors and then transferred to neural structures which produce circadian rhythms; in mammals the suprachiasmatic nucleus (SCN) of the hypothalamus plays an essential role in generating and maintaining these rhythms (Rusak 1989): as regards to light, the receptor of photic information is retina which transmits directly the signal to SCN through retinohypotalamic tract (Pévet et al. 1996).

Patterns of activity at daily scale, despite characterized by a certain individual variability, are specie-specific. Some species display only a peak of activity during the 24-h, and patterns with three or more peaks also occurs (Aschoff 1966). However, many activity patterns studies on different taxa found as common pattern the one with two peaks; this pattern was displayed in various species of insects, reptiles, fishes, birds and mammals.

Activity pattern with two peaks in correspondence of dawn and dusk is common in wild ungulates, such as roe deer (Krop-Benesch et al 2013), mouflon (Pipia et al. 2008), mule deer (Eberhardt et al. 1984), and red deer (Georgii 1981; Clutton-Brock et al. 1982; Georgii et al. 1983; Carranza et al. 1991).

Ruminants have a typical foraging-resting-foraging activity pattern (Mysterud 1998). Activity time of ruminants is influenced by numerous external factors like temperature (Belovsky et al. 1986; Demarchi et al. 1995), human disturbance (Singer et al. 1991), quality of food (Cederlund 1989); time dedicated to activity decrease allometrically with increasing body weight but is also influenced by feeding type (Mysterud 1998).

Other rhythms

Ultradian rhythms are often generated by feeding physiology (Krop-Benesch et al. 2013) and they seem to be greatly influenced by exogenous factors, for example the quality of food (Aschoff et al. 1985); for this reason their characteristics may vary among species and seasons, as well as be influenced by external stressors or endogenous functional changes.

Seasonal or circannual rhythms are based on circadian rhythms, and they depend both on physiological parameters (as for example reproductive phases) and on environmental factors such as food resources (vegetation availability) and climate.

1.3 WILDLIFE, LIVESTOCK AND ACTIVITY STUDIES: LIMITS, OPPORTUNITIES AND IMPORTANCE

The study of ungulates behaviour has been a widely discussed subject as well as extensively documented. However, data collection has been frequently carried out by means of direct observations, which represent a difficult task when performed on free-ranging animals (especially on wild or elusive species); moreover, extensive observations over long periods of time are unlikely to be accomplished.

Finally, direct observation may induce in monitored animals behavioural variations, as well as determine several types of observer bias. Automated remote activity measurements represent a totally innovative way to collect and analyse an amount of information never possible before, and provides a potentially unbiased tool for the long-term and uninterrupted analysis of time budgets and rhythmic patterns. It would help biologists and wildlife managers to understand basic behavioural patterns and to identify aberrations induced from external changes, making feasible the elaboration of new strategies for the conservation of endangered species. Also animal production science and, in future, livestock managers could benefit these tools, in the perspective of pursuing precision livestock breeding as well as sustainable grazing systems.

Focus on the study species: Red deer

Genus *Cervus* is distributed in the Holartic region, specifically in North America, Europe, Asia and North Africa. Taxonomically recognized species are *C. elaphus* (Linneaus 1758) with Eurasiatic distribution, *C. nippon* (Temminck 1838) exclusively inhabiting East Asia, and *C. Canadensis* which has been considered a probable separated species from *C. elaphus* (Ludt et al. 2004). Eurasiatic red deer presents several subspecies; the Italian territory hosts three subspecies: the mainland European red deer (*C. e. hippelaphus*), Thyrrenian red deer (*C. e. corsicanus*) endemic to Sardinia and Corsica, and the Mesola red deer (*C. e. italicus*) recently proposed by Zachos et al. (2014).

Historical distribution of red deer in Europe has been vast, and at present the species is widespread and abundant in a great part of its current range, despite a tendency in fragmentation of populations in Central Europe; however, the population trend is positive. In Italy, red deer has been abundant until XVIII – XIX century, although being progressively relegated from plains and hills to mountainous areas of Alps and Apennines.

Hence, the species experienced a rapid decline, and at the beginning of 1900 was extinct in all the territories of the peninsula except in Mesola Wood, where currently resides the indigenous type of the peninsula, and in Sardinia. Red deer populations gradually restored in Central and Oriental Alps, through spontaneous migration from adjacent territories; Occidental Alps were repopulated in 1960s – 70s with deer from Central Europe, while in Northern Apennine the species was reintroduced in 1950s – 60s from a stock of Alpine deer, and from then the population rapidly increased. The latest estimates of the extent of the species in Italian territories (Fig. 1.2) reports about 68,000 deer in 2010 (Riga et al. 2012), with a growth tendency respect to 2000 of +54% (Raganella Pelliccioni et al. 2013); red deer inhabits 58 Provinces, and is managed through hunting programmes in 22 of them.

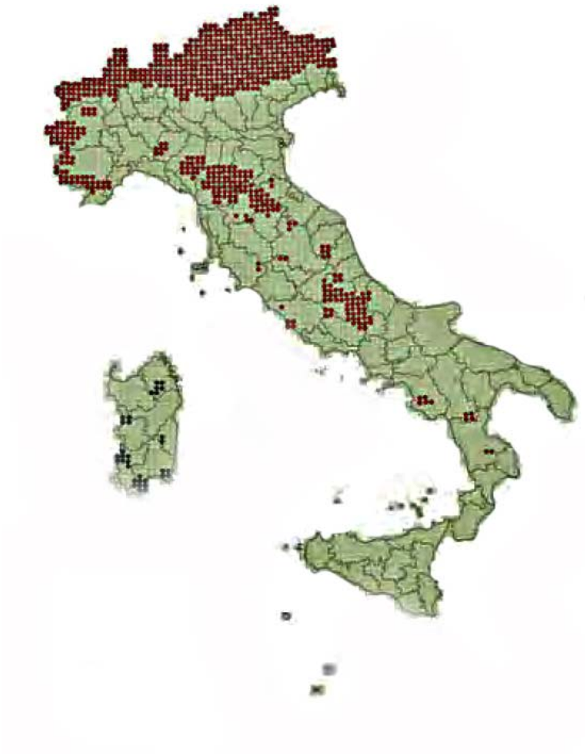

Fig. 1.2 Red deer (*Cervus elaphus*) distribution in Italian territory (red dots). Sardinia hosts *C. e. corsicanus* (black dots). (Raganella P. et al. 2013).

Red deer population of Northern Apennine hosts three reproductive cores, each one monitored and managed by ACATER (*Areale Cervo Appennino Tosco Emiliano Romagnolo*) since 2000; the estimated extent of this population in 2013 is about 6,500 deer.

Morphological traits

Red deer is the largest wild ungulate inhabiting the Italian territory. Live weight in stags is generally included between 160 and 220 kg, while in hinds it ranges from 90 to 120 kg (Boitani et al. 2003). Attainable size however depends greatly on habitat features and on density of the species and its competitors (Klein 1964); moreover, body weight and condition fluctuate during the year (Boitani et al. 2003). In deer living in Prato Province territory, which represents the core distribution of Northern Apennine population and hosts the deer studied in the present research, average weight is 168 kg for stags, and 99 kg for hinds, while height at withers is 125 cm and 110 cm, respectively (Ponzetta et al. 2013). Northern Apennine deer, however, appear larger than Alpine, Sardinian and Mesola deer (Mattiello et al. 2010).

Sexual dimorphism in red deer is relevant (Boitani et al. 2003), mostly due to selection forces operating through its reproductive strategy. Success in reproduction for males depends on direct competition (Clutton-Brock et al. 1982), which is displayed both by size and secondary sexual characters; indirect competition, instead, is of greater importance for females, since their reproductive success depends on the ability to rear offspring, and thus to acquire nutritional resources (Clutton-Brock et al. 1982).

Secondary sexual characters in stags represent the main distinctive features of the species: mane and antlers. Antlers are a common feature of all *Cervidae*, and they are present exclusively in males, with the exception of reindeers and caribou (*Rangifer spp.*) which have antlers both in males and in females.

Antlers are bone cranial appendices, with an annual cycle of development, maturation, velvet removal (July-August), casting (February-April) and renewal; this process is closely related to reproduction and in particular to hormonal cycles, mainly testosterone. Stags living in Prato Province territory have an average antler weight of 4.8 kg, average antler length of 88 cm and on average 6 tines per beam (Ponzetta et al. 2013).

Social behaviour and feeding habits

Red deer is a gregarious species, the size of the groups varies in relation to habitat features and vegetation, population density and season; however, the tendency is for sexual segregation. The family unit is usually composed by hind, her calf and the yearling female; however, yearlings of both sexes may join the family unit, forming broader groups. Stags, instead, tend to form temporary unisexual groups.

Females gather in larger groups during September and October, in correspondence of the mating period; red deer is a polygynous species: during rutting period stags compete each other, while assembling and defending a group of hinds ("*harem*"). The start of the mating period is linked to decreasing photoperiod and influenced by climatic conditions, thus it may vary according to latitude and climate; in Apennine the climax of reproduction lays in the second half of September (Mattiello et al. 2010). During mating season stags experience a period of high energy consumption and low food intake, thus they can lose as much as 20% of their body weight (Clutton-Brock et al. 1982).

Basing on the morphology and physiology of the digestive apparatus, red deer is considered an intermediate feeding type between concentrate selectors and grass eaters (Hoffmann 1989).

Diet composition is generally variable, comprising herbaceous species (especially *Graminaceae* and leguminous plants) as well as arboreal species and shrubs; these ones are mainly consumed during winter season.

Differences in diet composition and feeding strategy exist between periods of the year, but also between sexes; females tend to have a higher energy and protein diet, while males tend to consume a larger amount of herbage with higher quantity of fibre.

Number and duration of feeding bouts vary among seasons, sexes and reproductive period. Most feeding activity is concentrated during dusk and dawn. During the 24-hour, deer can display from 5 to 8 feeding bouts (Mattiello et al. 2010); duration of bouts in Rum isle deer ranged from about 70 minutes in summer and 130 minutes in winter (Clutton-Brock et al. 1982).

Ecology and criticalities

Spatial behaviour of mammals may be affected by several factors: metabolic requirements (McNab 1963), feeding and reproductive behaviour, age, sex, population density, competition and predation, anthropic disturbance (Lovari et al. 2007).

In red deer the *home range*, defined as the area utilised by the individual in its normal activities of food gathering, mating and caring of young (Burt 1943), varies its expansion among seasons and sexes. Home range extent in males tend to be higher than in females (Mitchell et al. 1977; Carranza et al. 1991, Bocci 2006, Lovari et al. 2007); *home range* in autumn and winter are larger than in spring - summer (Bocci 2006, Lovari et al. 2007, Crocetti 2011). Despite this variability, the extent of utilised territories is generally high, covering several hundreds of hectares.

The ample presence of red deer in a territory, considering the size of the species, its adaptability to various habitats and its remarkable capability of movement, determines frequently conflicts with anthropic activities;

when population density is substantial, the entity of damages caused to forests or cultivations may be considerable (Jarnemo et al. 2014). Moreover, frequently *home ranges* comprise wide portions of urban and extra-urban roads, increasing the probability of collision with vehicles; this is a raising phenomenon, counting several million events each year around the world (Rea 2003, Mysterud 2004, Dussault et al. 2007, Langbein et al. 2011), also in several Italian territories (Ponzetta et al. 2009).

Focus on the study species: Cattle

The two breeds employed in this study, Limousine and Chianina, are commonly reared for beef production; in Italy Limousine breed is widespread, especially in north-west and central parts of the peninsula. Chianina is a local breed, native of Tuscan territory, and is reared mainly in central Italy.

Beef cattle, including these breeds, is frequently reared in free-ranging systems or at confined pasture; monitoring animal activity and health, as well as utilization of land in these particular breeding conditions may be difficult. The study of landscape use by animals requires a record of the location of individuals over time (Ungar et al. 2005), thus GPS technology constitutes a useful technique for this purposes. Indeed, it was used to study grazing areas of hill sheep (Rutter et al. 1997), as well as tracking routes (Ganskopp et al. 2000) and pasture use (Turner et al. 2000; Ganskopp, 2001) of cattle. Incorporating information on activity in livestock behaviour studies, may improve management of animal husbandry and enhance breeding performances as for instance reproduction and lactation (Moreau et al. 2009). Precise models for behaviour prediction and spatial localization may also provide important information on diet selection and herbage intake (Guo et al. 2009); moreover, behaviour identification could allow to determine energy expenditure of grazing cattle.

The study of interactions between livestock and their ambience may be consequently enriched, permitting to understand how grazing animals modifies the environment (Guo et al. 2009) as well as their utilization on the basis of the available vegetation. Finally, the activity of animals may serve as a health indicator (Moreau et al. 2009); measuring stress directly (e.g. analysis of blood samples) in free-ranging animals is difficult, moreover additional stress may be caused during manipulation. Environmental stressors may be weak and persist for long time, thus long-term effects may become detectable with the analysis of the frequency and the structure of behaviour (Berger et al. 2003).

CHAPTER 2

AIM OF THE THESIS

The object of this research concerns the study of the possible applications of activity data recorded by accelerometers on GPS/GSM collars for the study of behaviour of wild and domestic ungulates. In particular, the research articulates in three main objectives and is focused on two species: red deer (*Cervus elaphus*) and two breeds of beef cattle, Chianina and Limousine.

The first theme pertains the possibility to identify the behaviour of monitored animals. The objectives for this theme is:

- Built classification models to predict the behaviour of red deer and beef cattle from data recorded by activity sensors in GPS collars.

The second objective regards the use of recorded data as a measure of intensity of the animal's activity, in particular:

- Studying some aspects of activity patterns of free-ranging red deer and cattle on pasture at different time scales.
- Provide an example of possible utilisation of this type of activity data, which could represent a useful tool for wildlife and livestock research.

In particular we present, to our knowledge, the first continuous and long-term study basing on remotely collected activity data performed on free-ranging red deer.

The third objective pertains the application of the models for the classification of activity, in particular:

- Use of the classification models to predict the behaviour of free-ranging deer, as well as Chianina and Limousine cows on pasture.
- Define the daily budget of activity for the two monitored species, and its variations among individuals and periods of monitoring.

CHAPTER 3
MATERIALS AND METHODS

3.1 GPS-GSM COLLARS: FEATURES AND DATA COLLECTION

Red deer and cattle were tracked using VECTRONIC Aerospace® GPS-GSM collars. The *GPS Pro-Light 4* collars employed in this study were from series 5900 and 6000; collar weight is about 980 grams (red deer collars) and 1700 grams (cattle collars), however weight is calibrated with respect to the body size of the animal (collar weight < 2% body weight).

The collars have 12 GPS channels and are equipped with sensors capable of recording acceleration by means of a three-axial activity sensor, ambient temperature and mortality; GSM communication allows the transmission of data and commands: GPS and mortality data are sent by the GSM modem as SMS to a ground station, and each SMS stores 7 GPS position data. Received data are automatically merged by GPS Plus® software (VECTRONIC Aerospace) and stored together with associated UTC/LMT date, time, altitude, DOP, *Navigation*, *Validation*, temperature. GPS data can also be downloaded directly from the collar; in this case the downloaded file includes additional information on the number of satellites used for each fix. *Navigation* specifies the type of fix obtained (1S, 2S, 2D, 3D), whereas *Validation* provides an information on the quality of the data: a fix is considered validated if the receiver uses five or more satellites to calculate the position, and the DOP is less than 10.0 (Vectronic Aerospace – GPS Plus Collar Manager user's manual).

Activity sensor

Each collar unit is provided with an accelerometer which measures activity on three axes perpendicularly oriented (Fig. 3.1).

The true acceleration experienced by the device is thus measured on *X* axis (forward-backward movements), *Y* axis (sideways and rotary movements) and *Z* axis (vertical movements).

Changes in acceleration, calculated as difference between two consecutive measurements, are measured 4 - 8 times per second simultaneously on the three axes; recorded values are reported on a scale ranging from 0 to 255, where 0 is assigned when the collar experiences no changes in acceleration (i.e. the animal is immobile) and 255 is assigned to maximum variation recordable. The dynamic range of the accelerometers is from +2G to -2G. Average activity measurements for the user-defined sampling interval are stored in the collar, together with associated UTC/LMT date, time and temperature.

Fig. 3.1 GPS/GSM collar and axes of acceleration measurement

Different activity measurement modes are selectable, depending on the purposes of the study and the duration of tracking period;

since the memory capacity of the collars allows to store approximately 315.000 data, in our study we decided to test two different sampling intervals. In both sampling modes, acceleration is measured only on the X and Y axis, and activity measurements are stored as two separate values.

- **Sampling interval 1.** Activity data are stored in five minutes intervals; since storing is based on eight seconds intervals, the actual activity interval is 296 seconds. The resulting deviation is adjusted by inserting a gap every 74 datasets. This mode was used for red deer monitoring.
- **Sampling interval 2.** Activity data are stored in 152 seconds intervals. This mode was used on cattle.

Activity data can only be downloaded directly by the collar; recorded activity values are stored together with associated UTC and LMT date, time and temperature.

Sources of error

GPS position and activity recording can give detailed and high-frequency information about the behaviour of animals, however errors may occur due to technical and functioning reasons.

With regards of activity measurements, there are several causes of such errors which can affect in a different manner the accuracy of the recorded data. A potential error in activity values is the occurrence of intervals duplicates. For older collars the most common origin of duplicates is owed to the fact that during a GPS fix, activity is measured but not stored; after the recording of the GPS fix, the average activity values of previous intervals are stored with the same timestamps (e.g. 12.35.00 – 12.45.00 – 12.45.00 – 12.50.00). In this case, the duplicate data was deleted.

Moreover, duplicates with different activity values may also occur; here, the second value is the more reliable (VECTRONIC Aerospace, personal communication), thus the first duplicate was deleted. The deletion of one of the duplicate values is performed in order to prevent erroneous classification of behaviour (Heurich et al. 2011). Another common source of error is the collar internal clock; all timekeeping devices are subject to drift, and in collars drift is corrected every time a GPS location fix occurs; thus the user-programmed GPS fix rate will determine the frequency of correction and the amount of drift accumulating between corrections (Gaylord 2013).

In addition, the clock that triggers the activity logger does not run synchronous with the internal clock of the collar, but is exposed to time lags (Löttker et al. 2009).

The combination of these particular types of error determines, for the purpose of our study, a lower accuracy when trying to identify the actual activity performed by the animal, leading to an incorrect match between observed behaviour and recorded activity.

3.2 STUDY ANIMALS AND STUDY SITES

3.2.1 Free-ranging deer

Nine female red deer were captured between November 2008 and January 2009 in Acquerino-Cantagallo Natural Reserve (Prato, Italy). Captures were carried out within a research project named “Uso dello spazio e dell’habitat da parte del Cervo nella Riserva Naturale dell’Acquerino-Cantagallo”, with financial support of Provincia di Prato and Regione Toscana, and within a PRIN 2008 project named “Monitoraggio a distanza di ungulati selvatici e domestici in ambiente appenninico”. The animals were narcotized using a mixture of Zoletil® and Xylazine with a ratio of 1:1.

The animals were tracked with the GPS/GSM collars for a minimum of 10 months (F6) and a maximum of 32 months (F1). Detailed information on hinds and related study periods are reported in Table 3.1 and Fig 3.2. Sampling interval for GPS position receiving was of 1 fix every hour; the duration of activity measurements sampling interval was, as previously mentioned, 5 minutes (300 seconds).

Individual	**Sex**	**Age (yrs)**	**From**	**To**	**Days (no.)**	**Activity intervals (no.)**
F1	female	-	30/01/2009	15/10/2011	988	279536
F2	female	2-3	04/12/2008	24/02/2011	812	230157
F3	female	5-6	22/12/2008	20/12/2010	728	205594
F4	female	9	19/11/2008	14/02/2010	452	127374
F5	female	4-5	19/12/2008	16/12/2010	728	205733
F6	female	4-5	26/11/2008	16/09/2009	294	82994
F7	female	2-3	23/12/2008	20/12/2010	727	204376
F8	female	3	12/11/2008	10/11/2010	728	205637
F9	female	8-9	30/01/2009	15/03/2010	408	115462

Table 3.1 *Red deer.* Study animals and activity measurement periods

The analysis of the quality of GPS receiving in the study area, the study of deer spatial behaviour, mobility as well as habitat selection, were the object of a previous research (Crocetti 2011). Our study, thus, focuses only on aspects related to red deer activity.

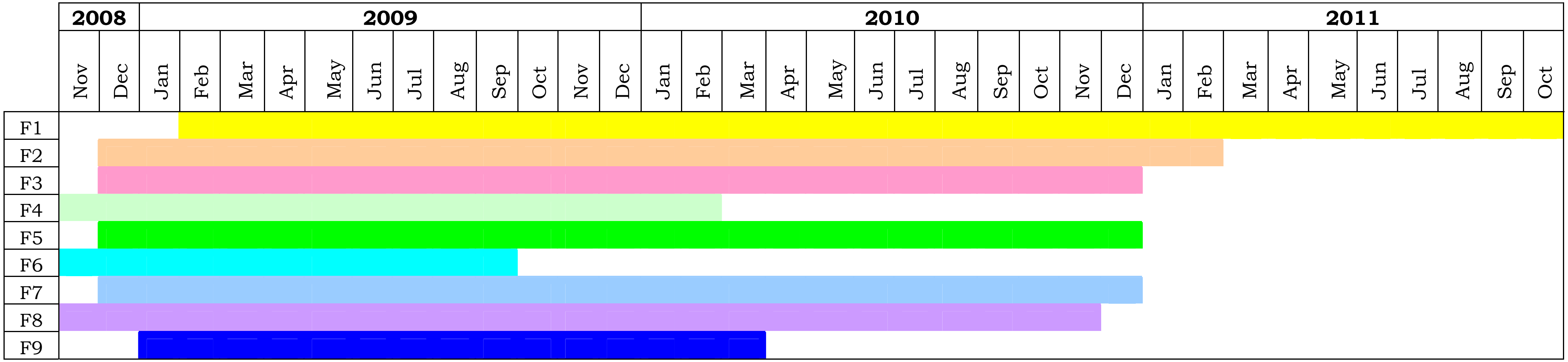

Fig. 3.2 Activity measurement periods for free-ranging hinds

Study area

The Reserve is situated in the core distribution of Apennine red deer, where is located part of its historic reproductive area; this core originated by the reintroduction of 7 deer in 1950s and 1960s (Mazzarone and Mattioli 1996), and hosts at present the largest Apennine population (about 3800 deer) that extends over four Provinces (Prato, Pistoia, Bologna, Firenze). The population is monitored since 1993 (Nicoloso et al. 2007) and managed by ACATER (Areale Cervo Appennino Tosco Emiliano Romagnolo) since 2000. Northern and Central Apennine red deer populations increased during the last decade, expanding also their territories. Despite a positive trend in number of deer within the study site during the decade 2000-2010, their density reduced from 2,60 deer/100 ha to 2,05 deer/100 ha, due to a greater expansion of populated territories (Nicoloso et al. 2013). The boundaries of Acquerino-Cantagallo Natural Reserve and the extent of territories frequented by the tracked hinds are reported in figure 3.3.

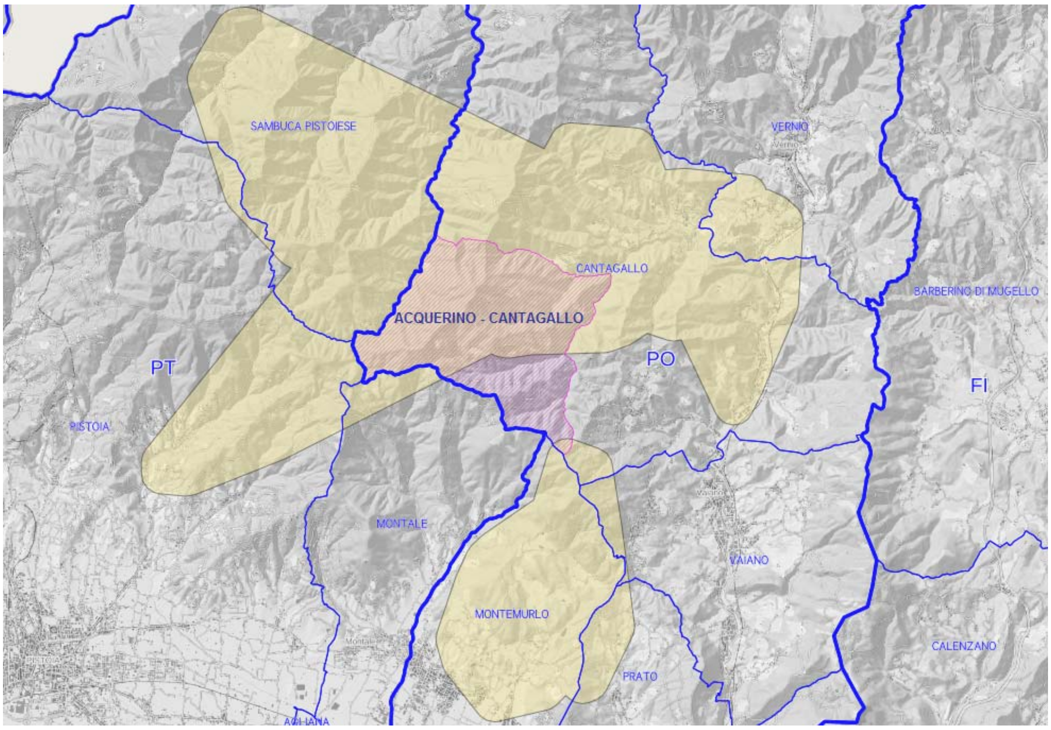

Fig. 3.3 Acquerino-Cantagallo Natural Reserve (pink) and boundaries of tracked hinds home ranges (yellow).

The Natural Reserve is located in correspondence of Apennine Mountains, in Central Italy; altitude ranges from 450 and 1204 m a.s.l., however monitored hinds covered also territories at lower altitudes (200 m a.s.l.).

Sub-mountain belt, that ranges from 400-600 to 800-1200 m a.s.l., is characterized by oak woods and mixed broad-leaved woods; mixed *Quercus cerris* L. and hornbeam (*Ostrya carpinifolia* Scop.) woods are prevalent, while oak woods are most commonly dominated by *Quercus cerris* L. and *Quercus pubescens* Willd. Sub-mountain belt and lower altitudes of mountain belt are dominated by chestnut (*Castanea sativa* Mill.) woods. In mountain belt, between 800 and 1600 m a.s.l., the most abundant tree species is beech (*Fagus sylvatica* L.), which is mainly present in monospecific woods, but is sporadically associated to Norway maple (*Acer platanoides* L.), sycamore maple (*Acer pseudoplatanus* L.), rowan (*Sorbus aucuparia* L.) and ash tree (*Fraxinus excelsior* L.). In reafforestated areas are present firs (*Pseudotsuga* spp.), *Abies alba* Mill., *Pinus nigra* Arn., *Pinus sylvestris* L. and *Alnus cordata* Loisel.

Herbaceous communities of these vegetation belts are mainly secondary grasslands; at woodland borders and in clearings are also common bracken (*Pteridium aquilinum* (L.) Kuhn.), heather (*Calluna vulgaris* (L.) Hull), and broom (*Sarothamnus scoparius* (L.) Koch).

3.2.2 Captive deer

In order to perform the calibration of the activity sensors, four captive red deer (two males and two females) were collared in different sites. All collar fitting procedures and manipulation of deer were performed in observance of the normative in force. Collars were kept on the experimental subjects for the necessary period that allowed us to collect behavioural observations encompassing all behavioural categories described in the sampling protocol used during field observations (see paragraph 3.3). Details of monitoring periods and information on the observed deer are reported in table 3.2. Differences in levels of activity between individuals and, within the same animal, between seasons are likely to occur (Stache et al. 2013). However collars couldn't be kept on deer for an extended period in order to account for seasonal differences; moreover, deer could not be collared in each season or period of the year: critical phases of biological cycle such as gestation and lactation periods for hinds, as well as antler growth and rutting period for stags represent moments of high sensitivity to external stresses, therefore we intentionally avoided manipulation of the animals during these phases.

Individual	**Sex**	**Age**	**Site**	**From**	**To**
F1	female	4	Marradi	23/10/2012	09/11/2012
F2	female	5	Sondrio	05/10/2014	09/10/2014
M1	male	2÷3	Scandicci	24/07/2013	15/08/2013
M2	male	1	Poppi	23/05/2014	01/07/2014

Table 3.2 *Red deer.* Study animals and monitoring periods

Experimental sites

For the purposes of the calibration, we selected deer living as close as possible to natural conditions. The size of the enclosures (about1-2 hectares) allowed experimental subjects to perform all activities and movements; deer lived together with conspecifics, so that they could display gregarious behaviour as well as social interactions. Enclosures encompassed wooded areas and grassland; deer fed mainly on grassland or chestnuts, however supplemental food was available, primarily hay, corn and fruit. Collared deer were observed in the following sites:

- F1 in a breeding farm “I Cancelli” located in Marradi (Tuscan Apennine)
- M1 in an enclosure located in a hunting reserve “I Lami” in Scandicci (Florence)
- M2 in a private zoo “Parco zoo della Fauna Europea di Poppi” situated in Poppi (Tuscany)
- F2 in a wildlife aid centre “Centro assistenza fauna selvatica – Ponte in Valtellina” in Sondrio (Central Alps)

3.2.3 Beef cattle

Six cows were monitored with GPS collars during two grazing seasons in 2012 and 2013, and behavioural observations were performed on the same animals in order to calibrate activity sensors. In 2012 two cows were collared in a breeding farm situated in the Tuscan Apennine (San Marcello Pistoiese), while in 2013 four cows were collared in a breeding farm located in Borgo San Lorenzo. Animals belonged to two breeds, commonly used for beef production: Limousine and Chianina, a local breed. The animals were farmed on confined pasture from late spring to autumn; during winter and early spring, they were kept in cattle sheds. In pasture season 2013, the four monitored cows were kept on confined pasture for several days also in December.

Collars were fitted on the cows before the beginning of grazing season (in May – June), and were kept on the animals until they returned permanently into stables (in November–December); for the Limousine cow C2, monitoring period was interrupted in August due to technical problems on the collar. Behavioural observations, according to the adopted sampling protocol, were performed when the cows were on confined pasture. Detailed sampling periods are displayed in table 3.3; experimental sites are shown in figure 3.4.

Individual	**Sex**	**Breed**	**Breeding site**	**From**	**To**	**Days (no.)**	**Activity intervals (no.)**
C1	female	Limousine	A	23/04/2012	29/11/2012	225	127248
C2	female	Limousine	B	07/06/2013	20/08/2013	105	58771
C3	female	Limousine	B	31/05/2013	15/10/2013	180	101298
C4	female	Chianina	A	23/04/2012	24/09/2012	186	104975
C5	female	Chianina	B	31/05/2013	18/11/2013	189	106475
C6	female	Chianina	B	31/05/2013	18/11/2013	196	110801

Table 3.3 *Cattle*. Study animals and continuous activity measurement periods; site A: San Marcello Pistoiese, site B: Borgo San Lorenzo.

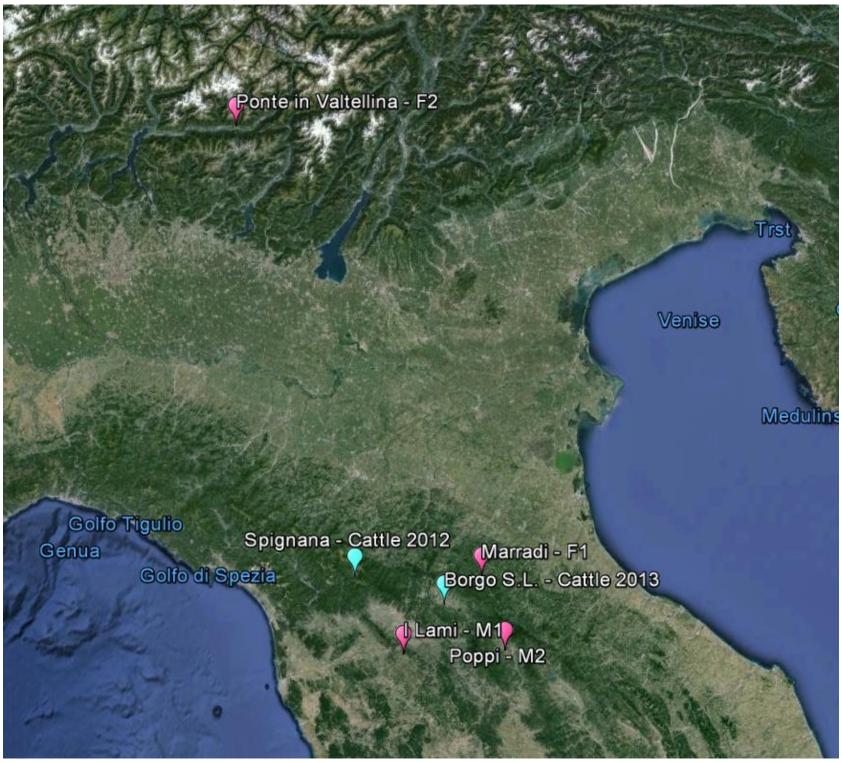

Fig. 3.4 Location of experimental sites for deer and cattle.

3.3 FIELD OBSERVATIONS AND DATA PREPARING

The sampling protocol used for behavioural observations was the same for deer and for cattle. The calibration of predictive models to discriminate among activities requires synchronized visual observations of collared individuals (Ungar et al. 2005); as first step, we defined an ethogram encompassing all performable behaviours. The description of the activities comprised in every behavioural class is reported in table 3.4.

Every 30 seconds the animals were observed, and the dominant activity, as categorized in the ethogram, was noted on a sampling form (Appendix I, figure I.1); in order to collect data of different behavioural sequences as well as the transition between sequences, the duration of observation sessions on each animal ranged from a minimum of 1 to 7 consecutive hours. Observations were performed at different times of the day, both at daylight and at dark, when possible. Furthermore, during the sessions, ambient temperature and humidity as well as weather conditions (sunlight, clouds, rain, wind) were also recorded (Appendix I, figure I.2).

Behaviour class [a]	**Description**
Bedded	Lying on ground, either sleeping or awake, with head up or head down
Bedded-ruminating	Lying on ground, chewing cud
Standing	Standing without leg movement, including breast-feeding
Standing-ruminating	Standing without leg movement, chewing cud
Grooming [b]	Licking or scratching oneself or conspecifics
Feeding	Standing, either with or without leg movement, and feeding on herbaceous/woody vegetation or hay
Walking	Walking without feeding
Fast moving	Trotting, galloping or running

Table 3.4 Behavioural categories recorded during surveys.

[a] Each behavioural category may include head movements.

[b] Grooming behaviour may occur in each other behavioural category.

In order to match correctly the observed activities with the activity data recorded by the devices, we synchronized a chronometer with the internal clock of the collars (UTC time). Total time of observation and days of surveys are reported in table 3.5 for red deer and in table 3.6 for cattle.

For red deer, rumination while standing was never observed; therefore for this species the *ruminating* behavioural class refers solely to rumination while bedded. G*rooming* behavioural class was never (for red deer) or rarely (for cattle) observed for the entire duration of sampling interval; therefore, for cattle, *grooming* intervals were assigned to the main activity displayed at the time (e.g. bedded, standing, feeding).

Also for *fast moving*, only few intervals were recorded both in cattle and in red deer; thus, this behavioural class was joined to the *walking* class, and they were reported together as *moving*.

ID **Month**	**F1**	**F2**	**M1**	**M2**
June	-	-	-	1200
August	-	-	600	-
October	257	1045	-	-
November	700	-	-	-
∑ **(ID)**	957	1045	600	1200

Table 3.5 Red d*eer*. Total amount of behavioural observations (minutes)

	ID	LIMOUSINE			CHIANINA			
	Date	1	2	3	4	5	6	Σ (Date)
2012	April	317	-	-	339	-	-	656
	May	56	-	-	43	-	-	99
	June	256	-	-	256	-	-	512
	July	-	-	-	81	-	-	81
	September	198	-	-	114	-	-	312
	October	301	-	-	304	-	-	605
2013	July	-	208	230	-	524	524	1486
	October	-	-	218	-	-	-	218
	December	-	1152	1200	-	580	580	3512
	Σ (ID)	1128	1360	1648	1137	1104	1104	**7481**

Table 3.6 *Cattle*. Total amount of behavioural observations (minutes)

After retrieving the devices, activity data in *.adf* format (storage format of the collars) were checked for gaps and duplicates using *ADF Merge*® software (VECTRONIC Aerospace), and subsequently exported in *.xls* format. Furthermore, a spreadsheet was created, where the recorded values for X and Y axis were joined to their associated observed activities. Although behaviour was observed every 30 seconds, sampling intervals were longer (152 seconds for cattle, 300 seconds for deer), therefore the dominant activity was determined using the modal (i.e. the most frequent) class.

In addition, sampling intervals entirely composed by a unique behavioural class were labelled as “*Pure* intervals” whereas intervals comprising different behavioural classes were marked as “*Modal* intervals”.

3.4 CLASSIFICATION OF BEHAVIOUR AND MODEL BUILDING

The resulting *calibration database*, where each activity interval is matched with observed behaviour classes, was employed to produce statistical models predicting dominant activity for each time unit, using X and Y values as variables. All types of intervals, both pure and modal, were employed to build the classification models; these models were used to predict the behaviour from a dataset composed by both pure and modal intervals. Two different statistical procedures were employed: Discriminant Analysis applied to Gaussian mixture models (DAGMM) and recursive partitioning (RPART); for GMM models, we used as predictor variables untransformed (real) and log-transformed (log) X and Y values; for log-transformed values the following transformation was applied: log(value + 0.5). We compared the performances of the models, evaluated by percentage of correct classification (%CC); the models with the highest %CC were chosen for each species. The %CC for single behaviour classes was considered acceptable if > 70%. Different models were initially tested, starting from a general model including all behavioural classes, months and breeds; subsequently, specific models were built, depending on the following factors:

- *Breed.* We adopted separate models for Chianina and Limousine cows.
- *Sampling season.* Cattle were monitored and observed throughout several months during pasture season; thus two periods were identified on the basis of average temperatures and by graphical examination of activity data: the *warm period* (from May to September) and the *cool period* (from October to December).

- *Behavioural classes.* Models with different groupings of behavioural classes were preliminary tested, in order to detect which behaviours were effectively recognizable. Observed behavioural classes were grouped basing on similarity of behaviour (e.g. bedded and ruminating were grouped into *resting* behavioural class for red deer). In order to display and compare the results of classification models built with different numbers of behavioural classes, the performances of both models with least aggregated behavioural categories and models built with the partition "*active*/*resting* behaviour" will be presented.

Frequently in literature models built with only *pure* intervals have been used to classify datasets of *pure* intervals (Naylor and Kie 2004; Coulombe et al. 2006; Bourgoin et al. 2008; Löttker et al. 2009; Grünewälder et al. 2012); however, this procedure neglects intervals composed by a mixture of different behaviours. In order to test the effects on %CC of using solely *pure* intervals, we also built models with these data and used them to predict the behaviour from a dataset composed both by pure and modal intervals.

3.5 ACTIVITY PATTERN ANALYSIS

RED DEER

Activity data as recorded by the collars, were used to study the activity patterns of free-ranging red deer at different time scales. Considering the number of monitored individuals (N=9), we did not focus on age-related differences, instead we aimed to identify the general species-specific activity patterns. Both activity channels (*X* and *Y*) recorded similar values in our monitored deer; moreover, activity values of *X* axis are significantly correlated to resting and feeding/moving, both in our data analysis and in other studies performed on red deer (Löttker et al. 2009) and on roe deer

(Heurich et al. 2011). For this reason, the following activity pattern analysis were performed using only data from the forward-backward channel (as in Krop-Benesch et al. 2013). For all activity pattern analysis, time was calculated an reported as Central European Time (CET).

3.5.1 Actograms

Activity data were plotted for the entire data collection period of hinds, in order to show annual patterns; graphical output was performed with the Activity Pattern® software (VECTRONIC Aerospace). The vertical axis reports the date, while the horizontal axis is the time of day in CET. Activity is coded in a coloured gradient, and reported in horizontal daily records displayed one beneath the other. Dawn and dusk are reported as black lines, representing three sun positions:

- *Nautical dawn* and *nautical dusk*. Begins in the morning and ends in the evening when the Sun is 12° below the horizon.
- *Civil dawn* and *civil dusk*. Begins in the morning and ends in the evening when the Sun is 6° below the horizon.
- *Sunrise* and *sunset*. Instant in which the upper edge of the sun appears or disappears above the horizon.

As referential latitude and longitude for the calculation of Sun positions, the geographical coordinates of the centroid of the home ranges calculated for the nine hinds were used (E: 11° 2' 52"; N: 44° 0' 38").

3.5.2 Annual activity patterns

Variations in patterns and intensity of activity between consecutive years were investigated; the following temporal boundaries were defined:

- Year 1: from December 2008 to November 2009
- Year 2: from December 2009 to November 2010

For this analysis, the data of the entire deer sample (N=9) was used. Furthermore, variations of activity between February 2009 and January 2010 were also examined; this yearly period was chosen as representation of a monitoring time span shared by almost the entire sample (N=8; F6 excluded).

Additionally, temperature data as well as forage productivity were considered. Average monthly temperatures from Acquerino weather station were used (database of “Centro funzionale Regione Toscana”). The start of vegetation period and the productivity of natural grasslands were derived from a study on growth intensity of natural vegetation in Central Apennine (Sarno et al. 1989), in areas with similar altitudes, temperatures and dominant species.

3.5.3 Single cosinor

Cosinor analysis was performed for each individual in order to verify the existence of an annual rhythm in deer activity. The analysis, carried out with Activity Pattern® software and according to Nelson et al. (1979), imply the least-squares fit of a 365-day cosine to test the assumption of zero-amplitude (i.e. absence of a rhythm); when this assumption is rejected, the three parameters of the model describing the annual rhythm are estimated: *amplitude* (A), which represents one half of the difference between the highest and lowest point in the cosine curve; *acrophase* (ϕ), a measure of the timing of the highest value of the cosine curve, expressed as a lag from a chosen zero time;

mesor (M), (<u>m</u>idline <u>e</u>stimating <u>s</u>tatistic <u>o</u>f <u>r</u>hythm) represents the midpoint value between the highest and lowest point of the sinusoidal curve (Moore & Halberg 1986). Moreover, estimates of confidence regions for amplitude, acrophase and mesor are provided. The cosinor uses a cosine function as pattern for the rhythm:

$$y(t) = M + A \cos(\omega/t + \phi) + e(t)$$

y(t) is the value of the variable at time t; ω is the angular frequency of the cosine model. Period length is 365 days.

Cosinor analysis was applied to the entire sample except for F6 (N=8), for the period February 2009 - January 2010. Estimated parameters are presented graphically in a “clock” cosinor-plot, where 0° point refers to midnight between 31 December and 01 January; *acrophase* and *amplitude* are reported as a vector, respectively as its direction and intensity. The circle around the tip of the vector represents the 95% confidence region of *acrophase* and *amplitude*.

3.5.4 Diurnality index

The relation of intensity of activity during light hours of the day and activity during dark hours, is reported through *diurnality index* (DI). This index was calculated in the period February 2009 - January 2010 for all hinds (N=9) with Activity pattern® software, according to the following equation introduced by Hoogenboom et al. (1984):

$$DI = \frac{\frac{a_d}{i_d} - \frac{a_n}{i_n}}{\frac{a_d}{i_d} + \frac{a_n}{i_n}}$$

where a_d is the sum of activity values recorded during light hours (day), a_n is the sum of activity values recorded during dark hours (night), i_d and i_n are the number of activity intervals corresponding to day and night, respectively.

The ratio between activity values and duration of light/dark hours automatically corrects for seasonal changes in daylight length.

The index produces for each day a value included between -1 and +1; if the animal is active exclusively during day or night, the corresponding values of the index will be +1 or -1, respectively. ID equals 0 when the animal is equally active during night and day.

3.5.5 Daily activity patterns

Variations in patterns and intensity of activity were investigated at daily scale for each month of the period February 2009 – January 2010, in order to account for differences in Sun position throughout the year. Median activity values were calculated hourly; the entire sample (N=9) was used.

Sunrise and sunset time were calculated, using the home ranges centroid as reference point for longitude and latitude, from US Naval Observatory website (http://www.usno.navy.mil/USNO/).

BEEF CATTLE

In accordance with as previously established for red deer, the analysis of activity for cattle was carried out using values recorded along the X axis. Time was calculated and reported in CET.

3.5.6 Actograms

The plotting procedure is the same as described for red deer in paragraph 3*.5.1.*

As referential latitude and longitude for the calculation of sun positions, coordinates of pasture enclosures were utilized. Activity recorded for the entire period of pasture was reported.

3.5.7 Seasonal activity patterns

Variations in intensity of activity during pasture season (*warm* period) were investigated; median activity values were calculated at intervals of 15 days, separately for Limousine cows (N=3) and Chianina cows (N=3).

3.5.8 Daily activity patterns

Variations in patterns and intensity of activity were investigated at daily scale for each month of the grazing *warm* period, in order to account for differences in sun position. Median activity values were calculated hourly, separately for Limousine and Chianina. Sunrise and sunset time were calculated, using the coordinates of pasture enclosures as reference point for longitude and latitude, from US Naval Observatory website (http://www.usno.navy.mil/USNO/).

3.6 MOBILITY OF BEEF CATTLE

The sampling interval for position acquisition from GPS collars was 1 fix per hour. Mobility at pasture was calculated hourly, as Euclidean distance between two consecutive fix. Only validated fix were employed for the calculation; cattle frequented almost exclusively open areas, thus both position acquisition rate and percentage of validated fixes were high (99.1% and 96.5% respectively). Detailed data on the performances of GPS fix acquisition rate are reported in Appendix II.

Therefore, the selection of validated fixes only led to a minimal data loss. Median values of interfix distances (metres) were reported for the 15-days periods during *warm period* of pasture.

Interfix distances were calculated using *Animal movement* extension for ArcView 3.2.

3.7 CLASSIFICATION OF ACTIVITY DATA

The classification models which displayed the best performances were used to predict the behaviour of monitored animals. Models calibrated on the four captive deer were employed to perform the classification of behaviour of the nine free-ranging hinds; for each individual, the duration of daily resting and active behaviours was calculated.

Moreover, models calibrated on cattle on confined pasture were employed to predict the behaviour of the same animals; here, duration of daily resting and active behaviours was calculated.

3.8 STATISTICAL ANALYSIS

All statistical analyses were performed using the R programming environment (R 3.0.2, R Core Team). For the classification of behaviour, models were built using both recursive partitioning and Discriminant Analysis applied to Gaussian mixture models; R packages *rpart* and *mclust* were employed.

Recursive partitioning is an algorithm which can be applied to the non-parametric statistical method of *classification and regression trees* (CART) (Breiman et al. 1984); it produces a decision tree through a step-by-step process, by splitting or not splitting each node on the tree into two children nodes.

A Gaussian Mixture Model (GMM) is a parametric probability density function represented as a weighted sum of Gaussian component densities.

Models were trained on a random subset (75% of the *calibration database*) and validated on remaining data with cross-validation.

Friedman Test was used to test for differences between months in annual activity patterns (paragraph 3.5.2) and between hours in daily activity patterns (par. 3.5.5) for deer; it was also used to test for differences between 15-days periods in seasonal mobility (par. 3.6) and between hours in daily activity patterns (par. 3.5.9) for cattle.

After Friedman test, pairwise comparisons with Bonferroni correction were performed.

Mann-Whytney U test was used to test for differences between months in consecutive years (deer).

Analysis of variance was used to test for differences in diurnality index (deer); it was also used to test for differences in duration of activity (calculated after classification of behaviour) in deer and cattle; after analysis of variance, Tukey post hoc test was performed. Student's t-test was employed to detect differences in the number of hours of activity between breeds (Chianina and Limousine) in each 15-days period.

CHAPTER 4

RESULTS

4.1 CLASSIFICATION OF UNGULATE BEHAVIOUR

RED DEER

The behavioural observations yielded the following number of intervals for each behavioural class (Table 4.1), which composed the classification database used for models building. In total, the number of intervals utilised to build the models for red deer was 633. As formerly observed in other studies on activity carried out with the same devices, activity values recorded on X-axis and Y-axis resulted highly correlated, both in free-ranging deer ($r_{pearson} = 0.92 – 0.96$) and in captive deer ($r_{pearson} = 0.96 – 0.99$).

The performances of the models built with the following groupings of behavioural classes were reported and compared:

- *5 behavioural classes group*: bedded, ruminating (only bedded), standing, feeding, moving. Used in DAGMM procedure.
- *3 behavioural classes group:* passive (encompassing bedded and ruminating), standing and active (encompassing feeding and moving). Used in RPART procedure.
- *2 behavioural classes group*: resting, active. Used both in DAGMM and RPART procedures.

	N° behavioural classes		
	5	3	2
Bedded	250		
Ruminating	94		
Resting		344	344
Standing	69	69	
Feeding	179		
Moving	41		
Active		220	289

Table 4.1 Number of sampling intervals for behavioural classes obtained from direct observations.

N° behavioural classes	Best Model	Behavioural classes							Total	Classes Average
		Bedded	**Ruminating**	**Resting**	**Standing**	**Feeding**	**Moving**	**Active**		
5	GMM_{log}	79.8%	11.7%		35.5%	84.1%	9.9%		65.5%	44.2%
2	GMM_{real}			94.2%				88.1%	91.4%	91.2%

Table 4.2 Percentage of correct classification for behavioural classes after cross-validation for discriminant analysis applied to Gaussian mixture models. The total % represents the percentage of all intervals correctly classified; the classes average represents the average % classification among behavioural classes.

The performances of the best models (i.e. models with the highest %CC) built with DAGMM procedure for the two groups of behavioural classes are reported in table 4.2.

The model with 5 behavioural classes was not able to amount to the target %CC for each class of 70%. *Ruminating* and *Moving* were the most misclassified classes, with %CC of 11.7% and 9.9%, respectively; the largest part of *Ruminating* intervals were classified as *Bedded*, while *Moving* intervals were mainly classified as *Feeding. Standing* behaviour instead, was classified both as *Bedded* and as *Feeding*. Average %CC between behavioural classes indicates with more precision the performances of the model; total %CC, indeed, is influenced by the number of intervals within each category of behaviour. The DAGMM model with 2 behavioural classes built with untransformed variables produced the best performances; it classified correctly 94.2% of *Resting* intervals and 88.1% of *Active* intervals.

Recursive partitioning models were able to classify exclusively *Passive*, *Standing* and *Active* behaviours; models with the highest %CC were those built with X-axis activity values as variable (Table 4.3). However, a high percent (60%) of *Standing* behaviour was misclassified.

N° behavioural classes	**Best Model**	**Behavioural classes**				**Total**	**Classes Average**
		Passive	**Resting**	**Standing**	**Active**		
3	RPART$_x$	95.3%		40.0%	85.5%	85.8%	73.6%
2	RPART$_x$		95.3%		84.8%	90.5%	90.2%

Table 4.3 Percentage of correct classification for behavioural classes after cross-validation for recursive partitioning models. The total % represents the percentage of all intervals correctly classified; the classes average represents the average % classification among behavioural classes.

Also with the RPART procedure, the model which has reached the target %CC for behavioural class is the one with 2 classes. This model was able to classify correctly 95.3% of *Resting* intervals and 84.8% of *Active* intervals.

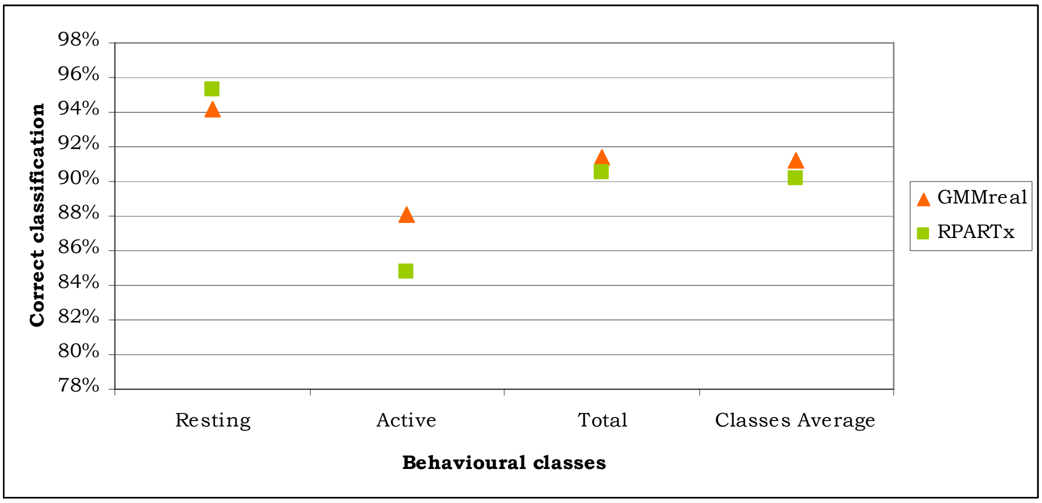

Figure 4.1 Comparison of percentage of correct classification for behavioural classes after cross-validation for recursive partitioning models and for Discriminant Analysis applied to Gaussian mixture models.

When comparing the performances of the GMM_{real} model with 2 behavioural classes and the $RPART_x$ model with 2 behavioural classes (Fig. 4.1), the first one produces the highest total and classes average %CC. Although the $RPART_x$ model classifies correctly a slightly higher number of *Resting* intervals (95.3% vs 94.2%), the percentage of correctly classified *Active* intervals is lower (84.8% vs 88.1%). The GMM classification model, however, tended to underestimate the active behavior of 5%.

Therefore, the GMM_{real} model will be used to perform an attempt to classify the activity database of free-ranging hinds.

However, for comparison purposes with the results of the only study performed with recursive partitioning on red deer, as well as for comparison with further attempts, threshold values for the $RPART_x$ and $RPART_y$ models are reported in table 4.4.

	X axis	Y axis
Threshold value	25.5	14.5

Table 4.4 Threshold values between active and inactive behaviour in red deer for the X-axis and Y-axis activity values, calculated with recursive partitioning models.

The main features of GMM_{real} model are reported in table 4.5, while graphical representation of classification dataset and mixture components of the model are shown in figure 4.2.

Classes	**n**	**Model**	**G**
Resting	344	VEV	5
Active	289	VEV	4

Table 4.5 Summary of the main features of GMM_{real} model for classification of red deer behaviour. In model column the abbreviation VEV summarizes the parametrization of the covariance matrix, with ellipsoidal distribution, variable volume, equal shape and variable orientation. G represents the number of mixture components.

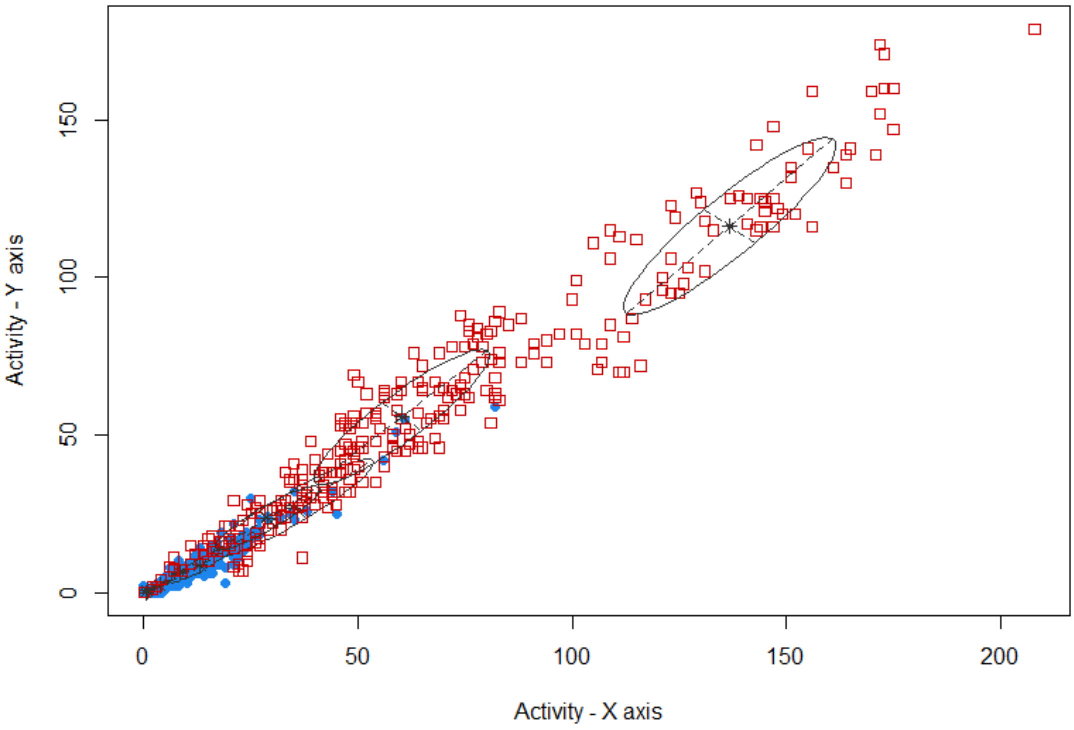

Figure 4.2 Plot of the classification database and graphical representation of mixtures components for the $\mathrm{GMM_{real}}$ model. Blue data points are referred to *Resting* behaviour, red data points to *Active* behaviour.

BEEF CATTLE

The behavioural observations on cattle yielded the following number of intervals for each behavioural class (Table 4.6), which composed the classification database used for model building. Separate models were adopted for the two breeds, as well as for different periods (*warm* and *cool*) within each breed. Also in this species activity values recorded on X-axis and Y-axis resulted highly correlated ($r_{pearson}$ = 0.82 – 0.94).

In total, the number of intervals used for cattle model building was 387 for Chianina-*cool* period, 534 for Chianina-*warm* period, 629 for Limousine-*cool* period and 347 for Limousine-*warm* period.

Here are compared models built using 4 and 2 groups of behavioural classes; additional groupings of behavioural classes were preliminary tested in order to achieve the optimal final model structure, however they have not been reported.

- *4 behavioural classes group (1)*: bedded (encompassing bedded and ruminating bedded), stationary (encompassing standing and ruminating standing), feeding, moving. Used in DAGMM procedure.

- *4 behavioural classes group (2)*: passive (encompassing bedded, ruminating bedded and ruminating standing), standing, feeding, moving. Used in the RPART procedure.

- *2 behavioural classes group*: resting, active. Used in DAGMM and RPART procedures.

N° behavioural classes		Chianina		Limousine	
		Cool	Warm	Cool	Warm
2	Resting	295	410	375	147
	Active	92	124	254	200
4(1) DAGMM	Bedded	37	277	93	44
	Stationary	258	133	282	103
	Feed	81	115	210	185
	Moving	11	9	44	15
4(2) RPART	Passive	147	350	185	62
	Standing	148	70	190	85
	Feed	81	115	210	185
	Moving	11	9	44	15

Table 4.6 Number of sampling intervals for behavioural classes obtained from direct observations.

The performances of the best models (models with the highest %CC) built with DAGMM procedure for two groupings of behavioural classes are reported in table 4.7. The model with 4 behavioural classes was not able to amount to the target %CC for each class.

The DAGMM procedure applied to the 2 behavioural classes dataset was able to reach the target %CC for every behavioural class. The examination of the performances of the models shows differences related to the period of monitoring: %CC is higher, both for Chianina and Limousine, during warm period.

Breed	Period	N° behavioural classes	Best Model	Bedded	Standing	Resting	Feeding	Moving	Active	Total	Classes Average
Chianina	Cool	4	GMM_{log}	0%	84.6%		45.0%	0%		67.6%	32.4%
Chianina	Cool	2	GMM_{real}			75.6%			70.1%	74.3%	72.9%
Chianina	Warm	4	GMM_{log}	64.4%	19.2%		87.6%	0%		77.8%	42.8%
Chianina	Warm	2	GMM_{real}			98.2%			93.6%	97.1%	95.9%
Limousine	Cool	4	GMM_{log}	75.3%	31.7%		85.7%	41.0%		65.4%	58.5%
Limousine	Cool	2	GMM_{real}			86.1%			86.8%	86.4%	86.3%
Limousine	Warm	4	GMM_{log}	73.9%	84.5%		92.2%	3.3%		84.0%	63.5%
Limousine	Warm	2	GMM_{real}			91.5%			88.3%	89.6%	89.9%

Table 4.7 Percentage of correct classification for behavioural classes after cross-validation for Discriminant Analysis applied to Gaussian mixture models. The total % represents the percentage of all intervals correctly classified; the classes average represents the average % classification among behavioural classes.

In regard to breed, performances vary according to season: during the *warm* period the highest percentage of correct classification results for Chianina, conversely during *cool* period the highest %CC results for Limousine breed. The best performances are achieved with Chianina dataset collected during warm season, with a total percentage of correct classification of 97.1%.

The performances of RPART models are reported in table 4.8. As with the previous procedure, the target %CC is reached by the 2 behavioural class model, with the exception of Chianina during *cool* period (25.5% correct classification for *Active* behaviour). This scarce performance of classification could be explained by the fact that in data collected on Chianina during cool season, distribution of values for active and resting intervals is rather overlapping for data recorded on Y axis; thus the threshold value determined with RPART procedure probably encompasses in *Resting* behaviour a large part of *Active* intervals. In this case GMM model applied on a bivariate distribution appear to perform a better classification.

Also with RPART procedure %CC results higher during *warm* period for both breeds; similarly, highest %CC in *warm* period are associated with Chianina, while highest %CC in *cool* period are found in Limousine breed.

The best performances are achieved by Chianina dataset collected during the *warm* period, with a total percentage of correct classification of 97.4%, and a 99% of correct classification for resting behaviour.

Comparisons between classification performances of the two procedures for the different models are presented in figures 4.3, 4.4, 4.5 and 4.6.

Breed	**Period**	**N° behavioural classes**	**Best Model**	**Passive**	**Standing**	**Resting**	**Feeding**	**Moving**	**Active**	**Total**	**Classes Average**
Chianina	Cool	4	RPARTy	84.7%	60.8%		26.8%	0%		60.6%	43.1%
Chianina	Cool	2	RPARTy			92.5%			25.5%	76.2%	59.0%
Chianina	Warm	4	RPARTy	97.7%	5.7%		89.5%	25.0%		82.8%	54.5%
Chianina	Warm	2	RPARTy			99.0%			91.8%	97.4%	95.4%
Limousine	Cool	4	RPARTy	91.3%	12.6%		80.0%	59.1%		61.5%	60.8%
Limousine	Cool	2	RPARTx			81.8%			91.3%	85.7%	86.6%
Limousine	Warm	4	RPARTx	52.9%	92.9%		93.4%	0%		82.1%	59.8%
Limousine	Warm	2	RPARTx			96.1%			91.8%	93.6%	94.0%

Table 4.8 Percentage of correct classification for behavioural classes after cross-validation for recursive partitioning models. The total % represents the percentage of all intervals correctly classified; the classes average represents the average % classification among behaviours.

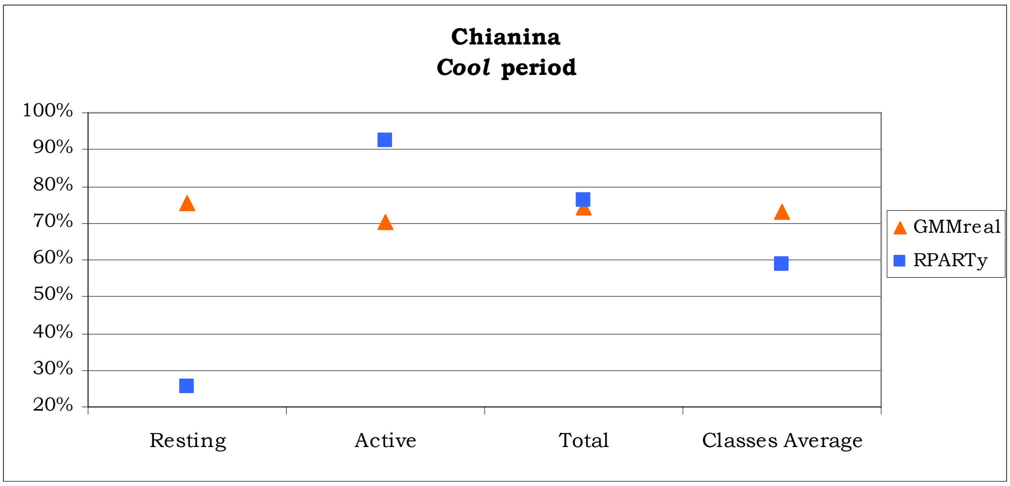

Figure 4.3 Chianina breed, cool period. Comparison of percentage of correct classification for behavioural classes after cross-validation for recursive partitioning models and for Discriminant Analysis applied to Gaussian mixture models.

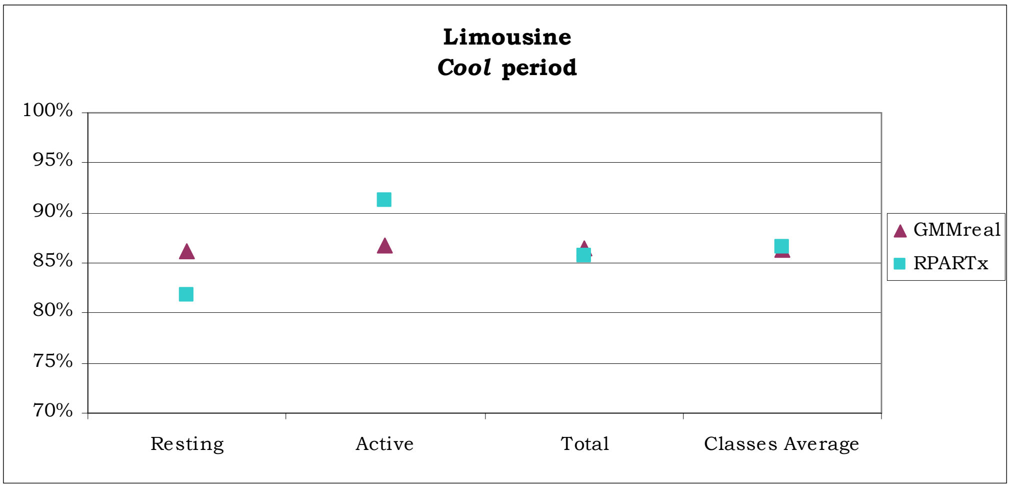

Figure 4.4 Limousine breed, cool period. Comparison of percentage of correct classification for behavioural classes after cross-validation for recursive partitioning models and for Discriminant Analysis applied to Gaussian mixture models.

RPART models on average performed better on Limousine data, while GMM models provided better results on Chianina datasets.

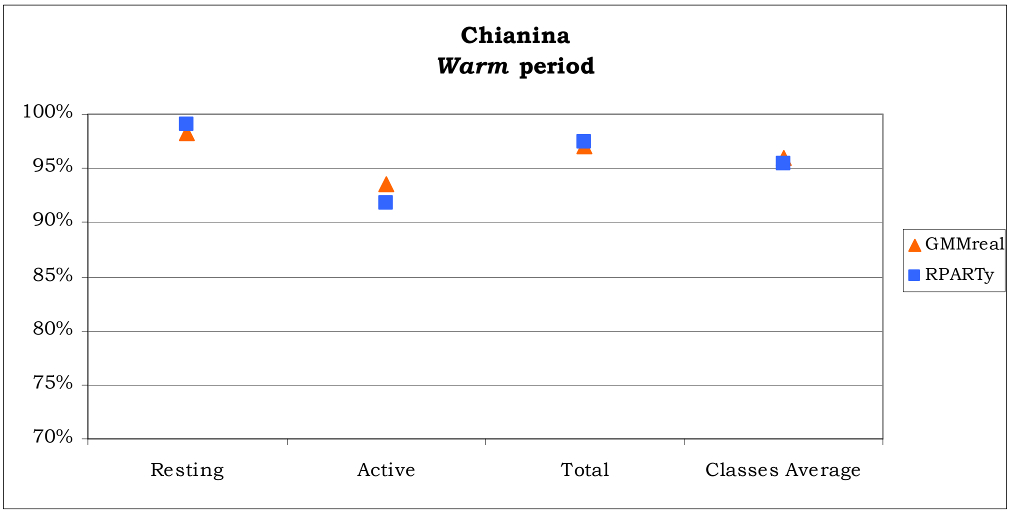

Figure 4.5 Chianina breed, warm period. Comparison of percentage of correct classification for behavioural classes after cross-validation for recursive partitioning models and for Discriminant Analysis applied to Gaussian mixture models.

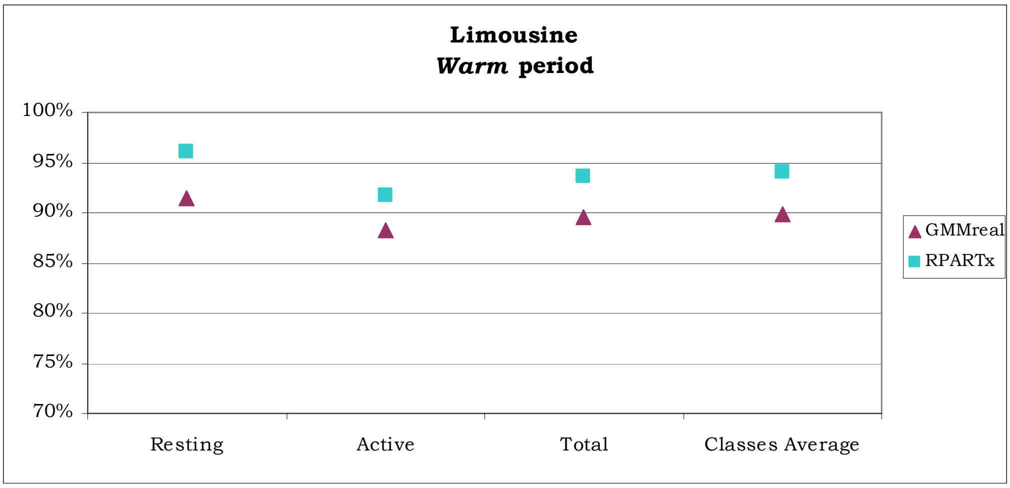

Figure 4.6 Limousine breed, warm period. Comparison of percentage of correct classification for behavioural classes after cross-validation for recursive partitioning models and for Discriminant Analysis applied to Gaussian mixture models.

Threshold values between active and inactive behaviour for Chianina (Y-axis) and Limousine (X-axis) calculated with recursive partitioning are reported in table 4.9.

Period	Breed	X axis	Y axis
Warm	Chianina		72
Warm	Limousine	53.5	

Table 4.9 Threshold values between active and inactive behaviour for the X-axis and Y-axis activity values, calculated with recursive partitioning models.

The main features of GMM_{real} model are reported in table 4.10. Graphical representations of classification dataset and mixture components for the model are shown in figures 4.7, 4.8, 4.9, 4.10.

Period	Breed	Classes	n	Model	G
Cool	Chianina	Resting	295	VEI	2
		Active	92	VEV	2
Cool	Limousine	Resting	375	EEV	5
		Active	254	VEV	5
Warm	Chianina	Resting	147	VEV	2
		Active	200	EEV	3
Warm	Limousine	Resting	410	VEV	4
		Active	124	EEV	2

Table 4.10 Summary of the main features of GMM_{real} model for classification of cattle behaviour. In model column the abbreviations summarize the parametrization of the covariance matrix, in the order: volume, shape and orientation; V = variable, E = equal, I = coordinate axes. G represents the number of mixture components.

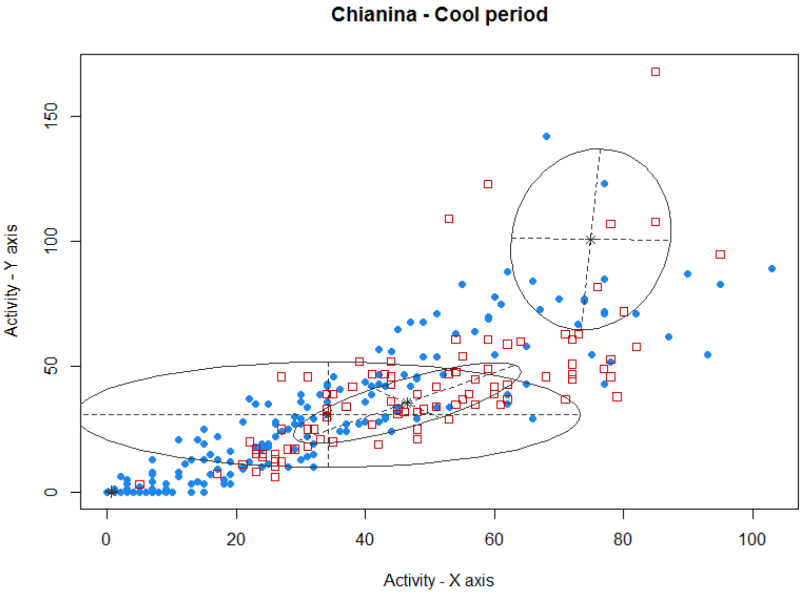

Figure 4.7 Chianina breed, cool period. Plot of classification database and graphical representation of mixtures components for the $\mathrm{GMM}_{\mathrm{real}}$ model. Blue data points are referred to *Resting* behaviour (G=2), red data points to *Active* behaviour (G=2).

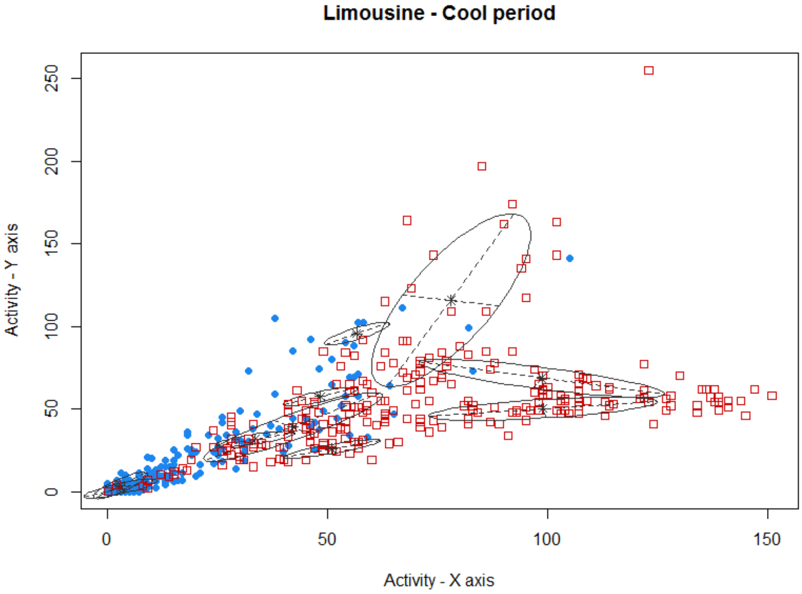

Figure 4.8 Limousine breed, cool period. Plot of classification database and graphical representation of mixtures components for the $\mathrm{GMM}_{\mathrm{real}}$ model. Blue data points are referred to *Resting* behaviour (G=5), red data points to *Active* behaviour (G=5).

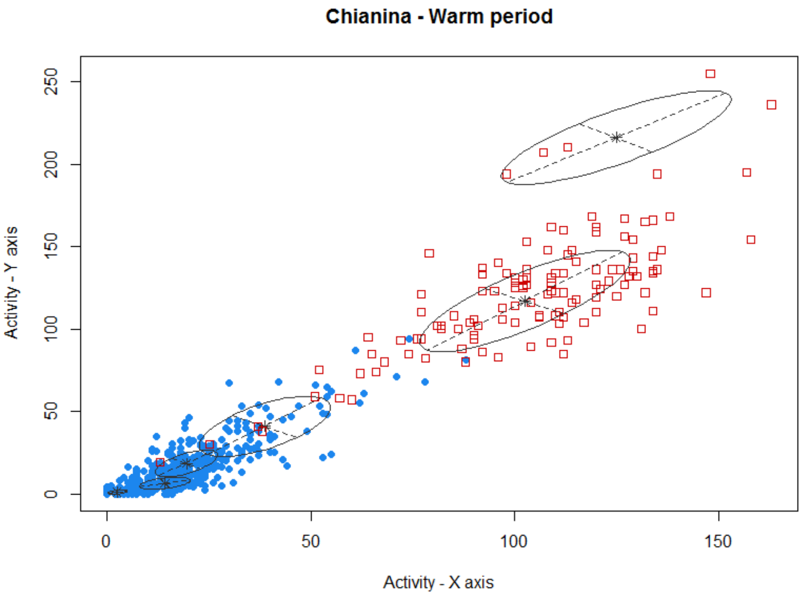

Figure 4.9 Chianina breed, warm period. Plot of classification database and graphical representation of mixtures components for the $\mathrm{GMM}_{\mathrm{real}}$ model. Blue data points are referred to *Resting* behaviour (G=2), red data points to *Active* behaviour (G=3).

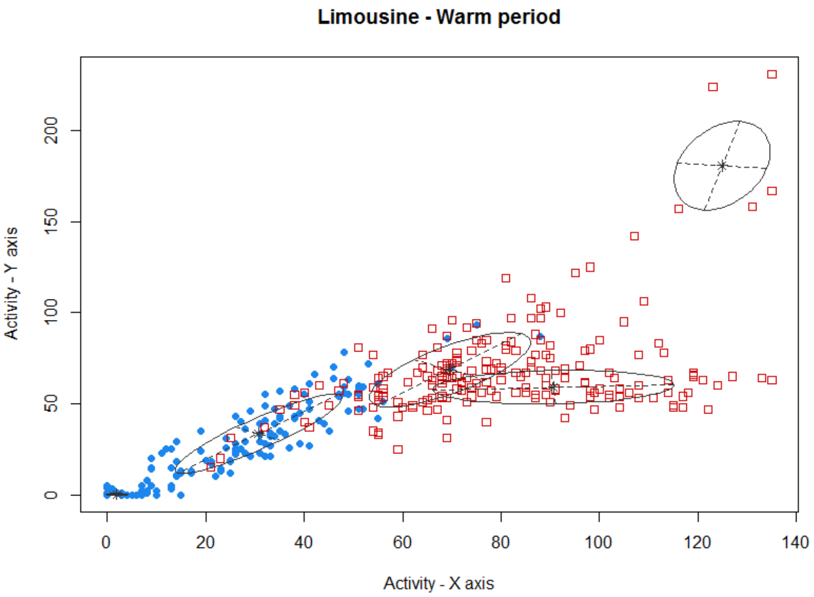

Figure 4.10 Limousine breed, warm period. Plot of classification database and graphical representation of mixtures components for the $\mathrm{GMM}_{\mathrm{real}}$ model. Blue data points are referred to *Resting* behaviour (G=4), red data points to *Active* behaviour (G=2).

For the purposes of this study, being interested in investigating the behaviour and time budget of cattle during pasture season in warm period, we chose RPART models, which provided similar results as GMM for Chianina (Fig. 4.5), but better performances for Limousine (Fig. 4.6). The $RPART_y$ model built for Chianina underestimated activity of 4.9%, while $RPART_x$ model built for Limousine underestimated activity of 5.2%.

PURE INTERVALS MODELS

The utilisation of only pure intervals determined a remarkable reduction of available data to be used for building the classification models. Indeed, pure intervals represent 44% of deer database, 53% of Limousine database (*warm* period) and 82% of Chianina database (*warm* period).

In addition, the performances of the pure intervals models (Table 4.11) resulted in every case lower than the ones of mixed intervals models (Table 4.2 and 4.8). However %CC for behaviour classes in pure intervals models were in general high, never being lower than 83.7%; for these models, the highest misclassification rates occur in active intervals.

N° behavioural classes	Species/Breed	Period	Model	Behavioural classes		Total	Classes Average
				Resting	Active		
2	Red deer		GMMreal	91.3%	86.1%	88.7%	88.7%
2	Chianina	Warm	RPARTy	92.6%	88.0%	92.1%	90.3%
2	Limousine	Warm	RPARTx	90.1%	83.7%	86.7%	86.9%

Table 4.11 Percentage of correct classification for behavioural classes after cross-validation for chosen models calibrated using only pure intervals and validated on mixed intervals. The total % represents the percentage of all intervals correctly classified; the classes average represents the average % classification among behaviours.

4.2 ACTIVITY PATTERNS

RED DEER

4.2.1. Actograms

The graphical representation of activity performed by actograms displayed a similar pattern in intensity of activity for all monitored hinds. Below are reported two actograms illustrative of the entire sampling period, related to F1 (Fig. 4.11) and F2 (Fig. 4.12). Actograms of all hinds are reported in Appendix III.

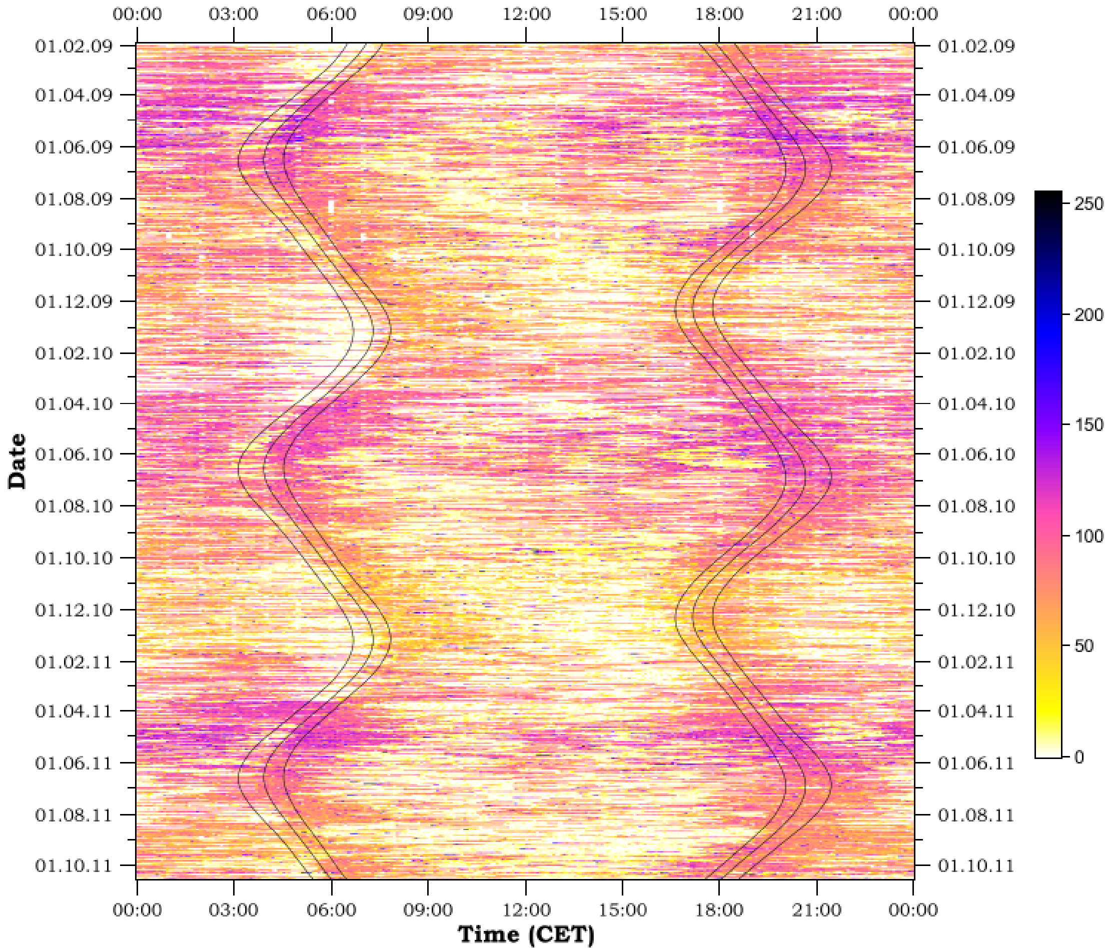

Fig. 4.11 Activity plot of F1 for the entire monitoring period. Vertical lines indicate from left: nautical dawn, civil dawn, sunrise, sunset, civil dusk and nautical dusk.

The activity plots reveal the succession, during the various seasons, of periods of major activity (pink-purple areas) which occurs regularly in correspondence of spring and early summer. The alternation of active and inactive bouts is noticeable also at daily level; high levels of activity occur in correspondence with twilight: civil dawn seem to correspond with the beginning of crepuscular activity, while the following restart anticipates sunset.

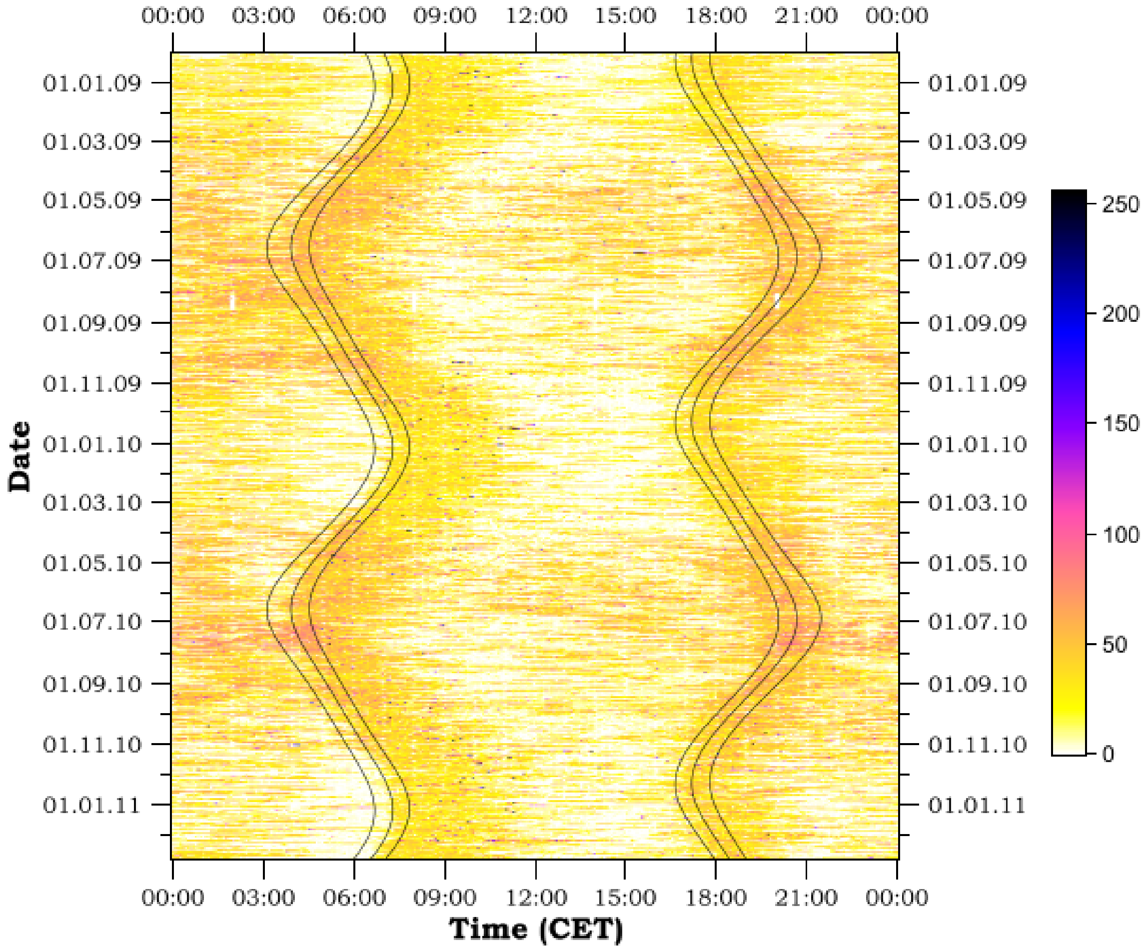

Fig. 4.12 Activity plot of F2 for the entire monitoring period. Vertical lines indicate from left: nautical dawn, civil dawn, sunrise, sunset, civil dusk and nautical dusk.

The comparison of the activity plots denotes inter-individual differences in overall intensity of activity; however, this difference appears marked only between F1 and the other hinds.

4.2.2. Annual activity patterns

Despite slight inter-individual differences, the seasonal pattern of activity was similar for all monitored hinds in the study period. Intensity of activity displayed a similar pattern in the two consecutive years of monitoring (Fig. 4.13). Significant differences in median activity between months resulted both in year 1 (χ^2 = 38.37, df =11, p<0.001) and in year 2 (χ^2 = 45.42, df =11, p<0.001).

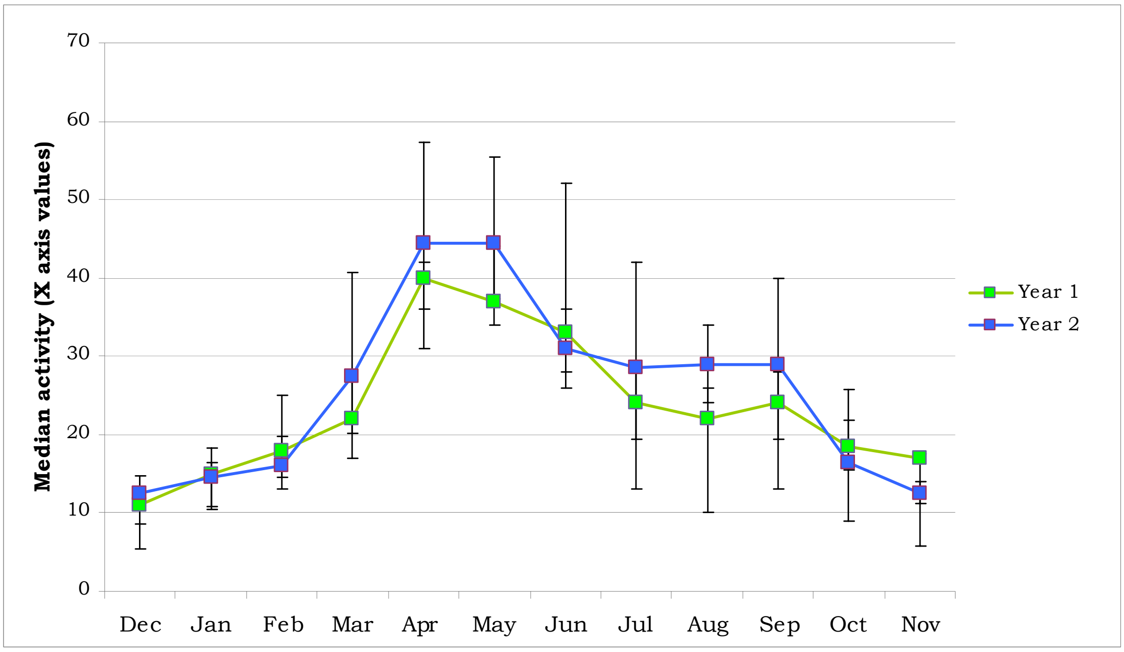

Fig. 4.13 Monthly activity (Median, Q1, Q3) of free-ranging hinds (F1-F9) for two consecutive years.

However, no significant differences were detected between months of consecutive years (U=13.5 - 33.5, N_{y1}= 7-9, N_{y2}= 6-8, p=N.S.); although longer time series of activity measurements should be required to confirm circannual rhythms, the general pattern appeared consistent among sampled animals and constant among the study period.

The highest activity levels occur between April and May; from June activity display a decreasing trend with reduced activity during winter, except for a slight increase in September, which in Apennine red deer corresponds to the climax of mating period. With regard to the annual activity pattern in the monitoring period shared by the entire sample, significant monthly variations were detected (χ^2 = 43.76, df =11, p<0.001); in particular, intensity of activity in April and May resulted significantly different (multiple comparisons, p< 0.05) from November, December and January.

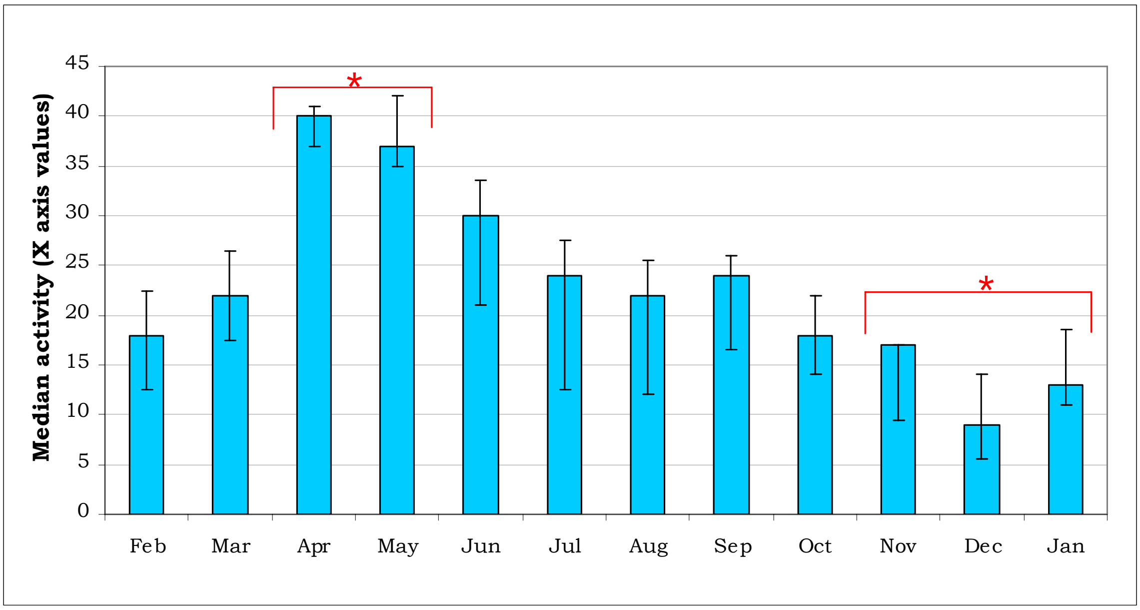

Fig. 4.14 Monthly activity (Median, Q1, Q3) of free-ranging hinds (N=8) for the common year of monitoring (Feb 09 – Jan 10). *significant differences p< 0.05

Monthly activity resulted not significantly correlated with average monthly temperatures (Appendix IV, Fig. IV.1); however, there is a correspondence between intensity of activity and productivity of natural grasslands in Apennine. The start of herbaceous vegetation growth approximately occurs between the first week of March and the first week of April, while maximum growth occurs between the first week of May and middle June, with a peak of dry matter production between the middle of May and the middle of June (Sarno et al. 1989).

4.2.3. Single cosinor

Significant acrophases ($p < 0.001$), i. e. 365-days periods, resulted for all examined hinds. The estimates of mesor were similar for all the sampled animals (Table 4.12), ranging from 23.19 in F9 to 31.57 in F3, with the exception of F1; this result is consistent with inter-individual similarities observed in intensity of activity: all monitored hinds presented similar ranges. Only F1 displayed higher activity values, but limited to the period between February and October; in the remaining months, the observed values matched the ones of the other hinds. This fact suggest that differences observed for F1 are probably not due to variability in sensitivity of accelerometers or to a different fit (i.e. more or less tighten to the animal's neck), but rather they can be more likely interpreted as inter-individual behavioural differences.

ID	Mesor	Mesor Lower CI	Mesor Higher CI	Amplitude	Amplitude Lower CI	Amplitude Higher CI
F1	51.12	39.12	63.12	14.60	14.12	15.08
F2	24.96	21.60	28.33	5.17	4.91	5.42
F3	31.57	26.16	36.98	5.78	5.45	6.10
F4	23.35	20.09	26.62	7.26	7.01	7.51
F5	23.94	19.79	28.09	5.86	5.58	6.14
F7	27.69	22.83	32.54	8.11	7.81	8.42
F8	27.90	22.85	32.95	4.97	4.66	5.28
F9	23.19	19.46	26.92	7.12	6.85	7.39

Table 4.12 Parameters of single cosinor: mesor and amplitude with 95% confidence intervals. Free-ranging hinds (N=8)

Similar are the results for amplitude values (Table 4.12): variability between hinds for this parameter is moderate except for F1, which displays an higher peak (Fig. 4.15).

Acrophases are similar for almost the entire sample, and centred on spring season; dates of maximum estimated activity (Table 4.13) range from April 17 (F8) to June 2nd (F4). Here the exception is represented from F2, which displays the latest peak, in correspondence of July 4.

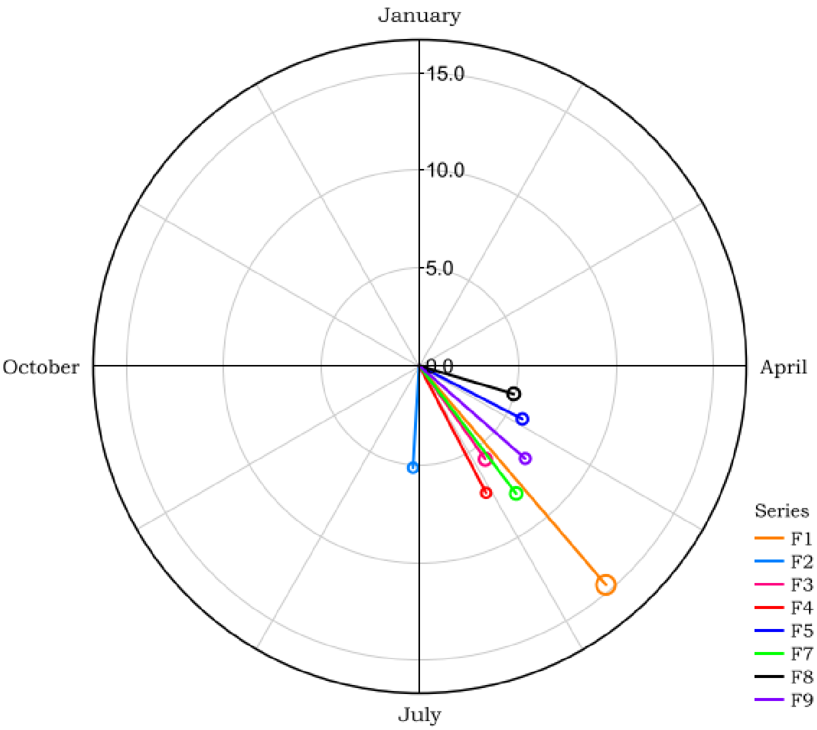

Fig. 4.15 Cosinor plot for free-ranging hinds (N=8). The length of the vector represents amplitude, its orientation represents acrophase, the circle around the vector the 95% confidence region of acrophase and amplitude.

Despite isolated differences in intensity of activity (F1) and timing of activity peak (F2), annual pattern of the sampled deer resulted rather homogeneous.

ID	Acrophase	Date	Acrophase Lower CI	Date	Acrophase Higher CI	Date
F1	-139.57°	May 20	-137.69°	May 18	-141.45°	May 22
F2	-183.88°	July 4	-181.08°	July 2	-186.69°	July 7
F3	-144.78°	May 26	-141.59°	May 22	-147.97°	May 29
F4	-152.37°	June 2	-150.40°	May 31	-154.34°	June 4
F5	-117.14°	April 28	-114.39°	April 25	-119.90°	April 30
F7	-142.85°	May 24	-140.70°	May 22	-145.00°	May 26
F8	-106.33°	April 17	-102.76°	April 13	-109.91°	April 20
F9	-131.17°	May 12	-129.02°	May 10	-133.31°	May 14

Table 4.13 Parameters of single cosinor: acrophase in degrees and corresponding dates, with 95% confidence intervals. Free-ranging hinds (N=8)

4.2.4. Diurnality index

Average values of diurnality index calculated monthly for each hind are reported in table 4.14. The values of the index range from a minimum of -0.56 for F8 in March, to a maximum of 0.45 for F4 in the same month. Individual differences in distribution of activity during the 24h are remarkable (F = 154.41, df = 8, p < 0.001).

ID	Month											
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
F1	-0.07	-0.16	-0.15	-0.11	-0.19	-0.19	-0.17	-0.05	-0.23	-0.03	0.07	-0.03
F2	0.01	-0.16	-0.06	-0.12	-0.18	-0.30	-0.41	-0.24	-0.31	-0.18	-0.03	-0.12
F3	-0.43	-0.51	-0.34	-0.20	-0.22	-0.29	-0.43	-0.31	-0.33	-0.19	-0.08	-0.13
F4	0.45	0.45	0.21	-0.08	-0.13	-0.07	0.03	0.12	0.04	0.12	0.17	0.00
F5	0.16	-0.06	0.01	-0.11	-0.23	-0.22	-0.27	-0.02	-0.24	-0.16	0.05	0.07
F6	-0.41	-0.44	-0.25	-0.28	-0.42	-0.53	-0.40	-0.01	-	-	-	-
F7	0.09	0.10	0.00	-0.27	-0.32	-0.26	-0.07	0.02	-0.13	0.05	0.00	0.17
F8	-0.49	-0.56	-0.16	-0.16	-0.29	-0.40	-0.40	-0.39	-0.19	0.02	-0.02	0.06
F9	-0.14	-0.50	-0.18	-0.18	-0.26	-0.37	-0.29	-0.24	-0.21	0.20	0.09	-0.04

Table 4.14 Monthly average values of diurnality index calculated with Hoogenboom (1984) equation for the whole sample of hinds (N=9). Study period: Feb 2009 – Jan 2010.

F4 proved to be the most active hind during daylight hours (p = 0.001) as can be observed in figure 4.16, with an overall average diurnality index of 0.11. Conversely, the hind displaying the major activity during night hours results F6 for which, however, monitoring period interrupts in September; indeed, late autumn and winter months are the periods in which activity during daylight hours is more frequent, as displayed by figure 4.17 and explained in detail in the following paragraph.

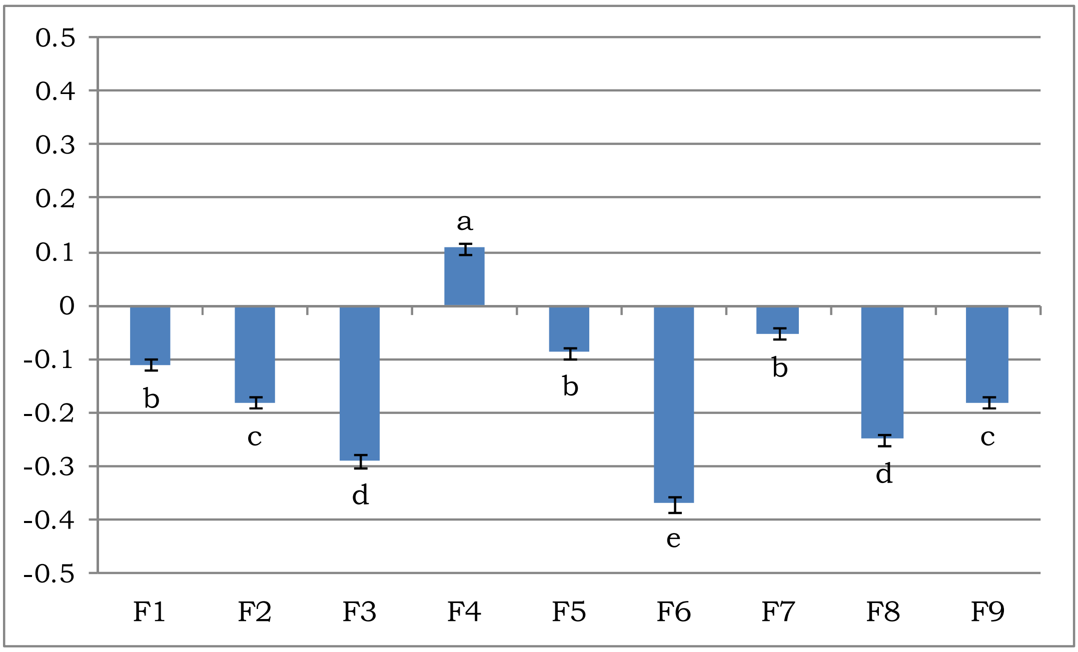

Fig. 4.16 Average values of diurnality index calculated with Hoogenboom (1984) equation for each hind (N=9) in the analysis period (Feb 2009 – Jan 2010). Letters indicate differences at p = 0.001 (Tukey post hoc test).

The distribution of intensity of activity, as summarized by the index, varies also among months (F = 62.48, df = 11, p < 0.001); moreover also hinds, taken individually, vary their behaviour along the year (F = 11.96, df = 84, p < 0.001) as observable by figure 4.17. From late autumn (November) to middle spring (April) some hinds, particularly F4, F5 and F7, tend to be more intensively active during daily hours.

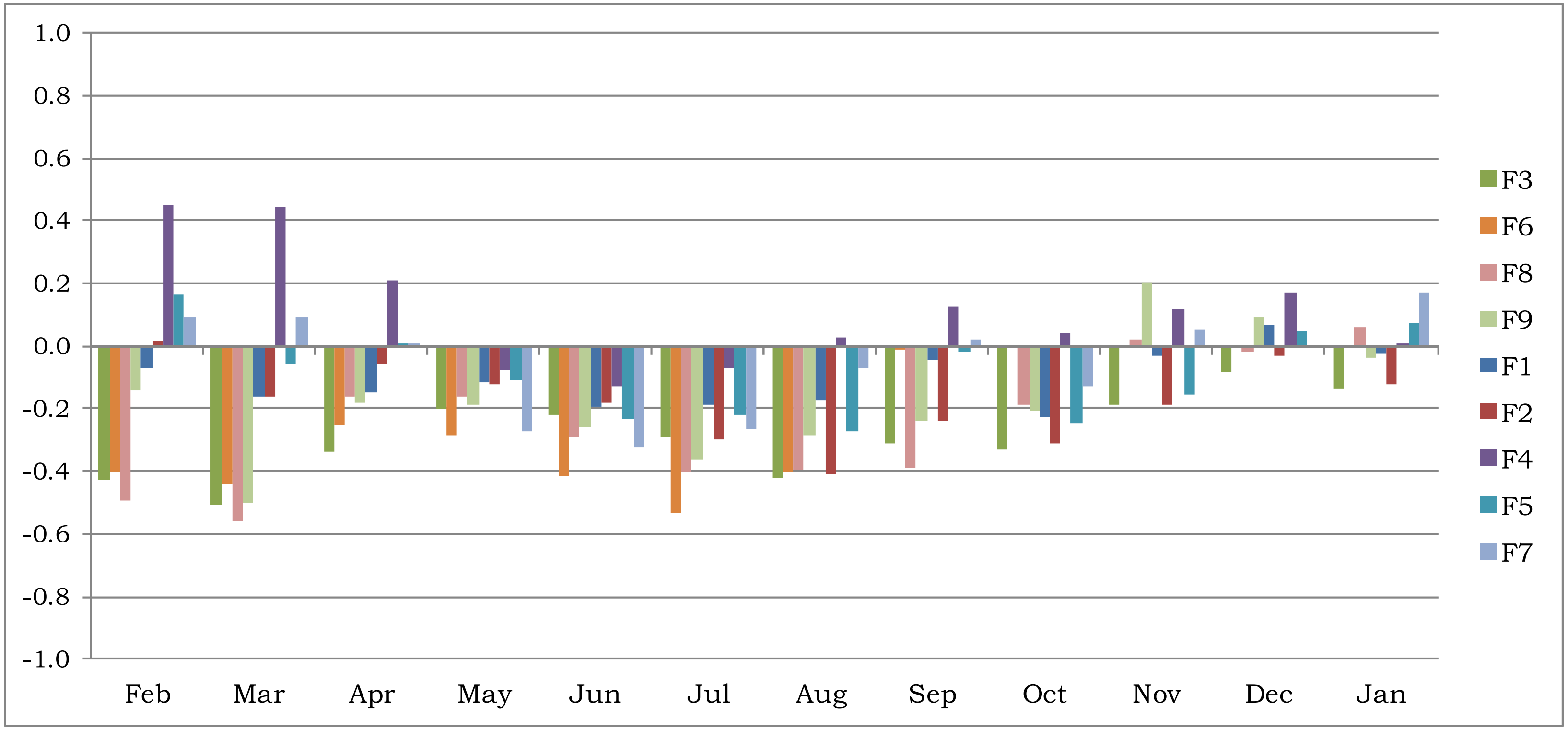

Fig. 4.17 Monthly variation of average values of diurnality index calculated with Hoogenboom (1984) equation for the whole sample of hinds (N=9). Study period: Feb 2009 – Jan 2010.

The other hinds conversely, despite slight variations, tend to be more intensively active during night hours; only F9 (in November and December) and F8 (in November and January) appear to increase activity during daily hours.

4.2.5. Daily activity patterns

Median intensity of activity for each time of the day, in separated months, is shown below; for layout clarity, monthly graphics are presented grouped according to meteorological seasons.

Daily patterns display two distinct peaks throughout the entire year; a third peak, however, appears between 1.00 and 3.00, soon before the increase of activity in correspondence of dawn. The two main peaks occur regularly around sunrise and sunset, consequently their timing varies throughout the seasons. For all considered months, the second of the "twilight" peaks is higher than the first; from late spring (May - June) to the whole summer period, the main peaks are more pronounced and activity during daylight is lower.

General activity patterns vary across the seasons; during spring (Fig. 4.18), autumn (Fig. 4.20), and winter (Fig. 4.21) a profound nadir in activity occurs between 4.00 and 6.00, highlighting the nocturnal peak of activity. In addition, during these seasons activity extends after dawn until late morning (10.00). Conversely, during summer period (Fig. 4.19) activity seems anticipated towards night hours, and the night nadir almost disappears.

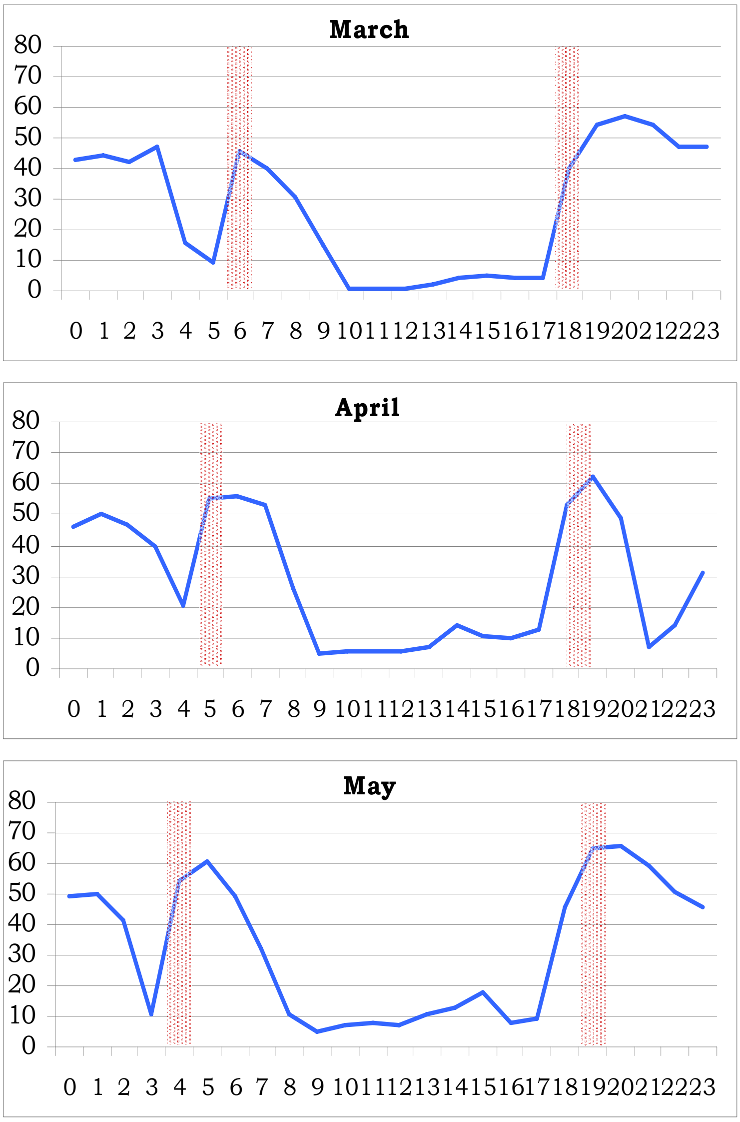

Fig. 4.18 Spring months: median intensity of activity during time of the day for the entire sample of hinds (N=9). Vertical red bars indicate sunrise and sunset.

Median activity during daylight hours is low in every season (median X axis value < 10) except for spring (Fig. 4.18); in this period, particularly in May and June, sampled animals display an increase in activity a few hours after noon. This slight increase appears, although less evidently, also in April and July (Fig. 4.18 and 4.19).

In spring, dusk peak of activity occurs between 19.00 and 20.00; in May median activity at 19.00 and 20.00 resulted to differ significantly from activity at 9.00, 10.00, 12.00 and 16.00 ($p < 0.05$); dawn peak occurs between 5.00 and 6.00.

Detailed tables of significance for this and the following comparisons between hours of the day are given in Appendix V.

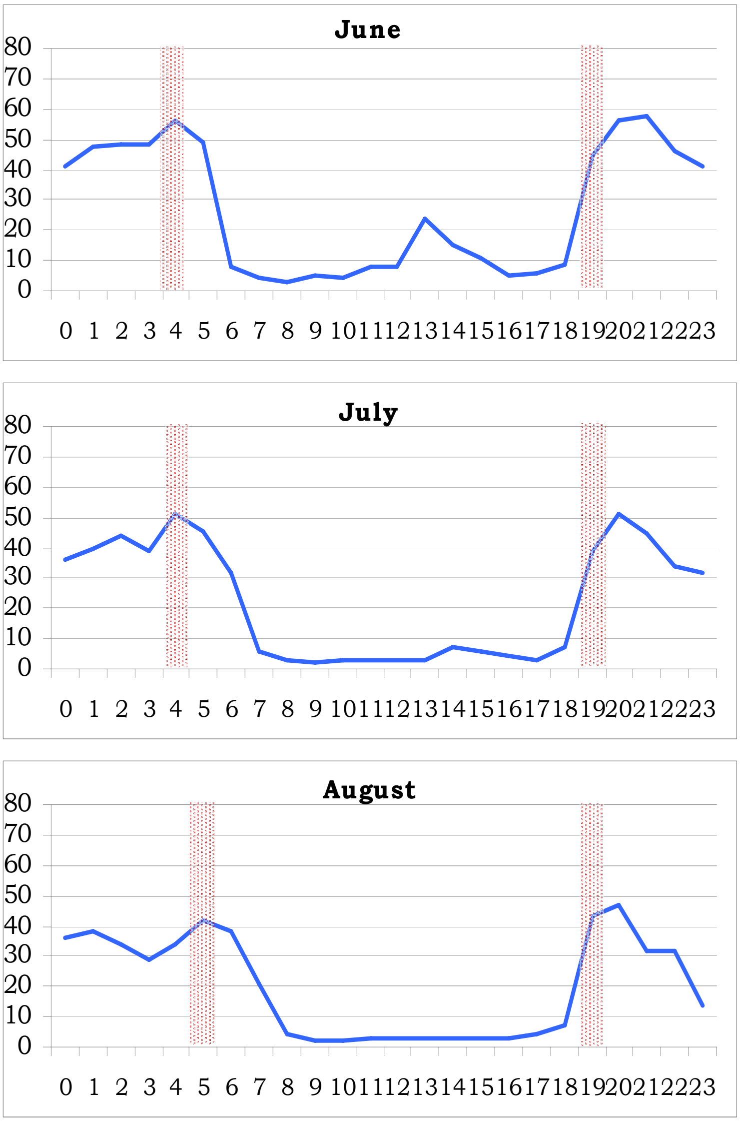

Fig. 4.19 Summer months: median intensity of activity during time of the day for the entire sample of hinds (N=9). Vertical red bars indicate sunrise and sunset.

During summer, the peak of dawn arises at 4.00 in June and July, where activity differs from the nadir occurring at 8.00 and 9.00 ($p < 0.05$); these nadirs also differ from the peak occurring at dusk between 20.00 and 21.00 ($p < 0.05$).

In August, the dusk peak arises at 20.00, resulting different from morning nadir occurring at 9.00 and 10.00 ($p < 0.02$).

During this month, when average temperatures reach the year maximum (see Appendix IV), activity during daylight is the lowest.

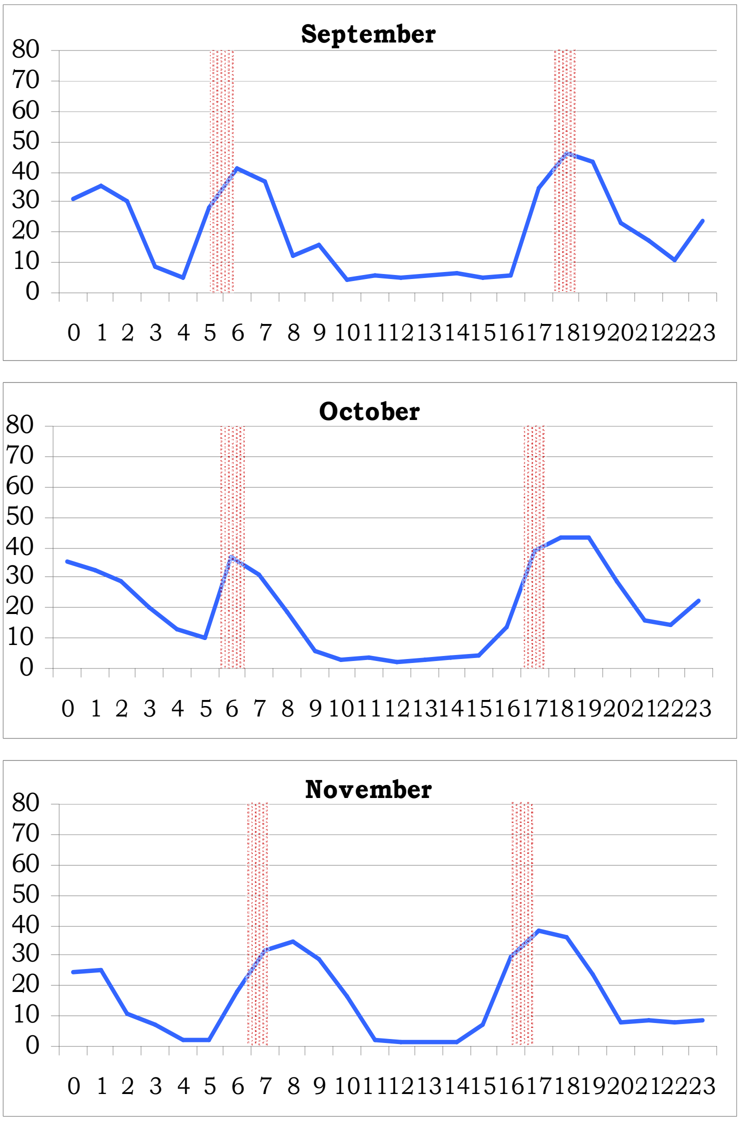

Fig. 4.20 Autumn months: median intensity of activity during time of the day for the entire sample of hinds (September: N=9; October – November: N=8). Vertical red bars indicate sunrise and sunset.

In autumn intensity of activity in daily peaks gradually decreases; in September and October the highest intensity arises at 18.00 and 19.00; it differs from morning nadir ($p < 0.05$) occurring between 11.00 and 14.00, which results postponed respect to previous months. In November the nocturnal nadir, occurring at 4.00, is emphasized and results different from the peak that arises at 17.00 ($p = 0.03$).

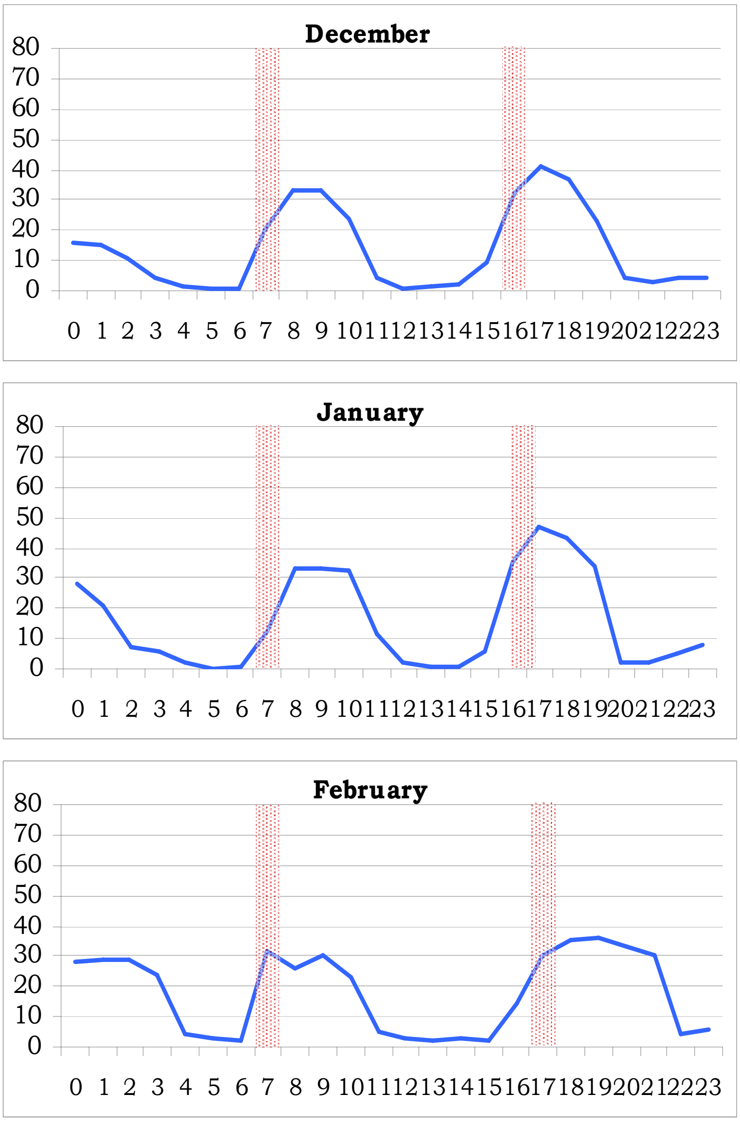

Fig. 4.21 Winter months: median intensity of activity during time of the day for the entire sample of hinds (December – January: N=8; February: N=9). Vertical red bars indicate sunrise and sunset.

During winter, particularly in December and January, the lowest intensity of activity arises before dawn, between 4.00 and 6.00; here, median activity results different from the dusk peak occurring at 17.00 and 18.00 ($p < 0.04$). Diurnal nadir occurs between 12.00 and 14.00.

4.2.6. Actograms

The graphical representation of activity in cattle is reported for each animal for the entire period of pasture available. Exemplary graphics are reported in figures 4.22 and 4.23; graphics of all monitored animals are reported in Appendix III.

All individuals display similar general patterns, with low intensity or absence of activity before dawn and after dusk; a resting phase appears also during early morning in both breeds.

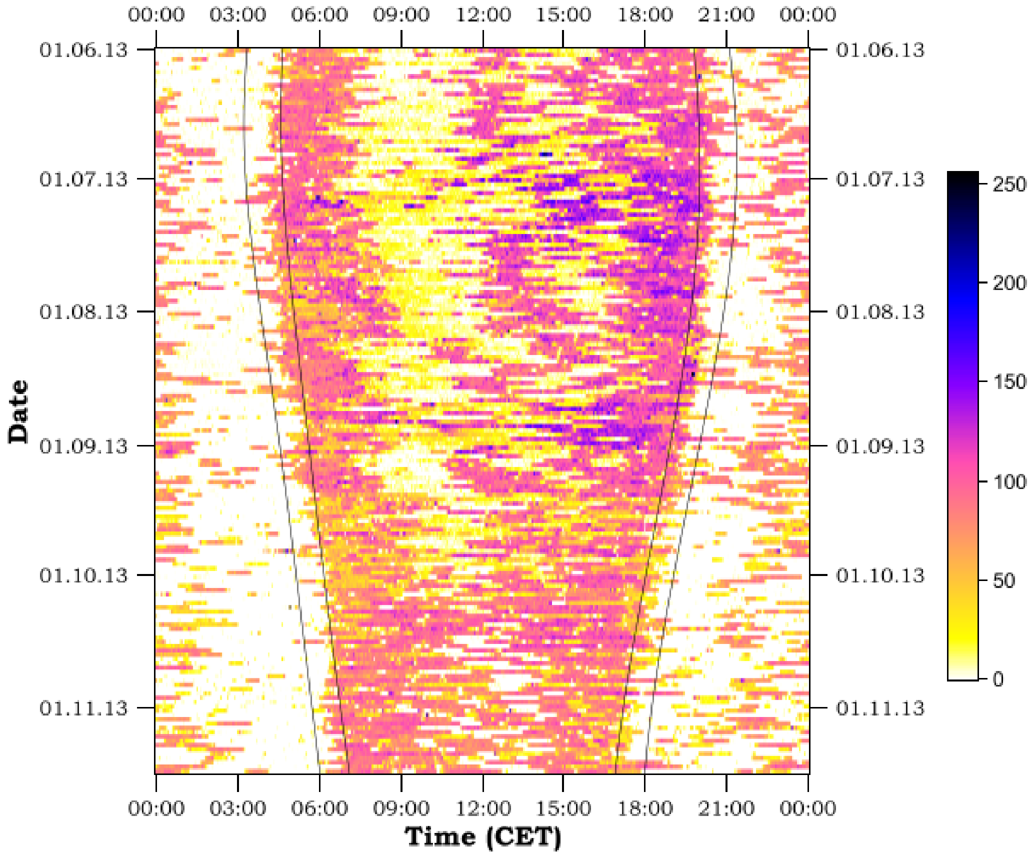

Fig. 4.22 Activity plot of C5 (Chianina) for the entire monitoring period. Vertical lines indicate from left: nautical dawn, sunrise, sunset, nautical dusk.

Chianina cows display a general higher intensity of activity; at daily level, the two breeds show a greater level of activity in the hours after noon preceding sunset.

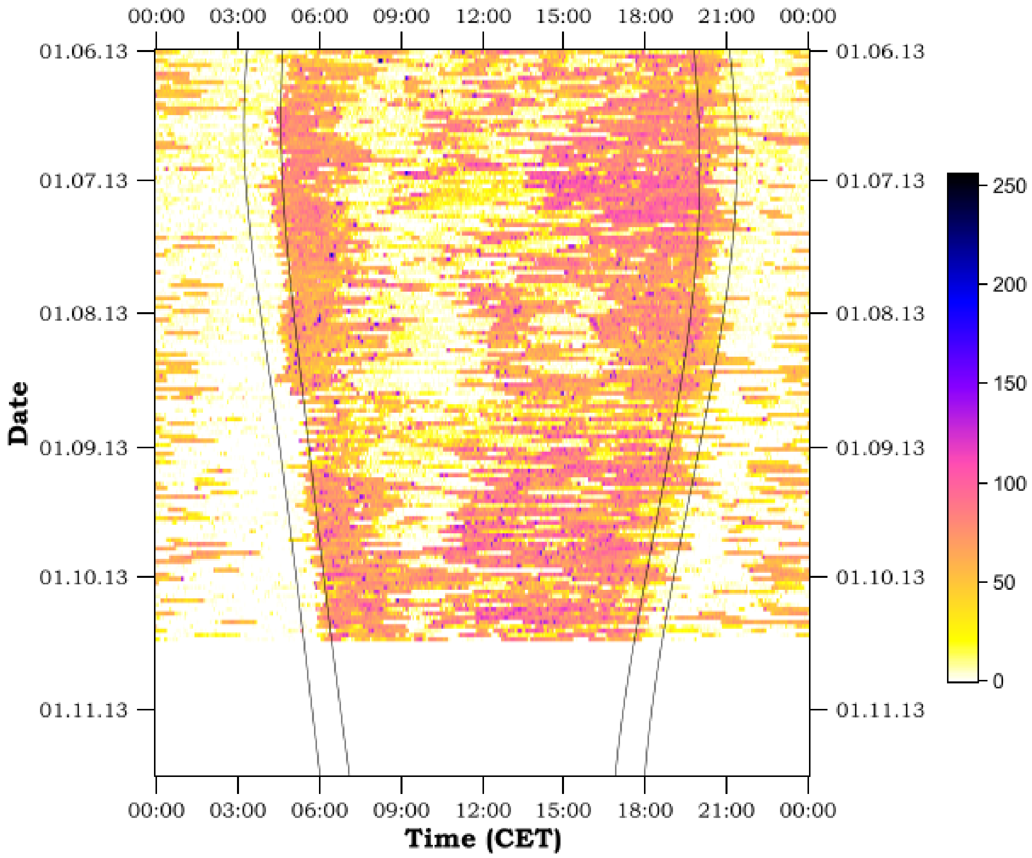

Fig. 4.23 Activity plot of C3 for the entire monitoring period. Vertical lines indicate from left: nautical dawn, sunrise, sunset, and nautical dusk.

4.2.7. Activity patterns during pasture season

The individual pattern of activity levels for the monitored cattle resulted similar for all animals, with two higher levels of activity occurring for all individuals during the periods 29 Jun – 13 Jul and 28 Aug – 11 Sep (Fig. 4.24).

However, no significant differences in activity were detected during these periods, both considering the whole sample (χ^2 = 11.09, df = 8, p = N.S.) and the two breeds, Chianina (χ^2 = 11.21, df = 8, p = N.S.) and Limousine (χ^2 = 8.81, df = 8, p = N.S.) , separated.

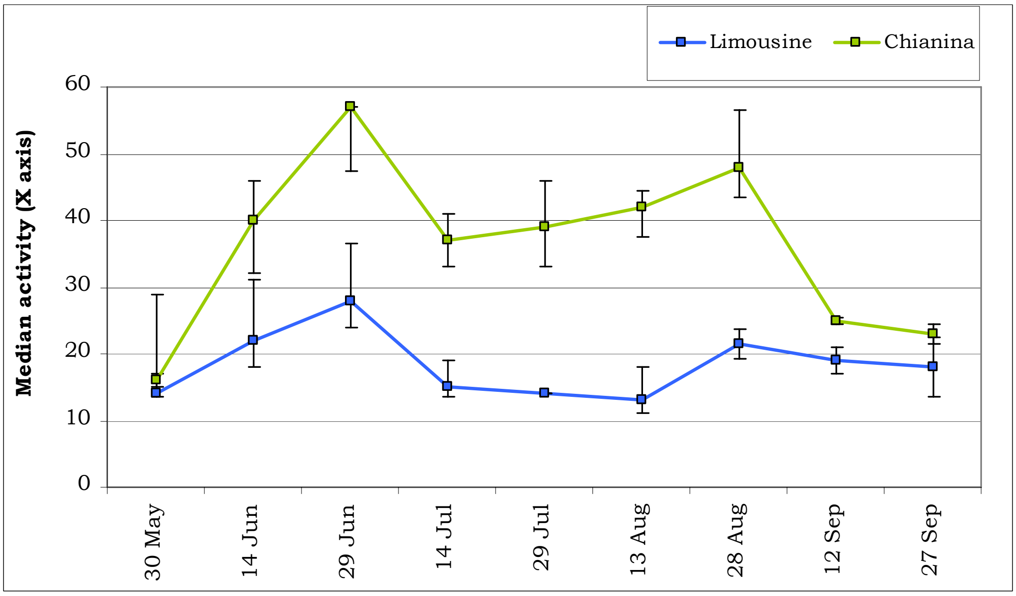

Fig. 4.24 Intensity of activity (Median, Q1, Q3) for 15-day periods during the warm period of pasture for cattle on confined pasture. Limousine (N=3) and Chianina (N=3) are displayed separately.

Intensity for Chianina is always higher than for Limousine from middle June to middle September.

Mobility represents the distance (meters) covered by the animal in 1 hour; mobility of monitored animals during the grazing period is reported in figure 4.25.

It varies on the whole between a minimum of 31.3 metres in the period 14 Jun – 28 Jun, to a maximum of 61.7 metres during the period 28 Aug – 11 Sep. Minimum and maximum individual values (median) were registered for C4 (Chianina), ranging from 23.2 metres to 82.1 metres.

Also for mobility, the general pattern during grazing period was similar for all sampled animals. Mobility differed significantly through pasture season ($\chi^2 = 19.33$, df = 8, $p = 0.013$); in particular, in the period 28 Aug – 11 Sep mobility resulted higher than the period 30 May – 13 Jun ($p < 0.05$) and the period 14 Jun – 28 Jul ($p < 0.01$).

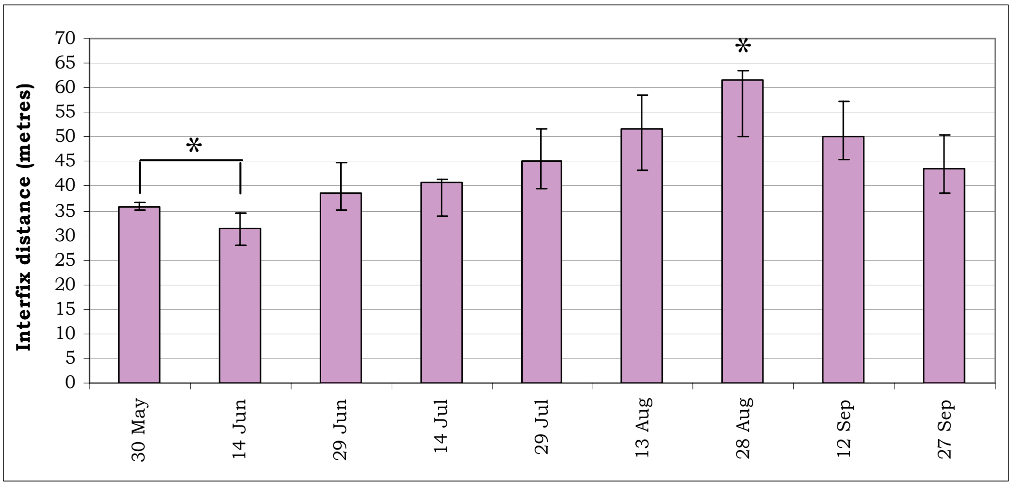

Fig. 4.25 Distance (Median, Q1, Q3) covered between two consecutive fix (meters/hour) for 15-day periods during warm period of pasture. 30 May – 27 Aug, N=6; 28 Aug – 30 Sep, N=5.

Distances covered by cattle on pasture are known to vary with abundance of grass (Houpt 1982), thus the increase of mobility is justified by the progressive reduction in forage availability at pasture.

Median intensity of activity for each time of the day, in separated months of warm period of pasture, is presented below. Daily patterns of cattle display two main peaks throughout the pasture season; a third one occurs in June (figure 4.26), July (figure 4.27) and August (figure 4.28) around midday.

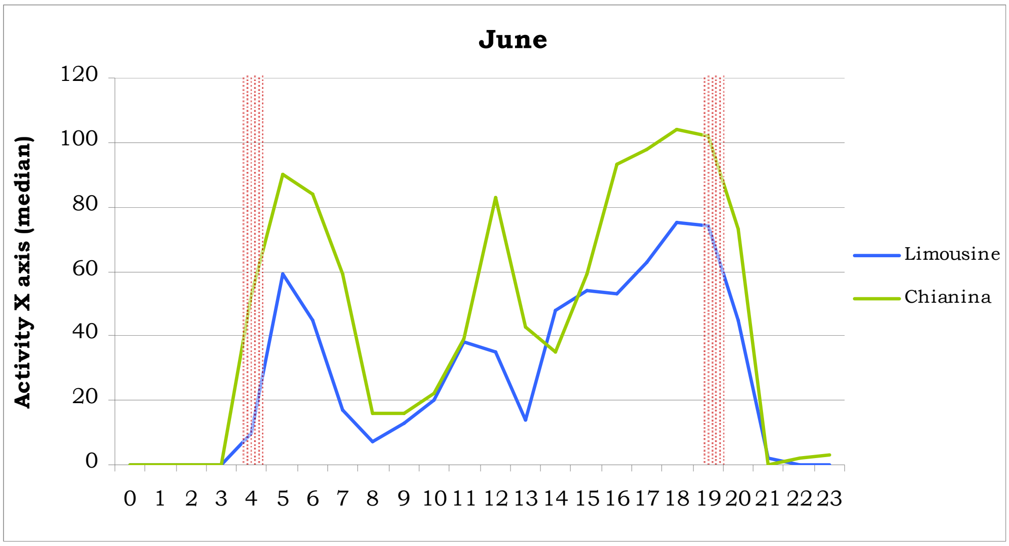

Fig. 4.26 June. Median intensity of activity during time of the day for two breeds (N=6). Vertical red bars indicate sunrise and sunset.

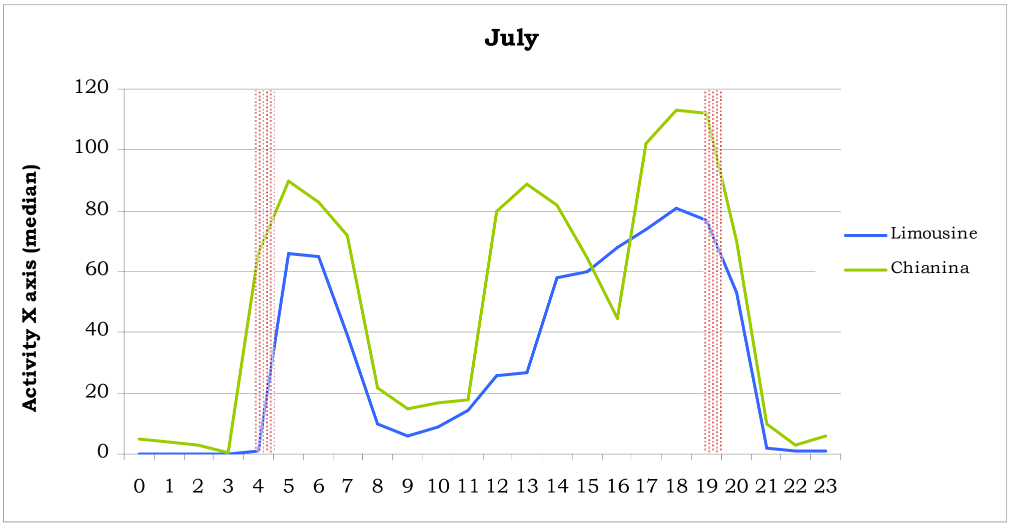

Fig. 4.27 July. Median intensity of activity during time of the day for two breeds (N=6). Vertical red bars indicate sunrise and sunset.

The first of the two main peaks starts slightly after sunrise, while in the latter activity cease before sunset. Also in this species, for all considered months, the second peak corresponding to dusk is always higher than the first.

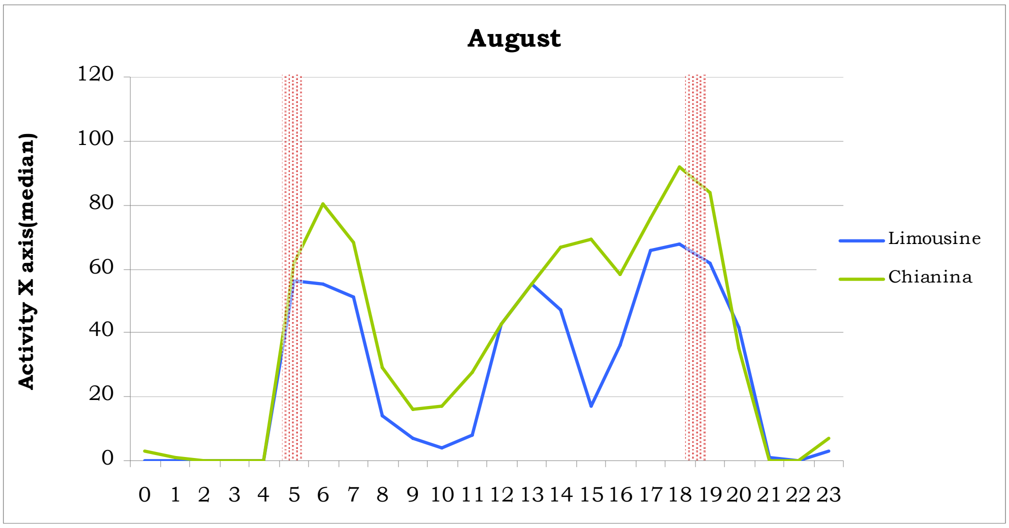

Fig. 4.28 August. Median intensity of activity during time of the day for two breeds (N=6). Vertical red bars indicate sunrise and sunset.

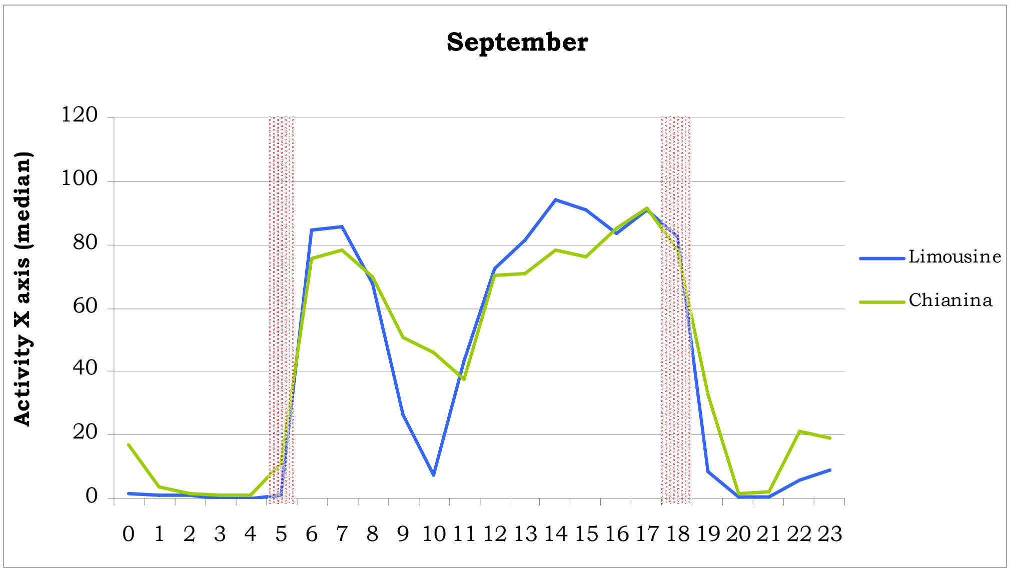

Fig. 4.29 September. Median intensity of activity during time of the day for two breeds (N=5). Vertical red bars indicate sunrise and sunset.

When considering the two breeds together, significant peaks are found in June, July and August at 18.00 and 19.00, when intensity of activity results different ($p < 0.05$) from nadirs occurring at 2.00, 3.00 and 4.00.

4.3 CLASSIFICATION OF ACTIVITY DATA

RED DEER

The Gaussian mixture model built on captive deer data (paragraph 4.1) was used to classify activity data recorded on free-ranging hinds. The model was used to predict behaviour for the period relative to months in which observation were performed on captive deer. Indeed, the period of the year proved to influence markedly animal behaviour, and thus activity levels (as observed on cattle).

The amount of daily time dedicated to active behaviours (n° hours of activity per day) varies among individuals (F = 9.18, df = 8, $p < 0.001$), as in figure 4.30, but not between examined months (F = 2.42, df =4, p = 0.06), as displayed in figure 4.31.

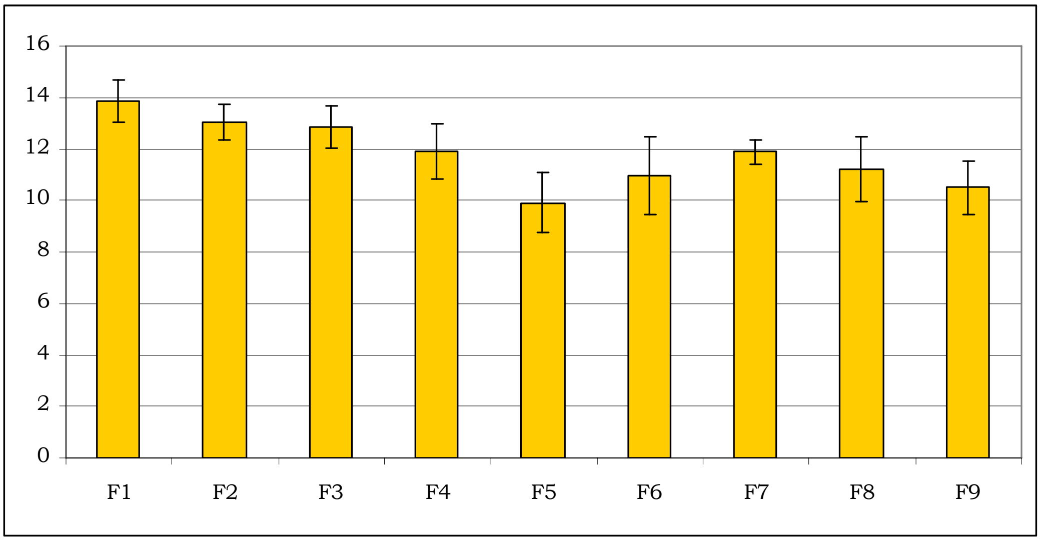

Figure 4.30 Amount (n° hours) of daily time (mean ± standard deviation) dedicated to activity in sampled animals. Significant differences resulted between individuals ($p < 0.001$).

ID	Hours
F1	13.8 ^{a}
F2	13.0 ^{ab}
F3	12.8 ^{ab}
F4	11.9 ^{abc}
F5	9.9 ^{c}
F6	11.0 ^{abc}
F7	11.9 ^{abc}
F8	11.2 ^{abc}
F9	10.5 ^{bc}

Table 4.15 Amount of daily hours (monthly average) dedicated to active behaviour, from June 2009 to October 2009. Letters indicate differences ($p < 0.001$) according to Tukey test.

The monthly pattern of activity budget (Fig. 4.31) is similar, for the considered period, to the one of intensity of activity (Fig. 4.14); however, the slight peak in intensity of activity occurring in September does not have a correspondence in duration of activity. Thus, the observed increase probably reflects the higher state of agitation connected with reproductive activity.

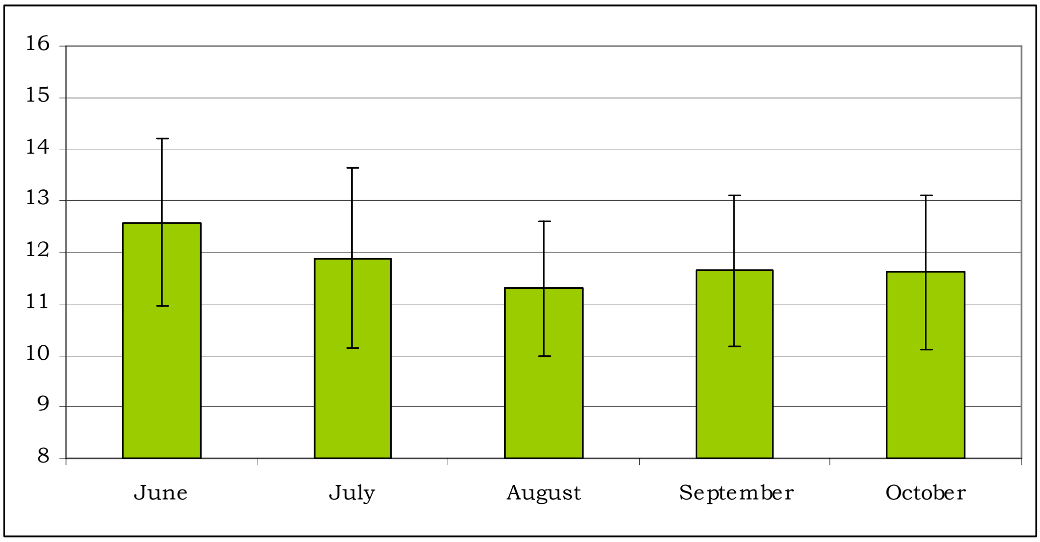

Figure 4.31 Amount (n° hours) of daily time (mean ± standard deviation) dedicated to activity during each month. No significant differences were detected among considered months ($p = 0.06$).

Considering activity time individually, F1 appears to be the hind spending more time active (55-63% of daily time), as displayed in Table 4.16, while F5 displays the lowest amount of time dedicated to active behaviours (36.6%-45.1%). On the average hinds spent 50.1% of the time feeding and moving.

	June	**July**	**August**	**September**	**October**
F1	63.0%	61.7%	56.2%	57.8%	55.0%
F2	59.3%	54.5%	51.4%	55.9%	54.7%
F3	58.3%	57.9%	52.6%	50.4%	53.4%
F4	54.2%	52.9%	51.5%	51.9%	42.8%
F5	39.4%	36.6%	40.2%	49.0%	45.1%
F6	54.6%	48.6%	43.5%	39.2%	-
F7	51.4%	50.4%	50.3%	53.4%	47.9%
F8	49.4%	45.9%	42.2%	44.3%	55.8%
F9	51.2%	46.1%	43.9%	42.3%	39.4%

Table 4.16 Percentage of daily time (monthly average) dedicated to active behaviour, from June 2009 to October 2009.

The predicted daily activity budget displays that average activity duration in the 24-h ranges between a minimum of 8.6 (F5) and a maximum of 14.8 (F1) hours (Table 4.17). Considering pooled animals, the lowest activity time is recorded in August (11.3 hours), while highest duration of activity occurs in June (12.6 hours), as in figure 4.31. F5 and F9 resulted the least active hinds (Table 4.15), compared to F1 ($p < 0.001$).

	June	**July**	**August**	**September**	**October**
F1	14.8	14.5	13.2	13.6	13.0
F2	14.0	12.9	12.1	13.2	12.9
F3	13.7	13.6	12.4	11.9	12.6
F4	12.8	12.5	12.1	12.2	10.1
F5	9.3	8.6	9.5	11.6	10.6
F6	12.8	11.5	10.3	9.3	-
F7	12.1	11.6	11.8	12.6	11.3
F8	11.7	10.8	9.9	10.5	13.2
F9	12.1	10.9	10.3	10.0	9.3

Table 4.17 Amount of daily hours (monthly average) dedicated to active behaviour, from June 2009 to October 2009.

BEEF CATTLE

Threshold values estimated with recursive partitioning models (Table 4.9) for each breed were employed to predict cattle behaviour during *warm* pasture period, and limited to behavioural observation period. The amount of time during the 24 h dedicated to active behaviour (namely feeding and walking) varies significantly between individuals (F = 34.43, df = 5, p < 0.001) and 15-days periods (F = 36.67, df = 5, p < 0.001); moreover, differences in activity budget also exist among the two breeds (F = 7.60, df = 1, p = 0.006); also the interaction between breed and 15-days period resulted significant (F = 8.92, df = 5, p < 0.001).

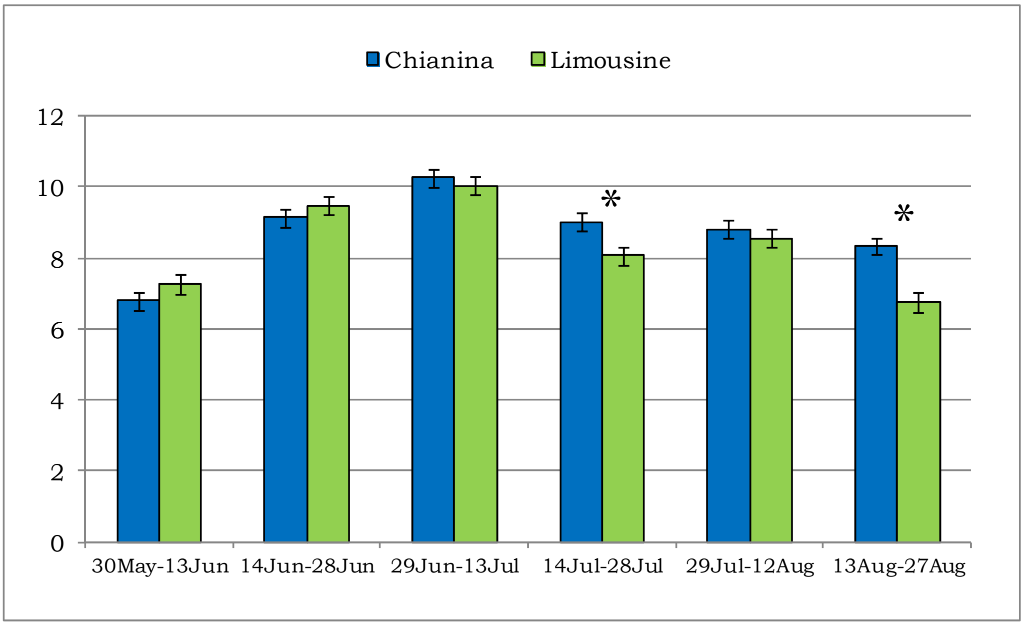

Figure 4.32 Amount (n° hours) of daily time (mean ± standard deviation) dedicated to activity during each 15-days period.

The maximum duration of activity occurs during the period 29 Jun - 13 Jul (10.0 – 10.3 hours) both for Limousine and Chianina breed (figure 4.32). Peak of duration of activity is consistent with the maximum level of activity (figure 4.24).

In absolute, the minimum duration of daily activity was 4.2 hours for Chianina and 3 hours for Limousine; the maximum duration in the two breeds was 12.7 and 13.5, respectively.

The lowest time spent grazing and moving occurred in Limousine breed between 13 and 27 August (6.8 hours), but also during the early grazing period (30 May – 13 June) in both breeds (6.8 – 7.3 hours), as displayed in table 4.18.

Average daily duration of activity within each 15-days period differs between breeds (Fig. 4.32) only in the second mid of July (14-28 Jul; $t = 2.37$, $df = 87$, $p = 0.02$) and mid August (13-27 Aug; $t = 3.04$, $df = 60$, $p = 0.003$).

	Chianina		**Limousine**	
	N° hours	**Activity budget**	**N° hours**	**Activity budget**
30May-13Jun	6.80	28.3%	7.25	30.2%
14Jun-28Jun	9.13	38.0%	9.47	39.5%
29Jun-13Jul	10.27	42.8%	10.04	41.9%
14Jul-28Jul	9.02	37.6%	8.07	33.6%
29Jul-12Aug	8.80	36.7%	8.56	35.7%
13Aug-27Aug	8.34	34.8%	6.75	28.1%

Table 4.18 Amount of daily hours (15-days average) dedicated to active behaviour, from May to August.

On average, Chianina cows spent more time ($p < 0.05$) being active (8.8 hours) than Limousine cows (8.5 hours). The major part of active behaviours occurs during daylight hours: approximately 94% for Chianina (8.1 hours) and 91% for Limousine (7.9 hours).

CHAPTER 5
DISCUSSION

The sampling protocol and the procedures adopted to calibrate the activity sensors located on GPS/GSM collars (Vectronic Aerospace) allowed to correctly classify on average 91.2% of observed behaviours in captive red deer, and from 72.9% to 94.0% in beef cattle on confined pasture. The minimum percentage, considering both cattle and red deer, of correct classification for the best models occurred in Chianina breed during cool period (70.1% - active behaviour -); the best performances occurred in the same breed during warm period (99.0% - resting behaviour -). The achieved performances of classification are similar to the percentages of correct classification reached in comparable studies, which are generally included between 60% and 100%. Active behaviours resulted underestimated of about 5% both for red deer and for cattle.

Activity data recorded by the utilised GPS collars allow to discriminate in these two species between two states, resting and active, when is not possible to collect a large amount of data on movements with high intensity of activity, such as running or galloping.

A fact that has previously been remarked, which may affect the informative level of recorded data and thus the possibility to identify further behaviours, is the high correlation and similarity of activity values recorded between the X-axis (in forward-backward movements) and the Y-axis (in sideways movements); average Pearson correlation coefficient calculated on data recorded on these two activity channels resulted 0.95 for red deer. The extent of correlation is comparable to the one detected for roe deer ($r_{pearson}$ = 0.95) monitored with similar equipment (Krop-Benesch et al. 2013, Stache et al. 2013);

in cattle, average correlation between the activity values is slightly lower ($r_{pearson} = 0.90$), however still high. The possibility to collect less correlated or dissimilar activity values would eventually allow a better identification of certain behaviours when using both X and Y values to build the classification models, and possibly discern more behavioural states.

The number of animals employed to calibrate the sensors is not sizeable (4 deer, 3 Chianina cows, 3 Limousine cows), however the majority of such studies utilized the same, or even lower, sample sizes (Pépin et al. 2006a; Moreau et al. 2009; Löttker et al. 2009; Heurich et al. 2012); this reflects, for wild species as red deer, the difficulty of raising wildlife in captivity especially in quasi-natural conditions.

The number of recognizable behavioural classes resulting from our experimentation is consistent with the results achieved in analogous studies: most of the attempts to identify animal behaviour from activity sensors produced models capable of identifying 2 (Adrados et al. 2003; Gervasi et al. 2006; Pépin et al. 2006a; Adrados et al. 2008; Yamazaki et al. 2008; Gottardi et al. 2011), 3 (Naylor et al. 2004; Ungar et al. 2005; Löttker et al. 2009; Grünewälder et al 2012; Heurich et al. 2012) or less frequently 4 behavioural categories (Moreau et al. 2009; McClune et al. 2014); in the latter case however, the utilised activity sensors record and provide the raw acceleration data, without scaling and averaging the measured values.

From this consideration, we can suggest that averaged and scaled activity data do not allow to distinguish successfully detailed movements, neither using different sampling protocols or classification procedures. In our case both utilized procedures (RPART and DAGMM) permitted to discern activities as rumination and standing; however, correct classification rates resulted low in some circumstances, while high in other.

This occurrence depends on the fact that actual behaviour may produce different activity values in diverse situations or individuals. As previously highlighted by Gervasi et al. (2006), the position of the sensor on the collar is the main reason for the fine-scale inefficiency: the motion sensor is placed around the animal's neck thus the sensor is mainly affected by head movements; occasional head movements due for instance to insects harassment, which occurs frequently during summer (Colman et al. 2001, Hagemoen et al. 2001), as well as to grooming behaviour, are likely to increment recorded activity, as also pointed out by Pépin et al. (2006a), thus masking the performed activity. Moreover, the recorded values may be affected also by factors not directly dependent from movement, especially the tightness of the collar (Coulombe et al. 2006, Gervasi et al. 2006).

The detection of ruminating activity, for instance, would permit to calculate rumination time and number and distribution of bouts. However, in our experimentation, ruminating behaviour for red deer was assigned in approximately 80% of cases to bedded behaviour, and in about 7% cases to standing behaviour; similarly this occurred in cattle models. We could notice that, when undisturbed, rumination activity produced in both species X values equals to 0 - 1, and corresponding Y values included between 1 and 10; nevertheless, this behaviour is not regularly identifiable due to the above-mentioned reasons. Thus, the detection of this behaviour could possibly be achieved by avoiding the averaging process and displaying the row acceleration data, as already clarified by Löttker et al. (2009); furthermore, in future, new features in sensors appositely designed to detect such behaviours may complement GPS collars, allowing to deepen this research topic. Regarding the lack of distinction between feeding and movement, we have to consider that in ruminants most active time is spent feeding (Geist 1963, Beier et al. 1990);

in red deer feeding amounts to almost 95% of active time (Berger et al. 2002), and locomotion is mainly linked with feeding (Löttker et al. 2009).

In regard to possible differences in behaviour - and thus in activity values - among individuals, dissimilarities between sexes or age classes may occur (Coulombe et al. 2006, Gervasi et al. 2006, Löttker et al. 2009); however, so far this effect on classification of behaviour have not been investigated yet, since sample sizes of wild species were in every case low. In any case, the definition of absolute cut-off values obtained with tree based models may be less adaptable to subjects displaying different levels of activity; mixture models appear thus a more adaptable procedure, indeed it demonstrated to perform slightly better on captive deer data collected on individuals of different sexes and age classes. When comparing cut-off values calculated on our red deer sample with recursive partitioning with the thresholds values obtained for the same species (N=4, 1 male, 3 females) with this classification procedure by Löttker et al. (2009), a strong similarity appears: our cut-off values were 25.5 for X axis and 14.5 for Y axis, while their values were 15 (X) and 27 (Y). This result suggest that on a sample of mixed ages and sexes of captive deer, thresholds between active and inactive bouts are quite constant, and also markedly different from the ones calculated for other ungulates as roe deer (X: 3; Y: 2; Heurich et al. 2012) or cattle (Chianina, Y: 72; Limousine, X: 53.5; see table 4.9). In the above-mentioned experiments, as well as in numerous other attempts, the models were both trained and tested on *pure* intervals. As also pointed out by other authors (Gottardi et al. 2011, Body et al. 2012, Gaylord 2013) this represents a simplification, although the so built model provides better performances when employed to classify only pure intervals; this procedure, however, has to be considered inappropriate to classify the whole dataset, being composed both by *pure* and *mixed* intervals.

We therefore discourage to build classification models solely on *pure* intervals: performances of classification on mixed intervals dataset are lower, as also observed by Gaylord (2013), and the loss of data suitable for training dataset may be considerable (up to 56% in our case study on captive deer). The use of shorter sampling intervals reduces the occurrence of mixed intervals, as can be observed for cattle data, however it is not recommendable when planning long-term studies due to the limits of devices in storage capacity of data.

The continuous monitoring carried out on beef cattle allowed to perform behavioural observation in different seasons; differently from the majority of previous attempts of behaviour classification from activity data, we were thus able to prove that the monitoring period affect considerably recoded values, as a consequence of modification in specific behaviour and intensity of activity. In contrast with Löttker et al. (2009), who consider more reasonable the definition of universal thresholds that can be used year round and for all individuals, we believe that the effect of the monitoring period should be more carefully analysed also for other species, in particular for wildlife; this could lead, as for beef cattle, to the building of different classification models specific for each season.

Finally, not only different species require separate classification models or thresholds, but also diverse breeds in domestic livestock may display remarkable dissimilarities in behaviour according to their respective temperament or habits.

The present activity pattern analysis from activity data represents, to our knowledge, the first continuous and long-term study on data collected on free-ranging red deer (*Cervus elaphus*) monitored with GPS collars. Therefore, no direct comparisons can be performed for this species with similar type of data; numerous investigation on red deer activity budgets have been performed with VHF-telemetry, but no one in the area of Northern Apennine.

Despite studied animals belonged to different age classes and, in certain cases, lived in diverse territories within the same area, patterns of activity both at annual and at daily level were similar, although a certain inter-individual variability was detected. Both in captive hinds (Berger et al. 2002, Pépin et al. 2006b) and in free-ranging red deer (Georgii 1981, Georgii et al. 1983), as well as in other cervids (roe deer: Krop-Benesch et al. 2013), regularities of general patterns beyond individual differences are common and reflect the specie-specific behaviour.

Also the patterns of intensity of activity displayed by the overall sample monitored during two consecutive years, are almost overlapping; moreover, at this time scale, inter-individual differences in the pattern are limited; this suggests that, for deer living this particular territory, the general structure of behaviour at the annual level is remarkably definite and constant.

Intensity of activity steady increases in spring, starting from April, it begins to decline in summer with a relative peak during mating season, and reaches the lowest values between November and January; a similar pattern of intensity of activity was found for roe deer (Krop-Benesch et al. 2013), with the lowest values recorded in winter and the maximum levels activity during spring and summer. Several studies have displayed seasonal changes in activity time of red deer in different habitats; although direct comparison with our data would not be appropriate, some aspects of physiology and reproduction of this species allow to explain variation of both patterns. During mating period hinds gather in larger groups defended by mature stags, which become progressively more aggressive and prevent hinds from leaving the group as well as herd them (Clutton-Brock et al. 1982, Carranza et al. 1990); this particular behaviour explains the raise of activity levels recorded during the rutting month. After the mating period and during the whole winter season, a progressive decline of intensity of activity was detected.

During this period the daily amount of total activity has been shown to reduce, in some cases up to 30-50% respect to summer (Craighead et al. 1973, Georgii 1981, Georgii et al. 1983, Hanley 1982, Berger et al. 2002, Arnold et al. 2003); the decrease of activity occurring during winter can be interpreted as an adaptation for energy conservation in regard of the unfavourable conditions of this season. This explanation is also supported by the findings of Arnold et al. (2003), which observed that red deer decreased energy expenditure during winter by reducing locomotor activity and moving more slowly, as well as reducing metabolic rate. This phenomenon also comply with the decrease of mobility and extension of home ranges observed our sampled animals during this season with respect to autumn (Crocetti 2011); a reduction of winter extent of home ranges was found also by Georgii (1980) in Alpine territory. The increase in activity levels occurring in spring may probably due jointly to the start of growth of herbaceous vegetation (which occurs during April) and to the higher energy demand during the last period of gestation. Indeed, annual patterns of behaviour follow the variations in energy consumption, quantity and quality of available food resources and weather conditions (Berger et al. 2002).

The single Cosinor, employed to test for circannual rhythmicity, detected significant amplitudes for the 8 hinds which were monitored for at least 1 year. The results match with the previously commented annual activity patterns, showing acrophases comprised between April 17 and June 2nd, with the exception of one hind which displayed the activity peak in July. To our knowledge, this represents the first Cosinor analysis performed on wild red deer.

The budget of activity calculated in the attempt to identify free-ranging deer behaviour, by using the classification model built in chapter 4.1, showed that the amount of time dedicated to feeding and moving varied among sampled animals;

individual average durations of activity ranged from 10 to almost 14 hours. Georgii (1981) found that individual differences in the total daily activity could amount up to 6 hours. No significant variations in the activity budget were detected from June to October, with average monthly activity time of 11.3 – 12.6 hours; other activity studies on the species reported pronounced variations in activity budget during the year, however the major differences were observed between summer and late winter. In our case data collected on free-ranging hinds from November to May were intentionally not interpreted, and thus comparisons between seasonal trends cannot be performed; however we found similar, yet slightly lower, daily activity time of red deer monitored in the same periods. Georgii (1981) found that, during summer, deer of the Bavarian Alps were active for about 12-16 hours; red deer of Velčice (Slovak Republic) were estimated to be active from June to October nearly 66% of daily time, namely about 16 hours (Arnold et al. 2003), while Rum hinds spent nearly 14 hours (58%) grazing and moving (Clutton-Brock et al. 1982). Elk (*Cervus canadensis*) of the Yellowstone National Park were active during summer almost 54% of the time, i. e. 13 hours (Craighead et al. 1973). Activity budget of our sample of Apennine red deer is thus lower (51%); however, in the calibration dataset for the classification model, active behaviours were underestimated by 5%: thus we might reasonably think that also in the case of the free-ranging deer the actual activity budget might be slightly higher.

Daily patterns of activity, in particular the relationship between diurnal and nocturnal activity, has been used in red deer as an indices of disturbance (Georgii et al. 1983). The calculated Diurnality Index (DI), which is a measure of the tendency to be more active during daylight hours or during night, never approached its extreme values; also Krop-Benesch et al. (2013) observed in roe deer a limited range of the index, despite seasonal differences.

Our results showed that for almost the entire sample (8 hinds out of 9) the index displayed negative values, which underlines the general tendency in nocturnality; however, in one case (F4) the overall index had a positive value (0.11) and, by considering the behaviour of this individual, it appears that this difference is due to the higher levels of activity during daytime between February and April, when the DI ranged from 0.21 to 0.45. Giving an explanation to the particular behaviour of this hind is not simple, however we can meditate on a fact; three hinds of the sample (F4, F5 and F7), show a major tendency to diurnality during winter. In effect, we would expect deer to be mostly active in warmer hours during cold seasons, as has been already observed in situations of low disturbance (Berger et al 2002); the other 6 hinds, conversely, tend to be more nocturnal during this period. This behaviour has been interpreted by Georgii (1981) in his case study as consequence of human disturbance; indeed, this hypothesis could hold for our case study, since the home ranges of the more "nocturnal" hinds appear to be closer to residential areas. However this is an hypothesis that has to be more deeply examined.

In general the monitored hinds display a daily pattern of behaviour which varies throughout the year (chapter 4.2.5). The daily pattern of activity appear mostly shaped by the dark-light seasonal regime, with the peaks of activity occurring in correspondence of twilights; this patterns was already found for red deer with regards to activity time (Georgii 1981, Georgii et al. 1983), but also in other ungulates as elk (Craighead et al. 1973, Wichrowski et al. 2005), roe deer (Cederlund 1989, Jeppensen 1989, Krop-Benesch et al. 2013), moose (Cederlund 1989). Additionally to the two main peaks, a third raise in activity levels occurs around midnight from September to February, except for December, and in April; this particular pattern was detected also in elk living in Kentucky (Wichrowski et al. 2005).

Beyond the timing of the two main peaks, also the distribution of activity levels during daylight and night varies; during summer months (June-August) the intensity of activity during light hours is very low, and activity seems concentrated during night. Conversely, in winter the first peak of activity is shifted towards morning and noon: thus, in general, hinds tend to be more active during the day; this behaviour was detected for red deer also by Clutton-Brock et al. (1982), Carranza et al. (1991) and Kamler et al. (2004). In contrast, as previously reported, studied deer living in other regions displayed more activity during night in winter; these opposite results suggest that seasonal patterns of red deer during the 24-h depend on environmental conditions, in particular on human disturbance (e.g. hunting) as pointed out by Pépin et al. (2006a).

The results of activity patterns and activity budget reported for beef cattle has to be considered as an example of possible information derivable from this type of sensors, in particular for future employment in monitoring for research purposes of free-ranging cattle, as well as possibly on other species; however, some indications and data can be obtained.

Despite differences in breed and year of monitoring, the seasonal pattern of both intensity of activity and activity budget, were similar for all sampled animals. Moreover, the seasonal pattern of activity levels follows the seasonal pattern of duration of activity; this suggests that the general pattern of activity budget can be drawn from that of activity levels for this species, but possibly also for others.

Both in seasonal and in daily patterns of intensity of activity, breed differences in behaviour proved to influence notably the extent of recorded values, as also observed in the building process of the behaviour classification models. Conversely, this difference is far less marked, although significant, when considering breed-specific activity budgets.

Total duration of activity in the 24-h resulted greatly variable (from 3 to 13.5 hours). On average Chianina, the traditional breed, spent more time grazing and moving than Limousine, the commercial breed; this result is consistent with the findings of earlier studies, where less specialized production breeds of livestock were more active and more inclined to explored their environment during foraging than livestock of production breeds (Gustafsson et al. 1999, Sæther et al. 2006).

Over the warm grazing period, the cows increased their activity time until July; subsequently, time spent feeding and moving decreased until the end of August. Distance travelled, however, increased progressively during this period in all animals, reaching its maximum at the end of August.

The anti-predator theory predicts large herbivores will avoid foraging during the hours of darkness due to a perceived risk of predation (Rutter 2006, Hessle et al. 2008). In cattle, grazing time during light hours varies between 70% (Hassoun 2002, Hessle et al. 2008) and 90%. Our results showed that above 90% of activity for both breeds time was distributed during daylight hours, indicating that cows avoided grazing in darkness; indeed, the grasslands grazed by the studied cattle are located in territories where natural predators (i.e. wolf) are present, and attacks to grazing livestock are not infrequent.

Activity patterns and budgets in all species are influenced by exogenous factors, such as temperature, quality and availability of food resources, disturbance by predators or humans, insects and dominant conspecifics. A better understanding of these patterns can thus be achieved by the contextual study of several environmental variables.

CHAPTER 6
CONCLUSIONS

Activity sensors in GPS collars represent a tool with an enormous potential for research applied both to wildlife management and conservation, as well as to livestock breeding; however, until now they have been scarcely employed for long-term studies on wild and domestic fauna. The reason for their limited utilisation may be the difficulty in interpretation of this enormous amount of data. The quick diffusion of GPS telemetry has widened the range of application of remote tracking to many research fields, enabling the collection of a remarkable amount of behavioural data; yet the advancements in the analysis of such data have been less fast.

This work intended to illustrate an approach to the interpretation of activity data for behavioural studies of wild elusive species and for possible applications in free-ranging livestock research; it also allowed to provide some practical indications for future deepest investigations on identification of behaviour, as well as to highlight possible synergies with other fields of environmental research.

The recording of activity data, given as average value over a sampling time interval and reported on an arbitrary scale, proved to be able to distinguish between two (potentially three, when including fast movements) behavioural states: active and inactive. Sampling intervals of five minutes proved to be a reasonable compromise in long-term monitoring between the collection of fine-scale data, the interpretability of behaviour and the memory capacity of the collars; in seasonal monitoring (for tracking periods up to 6-8 months), the 152 seconds intervals revealed to be also profitably applicable.

Differences in behavioural expressions may lead to the necessity of adapting specific models of classification, not only at species level but also among breeds;

further research with wider samples should allow to establish the possible effects of sex and age class. Moreover, the seasonal variations in the activity state of animals require the adoption of different models; thus, the sampling protocol for behavioural observations utilised to calibrate the activity sensors, should consider continuous monitoring throughout the year. Given the numerous sources of variability in individual activity, mixture models appear more capable to account for such differences than the definition of absolute threshold values, especially when building overall classification models.

We believe that GPS collars producers should consider to make available the raw acceleration data in addition to scaled data, in order to increase the possibilities of behaviour recognition.

Activity patterns analysis proved to provide new and interesting insights into wildlife behaviour. We believe that a more profound explanation of certain aspects of activity could be possible when studying such patterns in combination with other environmental information. The combined study of activity patterns with variations of climatic parameters, quality and quantity of feeding resources, presence of predators, external stressors and human disturbance, could lead to a new level of understanding of animal ecology and livestock production and welfare. Biological rhythms are the results of adaptations of the animals to environment; the capability of detecting and interpreting variations in such rhythms, may offer a key to understand the effects of future environmental changes. This is crucial in the perspective of conservation and sustainable management of wildlife, and for enhancing livestock welfare, its productivity as well as the quality of their products.

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APPENDIX I

							NAME		COLLAR			DATE					
UTC TIME	BED	RUM	STAT	GRO	FEED	WAL	FM	NOTES	UTC TIME	BED	RUM	STAT	GRO	FEED	WAL	FM	NOTES
5.40.00									6.20.00								
5.40.30									6.20.30								
5.41.00									6.21.00								
5.41.30									6.21.30								
5.42.00									6.22.00								
5.42.30									6.22.30								
5.43.00									6.23.00								
5.43.30									6.23.30								
5.44.00									6.24.00								
5.44.30									6.24.30								
5.45.00									6.25.00								
5.45.30									6.25.30								
5.46.00									6.26.00								
5.46.30									6.26.30								
5.47.00									6.27.00								
5.47.30									6.27.30								
5.48.00									6.28.00								
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5.49.00									6.29.00								
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6.01.00									6.41.00								
6.01.30									6.41.30								
6.02.00									6.42.00								
6.02.30									6.42.30								
6.03.00									6.43.00								
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6.06.00									6.46.00								
6.06.30									6.46.30								
6.07.00									6.47.00								
6.07.30									6.47.30								
6.08.00									6.48.00								
6.08.30									6.48.30								
6.09.00									6.49.00								
6.09.30									6.49.30								
6.10.00									6.50.00								
6.10.30									6.50.30								
6.11.00									6.51.00								
6.11.30									6.51.30								
6.12.00									6.52.00								
6.12.30									6.52.30								
6.13.00									6.53.00								
6.13.30									6.53.30								
6.14.00									6.54.00								
6.14.30									6.54.30								
6.15.00									6.55.00								
6.15.30									6.55.30								
6.16.00									6.56.00								
6.16.30									6.56.30								
6.17.00									6.57.00								
6.17.30									6.57.30								
6.18.00									6.58.00								
6.18.30									6.58.30								
6.19.00									6.59.00								
6.19.30									6.59.30								

Fig. I.1 Sampling form used for behavioural observations

COLLAR	SEX	AGE	BIRTH DATE	WEIGTH	NOTES

METEOROLOGICAL DATA								
DATE	UTC TIME	TEMPERATURE	SUN	RAIN	WIND	OVERCAST	HUMIDITY	NOTES

Fig. I.2 Sampling form for animal information and weather conditions

APPENDIX II

	LIMOUSINE			CHIANINA		
ID	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**
EXPECTED FIX	4877	1864	3617	3484	4122	4119
OBTAINED FIX	4867	1811	3548	3481	4118	4107
VALIDATED FIX	4774	1719	3363	3454	3955	3944
PAR	99.8%	97.2%	98.1%	99.9%	99.9%	99.7%
VALIDATED	98.1%	94.9%	94.8%	99.2%	96.0%	96.0%

Table II.1 Performances of fix acquisition for GPS collars mounted on cattle on confined pasture. Expected fix: number of fix receivable if all positions were successfully obtained basing on scheduled fix interval. Obtained fix: number of fix actually obtained. Validated fix: number of fix obtained with ≥ 5 satellites and DOP < 10. Position Acquisition Rate (PAR): (obtained fix/expected fix)*100. Validated: (validated fix/obtained fix)*100.

	Number of used satellites									
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
C1	0.0%	0.3%	5.7%	34.1%	31.7%	17.1%	8.0%	2.8%	0.2%	0.0%
C2	0.0%	1.0%	7.5%	31.1%	34.9%	16.1%	6.9%	1.5%	0.9%	0.1%
C3	0.1%	1.7%	7.7%	32.4%	32.0%	16.6%	6.0%	2.6%	0.8%	0.1%
C4	0.0%	0.1%	4.3%	29.9%	29.8%	18.8%	11.7%	4.8%	0.5%	0.0%
C5	0.0%	0.7%	7.1%	30.7%	31.4%	17.1%	8.4%	3.1%	1.2%	0.3%
C6	0.0%	0.7%	7.8%	31.1%	30.8%	17.8%	7.9%	2.7%	0.9%	0.1%

Table II.2 Frequency (%) of fix obtained, for each animal, using different numbers of satellites.

APPENDIX III

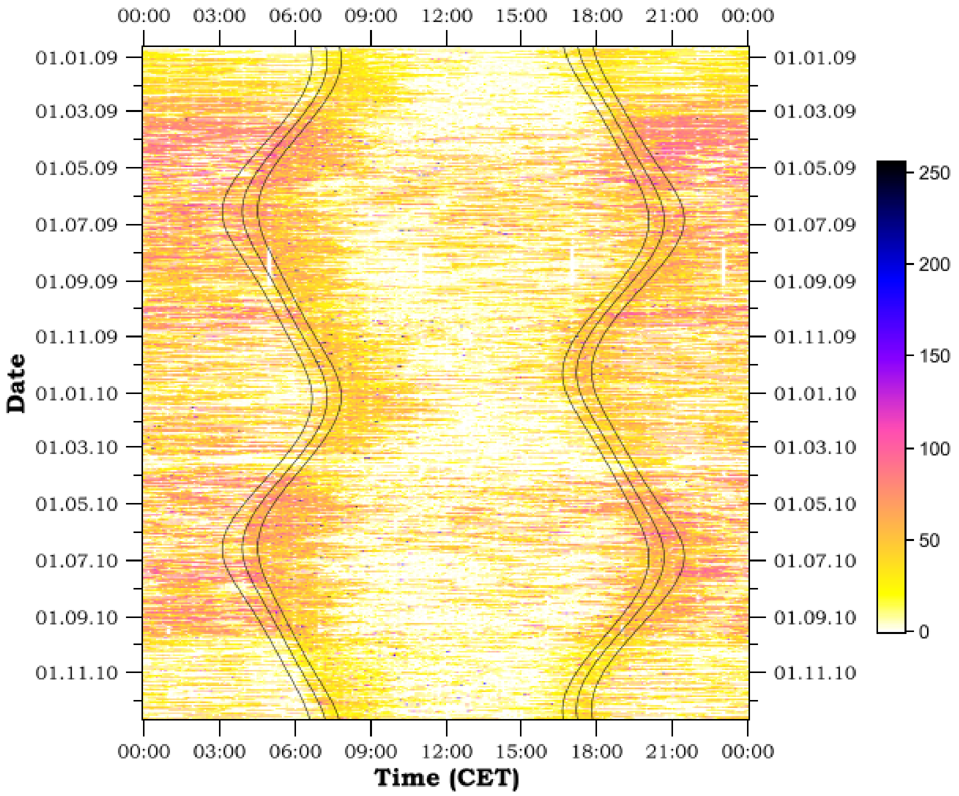

Fig. III.1 Activity plot of F3 for the entire monitoring period. Vertical lines indicate from left: nautical dawn, civil dawn, sunrise, sunset, civil dusk and nautical dusk.

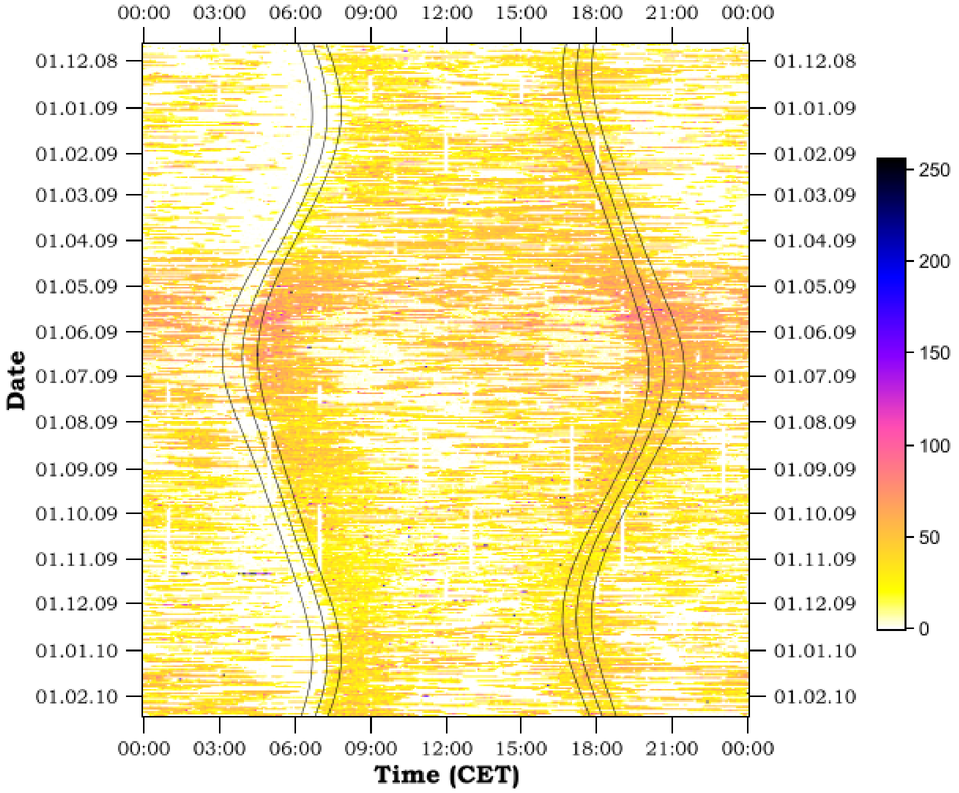

Fig. III.2 Activity plot of F4 for the entire monitoring period

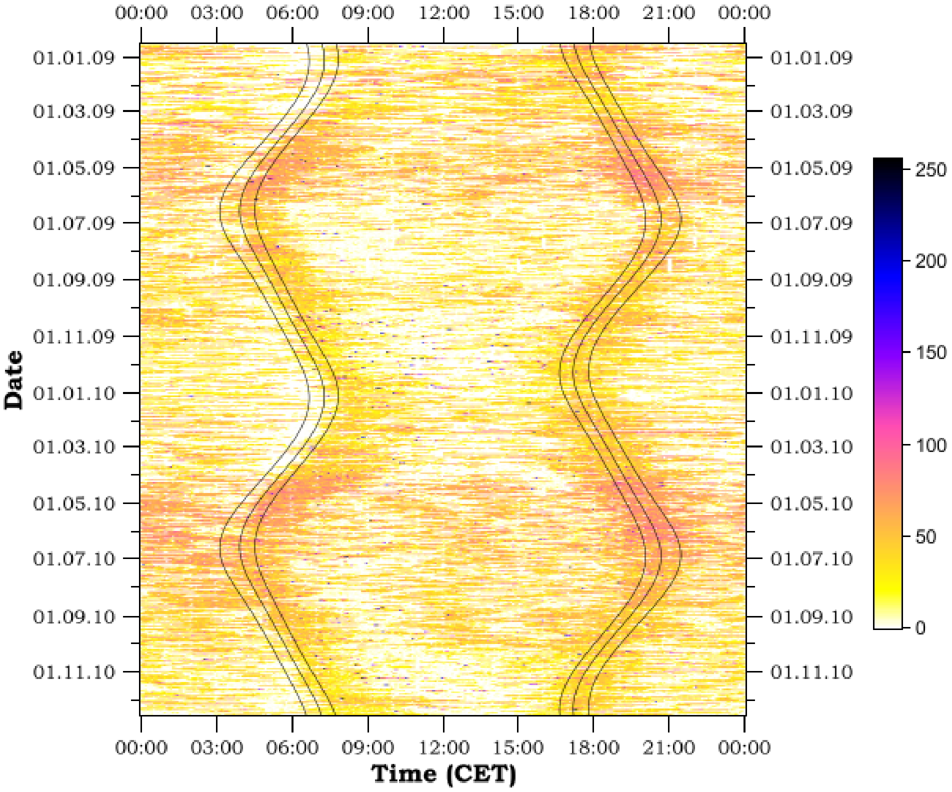

Fig. III.3 Activity plot of F5 for the entire monitoring period

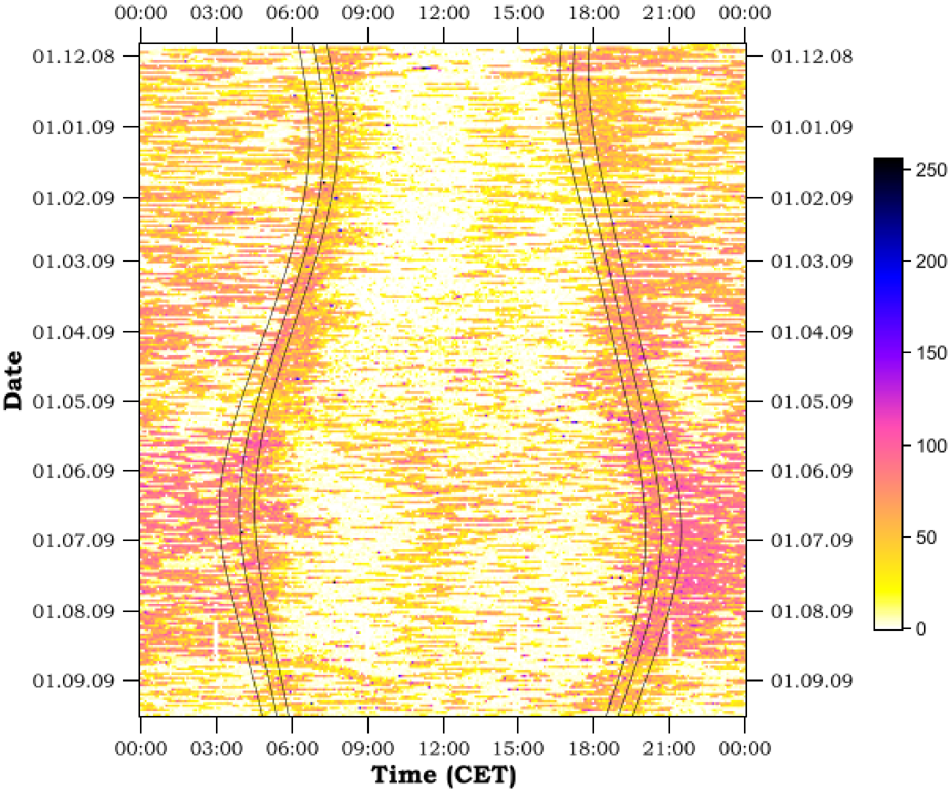

Fig. III.4 Activity plot of F6 for the entire monitoring period

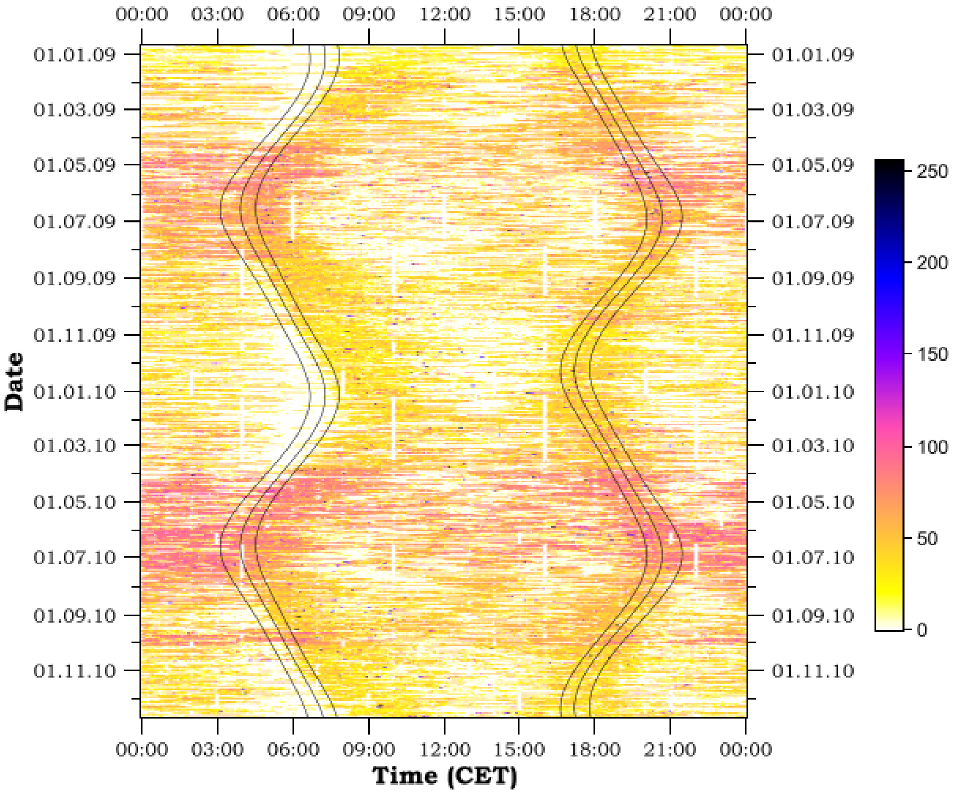

Fig. III.5 Activity plot of F7 for the entire monitoring period

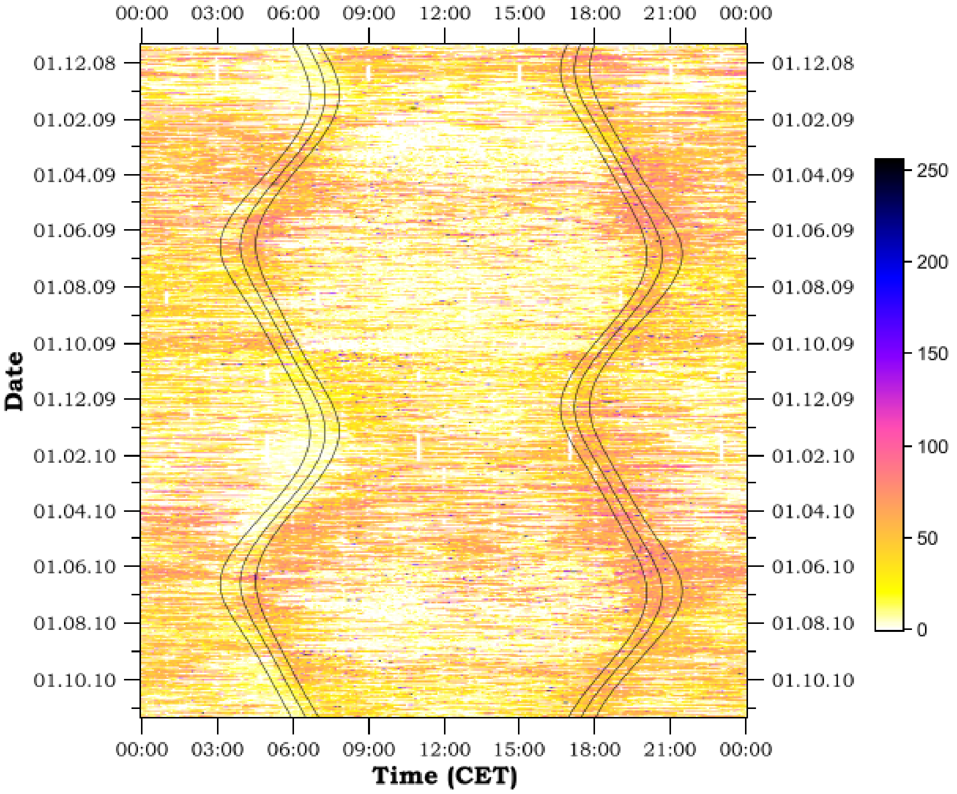

Fig. III.6 Activity plot of F8 for the entire monitoring period

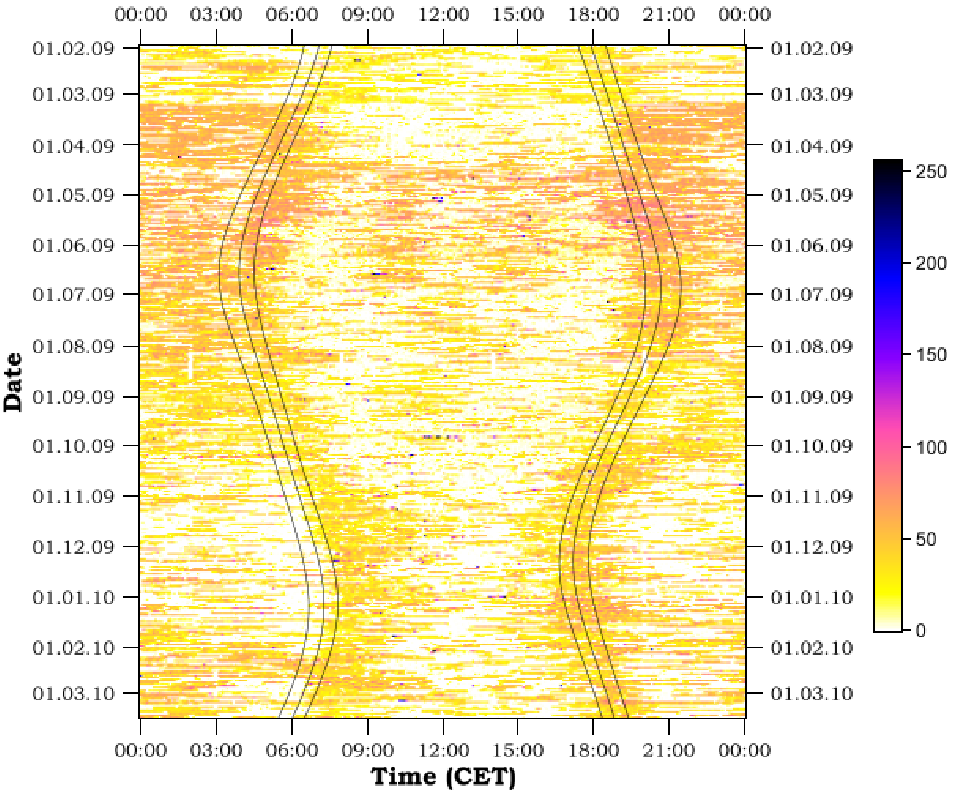

Fig. III.7 Activity plot of F9 for the entire monitoring period

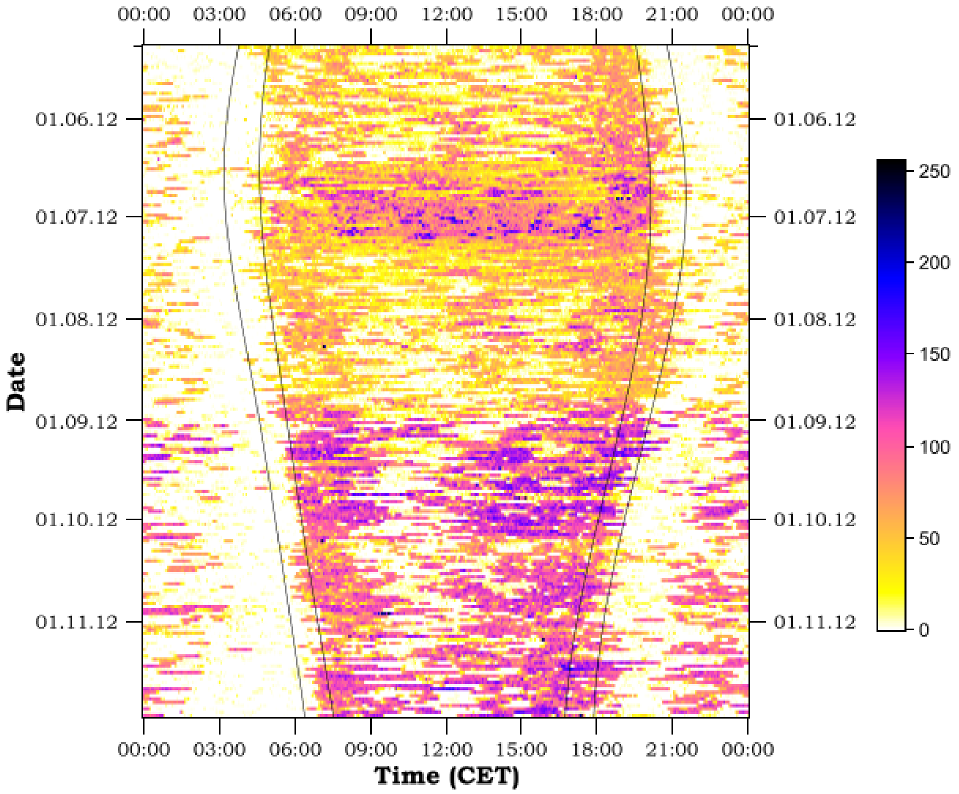

Fig. III.8 Activity plot of C1 for the entire monitoring period. Vertical lines indicate from left: nautical dawn, sunrise, sunset, and nautical dusk.

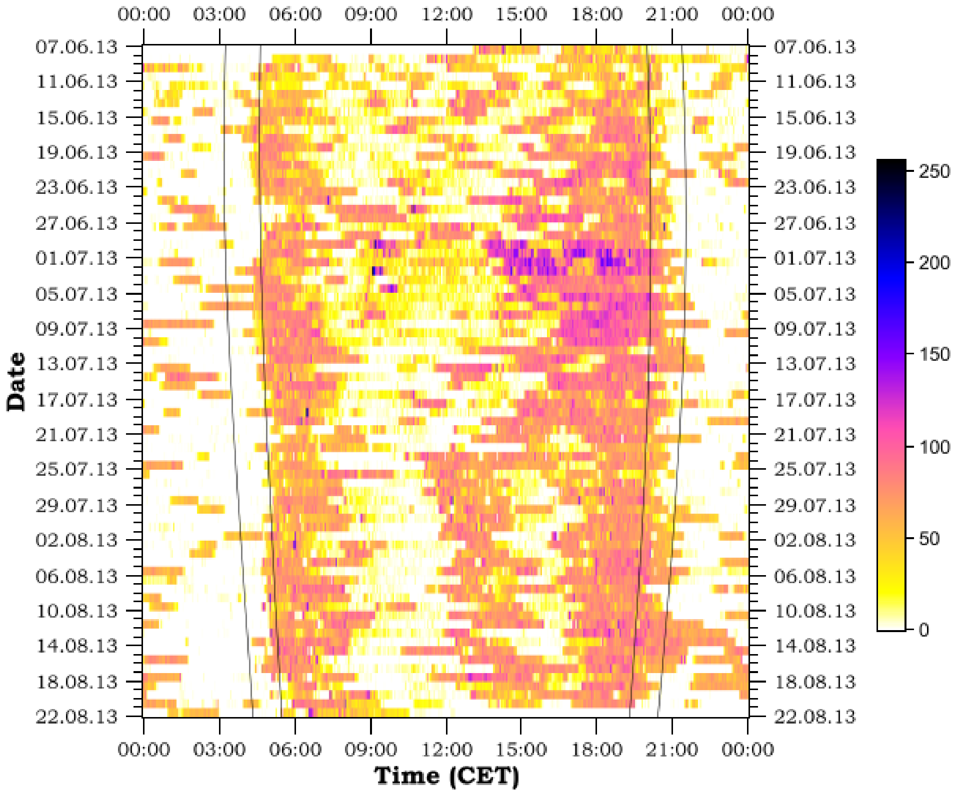

Fig. III.9 Activity plot of C2 for the entire monitoring period.

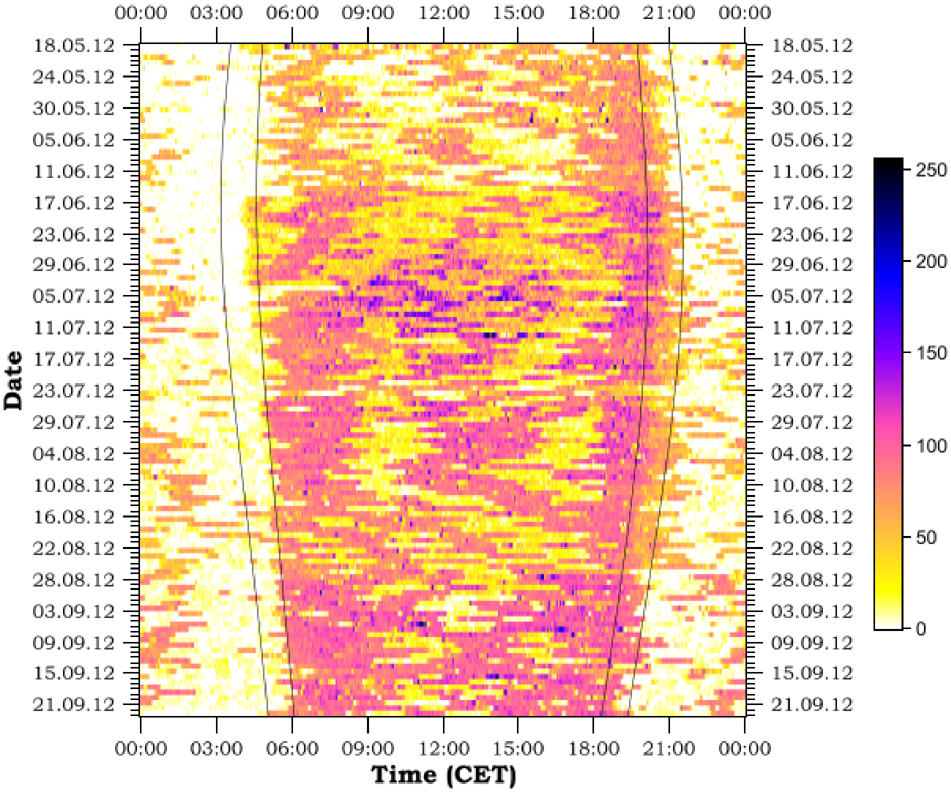

Fig. III.10 Activity plot of C4 for the entire monitoring period.

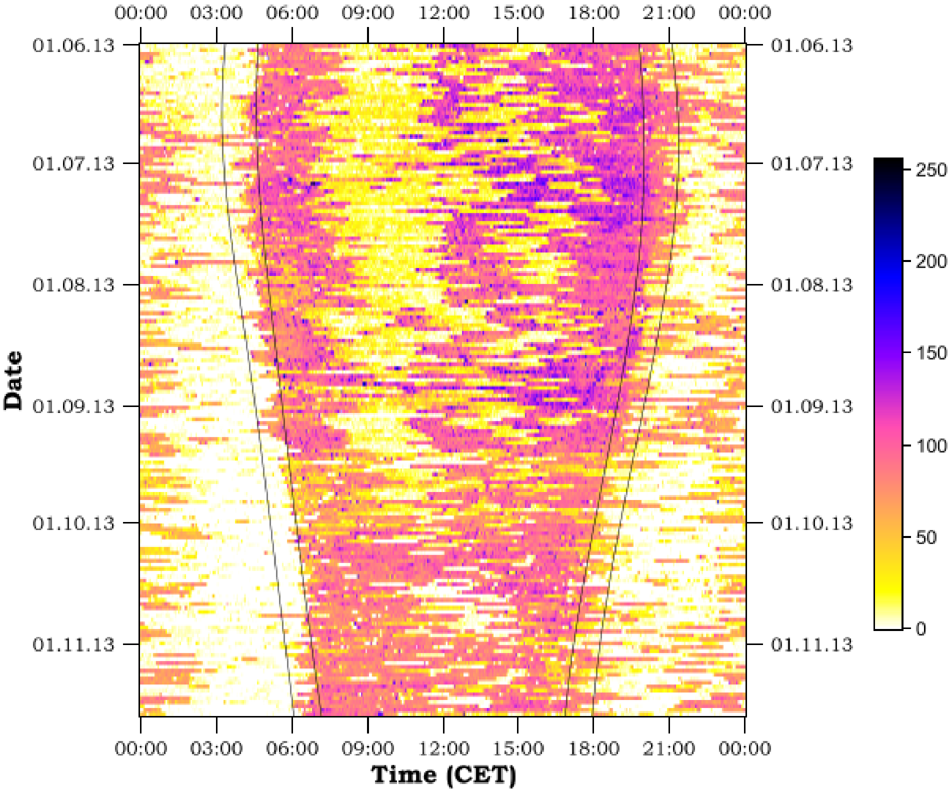

Fig. III.11 Activity plot of C6 for the entire monitoring period.

APPENDIX IV

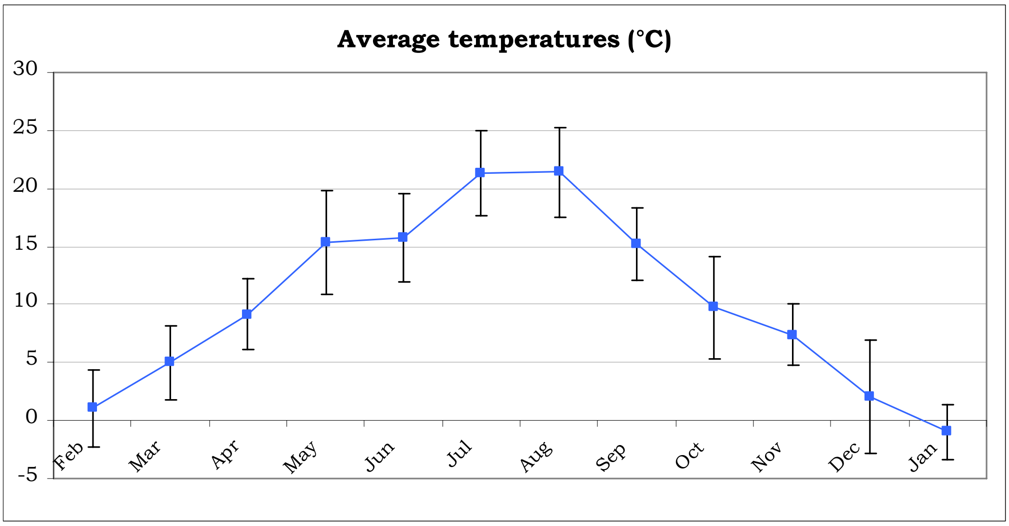

Fig. IV.1 Average monthly temperatures (°C) for the period Feb 09 - Jan 10 recorded by Acquerino weather station.

APPENDIX V

Month	Time of the day		p
Jan	5.00	17.00	0.021
	5.00	18.00	0.013
May	9.00	19.00	0.010
	9.00	20.00	0.001
	10.00	19.00	0.049
	10.00	20.00	0.006
	12.00	19.00	0.047
	12.00	20.00	0.006
	16.00	20.00	0.047
June	4.00	9.00	0.042
	8.00	4.00	0.007
	8.00	20.00	0.012
	8.00	21.00	0.019
Jul	8.00	4.00	0.047
	8.00	20.00	0.026
	9.00	4.00	0.008
	9.00	20.00	0.005
	9.00	21.00	0.047
Aug	9.00	20.00	0.008
	10.00	20.00	0.015
Sep	11.00	18.00	0.05
	11.00	19.00	0.05
Oct	12.00	18.00	0.021
	13.00	18.00	0.013
	13.00	19.00	0.037
	14.00	18.00	0.031
Nov	4.00	17.00	0.034
Dec	4.00	17.00	0.037
	12.00	17.00	0.013
	13.00	17.00	0.029

Table V.1 Free-ranging red deer. Multiple comparisons with Bonferroni adjustment for intensity of activity during times of the day. February 2009 – January 2010.