The assessment of activity in colony-housed domestic cats (*Felis catus*)

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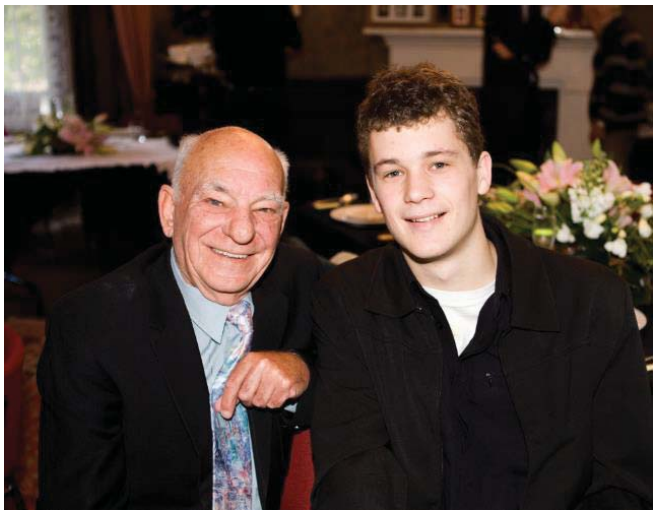
Dedication

This thesis is dedicated to my grandfather, Dale Petersen, who passed away half-way through this work. Poppa, you taught me to live life to the full and strive for success in all aspects of my life. Your unwavering belief in me gave me, and continues to give me, the confidence and determination to pursue all of my current and future goals and ambitions. For that I am eternally grateful. You set the standards pretty high for what can be achieved in a lifetime, but I am determined to give you a run for your money. Consider this thesis an official start to our little competition!

I will forever cherish the times we spent together. Love always.



Dale Petersen (18/12/1934 – 23/05/2014) (left) and Chris Andrews - Author (right).

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Synopsis

Monitoring and quantifying the overall physical activity (OPA) of cats can provide insight into their health, wellbeing, behaviour and physiology, but the accurate quantification of OPA is difficult without labour-intensive behavioural observation. Recent advances in remote sensing technologies such as accelerometry offer an automated method for continuously assessing OPA. A single study has validated Actical® 'MiniMitter' accelerometers (MMAs) to assess OPA in domestic cats (*Felis catus*), but their sample size precluded investigation of inter-individual variation. The first aim of this thesis (Chapter 2) was to compare the MMA and observed activity data of a larger number of cats (n = 12). While the MMA activity counts provided an accurate representation of observed activity for each cat, there was considerable variation between cats, so care is required when comparing the MMA activity data of different cats. Also the underlying factors affecting the OPA of individual animals need to be understood. The second aim (Chapter 3) investigated the effects of abiotic factors (day of the week, temperature, rainfall, and humidity) and one biotic factor (behavioural oestrus) on the OPA of domestic cats using MMAs. Day of the week did not appear to affect the activity of the cats despite lower levels of human interaction over weekends. This suggested that the group housing of the cats, with associated high intra-group interactions, outweighed human interactions. Temperature, rainfall and humidity all affected the OPA of the cats, but with considerable inter-cat variation. Reproductive state (anoestrus or oestrus) had a major effect on the OPA of the cats, with this study providing the first quantitative support for observations that cats are typically more active during oestrus. However, the behavioural detection of oestrus used in this thesis is challenging and may have led to the inaccurate categorisation of oestrus in some cats. Daily saliva samples were collected from all of the cats throughout the study, with the aim of using salivary oestradiol (E_2) concentrations to monitor ovarian activity and more accurately identify periods of oestrus in the cats. An addition study (Appendix 2) was conducted to validate a liquid chromatographic (LC) assay for salivary E_2, but further development of this assay is required to improve sensitivity. It is clear that the OPA of cats is affected by ovarian cyclicity, and that this should be considered and accounted for when conducting activity-based research in cats.

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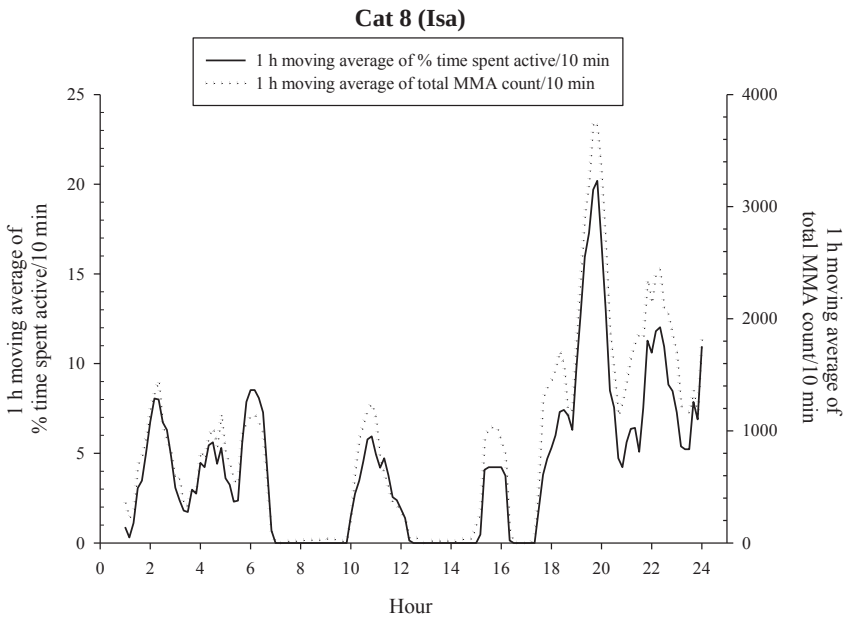


Chapter 1: Introduction - A review of the quantification of activity in animals

1.1 Introduction

Monitoring and quantifying the overall physical activity (OPA) of animals can provide insight into their health, wellbeing, behaviour and physiology. Activity assessment has now been used in a range of biological (e.g. studying stress-related behaviour, reproductive behaviour, hunting/foraging behaviour, and energy expenditure) and clinical (e.g. joint disease and obesity) studies (Watanabe *et al.*, 2005; Hansen *et al.*, 2007; Lascelles *et al.*, 2007; McGowan *et al.*, 2007; Papailiou *et al.*, 2008; Takahashi *et al.*, 2009; Rothwell *et al.*, 2011; Piccione *et al.*, 2013; Wilson *et al.*, 2013; Deng *et al.*, 2014; Jones *et al.*, 2014). The assessment of activity in animals has traditionally been achieved through direct visual observation, but recent technological advances have provided researchers with a number of automated methods for assessing OPA (Lascelles *et al.*, 2001; Siwak *et al.*, 2002; Siwak *et al.*, 2003; Hansen *et al.*, 2007; Lascelles *et al.*, 2008). As a result, there has been a rapid increase in the number of scientific publications assessing activity over the past decade.

The aim of this chapter is to review the current methods for quantifying the activity of companion animals, specifically domestic cats (*Felis catus*) and dogs (*Canis familiaris*). It will also focus on accelerometry and its potential applications, since accelerometers are now the most widely used tool for assessing OPA in animals (Watanabe *et al.*, 2005; Hansen *et al.*, 2007; Lascelles *et al.*, 2007; Lascelles *et al.*, 2008; Preston *et al.*, 2012; Piccione *et al.*, 2013; Alexander *et al.*, 2014; Deng *et al.*, 2014). The review concludes with an outline of the aims and structural plan of this thesis.

1.2 Methods for assessing the physical activity of animals

1.2.1 Visual observation

The OPA of animals has traditionally been assessed through real-time or recorded behavioural observations. This has certainly been the case in companion animals, with most activity-related studies using owner-based, subjective assessments of behaviour, gait and OPA (Lascelles *et al.*, 2001; Siwak *et al.*, 2002; Siwak *et al.*, 2003; Lascelles *et al.*, 2007; Brown *et al.*, 2010). Such subjective assessment of activity has the potential

for bias that may cause either an overestimation or underestimation of OPA, especially if the assessment is conducted by owners (who generally have a vested interest in the outcome of the study) or a number of different untrained observers (Lascelles *et al.*, 2008; Dow *et al.*, 2009; Morrison *et al.*, 2013). If observers are provided with sufficient training, then the visual assessment of OPA can be very accurate and reliable, but it is time consuming and difficult to monitor over a prolonged period or in multiple subjects simultaneously. The use of this method is therefore limited to assessing the activity of a single individual or multiple individuals for pre-defined periods (Siwak *et al.*, 2002; Siwak *et al.*, 2003), making the detection of subtle daily or weekly variation in activity difficult.

1.2.2 The automated assessment of observed activity: Ethovision

A proprietary software program called Ethovision (EV; Noldus, Wageningen, Netherlands) has been developed for the automated assessment of activity from a live video feed or time-lapse recording (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). Ethovision identifies an animal based on its videoed grey scale density (GSD) with respect to the surrounding environment (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). It also calibrates pixels against a standardised measure so that each pixel represents a defined distance (Lascelles *et al.*, 2008). Ethovision then calculates observed activity (e.g. velocity, distance moved and/or time spent moving) based on the movement of the animals' GSD with respect to individual pixels (Lascelles *et al.*, 2008).

Ethovision has since been used successfully to assess the observed activity of both cats and dogs, and can also be used to monitor behaviours such as feeding, drinking, and sleeping (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). This is possible because certain areas (e.g. food, water bowl or bed) in the video footage can be manually designated as points of interest and the frequency and duration of all visits to these areas recorded (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). While EV clearly has great potential for assessing the OPA and behaviour of animals, it has a number of limitations (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). Activity can only be assessed in a pre-defined arena that can only be monitored with a single overhead camera positioned perpendicular to the arena floor (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). The amount of furniture or equipment in the arena also needs to be restricted to ensure that the animal is visible at all times. Shadowing and variations in light intensity also need to be limited as they can

negatively influence activity estimates (Lascelles *et al.*, 2008). A relatively empty recording area, as is required, has the potential to influence the behaviour of the animal and offers minimal stimulation for the expression of normal active behaviours. For these reasons, EV cannot be used to monitor 'natural' activity and behaviour of animals or to study free-living animals.

The behaviour of free-living animals can differ greatly from that of animals in controlled settings. Factors such as food availability (e.g. prey density), interspecific and intraspecific interactions, environmental temperature, and photoperiod have all been found to affect the activity patterns of carnivores (Boulos and Terman, 1980; Zielinski, 1988; Tsutsui *et al.*, 2004; Piccione *et al.*, 2013). Furthermore, seasonal reproduction in the cat is regulated by photoperiod, with long day-length (~14h of daylight) promoting the onset of oestrus and thus the expression of reproductive behaviours (Graham *et al.*, 2004; Tsutsui *et al.*, 2004; Hoover and Vertes, 2007). Programs such as EV preclude investigation of the behaviour of animals under natural conditions or the effects of natural changes in photoperiod on animal activity due to the requirement for artificial lighting (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). As a result, EV has largely been used to validate other techniques for monitoring OPA (e.g. accelerometry) against measures of observed activity (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). However, the high price of the software and hardware of the EV system (~$10,000 NZD) has also limited its uptake and use in this context. Similar validation trials can be conducted using direct visual observations which, while far more time consuming, are much less expensive.

1.2.3 Pedometry

The application of pedometry in companion animal research has largely been limited to examining the effects of dog walking on the activity of their owners, with results suggesting that dog ownership promotes activity in humans if they regularly walk their dogs (Coleman *et al.*, 2008; Nijland *et al.*, 2010; Murray *et al.*, 2012). Interestingly, there appears to be a positive relationship between the weight of dogs and the body mass index (BMI) of their owners (Nijland *et al.*, 2010), possibly due to the fact that activity levels of dogs and their owners appear to be closely related (Dow *et al.*, 2009; Nijland *et al.*, 2010; Preston *et al.*, 2012). Despite this, few studies have used pedometry to directly measure the activity of the dogs themselves, with research

concentrating mostly on owner activity. One study that has validated the use of pedometry in dogs confirmed a close relationship between the number of pedometry-recorded steps (from collar-mounted pedometers) and the actual number of steps taken (determined from video footage) for a range of different sized dogs (small, medium and large dog breeds) (Chan *et al.*, 2005). The total number of pedometry-recorded steps decreased as the intensity of movement per unit of distance covered (i.e. gait) increased (Chan *et al.*, 2005). This is not surprising as the number of actual steps also decreased as gait changed from walking to trotting to running; however, it does raise concern about the efficacy of pedometry for quantifying the intensity of movement in dogs and other animals (Chan *et al.*, 2005). For this reason, pedometers have been replaced in more recent studies by more advanced, accelerometer-based activity monitors.

1.2.4 Accelerometry

Accelerometry provides a far more detailed assessment of activity than pedometry, with accelerometers being able to accurately detect the intensity, duration and direction of movement (Watanabe *et al.*, 2005; Hansen *et al.*, 2007; Lascelles *et al.*, 2008; Preston *et al.*, 2012; Gerencsér *et al.*, 2013). Despite this, accelerometers have only recently been used to assess the activity of cats and other companion animals, with the devices historically being too large to attach to the animals without influencing their movement or behaviour. Recent advances in accelerometer technology have greatly reduced the size of accelerometer devices, and thus permitted the use of these devices to monitor the activity of smaller animals such as cats. The first study of accelerometry on domestic cats used a device (M190-D2FT logger; Little Leonardo Co. Ltd., Tokyo, Japan) that continuously measured bi-axial acceleration and generated a temporal acceleration profile (Watanabe *et al.*, 2005). The acceleration profiles associated with certain behaviours (e.g. sleeping, grooming, scratching, eating, drinking and different locomotor gaits) were then compared, demonstrating that the device could be used to automatically identify the expression of these behaviours (Watanabe *et al.*, 2005).

Most accelerometer-based activity monitors contain a bi-axial or tri-axial accelerometer, which enables the omnidirectional detection of movement (Watanabe *et al.*, 2005; Lascelles *et al.*, 2008; Yam *et al.*, 2011; Gerencsér *et al.*, 2013; Piccione *et al.*, 2013; Jones *et al.*, 2014). This ultimately means that these activity monitors have the potential to detect both dynamic (e.g. locomotion) and static (e.g. body posture only) behaviours,

as changes in body posture would change the manner in which gravity (a constant acceleration of 9.8 ms^{-2}) affects the acceleration profiles of each accelerometer within the device (Watanabe *et al.*, 2005).

It is important to note that accelerometers can differ in how they collect activity data, with some devices recording acceleration continuously (continuous activity monitors), while others accumulating acceleration-based activity counts over a pre-defined and adjustable time interval (accumulative activity monitors) (Watanabe *et al.*, 2005; Lascelles *et al.*, 2008). Accelerometers that record acceleration forces continuously have the advantage of greater sensitivity for detailed behavioural assessments and for examining body posture (Watanabe *et al.*, 2005). This does, however, greatly limit the duration over which activity or behaviour can be monitored without the removal of the device and downloading of data due to the limited memory and battery life of such small the devices. While accumulative activity monitors provide less detailed activity data, they can be used to monitor activity for longer periods (>40 days) (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). Consequently, accumulative activity monitors are generally preferred when studying the OPA of animals in their natural environment.

Different types of accumulative activity monitors also differ in how they obtain activity counts from acceleration forces (Lascelles *et al.*, 2008). Lascelles *et al.* (2008) defined three different accumulative activity monitors as time above threshold devices (devices that quantify the accumulative time that acceleration exceeds a pre-set threshold), zero crossing devices (the number of times acceleration crosses the no activity, or zero, point) and digital integration devices (activity counts represent the duration and intensity of acceleration forces). Nonetheless, the versatility and accuracy of accumulative activity monitors has resulted in accelerometry becoming the most widely used technique for the objective assessment of activity in companion animals (Lascelles *et al.*, 2007; Brown *et al.*, 2010; Preston *et al.*, 2012; Piccione *et al.*, 2013; Alexander *et al.*, 2014; Deng *et al.*, 2014).

Most accelerometer devices are small and easily attached to animals via a collar or harness (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). Attachment of accelerometers to an existing cat or dog collar does not appear to interfere with normal daily behaviours (e.g. sleeping, eating, drinking, grooming, locomotion, playing and jumping), with the devices consistently being positioned on the ventral side of the neck due to the effects of

gravity (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). Harness attachment also appears to have a minimal effect on the behaviour of cats and dogs after an acclimatisation period; however, collar attachment is preferred as it is easier to implement and does not require the animal to wear additional harness equipment (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). The activity counts obtained from harness-attached accelerometers appear to show a slightly better correlation with observed activity than those attached to a collar, which probably relates to the reduced influence of stationary behaviours such as grooming or scratching on the devices (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). However, both provide a highly accurate representation of observed activity, thus the attachment of accelerometers to the existing collars of cats and dogs is recommended.

Actical® (also known as Actiwatch® or Actigraph®) accelerometers are the most commonly used accelerometer-based devices for quantifying the OPA of companion animals, and have been used for the vast majority of activity-related research in both cats and dogs (Hansen *et al.*, 2007; Lascelles *et al.*, 2007; Lascelles *et al.*, 2008; Brown *et al.*, 2010; Yam *et al.*, 2011; Preston *et al.*, 2012; Gerencsér *et al.*, 2013; Morrison *et al.*, 2013; Piccione *et al.*, 2013). These accelerometers have been thoroughly validated against measures of observed activity (e.g. distance moved or time spent moving) and varying intensities of induced physical activity (e.g. different treadmill inclines and speeds) in dogs (Hansen *et al.*, 2007; Michel and Brown, 2011; Yam *et al.*, 2011; Preston *et al.*, 2012; Gerencsér *et al.*, 2013). The literature in cats, however, is limited to a single study.

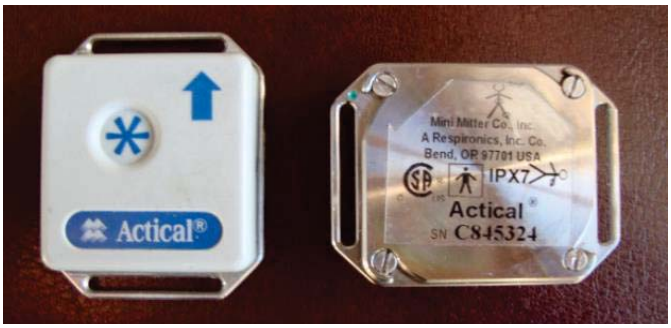
Lascelles *et al.* (2007) compared a total of 144 hours of concurrent accelerometer and observed activity data (distance moved and time spent moving as calculated using EV) from three domestic cats. While the study found a strong correlation between accelerometer-based activity counts and observed activity, the small sample size used (n =3) precluded investigation of inter-individual variation. Accordingly, the authors stated that each cat should be considered as its own control until the levels of variation between cats could be investigated (Lascelles *et al.*, 2008). Nonetheless, it is clear that collar-mounted Actical® accelerometers can be used to accurately assess the OPA of individual cats (Lascelles *et al.*, 2008; Piccione *et al.*, 2013; Alexander *et al.*, 2014; Deng *et al.*, 2014).

1.3 Actical® accelerometers

Actical® 'MiniMitter' accelerometers (MMAs; MiniMitter, Bend, OR, USA), initially designed to assess human activity, have now been validated for the automated quantification of activity in domestic cats, with the MMA and observed activity data (e.g. distance moved) of individual cats being highly correlated (Lascelles *et al.*, 2008). These MMAs are small (measuring 28 mm x 27 mm x 10 mm and weighing 17 g; Figure 1.1) and can easily be attached to the collars of cats without significantly affecting their behaviour (Lascelles *et al.*, 2007; Lascelles *et al.*, 2008).

The device is referred to as a tri-axial accelerometer as it uses a cantilevered rectangular piezoelectric bi-morph plate and seismic mass to detect movement in three planes (craniocaudal, mediolateral and vertical) (Lascelles *et al.*, 2008). An acceleration force causes the bi-morph plate to bend, generating a voltage output that is amplified and converted into a quantifiable digital value by an inbuilt microprocessor (Lascelles *et al.*, 2008). This digital value is then compared against a running baseline value and corrected for the effects of gravity (a constant acceleration of 9.8 ms^{-2}) (Hansen *et al.*, 2007; Lascelles *et al.*, 2008). The resulting digital output is recorded as an activity count that is the sum of activity over a defined period (referred to as an epoch), which can be set to 15 s, 30 s or 60 s (Lascelles *et al.*, 2008). The epoch selected determines the duration over which activity data can be continuously collected (45.0 days for 60 s epochs, 22.5 for 30 s epochs, and 11.3 for 15 s epochs), with battery life lasting approximately 180 days regardless of the epoch length (Hansen *et al.*, 2007; Lascelles *et al.*, 2007).

The raw activity data are downloaded from the MMAs using the Actireader® device (Figure 1.1) and software (Mini Mitter., Bend, OR, USA), which produces an Actogram of the raw activity counts from each of the devices used (Figure 1.2). The raw activity counts for each epoch over the total sampling period can also be exported to an excel spreadsheet for analysis. After sufficient experiment calibration, MMAs can be used to assess energy expenditure from the activity data.



(a)

(b)

Figure 1.1 Actical® 'MiniMitter' accelerometer (MMA; MiniMitter, Bend, OR, USA) (a) and the Actireader® (MiniMitter, Bend, OR, USA (b) used to download the data from the MMAs.

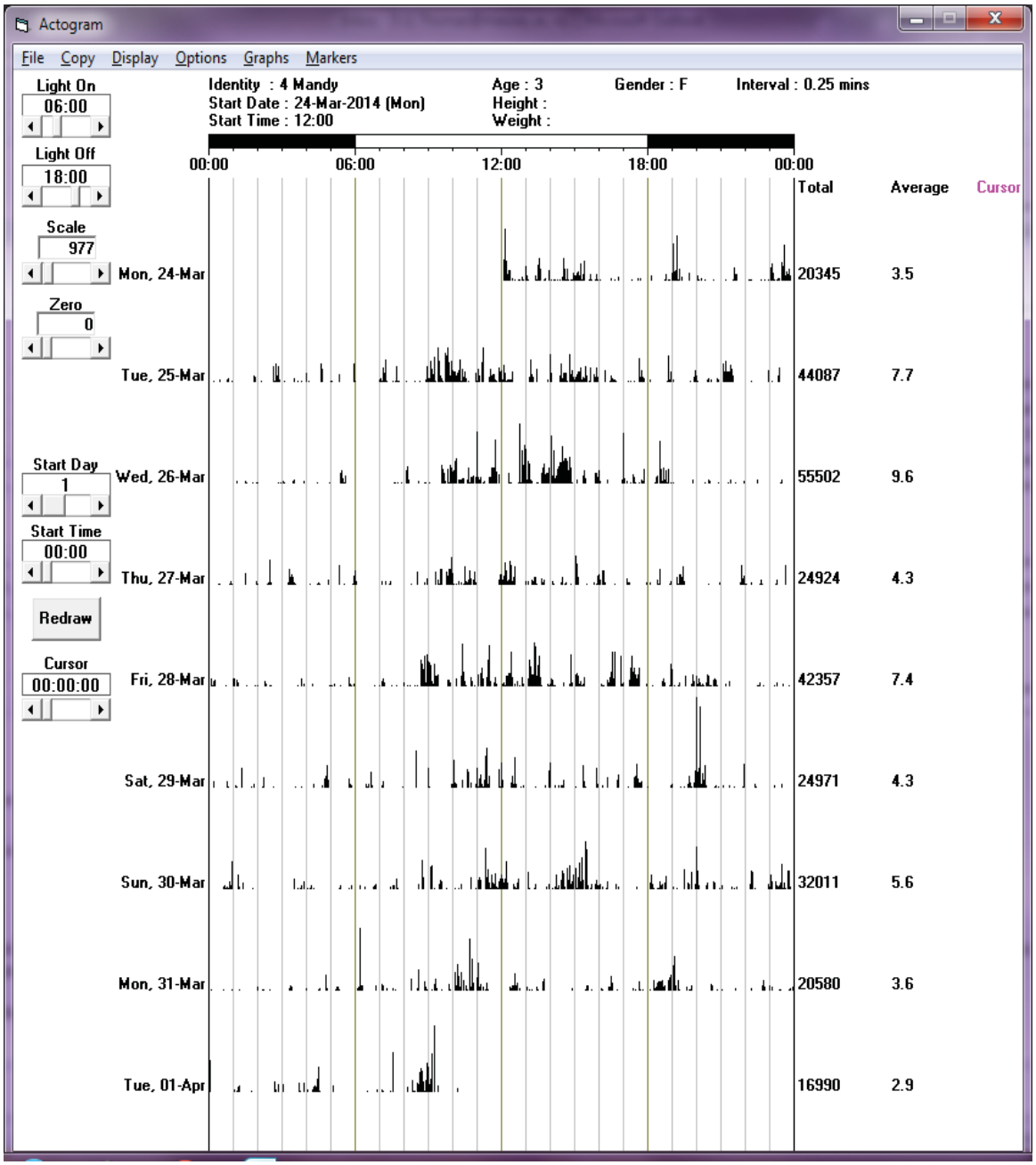


Figure 1.2 Actogram produced from the activity data collected from an Actical® accelerometer fitted to single female cat (Mandy).

Actical® devices have been calibrated for the assessment of activity-associated energy expenditure (AEE) in humans, with the MMA activity counts being strongly correlated with accurate measures of AEE (oxygen consumption (VO_2)) (Puyau *et al.*, 2002; Pfeiffer *et al.*, 2006). Actical® accelerometers have also been validated for this purpose in working farm dogs (Singh, 2013). This was achieved by calibrating the devices

using the doubly-labelled water (DLW) technique, which estimates carbon dioxide (CO_2) production based on the comparative loss of two intravenously injected isotopic labels, heavy oxygen (^{18}O; lost as water and CO_2) and deuterium (^{2}H; lost as water only), in the body water (Singh, 2013). The results of the study showed that the MMAs could be used to accurately assess AEE in working farm dogs (Singh, 2013). Such a calibration study has not yet been published in domestic cats.

1.4 Potential applications of accelerometry in cats and non-domestic felids

1.4.1 Behavioural assessments

As mentioned previously, the first study using accelerometry in domestic cats found that continuous activity monitors could be used to automatically identify behaviours such as sleeping, grooming, scratching, eating, drinking and different locomotor gaits (Watanabe *et al.*, 2005). Suggested further applications focused on wild felids, with the authors discussing the potential use of these activity monitors to study hunting behaviour (Watanabe *et al.*, 2005). Such a study was subsequently undertaken in cheetah (*Acinonyx jubatus*) using a continuous activity monitor coupled with a global positioning system (GPS) (Wilson *et al.*, 2013). This enabled researchers to compare the dynamics of cheetah hunting a range of different of prey species, including ostrich chicks (*Struthio camelus*), hares (*Lepus* spp.), gemsbok calves (*Oryx gazelle*), blue wildebeest calves (*Connocheates taurinus*), and common duiker (*Sylvicapra grimmia*) (Wilson *et al.*, 2013), but the limited duration over which continuous recording of activity can be sustained remains problematic due to the sheer volume of acceleration data obtained (Grünewälder *et al.*, 2012; Wilson *et al.*, 2013).

Consequently, the majority of studies that have used accelerometers to study the activity of felids, have used accumulative activity monitors (e.g. MMAs) (Lascelles *et al.*, 2007; Grünewälder *et al.*, 2012; Piccione *et al.*, 2013; Alexander *et al.*, 2014; Deng *et al.*, 2014). Even these accelerometers collect a large quantity of activity data, the manual processing of which can be difficult and time consuming. As a result, a recent study in cheetah examined the potential for the automated analysis of activity data (Grünewälder *et al.*, 2012). Grünewälder *et al.* (2012) used a Support Vector Machine (SVM) and hidden Markov approach (for details see Grünewälder *et al.* (2012)) to classify behaviours as stationary, feeding or mobile according to the accelerometer activity counts (5 min epoch). Comparisons with behavioural observations showed that this

method classified the 5 min activity counts with an accuracy of 83% - 94%, showing the potential for the use of such an automated technique (Grünewälder *et al.*, 2012). While a similar technique has been successfully used to study the behaviour of dogs (Gerencsér *et al.*, 2013), no such study has been reported on domestic cats. This is surprising given that, in terms of felids, the majority of accelerometer-based studies have been conducted on domestic cats due to their accessibility and reasonably accommodating behaviour (Watanabe *et al.*, 2005; Lascelles *et al.*, 2007; Piccione *et al.*, 2013; Alexander *et al.*, 2014; Deng *et al.*, 2014). Interestingly, very few studies have used accelerometry for the sole purpose of studying the 'normal' behaviour of cats or any other felids. Instead, accelerometers have predominantly been used to examine the effects of disease conditions (such as joint disease or obesity) on the OPA of cats (Lascelles *et al.*, 2007; Alexander *et al.*, 2014; Deng *et al.*, 2014).

1.4.2 Examining the effectiveness of pain therapy

Degenerative joint disease is common in older companion animals, causing chronic inflammation and pain that can be detrimental to their quality of life, and drastically affects their ability to perform normal daily activities (Crane, 1991; Lascelles *et al.*, 2001; Clarke *et al.*, 2005; Lascelles *et al.*, 2007; Brown *et al.*, 2010; Lascelles, 2010). It has been estimated that approximately 25 - 30% of dogs and 17 - 64% of cats are affected by osteoarthritis (OA), the most common degenerative joint disease in companion animals (Clarke *et al.*, 2005; Lascelles *et al.*, 2007; Brown *et al.*, 2010; Lascelles, 2010). Osteoarthritis is a multifactorial disease, which complicates the direct treatment of this condition (Lascelles, 2010). Most studies aim only to alleviate the pain associated with OA through treatment with non-steroidal anti-inflammatory drugs (NSAIDs) (Lascelles *et al.*, 2001; Lascelles *et al.*, 2007; Brown *et al.*, 2010).

An effective treatment for OA would alleviate chronic pain and likely lead to an increase in OPA (Lascelles *et al.*, 2007; Brown *et al.*, 2010). Historically, the efficacy of NSAID treatment has been tested through the owner-based subjective assessment of their pet's behaviour, gait and OPA (Lascelles *et al.*, 2001; Lascelles *et al.*, 2007; Brown *et al.*, 2010). However, such subjective assessment has the potential for bias and is heavily reliant on the observational astuteness of pet owners who receive little or no training. The use of accelerometry offers a far more accurate quantification of OPA and could be used alongside subjective assessment to provide a more definitive and

quantitative evaluation of the effectiveness of NSAID treatment for cats and dogs with OA.

Actical® accelerometers have been used for this purpose in both cats and dogs (Lascelles *et al.*, 2007; Brown *et al.*, 2010). The treatment of OA in cats with meloxicam, a NSAID, resulted in a significant increase in activity from baseline/pre-treatment activity levels and increased mobility compared to cats given a placebo (Lascelles *et al.*, 2007). In contrast, the activity levels of cats with OA given a placebo did not differ from baseline (Lascelles *et al.*, 2007). A similar finding has been reported in dogs affected by OA, whereby carprofen (another NSAID) resulted in a 20% increase in activity from baseline MMA counts recorded prior to treatment (Brown *et al.*, 2010).

While NSAID treatment limits the pain associated with this condition it does not limit the rate of joint degeneration (Crane, 1991; Lascelles, 2010), and thus drug therapy alone is currently not sufficient for the treatment and management of conditions such as OA in cats and dogs. The standard initial treatment recommendation for companion animals with OA or a similar joint condition is weight loss, with obesity being one of the leading contributors towards degenerative joint disease in companion animals. Prevention of obesity is also the key to minimising the susceptibility of animals to joint disease, and the severity of the disease once established (Crane, 1991; German, 2006; Lascelles, 2010).

1.4.3 Obesity and energy expenditure research

Obesity has become a significant problem for cats and dogs worldwide and has been linked to a range of conditions such as diabetes mellitus, cardiorespiratory complications, urinary and reproductive disorders, and orthopaedic diseases (e.g. OA) (German, 2006; Morrison *et al.*, 2013; Alexander *et al.*, 2014; Deng *et al.*, 2014). As with most species, obesity in cats is generally a consequence of excessive energy intake relative to the level of energy expenditure (i.e. exercise) (German, 2006; Alexander *et al.*, 2014; Deng *et al.*, 2014). Overfeeding can be easily corrected by offering a well-balanced diet and strict feeding regime, but it is much more difficult to promote and assess physical activity in cats (Alexander *et al.*, 2014; Deng *et al.*, 2014).

Accelerometry offers a means of automatically assessing activity and examining the effectiveness of approaches for promoting OPA in cats and other animals. Two recent

publications have reported that specialised feeding regimes and dietary changes can enhance the OPA of domestic cats (Alexander *et al.*, 2014; Deng *et al.*, 2014). Increasing the total moisture content of a dry kibble diet has been shown to increase the daily MMA counts of cats (Alexander *et al.*, 2014; Deng *et al.*, 2014). Furthermore, this is a form of energy dilution and thus limits energy intake, assuming energy intake is limited by meal volume (Alexander *et al.*, 2014). The provision of smaller, more frequent meals also appears to increase the OPA and hence energy expenditure of cats (Deng *et al.*, 2014). This seems to be a consequence of food anticipatory activity (FAA); that is, an increase in activity associated with anticipation of food 2 h before a defined meal time (Deng *et al.*, 2014). Ultimately then, small, frequent, meals of a diet with a high water content diet appears to be ideal for weight loss in cats (Alexander *et al.*, 2014; Deng *et al.*, 2014). The provision of *ad libitum* high moisture wet food may have a similar effect, as groups of overweight cats on this simple dietary regime lost weight, but it remains to be determined whether this weight loss was related to an increase in activity (Weidgraaf *et al.*, 2007).

As mentioned previously, accelerometers can also be used to indirectly monitor and assess activity-related energy expenditure (AEE) in individual animals (Wrigglesworth *et al.*, 2011; Singh, 2013). Such an application would be useful for determining the effectiveness of methods to promote activity and AEE in overweight animals. Of greater significance is the potential use of accelerometer-based estimates of AEE to determine the daily maintenance energy requirements (MER) of animals, since MER is a function of both bodyweight and OPA (Wrigglesworth *et al.*, 2011).

The use of accelerometers to assess strategies designed to combat obesity and promote and monitor AEE could also be applicable to the management of many non-domestic felids in captivity. Monitoring the activity of captive felids could provide insight into their energy expenditure and help researchers to improve enclosure design and dietary and management regimes to promote energy expenditure and reduce the risk of obesity and other associated conditions. Ultimately, this could help optimise the health and maximise breeding success in endangered felids and other animals.

1.4.4 Studying stress and the expression of stereotypical behaviours

Another major problem for captive non-domestic felids, or any captive animal for that matter, is the potential for captivity-related stress (Jurke *et al.*, 1997; Wielebnowski *et al.*, 2002a; Morato *et al.*, 2004; Terio *et al.*, 2004; Moreira *et al.*, 2007; Fanson *et al.*, 2012). In felids, this has largely been investigated by comparing the basal faecal glucocorticoid metabolites (FGM) of free-living and captive conspecifics (Wielebnowski *et al.*, 2002a; Terio *et al.*, 2004; Fanson *et al.*, 2012). Stress (defined as any predicted threat or physical challenge to homeostasis) leads to the activation of the hypothalamo-pituitary-adrenal (HPA) axis, which ultimately promotes the synthesis and secretion of glucocorticoids (e.g. cortisol) from cells within the zona fasciculata of the adrenal cortex (Miller and O'Callaghan, 2002). Plasma glucocorticoid or FGM concentrations are closely correlated to the degree of stress an individual animal experiences (Wielebnowski *et al.*, 2002a; Terio *et al.*, 2004; Fanson *et al.*, 2012). The captive environment is associated with a number of potential stressors, including small enclosure sizes leading to restricted movement, boredom, forced proximity to humans and other animals, and abnormal social groups, increased disease prevalence, novel sounds or smells, artificial lighting and photoperiod (Morgan and Tromborg, 2007).

It is not surprising to find then that captive cheetah, clouded leopards (*Neofelis nebulosa*) and Canadian lynx (*Lynx canadensis*), have all been shown to exhibit significantly higher basal FGM than their free-living conspecifics (Wielebnowski *et al.*, 2002a; Terio *et al.*, 2004; Fanson *et al.*, 2012). In fact, individuals on public display appear to exhibit even higher basal FGM concentrations than those off-display, implying additional stressors associated with public exhibition (Wielebnowski *et al.*, 2002a; Terio *et al.*, 2004; Fanson *et al.*, 2012). While this provides insight into the stress associated with captivity, the practical application of FGM analysis for monitoring stress levels in captive felids is limited. Faecal glucocorticoid metabolites can only be used to evaluate stress if compared against the FGM concentrations of free-living individuals of the same species, with basal FGM concentrations varying considerable between felid species (Graham *et al.*, 1995; Brown and Wildt, 1997; Brown *et al.*, 2002). Faecal glucocorticoid metabolites concentrations are also highly variable within an individual due to a circadian rhythm of cortisol secretion and a large number of other, non-stress related, factors that affect the section of cortisol (Veldhuis

et al., 1989). The prolonged assessment of FGM concentrations also becomes expensive due to the cost of labour and the required reagents, thus a cheaper and more efficient method would be preferred.

Captivity-related stress is often associated with the expression of certain "stress" behaviours, which in felids include stereotypies (e.g. pacing), excessive sleep, aggression, hiding, reduced exploratory behaviour, reduced appetite and self-mutilation (e.g. fur-plucking) (Wielebnowski *et al.*, 2002a; Bashaw *et al.*, 2003; Terio *et al.*, 2004; Szokalski *et al.*, 2012). Excessive pacing is probably one of the most indicative stress-related behaviours in felids, but it is difficult to determine the amount of pacing without extensive behavioural observation (Wielebnowski *et al.*, 2002a; Wielebnowski *et al.*, 2002b; Bashaw *et al.*, 2003; Resende *et al.*, 2011). A degree of pacing is expected among captive felids as it is associated with territorial behaviours such as patrolling and scent marking (Wielebnowski *et al.*, 2002a; Wielebnowski *et al.*, 2002b; Bashaw *et al.*, 2003; Szokalski *et al.*, 2012). It also offers a means of expending energy that would otherwise be spent hunting or patrolling large territories in the wild (Wielebnowski *et al.*, 2002a; Wielebnowski *et al.*, 2002b; Bashaw *et al.*, 2003; Szokalski *et al.*, 2012). Conversely, some individuals may respond to captivity-related stress by reducing their activity levels and sleeping excessively (Wielebnowski *et al.*, 2002a; Bashaw *et al.*, 2003; Terio *et al.*, 2004; Szokalski *et al.*, 2012). Accelerometers could be used to help differentiate between normal and excessive or insufficient amounts of activity in captive felids

Accelerometry has not yet been applied in the context of stress in felids, but it has been used in this context to study shelter dogs (Jones *et al.*, 2014). In these dogs, both unusually low and high levels of activity were shown to be indicative of stress (Jones *et al.*, 2014). This may also be the case with other canids and felids, although further investigation into the association between OPA and stress in these groups is needed to confirm this.

1.4.5 Obesity, stress and reproductive success

Captivity-related stress and obesity have both been linked to infertility and poor reproductive success in both male and female felids (Jurke *et al.*, 1997; Feldman and Nelson, 2004; Morato *et al.*, 2004; Terio *et al.*, 2004; Fanson *et al.*, 2012; Koester *et al.*,

2015), although the physiological mechanisms by which this occurs have not yet been studied. There has been extensive research into the negative effects of stress (i.e. high plasma glucocorticoid concentration) and obesity on the male and female hypothalamo-pituitary-gonadal (HPG axis) in a number of other mammalian species, particularly humans and rodents (Rivier and Rivest, 1991; Shaw *et al.*, 1997; Gore *et al.*, 2006; Hammoud *et al.*, 2008; Kirby *et al.*, 2009; Brewer and Balen, 2010; Whirledge and Cidlowski, 2010). A full discussion of this is beyond the scope of this literature review, but both obesity and stress have been shown to have a detrimental effect on the reproductive performance of captive felids as well as their health and wellbeing.

The application of accelerometry in the context of obesity, energy expenditure and captivity-related stress could also benefit captive breeding programs for non-domestic felids, with accelerometers offering a means of identifying stress and managing or preventing obesity. The results of accelerometer-based research could then be used to adapt and modify enclosures and management regimes to limit stress and promote adequate levels of exercise to prevent obesity, thereby improving the reproductive performance of captive felids.

1.4.6 Assessing ovarian cyclicity and oestrus detection in felids

The oestrous cycle of felids is summarised in Figure 1.3 and consists of four phases: anoestrus, pro-oestrus, oestrus and dioestrus (Feldman and Nelson, 2004; Brown, 2011). The reproductively active phase of the oestrous cycle is referred to as oestrus and is marked by the presence of mature tertiary follicles and peak oestradiol concentrations (Feldman and Nelson, 2004; Bristol-Gould and Woodruff, 2006; Brown, 2011; Malandain *et al.*, 2011). Felids are induced (or reflex) ovulators, with the tactile stimulation of sensory neurons within the cervix and vagina being required for ovulation; however, this only occurs in the presence of oestral plasma oestradiol concentrations (Bakker and Baum, 2000; Feldman and Nelson, 2004; Brown, 2011). The accurate and reliable detection of oestrus is thus critical to the captive management and breeding of non-domestic felids.

All felids (excluding lions (*Panthera leo*) and male cheetah coalitions) lead a solitary life, and hence they are typically housed individually in captivity (Mellen and Shepherdson, 1997; Wielebnowski and Brown, 1998; Swanson, 2003). In some

instances single sex conspecifics may be housed together, but individuals of opposite sex are rarely housed communally due to the potential risk of injury or death (Brown *et al.*, 1995; Mellen and Shepherdson, 1997; Swanson, 2003). Techniques that allow the accurate and reliable detection of oestrus are needed to determine when males and females should be introduced to one another for breeding purposes.

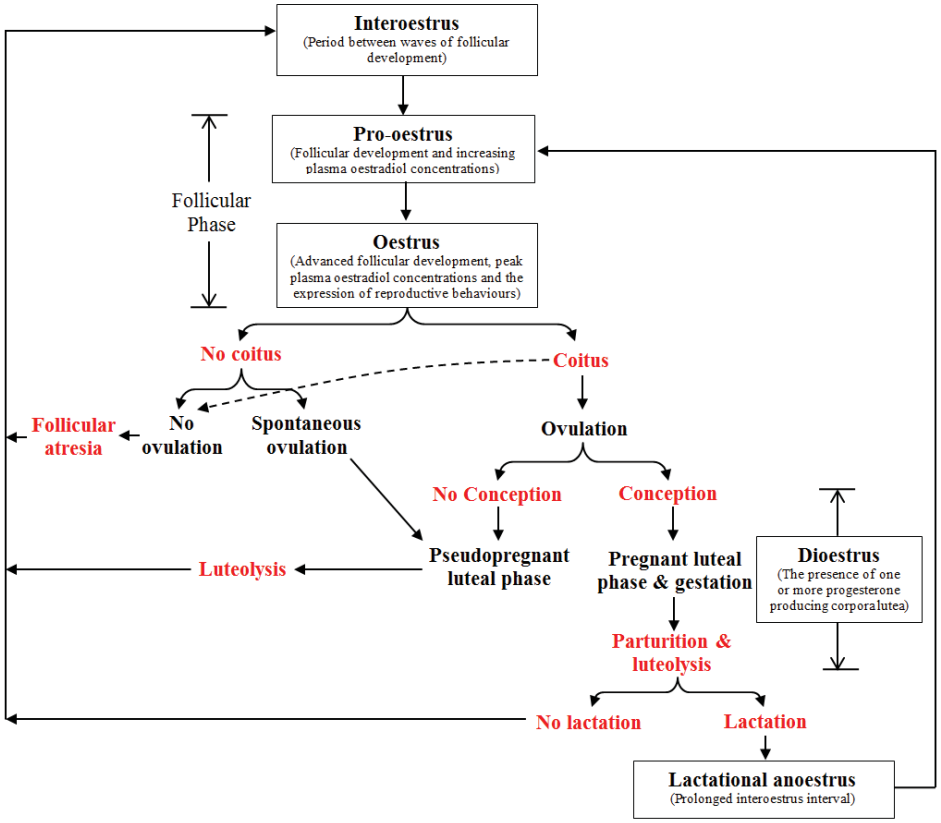


Figure 1.3 The oestrous cycle of felids that exhibit multiple periods of oestrus during a breeding season, or throughout the year if non-seasonal. The oestrous cycle of felids consists of four main phases: interoestrus, pro-oestrus, oestrus and dioestrus.

Faecal oestradiol-17β metabolites (FEM) (consisting of unconjugated and conjugated (3-sulphate, 17α-sulphate and 17β-sulphate) forms of oestradiol, oestrone and oestriol) have successfully been used to monitor the ovarian cyclicity of a wide range of felid species (Blomqvist and Sten, 1982; Brown *et al.*, 1994; Graham *et al.*, 1995; Brown and Wildt, 1997; Moreira *et al.*, 2001; Brown, 2006; Herrick *et al.*, 2010). This is an accurate method for monitoring the reproductive activity of felids; however, there are several major limitations of this technique. Firstly, FEM concentrations are not reflective of the animal's current physiological state since there is a 24 h delay between plasma and FEM concentrations (Brown *et al.*, 1994; Brown and Wildt, 1997; Kinoshita

et al., 2009). This is further exacerbated by the time taken to prepare the faecal sample and subsequently extract and analyse the FEM concentrations (Brown *et al.*, 1994; Brown and Wildt, 1997). As a result, FEM analyses are largely limited to the retrospective assessment of ovarian cyclicity, with limited application for the real-time detection of oestrus.

Oestrous behaviours are fairly consistent among felids and include lordosis, calling/prusten, grooming, allogrooming, scent marking, rubbing, rolling, and increased locomotor activity (Michael, 1961; Michael and Scott, 1964; Blomqvist and Sten, 1982; Mellen, 1993; Umapathy *et al.*, 2007; Kinoshita *et al.*, 2009; Groot, 2013). For many felids such as the domestic cat, clouded leopard, lion, snow leopard (*Pantehera uncia*) and leopard (*Panthera pardus*) these oestrous behaviours are generally overt and readily detectable (Blomqvist and Sten, 1982; Umapathy *et al.*, 2007; Kinoshita *et al.*, 2009; Groot, 2013). However the visual detection of oestrus has been difficult to achieve in many other felids including the cheetah (Asa *et al.*, 1992; Wielebnowski and Brown, 1998), margay (*Leopardus weidii*) (Moreira *et al.*, 2001), ocelot (*Leopardus pardalis*) (Moreira *et al.*, 2001), tigrina/oncilla (*Leopardus tigrinus*) (Moreira *et al.*, 2001), Eurasian lynx (*Lynx lynx*) (Henriksen *et al.*, 2005), Pallas' cat (*Otocolobus manul*) (Brown *et al.*, 2002) and Geoffroy's cat (*Oncifelis geoffroyi*) (Foreman, 1997).

While it has been suggested that oestrus is "silent" in these species this is not entirely true, since oestrus tends to be associated with subtle increases in expression of several typical oestrus behaviours (Asa *et al.*, 1992; Graham *et al.*, 1995; Wielebnowski and Brown, 1998). These subtle behavioural changes are unlikely to be detected without detailed and labour-intensive behavioural assessment, which is further complicated by the crepuscular or nocturnal nature of most felids. Furthermore, given the degree of inter-individual variability in the behaviours associated with oestrus it would likely require the long term observation of each animal (Michael, 1961; Michael and Scott, 1964; Wildt *et al.*, 1981; Schmidt *et al.*, 1988; Umapathy *et al.*, 2007). This observation is also valid for species that typically exhibit more obvious oestrous behaviours, as there still is considerable inter- and intra-individual variation in the expression of oestrus behaviours as well as the frequencies and intensities at which they are displayed.

Interestingly, many of the behaviours that felids express more frequently during oestrus appear to include an increase in overall activity (Foreman, 1997; Wielebnowski and

Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002). Thus it may be possible to improve oestrus detection in felids by monitoring their OPA using accelerometry, providing the activity data can be remotely download and monitored in real time. This technique has previously been used for this purpose in dairy cows (*Bos taurus*), and has successfully detected ~90% of oestrus events (At-Taras and Spahr, 2001; McGowan *et al.*, 2007). Significant increases in activity during oestrus have also been reported in a number of other species including mice (*Mus musculus*) (Kopp *et al.*, 2006), rats (*Rattus norvegicus*) (Gerall *et al.*, 1973), and pigs (*Sus scrofa*) (Cornou, 2006).

It appears that domestic cats and other felids are also more active during oestrus, and that this increase in activity correlates with females searching for male mates; however, the evidence for this is circumstantial and based entirely on subjective observations (Layhausen, 1979; Morali and Beyer, 1979; Asa *et al.*, 1992). Accelerometry offers a means of quantitatively examining the association between activity and oestrus in felids, and thus confirming whether the existing hypothesis that physical activity is higher during oestrus is true. The first step in investigating this would be to examine this association in the domestic cat as a model species, and subsequently apply the research methodology developed to non-domestic felids. If activity changes significantly during oestrus in felids then there is potential for the development of a novel, non-invasive technique for improving oestrus detection.

1.5 Aims and objectives of this thesis

This thesis set out to investigate the use of accelerometers to quantify the activity of domestic cats and examine the main underlying environmental and biological factors affecting their OPA. The main objectives of this thesis were: (1) to validate the use of Actical® accelerometers (MMAs) for monitoring the activity of domestic cats; (2) to examine environmental factors that may affect the overall physical activity of colony-housed domestic cats; and (3) to investigate the activity of domestic cats during periods of anoestrus and behavioural oestrus.

The thesis initially had a further two objectives: (4) to validate a sensitive and reliable salivary oestradiol assay for cats using liquid chromatography, and (5) to investigate the effects of reproductive state (as determined by monitoring salivary E_2 concentrations) on the OPA of domestic cats in a colony. Unfortunately, it was not been possible during

the time constraints of this study to achieve the assay sensitivity required to accurately detect oestradiol in saliva, thus these final two objectives were not achieved (see Appendix 2). As a result, the reproductive component of this thesis was restricted to behavioural assessments of reproductive state (anoestrus or oestrus) (Objective 3).

There is a paucity of research into the key factors affecting the OPA of cats, and no study to date has examined the effects of these factors on the accelerometer-based activity data of the cats. The overarching aim of this thesis was to improve our understanding of the main factors affecting the activity of colony-housed cats and, in turn, ensure that accelerometer-based activity data are not misinterpreted due to confounding variables such as temperature, rainfall, humidity, time of the day, level of human interaction, and reproductive cyclicity.

1.6 Study species and site: Massey University Centre for Feline Nutrition

All of the research in this thesis was conducted using domestic cats (*Felis catus*) from the Massey University Centre for Feline Nutrition. The colony currently consists of 150 adult short-haired domestic cats, specifically 44 entire females, 31 spayed females, 2 entire males and 73 neutered males. The cats in the colony are housed in 19 outdoor pens, with roofing providing shelter over two thirds of each pen (Figure 1.4). The cats were maintained in mix-sexed groups of seven to 10 individuals (the entire males are housed individually). The husbandry of the cats complied with the Animal Welfare (Cats) Code of Welfare (Anonymous, 2007) and Massey University Animal Ethics Committee (MUAEC) protocol number 12/12.

Pens were cleaned (washed and sawdust litter replaced) and the cats were provided fresh food and water daily. The cats were fed a complete and balanced (AAFCO, 2009) commercial moist (canned) feline diet (Heinz Wattie's Ltd., Hastings, New Zealand). The daily food allowance (adult energy maintenance requirement; MER) for each cat was determined using the equation: MER = 100 x kg $BW^{0.67}$ (NRC, 2006), where BW = body weight. Individual requirements were summed to calculate the daily food allowance per pen, which was sufficient to provide the cats with *ad libitum* access for approximately 24 h. Any refusals were recorded and the food allowance for each pen was adjusted weekly according to the changing bodyweight of the cats.

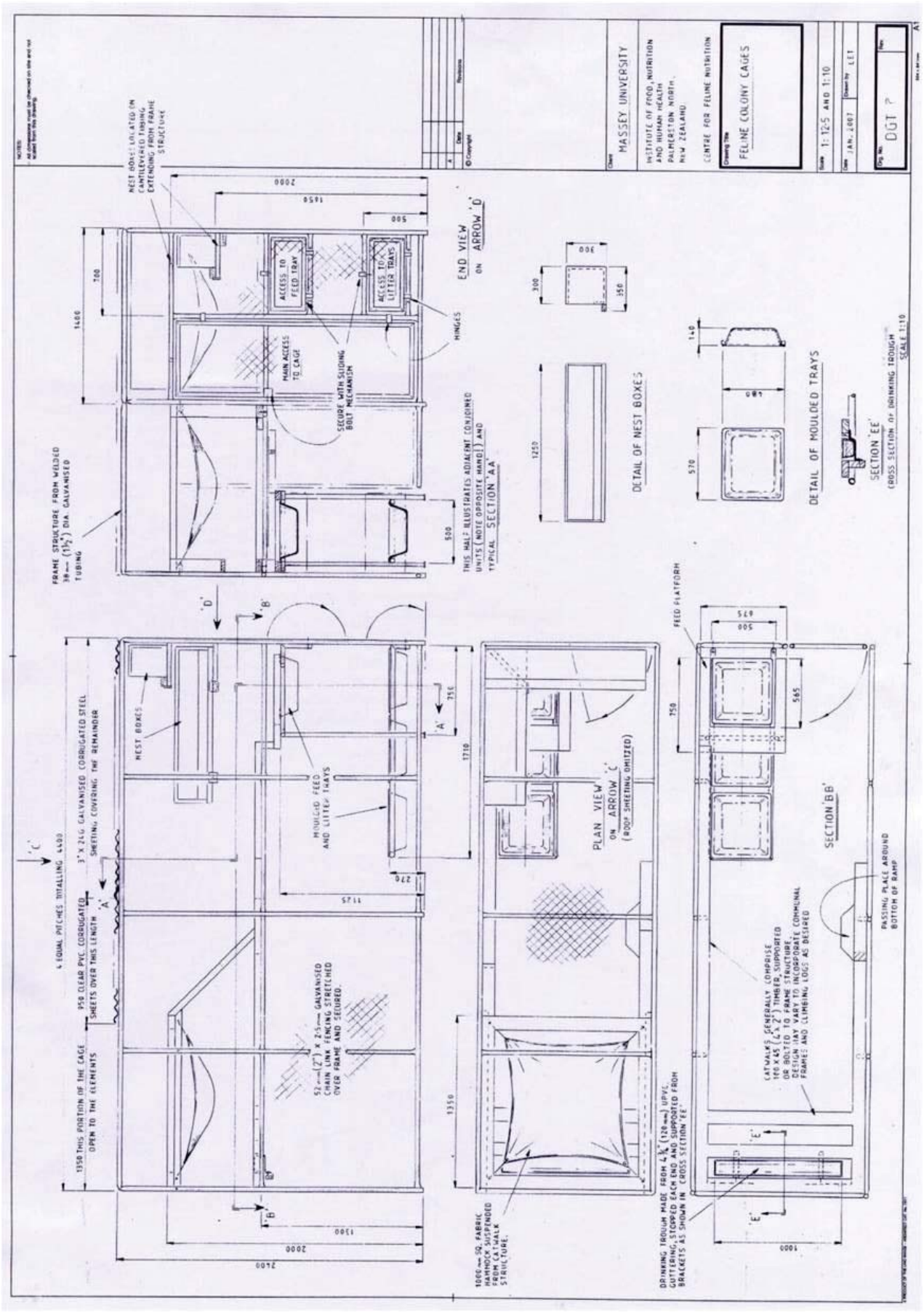


Figure 1.4 Blueprint for one of the pens at the Massey University Centre for Feline Nutrition.

The cats' routines were consistent from week to week, with staff being present from approximately 0730 - 1500 h on Monday to Friday. The pens were cleaned from 0730 - 0930 h and then food replenished from 1000 - 1100 h. Each pen of cats had a designated time during the working week (one day between 1130 - 1500 h) when they are moved to a "playroom" for an hour. During this time the cats interacted with staff and were observed for any general health issues, and were weighed. This did not occur during the weekends, when staff were generally only present at the unit from 0800 - 1200 h to clean the pens and replace food.

1.7 Thesis plan

*1.7.1 Chapter 2: Quantification of activity in domestic cats (***Felis catus***) by accelerometry*

The accurate and reliable quantification of OPA of domestic cats and other felids has been challenging due to the need for labour-intensive behavioural observations. However, over the past decade accelerometer technology has advanced considerably and now offers a means of remotely assessing the activity of cats. Chapter 2 validates the use of a small tri-axial accelerometer to quantify the OPA of cats by comparing accelerometer-based and video observations of activity (percentage of time spent active).

Actical® accelerometers (MMAs) have already been used to quantify the activity in small group of cats (n = 3), but the sample size used precluded investigations of inter-individual variation (Lascelles *et al.*, 2008). The specific aim of this chapter is to evaluate the extent of inter-cat variation in the association between concurrent accelerometer and observed activity data. In addition, this chapter investigates the best technique for assessing the activity data; that is, how can the data be assessed in a manner that enhances the accuracy of the activity data without compromising sensitivity? The ultimate goal of this chapter is to validate MMAs for use in Chapter 3 and ensure that these devices and the activity data obtained are analysed appropriately.

*1.7.2 Chapter 3: Factors affecting the overall activity of colony-housed domestic cats (***Felis catus***)*

It is important to understand how underlying environmental and biological factors affect the OPA of cats. Without fully understanding this, accelerometer-based activity data

have the potential to be misinterpreted with incorrect conclusions drawn. Accordingly, this chapter aims to investigate the effects of temperature, rainfall, humidity, time of the day, day of the week, and reproductive state on the MMA activity counts of colony-housed domestic cats, with the overall aim being to improve the validity of accelerometer-based research in cats.

An additional aim of this chapter was to examine the effects of behavioural oestrus on the OPA of female domestic cats. While it is widely believed that cats and other felids are more activity during oestrus, there is no known quantitative data to support this. Chapter 3 aims to compare the activity of individual cats during periods of oestrus and anoestrus to clarify the effects of reproductive cyclicity on the OPA of cats.

1.7.3 Chapter 4: Overall discussion and future directions

Chapter 4 summarises the main findings of the above research chapters and relates this to the use of MMA to monitor the activity of domestic cats. This chapter specifically discusses some of the challenges associated with the use of accelerometry in cats, particularly the significance of extensive inter-cat variation. It also considers how the sampling period needs to be carefully selected to provide an optimal balance between accuracy and the level of sensitivity required. This chapter also discusses the underlying factors affecting the OA of domestic cats and the implications this has on activity-based research in this species. Furthermore, it closely evaluates the potential for accelerometers to improve oestrus detection in cats and other felids. The chapter concludes by identifying and discussing where further research is needed in terms of using accelerometers to assess the activity of domestic cats.

1.7.4 Appendix 2: Overall activity as a predictor of oestrus in domestic cats

This section discusses two of the initial objective of this thesis (Objectives 4 and 5) and outlines the challenges associated with validating an accurate and reliable liquid chromatography-based assay for the detection of salivary oestradiol in cats. It also discusses the effects of behavioural oestrus on the OPA of colony-housed cats and the issues associated with the behavioural detection of oestrus for such studies. Ultimately, this section illustrates the need for examining the association between OPA and reproductive cyclicity using the endocrine detection of oestrus.

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Chapter 2

Quantification of activity in domestic cats (*Felis catus*) by accelerometry

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A published version of this chapter is presented in **Appendix 3**.

Chapter 2: Quantification of activity in domestic cats (*Felis catus*) by accelerometry

2.0 Abstract

*Accelerometers (Actical® 'Mini Mitter' (MMA)) have been used to assess the activity of domestic cats (*Felis catus*), and have been validated against measures of observed activity in this species; however, previous validation trials have had very small sample sizes and have not considered inter-individual variation. The present study aimed to quantify the magnitude of inter-individual variation by validating MMAs against observed activity in a larger number of cats. A total of 288 h of concurrent MMA and observed activity data (percentage of time spent active) were collected from 12 cats and assessed using four defined sampling periods: 1 min, 1 h, 6 h and the 1 h moving average at 10 min intervals. There was a strong linear correlation (*$P < 0.001$*) between MMA and observed activity data for the combined data set for all four sampling periods; however, there was a considerable amount of variation between cats. The MMA and observed activity data of individual cats were also highly correlated over the 10 min (range of Pearson's correlations: 0.65 - 0.98,* $P < 0.001$*), 1 h (0.68 - 1.00,* $P < 0.001$*), 6 h (0.92 - 1.00,* $P < 0.05$*, except for cat 12 where* $P > 0.05$*) and 1 h moving average (0.81 - 0.99,* $P < 0.001$*) sampling intervals. The 1 h moving average at 10 min intervals appeared to be the best sampling interval as it maximised the strength of the correlation while maintaining sufficient information to follow patterns of activity over the 24 h. Ultimately, the current study showed that MMAs can be used to accurately quantify the activity of domestic cats; however, there was a considerable amount of variation between cats, and thus each cat should be considered independently and serve as their own control when assessing any changes in activity levels.*

Key words

Accelerometry, accuracy, feline, motion-detector, oestrus, reliability.

Abbreviations

Actical® Mini Mitter accelerometer (MMA), infra-red (IR)

2.1 Introduction

Monitoring the physical activity of animals can provide an insight into their behaviour, health and wellbeing. Changes in overall activity have been linked to illness, injury, hunting, stress/stereotypical behaviours, energy expenditure and reproductive events such as oestrus (Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002; Henriksen *et al.*, 2005; Lascelles *et al.*, 2007; Brown *et al.*, 2010; At-Taras and Spahr, 2001; McGowan *et al.*, 2007; Kopp *et al.*, 2006; Cornou, 2006; Gerall *et al.*, 1973).

The activity of domestic cats (*Felis catus*) has predominantly been assessed in order to determine the effectiveness of pain therapy for conditions such as osteoporosis, with the overall activity of cats increasing following treatment with non-steroidal anti-inflammatory drugs (NSAIDs) (Lascelles *et al.*, 2007). There may also be an increase or change in the overall activity of domestic cats and other felids during oestrus (Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002), and while many felid species lack overt behavioural indicators of oestrus there are several behaviours that are generally expressed more frequently during oestrus and appear to correlate with an increase in activity (Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002; Henriksen *et al.*, 2005).

A logistical challenge when examining the association between overall activity and oestrus in felids has been the accurate quantification of activity without continuous, labour-intensive and potentially intrusive behavioural observation. However, recent advances in remote sensing technologies, such as accelerometry, have enabled the accurate quantification of activity. Over the past decade accelerometer technology has advanced considerably, with the devices becoming smaller, cheaper and easier to use. Consequently, accelerometers are increasingly used as a tool to quantify activity in a variety of species including humans (Hendelman *et al.*, 2000; Trost *et al.*, 2000; Kumahara *et al.*, 2004; Penpraze *et al.*, 2006; Staudenmayer *et al.*, 2009; Hartel *et al.*, 2011), rhesus monkeys (*Macaca mulatta*) (Papailiou *et al.*, 2008), dairy cows (*Bos taurus*) (At-Taras and Spahr, 2001; McGowan *et al.*, 2007), dogs (*Canis familiaris*) (Hansen *et al.*, 2007; Brown *et al.*, 2010; Yam *et al.*, 2011; Singh, 2013), and cats (Watanabe *et al.*, 2005; Lascelles *et al.*, 2007; Lascelles *et al.*, 2008).

Only one study has thus far specifically validated the use of accelerometers to quantify overall activity in cats (Lascelles *et al.*, 2008). These authors used Actical® 'Mini Mitter' accelerometers (MMA) to monitor the activity of cats, and then compared the results against simultaneously observed activity (distance moved and mobility). While the study found a strong correlation between MMA and observed activity, the small sample size used (n = 3) precluded investigation of inter-individual variation.

The current study aimed to quantify the magnitude of inter-individual variation by validating MMAs against observed activity with a larger number of cats (n = 13).

2.2 Methods

2.2.1 Animal husbandry

Thirteen healthy adult female cats (11 intact and two spayed) aged from 2-13 years (mean ± SD, 7.7 ± 3.6 years) and weighing 2.8 to 4.4 kg (mean ± SD, 3.3 ± 0.5 kg) were used. The cats were housed in three purpose-built colony cages at the Centre for Feline Nutrition, Massey University, Palmerston North, New Zealand (175°38'E, lat. 40°22'S, long.), in mixed-sex groups of seven to 10 animals, of which three to five were studied per cage. They were fed a complete and balanced (Association of American feed control officials (AAFCO), 2009) commercial moist (canned) feline diet (Heinz Wattie's Ltd, Hastings, New Zealand) and had *ad libitum* access to water. The husbandry of the cats complied with Massey University ethics committee protocol number 12/12.

2.2.2 Activity monitoring

Behavioural activity was recorded in real-time at 200 frames/s using a TechView H.264 digital video recorder security camera system (Electus distributions, Auckland, New Zealand) that could detect both visible and infra-red (IR) light, enabling continuous observation of the cats under a natural photoperiod. Behaviours were categorised as either active (locomotion, rolling, rubbing, playing, and climbing) or inactive (sleeping, resting/stationary, urinating/defecating, drinking, eating, grooming and scratching) and continuous duration sampling of the 24 h video footage (analysed in 10 min blocks) was used to determine the amount of time that each cat spent exhibiting active behaviours (i.e. time spent active). Activity was measured concurrently using MMAs (Mini Mitter,

Bend, OR, USA), which each measured 28 mm x 27 mm x 10 mm and weighed 17 g (Figure 2.1). These MMAs used an omnidirectional accelerometer to detect movement in three planes (craniocaudal, mediolateral and vertical). An acceleration force produced a voltage output that was amplified and converted into a digital value that was corrected for the effects of gravity (Lascelles et al., 2008). The values were then summed for a defined period (epoch), resulting in a total activity count for that period. A 15 s epoch was used for this study.

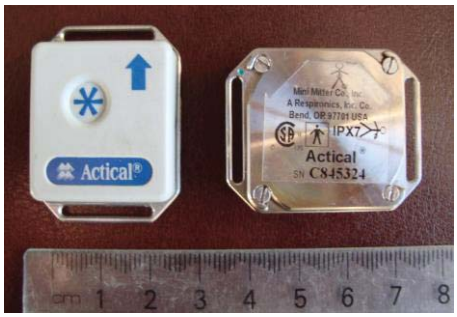

Figure 2.1 Actical® 'Mini Mitter' accelerometers (MMA) were used to monitor the activity of domestic cats.

2.2.3 Experimental design

A unique pattern of reflective tape was placed on each MMA so that the cats could be identified individually under IR light. The MMAs were then attached to the collars of the cats and positioned ventrally (Figure 2.2). The cats were returned to their normal enclosures which were under continuous video surveillance. Accelerometer and video data (observed activity) were collected from each cat over the following 24 h.

Figure 2.2 Actical® 'Mini Mitter' accelerometers (MMA) were attached to the cats' collars and positioned ventrally.

2.2.4 Data evaluation and statistical analysis

The raw activity data were downloaded from the MMAs using an Actireader device (Mini Mitter., Bend, OR, USA), and imported into an excel spreadsheet for analysis. Activity data (total MMA count/15 s) from each cat were summed to provide the total accelerometer counts for four sampling periods: 10 min; 1 h; 6 h; and a calculated 1 h moving average at 10 min intervals (McGowan *et al.*, 2007). The total accelerometer counts for these four sampling periods were then compared against the percentage of time spent active (observed activity) for the corresponding period of video data.

All statistics analyses were conducted using R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria.) and an $\alpha \leq 0.05$. Pearson's correlation coefficients were used to examine the relationship between the MMA counts and the percentage of time spent active for each cat and for the combined data set over the four sampling periods. The correlation coefficients for each of the four sampling periods were then compared to determine which sampling interval yielded the highest correlation. One cat (cat 3) had dramatically higher levels of activity when compared to the other cats, so analyses were conducted both including and excluding the data from this cat. In addition, one of the MMAs (fitted to cat 13) recorded activity counts that were substantially higher than recorded by any of the other devices and were not reflected in the cat's activity in the video data. It was concluded that the device had malfunctioned, so data from this cat were excluded from the study.

2.3 Results

A total of 288 h of concurrent MMA and videoed activity data were collected and are summarised in Table 2.1. The MMAs did not appear to modify the behaviour of the cats, with the behaviour of the cats being comparable to their pen mates that were not fitted with MMAs. The MMA activity data and the percentage of time spent active (videoed/observed activity) for the combined data set were strength correlated for all four sampling intervals ($P < 0.001$) (Table 2.2). Cat 3 had a significant ($P < 0.001$) effect on the strength of the correlation between MMA and observed activity. When the data from this cat were removed from the analysis the correlation coefficients between MMA and observed activity declined by 14.9%, 15.5%, 13.3%, and 12.4% for the 10 min, 1 h, 6 h and 1 h moving average sampling intervals respectively (Table 2.2).

Table 2.1 The percentage of time spent active and total MMA counts of individual cats for the 24 h study period. The data from cat 13 (Trixi) were excluded from the study as the MMA malfunctioned and recorded extremely high activity counts (*).

Cat	Total time spent active (min)/24 h	% time active/24 h	Total MMA count/24 h
1	16.87	1.17	18437
2	18.88	1.31	30369
3	285.08	19.80	421369
4	49.27	3.42	67364
5	38.25	2.66	81896
6	37.12	2.58	63883
7	27.03	1.88	68159
8	63.97	4.44	128252
9	20.35	1.41	49395
10	47.68	3.31	41804
11	37.92	2.63	79507
12	26.57	1.85	101271
-	-	-	4639737*

Table 2.2 Pearson correlation coefficients between the total MMA count and percentage of time spent active for individual cats and all cats combined over four different sampling intervals. All R^2 values are significant ($P < 0.001$) unless indicated otherwise (**P < 0.01, *P < 0.05, †P > 0.05).

Sampling interval	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6	Cat 7	Cat 8	Cat 9	Cat 10	Cat 11	Cat 12	All cats	All cats (Excluding Cat 3)
10 min	0.92	0.75	0.98	0.94	0.89	0.89	0.78	0.93	0.89	0.95	0.95	0.65	0.94	0.80
1 h	0.96	0.80	1.0	0.96	0.92	0.88	0.93	0.98	0.97	0.97	0.97	0.68	0.97	0.82
6 h	1.0	0.96*	1.0	0.99	1.0	0.92	0.99**	1.0**	1.0**	0.99*	1.0**	0.92†	0.98	0.85
1 h moving average at 10 min intervals	0.98	0.84	0.99	0.97	0.96	0.93	0.94	0.97	0.97	0.98	0.98	0.81	0.97	0.85

The MMA and observed activity data of individual cats were also highly correlated over the 10 min ($P < 0.001$), 1 h ($P < 0.001$), 6 h ($P < 0.05$, except for cat 12 where $P > 0.05$) and 1 h moving average ($P < 0.001$) sampling intervals. The correlation coefficients between the MMA and observed activity data of individual cats for the four sampling periods are also presented in Table 2.2. The R^2 values between the MMA and observed activity of individual cats ranged from 0.65 - 0.98 at 10 min intervals, 0.68 - 1.0 at 1 h intervals, 0.92 - 1.0 at 6 h intervals and 0.81 - 0.99 at 1 h moving average at 10 min intervals. The R^2 values for 10 min and 1 h sampling intervals did not differ; however, the R^2 values for the 6 h sampling interval were higher than obtained using the 10 min ($P < 0.05$) and 1 h sampling intervals ($P < 0.05$) (Figure 2.3). The correlation coefficients obtained using the 1 h moving average sampling period did not differ from those obtained using the 10 min, 1 h and 6 h sampling periods (Figure 2.3).

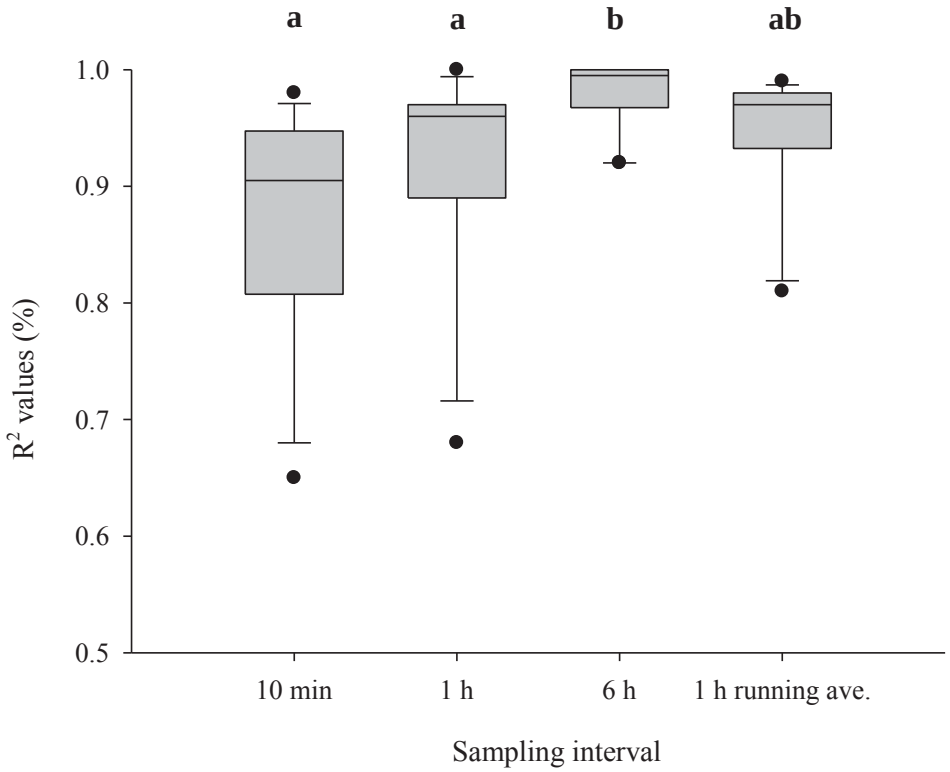

Figure 2.3 Pearson correlation coefficients between the total MMA count and percentage of time spent active for individual cats over four different sampling intervals (10 min, 1 h, 6 h and 1 h running average at 10 min intervals). Different letters above each sampling interval plot indicate statistical significance ($P < 0.05$).

Both the 6 h and 1 h moving average sampling periods resulted in the highest correlation coefficients between the MMA and observed activity data of individual cats (Table 2.2). Of these the 1 h moving average provided the greatest sensitivity and was more responsive to smaller bouts of activity (Figure 2.4) and was used in all further analyses. The 1 h moving average of the MMA and observed activity profiles of each cat for the entire 24 h study period are illustrated in Appendix 1.

The linear regression of the MMA and observed activity data of each cat over a 1 h moving average sampling period are given in Figure 2.5, with the associated correlation coefficients presented in Table 2.2. The slope of the regression lines differed considerably between cats, and there was also variation in the position in the y-intercepts (Figure 2.5), with the regression line of cat 12 for instance, exhibiting a higher y-intercept than the other cats due to more frequent grooming and scratching.

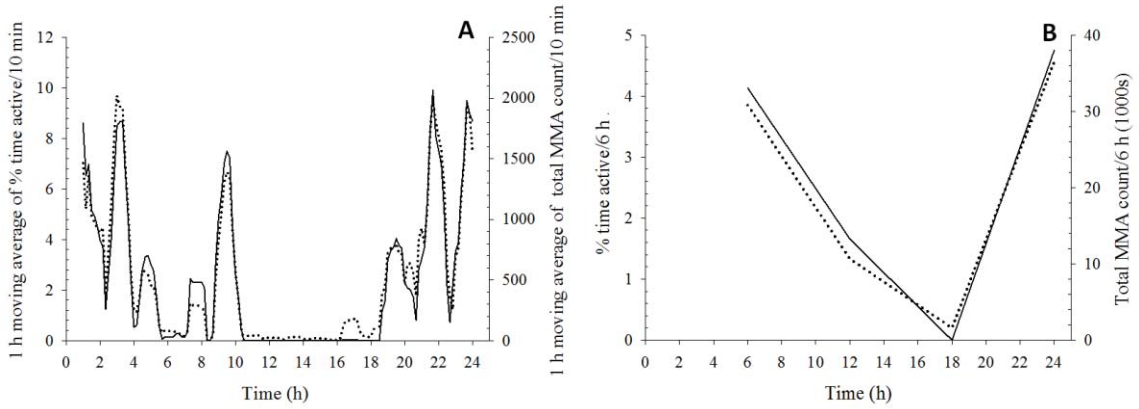

Figure 2.4 (A) 1-h running average and (B) 6 h sampling period plots of the total MMA counts (·····) and percentage of time spent active (——) for a single cat (cat 11) over 24 h.

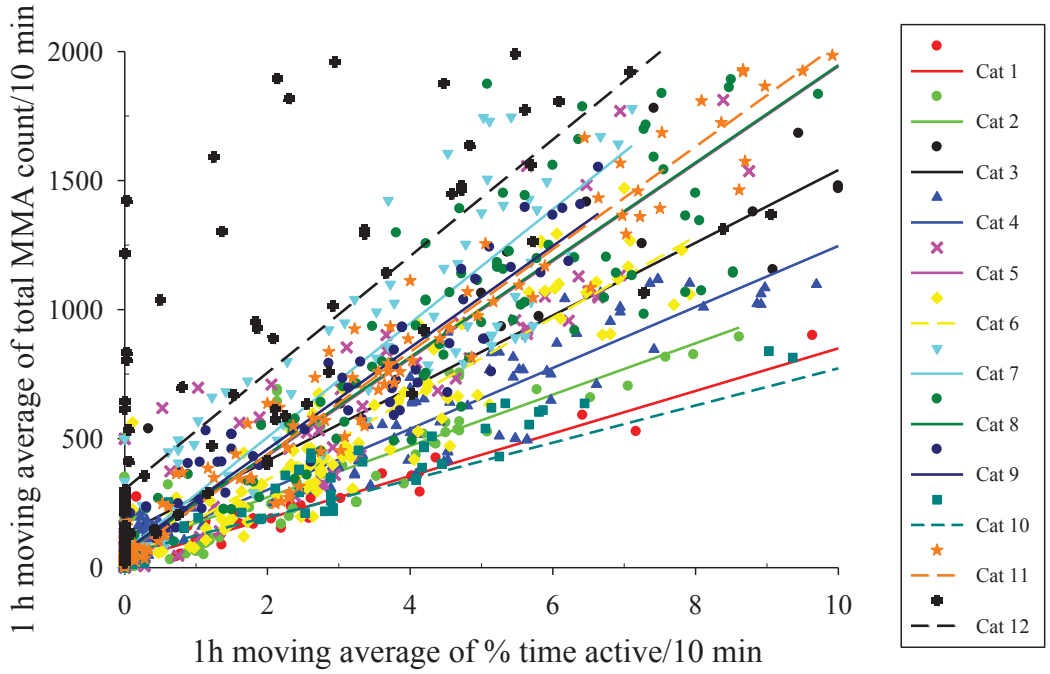

Figure 2.5 The linear relationships between the MMA and observed activity (percentage of time spent active) of individual cats determined using a 1-h running average at 10 min sampling intervals. The correlation coefficients associated with these regressions are presented in Table 2.2.

2.4 Discussion

This study validated the use of Actical® accelerometers as a tool for remotely quantifying activity in a large group of domestic cats, with correlation between MMA and observed activity (defined as the percentage of time spent active) being significant for all cats except cat 12 (6 h sampling interval. This result was consistent with the

findings of previous studies that used much smaller numbers of cats (Watanabe *et al.*, 2005; Lascelles *et al.*, 2008). Accelerometers have also been validated against observed activity in a number of other species, including dogs (Yamada and Tokuriki, 2000; Hansen *et al.*, 2007; Yam *et al.*, 2011; Preston *et al.*, 2012), rhesus monkeys (Papailiou *et al.*, 2008), humans (Trost et al., 2000; Penpraze *et al.*, 2006) and dairy cows (McGowan *et al.*, 2007), with varying levels of accuracy.

The correlation coefficients between the MMA-recorded and observed activity data of individual cats in the current study were considerably higher than previously documented in humans and monkeys (i.e. the MMAs provided a more accurate representation of observed activity), but were comparable to those reported in dogs and cattle (Yamada and Tokuriki, 2000; Penpraze *et al.*, 2006; Hansen *et al.*, 2007; Papailiou *et al.*, 2008; Preston *et al.*, 2012). This difference in the accuracy of activity assessment or measurement appears to be due to humans and monkeys exhibiting a much wider variety of 'active' behaviours, many of which are highly variable in intensity and often not involving locomotion. In quadrupeds, activity is much more consistent and predominantly locomotor-based, and hence, associations between accelerometer-recorded and observed activity data are generally stronger (Penpraze *et al.*, 2006; Hansen *et al.*, 2007; Lascelles *et al.*, 2008). Nevertheless, accelerometers appear to be an effective tool for remotely quantifying activity in most species studied to date, and provide an accurate representation of physical activity in domestic cats, at least within individual cats.

Despite the strong correlation between the MMA and video-observed activity of individual cats there was considerable variation between cats. A similar pattern has been reported for dogs, where differing body size appeared to account for some of this variation (Preston *et al.*, 2012). The causes of inter-cat variation observed here are unclear; however, the cats used in the current study were similar in body size, making this as an unlikely source of variation. Some of the disparity observed between the dog breeds may also reflect differences in their environments or daily routines (Dow et al., 2009; Preston et al., 2012). Again, this is an explanation that does not apply here because the cats experienced the same routine and almost identical environmental conditions. The variation in MMA data observed between cats was more likely due to behavioural differences associated with dominance rank or a consequence of how we defined and assessed activity.

Subjectively classifying behaviours as either 'active' or 'inactive' may have led to some inconsistencies between cats, as certain 'inactive' behaviours may have activated the MMAs. Lascelles *et al.* (2008) reported that grooming and scratching by cats caused high activity counts without affecting observed activity, with the impact being greater on collar-mounted MMAs than those attached via a harness. Both of these behaviours activated the MMAs in our study, so the cats that groomed and scratched more frequently exhibited a poorer correlation between MMA and observed activity data (particularly cat 12). In fact, these behaviours appeared to be the main source of disparity between the MMA and observed activity data of individual cats.

However, it is important to note that fleas are not present in the cat colony used for this study, so the levels of scratching we observed were likely to be lower than may be expected in free-living cats. Nevertheless, intense bouts of grooming or scratching lead to excessively high MMA activity counts relative to observed activity in most cats, and had a considerable influence on the position of the y-intercept and slope of the linear regression line between the MMA and observed activity data of individual cats. The effects of grooming and scratching on the variability of MMAs among cats may be augmented by differences in collar tightness. Accelerometers attached to loose collars might respond more strongly to grooming, scratching, and general activity (i.e. locomotion), but we found no evidence of this in the study.

Discrepancies associated with grooming and scratching can be mitigated in part by increasing the sampling period, with the strength of the correlation between the MMA and observed activity data of individual cats consistently improved by an increase in sampling period. However, the accumulation of activity data over a longer period removed a considerable amount of detail from the activity data (see Figure 2.5).

Maximising correlations between MMA data and observed activity has been achieved for dairy cows by using a moving average and this enabled the accurate identification of short bouts of activity and even specific behaviours (McGowan *et al.*, 2007). In the current study, the use of a 1 h moving average at 10 min intervals consistently improved the correlation between the MMA and observed activity data of individual cats whilst maintaining enough information to provide a detailed pattern of activity over 24 h.

Grooming and scratching were the main source of disparity between MMA-measured and observed activity data of individual cats. Static behaviours such as grooming and

scratching were excluded from our definition of activity; however, it may be beneficial for MMAs to detect grooming, and for grooming to be regarded as part of the suite of active behaviours. Treating these behaviours as activity certainly appears to be useful in studies of the effects of disease on activity. For example, Hansen *et al.* (2007) used MMAs to assess the effects of osteoporosis and associated drug therapy on the activity of dogs. They focused on locomotion when defining active behaviours, but indicated that scratching and shaking typically indicated a lack of joint pain and thus were relevant to this field of study. While categorising specific behaviours into two discrete groups, active or inactive, is a useful technique for validating MMA devices, it may be less appropriate for capturing all of the behaviours of relevance to a specific research question.

Our ultimate goal in validating the MMAs is to use them to examine the link between activity and oestrus in cats. Oestrus is highly variable in cats lasting from one to 23 days (Bell, 2009), so the resolution of the data required to accurately predict it needs to be considered as this determines whether a long sample interval or a moving average should be used when processing the MMA data. While the most accurate results were obtained in the current 24 h study using a 1 h moving average at 10 min intervals, our ultimate goal of accurately predicting oestrous in cats may require a longer sampling period.

2.5 Conclusions

In conclusion, MMAs provide a highly accurate representation of observed activity within an individual cat; however, the association between MMA and observed activity data differs considerably between cats. Thus each cat should be considered as its own control when examining changes in activity levels using MMAs. Grooming and scratching led to some discrepancy between the MMA and observed activity of individual cats; however, the effects of these behaviours were best minimised by analysing the activity data using a 1 h moving average at 10 min intervals, which enhanced the strength of the correlation between the MMA and observed activity individual cats without removing too much information/detail from the activity data.

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Chapter 3

Factors affecting the overall activity of colony-housed domestic cats

Chapter 3: Factors affecting activity patterns in colony-housed domestic cats (*Felis catus*)

3.0 Abstract

*Actical® 'MiniMitter' accelerometers (MMAs) have been validated for monitoring the overall physical activity (OPA) of individual domestic cats (*Felis catus*) and have now been used to study the behaviour, daily patterns of activity, efficacy of drug therapy for osteoarthritis, and monitor the effects of specialised feeding regimes aimed at promoting activity in overweight cats. Despite this, there is a paucity of published research on how environmental and biological factors affect OPA in cats. The present study aimed to investigate the effects of day of the week (human presence), temperature, humidity, rainfall and reproductive state (anoestrus or oestrus) on the OPA of colony-housed cats. A total of 414 days of activity data were collected from 10 entire female cats, which contained 135 and 279 days of anoestrous and oestrous activity data respectively. These activity data were then compared against concurrent temperature, humidity and rainfall data. Day of the week (reduced staff presence on the weekend) did not appear to influence the OPA of the cats (*$P = 0.214$*). This could be a consequence of the group housing of the cats, with intra-group interactions outweighing human interactions. On the other hand, the OPA of the cats was significantly affected by temperature (*$P = 0.014$*), rainfall (*$P = 0.006$*) and humidity (*$P < 0.05$*). A regression analysis of the mean activity values obtained from Tukey's post hoc tests showed that the activity of the cats decreased with increasing temperature, rainfall and humidity (*$P = 0.015$*, $P = 0.010$, and $P = 0.008$ respectively). Interestingly, the response of individual cats to these factors varied considerably which, in turn, would make it difficult to account for these factors in a research context. In contrast, reproductive state consistently affected the activity of the cats (*$P < 0.001$*), with this study providing the first quantitative support for anecdotal observations that cats are typically more active during oestrus. It would be worthwhile, however, investigating the effects of reproductive state on the OPA of cats using a more accurate method of oestrus detection (e.g. endocrine), since the behavioural detection of oestrus can be challenging in cats.*

Key words

Actical, accelerometer, humidity, inter-individual variation, obesity, oestrus, rainfall, temperature.

Abbreviations

Overall physical activity (OPA), Actical® 'MiniMitter' accelerometer (MMA), osteoarthritis (OA), non-steroidal anti-inflammatory drugs (NSAIDs), Massey University Animal Ethics Committee (MUAEC), analysis of variance (ANOVA), food anticipatory activity (FAA).

3.1 Introduction

Monitoring the overall physical activity (OPA) of animals can provide an extremely sensitive insight into their health, wellbeing, behaviour and physiology (Mellen, 1993; Lascelles *et al.*, 2001; Watanabe *et al.*, 2005; Lascelles *et al.*, 2007; McGowan *et al.*, 2007; Papailiou *et al.*, 2008; Brown *et al.*, 2010; Rothwell *et al.*, 2011; Piccione *et al.*, 2013; Wilson *et al.*, 2013; Alexander *et al.*, 2014; Deng *et al.*, 2014; Jones *et al.*, 2014). However, the assessment and quantification of an animal's OPA has proved to be difficult due to the need for labour-intensive behavioural observation. Recent advances in remote sensing technologies such as accelerometry and pedometry have provided researchers with an automated method for continuously assessing OPA, and have thus led to an increase in activity-based research over the past decade.

Accelerometry has now been used to accurately monitor the activity of a number of species, including humans (Hendelman *et al.*, 2000; Trost *et al.*, 2000; Penpraze *et al.*, 2006; Staudenmayer *et al.*, 2009; Hartel *et al.*, 2011), rhesus monkeys (*Macaca mulatta*) (Sullivan *et al.*, 2006; Hunnell *et al.*, 2007; Papailiou *et al.*, 2008), dairy cows (*Bos taurus*) (At-Taras and Spahr, 2001; McGowan *et al.*, 2007), dogs (*Canis familiaris*) (Yamada and Tokuriki, 2000; Hansen *et al.*, 2007; Brown *et al.*, 2010; Yam *et al.*, 2011), Eurasian badgers (*Meles meles*) (McClune *et al.*, 2014), African elephants (*Loxodonta africana*) (Rothwell *et al.*, 2011), koala (*Phascolarctos cinereus*) (Takahashi *et al.*, 2009), cheetah (Grünewälder *et al.*, 2012; Wilson *et al.*, 2013), and domestic cats (Watanabe *et al.*, 2005; Lascelles *et al.*, 2007; Lascelles *et al.*, 2008; Andrews *et al.*, 2015).

Collar-mounted Actical® (Mini Mitter, Bend, OR, USA) accelerometers (MMAs) have been shown to provide an accurate representation of observed activity (e.g. percentage

of time spent active or distance travelled) in cats, although the association between accelerometer-based and observed activity can differ considerably between cats (Chapter 2; Lascelles *et al.*, 2008; Andrews *et al.*, 2015). This inter-cat variation was possibly a consequence of 'inactive' behaviours such as grooming or scratching activity being recorded as movement by the MMAs (Chapter 2; Andrews *et al.*, 2015). The intensity and frequency at which these behaviours are displayed has been shown to affect the relationship between the MMA activity counts and observed activity of individual cats (Chapter 2; Andrews *et al.*, 2015). Thus, while accelerometry offers an accurate and reliable method for assessing the OPA of domestic cats, care is needed when comparing the MMA activity data of different cats.

The application of accelerometry in cats has largely been restricted to the validation of accelerometers against observed activity and the assessment of daily activity patterns (Watanabe *et al.*, 2005; Lascelles *et al.*, 2008; Piccione *et al.*, 2013; Andrews *et al.*, 2015). However, accelerometry has been used to remotely evaluate the efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) treatment for osteoarthritis (OA) in cats (Lascelles *et al.*, 2007). Lascelles *et al.* (2007) found that the treatment of osteoarthritic cats with meloxicam lead to an increase in overall activity, a result which was interpreted as a reduction in the pain associated with OA. MMAs have also been used to examine the effects of dietary water content and feeding frequency on the OPA of cats, with the results to date indicating that the OPA of cats can be increased by adding water to a dry kibble diet to increase its moisture content and providing smaller, more frequent meals (Alexander *et al.*, 2014; Deng *et al.*, 2014). This finding could have a particularly important role in combatting the growing obesity problem in domestic cats, a condition that has been linked to a number of other diseases in cats (e.g. diabetes mellitus, cardiorespiratory complications, urinary and reproductive disorders, and orthopaedic diseases) (German, 2006; Alexander *et al.*, 2014; Deng *et al.*, 2014).

Despite the realised and potential applications of accelerometry in cats, there has been no known published research on how environmental and biological factors affect the OPA of cats. Environmental factors such as temperature, rainfall and photoperiod have all been shown to affect the behaviour and OPA of other carnivores (Piccione *et al.*, 2013). In addition, biological factors such as reproductive state have been found to influence the activity patterns of several mammalian species including dairy cows (*Bos taurus*) (At-Taras and Spahr, 2001), mice (*Mus musculus*) (Kopp *et al.*, 2006), rats

(*Rattus norvegicus*) (Gerall *et al.*, 1973), and pigs (*Sus scrofa*) (Cornou, 2006). A thorough understanding of the underlying factors, both biotic and abiotic, affecting the OPA of cats is crucial for the accurate interpretation of activity data, and will help to ensure that the results of accelerometer-based studies are not influenced by these confounding variables.

The present study aimed to investigate the effects of abiotic factors such as day of the week, temperature, rainfall, and humidity on the OPA of domestic cats in a colony, and to compare the activity of domestic cats during periods of anoestrus and oestrus to determine the effects of reproductive state (anoestrus or oestrus) on the OPA of cats.

3.2 Methods

3.2.1 Animal husbandry

Ten clinically healthy, intact female cats from 1.8 to 10.1 years of age (mean ± SD, 5.6 ± 3.0 years) that weighed 2.3 to 4.0 kg (mean ± SD, 3.2 ± 0.6 kg) were used. The cats were housed in five purpose-built, outdoor colony pens (with roofing sheltering two thirds of the pen) at the Massey University Centre for Feline Nutrition, Palmerston North, New Zealand (175°38'E, lat. 40°22'S, long.), in mixed-sex groups of seven to 10 cats, of which one to five were studied per pen. They were fed *ad libitum* amounts of a complete and balanced (AAFCO, 2009) commercial moist (canned) feline diet (Heinz Wattie's Ltd, Hastings, New Zealand) once daily (between 1000 and 1100 h) and had access to water at all times. Each cage of cats was allowed 1 h in a play room per week (one day between 1130 - 1500 h) where they were observed for any general health issues and weighed. The routines of the cats were fairly consistent during the week, although staff were only present for cleaning and feeding purposes on the weekends. The husbandry of the cats complied with the Animal Welfare (Cats) Code of Welfare (Anonymous, 2007) and Massey University Animal Ethics Committee (MUAEC) protocol number 12/12.

3.2.2 Activity assessment

Activity was assessed using MMAs that measured 28 mm x 27 mm x 10 mm and weighed 17 g. These MMAs used an omnidirectional accelerometer to detect movement in three planes (craniocaudal, mediolateral and vertical). An acceleration force

produced a voltage output that was amplified and converted into a digital value that was corrected for the effects of gravity (Lascelles *et al.*, 2008). The activity count values were then summed for a defined period (epoch), resulting in a total activity count for that period. Approximately 64,800 recordings (epochs) could be made before memory capacity was reached, thus the epoch used affected the duration over which activity could be continuously monitored. A maximum epoch length of 60 s was selected here to maximise the duration over which activity data could be continuously collected (approximately 45 days). The other two smaller epochs, 15 s and 30 s, can only monitor activity for 12 and 23 days respectively.

3.2.3 Experimental design

Actical® accelerometers were attached to the collars of 10 cats and positioned ventrally. Activity data were then collected for a total of 39 - 43 days between the 31 October and 12 December 2013. Cat 4 (Mandy) was removed from the study prematurely due to the need for dental surgery, thus the activity of this cat was only assessed for 34 days. The behaviour of the cats was also monitored daily to determine reproductive state. This trial was conducted alongside a study examining the association between activity and oestrus in domestic cats, which involved daily saliva samples from each cat (see Appendix 2). The times when saliva samples were collected each day were recorded to account for any effect this had on the activity of the cats.

3.2.4 Data evaluation and statistical analysis

The raw activity data were downloaded from the MMAs using an Actireader® device (Mini Mitter., Bend, OR, USA), and imported into an excel spreadsheet. The data (total MMA count/1 min) were summed to provide the total MMA count per day. Previous research (Chapter 2) has shown a highly variable association between the MMA and observed activity of individual cats (Chapter 2; Andrews *et al.*, 2015). To reduce this variation, the daily activity counts were converted into the proportional difference from the mean total daily activity count for each cat over the entire study period (proportional activity data). This enabled the MMA activity data of the cats to be pooled for analysis.

The pooled proportional activity data were compared against concurrent temperature, humidity, and precipitation data obtained from MetService (Meteorological Service of New Zealand Ltd., Wellington, New Zealand). The effects of day (Monday to Sunday)

and reproductive state on the OPA of the cats were also examined. All statistical analyses were conducted using R Studio version 0.98.1091 (R Foundation for Statistical Computing, Vienna, Austria) and an $\alpha \leq 0.05$ (unless stated otherwise).

This study was conducted over a period of seasonal change (spring to summer) so day-length and temperature were both expected to increase as the study progressed. This was adjusted for by calculating the difference between the observed and expected (calculated from a linear regression analysis of temperature and day of the trial) temperatures for each day (i.e. residual temperature). Regression analyses were also conducted to determine if humidity and rainfall differed over the study.

Multiple two-way analyses of variance (ANOVA) and Tukey's post hoc tests were used to determine the effects of temperature, humidity, day of the week, and reproductive state on the OPA of the cats. Regression analyses were conducted using the mean activity data obtained from the Tukey's post hoc tests on temperature, humidity and rainfall to determine the overall effect of these factors on the OPA of the cats. All ANOVA and Tukey's post hoc tests were repeated for each of the cats individually using an $\alpha \leq 0.005$, as recommended by the Bonferroni correction method.

3.3 Results

A total of 414 days of activity data were collected from 10 cats, which contained 135 and 279 days of anoestrous and oestrous activity data respectively (Table 3.1). The activity profiles of the cats for the duration of the study are presented in Appendix 3. As expected, there was considerable inter-individual variation in the MMA activity counts of the cats ($P < 0.001$, Figure 3.1), and hence the proportional activity data were used for all subsequent analysis.

Table 3.1 A summary of the MMA activity data (total MMA count per day) collected from the cats.

Cat No.	Cat name	Age in years at start of trial	Average weight for trial (g)	Total duration of monitoring (days)	% of time in behavioural oestrus	Ave. daily activity count	Ave. daily activity count (oestrus)	Ave. daily activity count (anoestrus)
1	Blur	1.84	2255	43	100.0	82554	82554	N/A
2	Chika	4.04	2680	41	73.2	93343	105639	55225
3	Lana	6.59	3970	43	34.9	54566	57497	52829
4	Mandy	6.78	3740	34	79.4	469418	499129	330767
5	Milly	6.78	2820	43	37.2	154396	224164	113052
6	Nena	8.74	3230	43	86.0	192309	187031	232421
7	Nova	1.82	3140	43	37.2	53709	59997	49598
8	Opra	7.76	3469	39	35.9	102253	123742	88822
9	Poket	1.87	2920	42	100.0	476461	476461	N/A
10	Zeal	10.10	3960	43	74.4	13572	109033	91393
Total	-	-	-	414	67.4	1783939	1925246	1014108

Day of the week did not affected the OPA of the cats (P = 0.214), so it was removed from all subsequent analyses. Average daily temperature varied greatly over the study (mean ± SD, range: 15.9 ± 1.9˚C, 11.5 - 20.2˚C), with temperature following a highly cyclical pattern that, as expected, increased significantly over the duration of the study ($P < 0.001$). Humidity and rainfall also varied throughout the study (mean ± SD, range: 77.1 ± 5.6%, 70.0 - 89.8% and 2.4 ± 5.4 mm, 0 - 26.4 mm, respectively), but this variation was independent of date (P = 0.051 and P = 0.639 respectively). Despite this, the OPA of the cats was significantly affected by temperature (P = 0.014), humidity (P = 0.050), rainfall (P = 0.006), and reproductive state ($P < 0.001$) (Table 3.2).

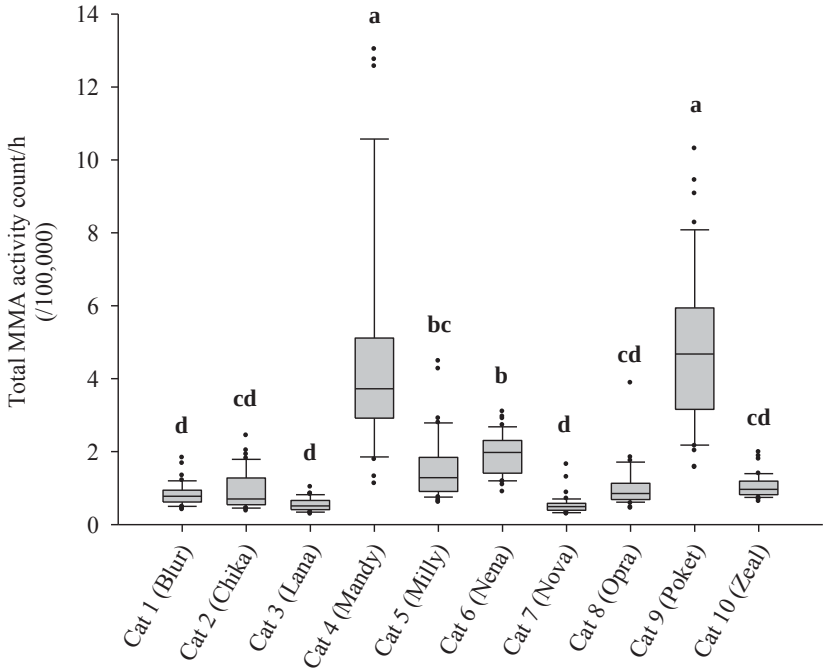

Figure 3.1 Variation in the total daily activity counts for each cat. Different letters above each plot indicate statistical significance ($P < 0.05$).

Table 3.2 Results of an analysis of variance (ANOVA) examining the effects of cat, day of the week, residual temperature, rainfall and humidity on the pooled proportional activity data of the cats.

ANOVA	Degrees of freedom	F-value	Pr (>F)
Cat	9	0.00	1.000
Residual temperature	1	6.14	0.014*
Rainfall	1	7.65	0.006**
Humidity	1	3.88	0.050*
Oestrus	1	40.67	<0.001***
Cat - Residual temperature	9	4.32	<0.001***
Cat – Humidity	9	2.27	0.018*
Cat – Oestrus	7	4.01	<0.001***
Residual temp – Oestrus	1	5.73	0.017*
Cat – Humidity – Oestrus	7	2.42	0.020*
Rain – Humid – Oestrus	1	9.32	0.003**
Residuals	281	-	-

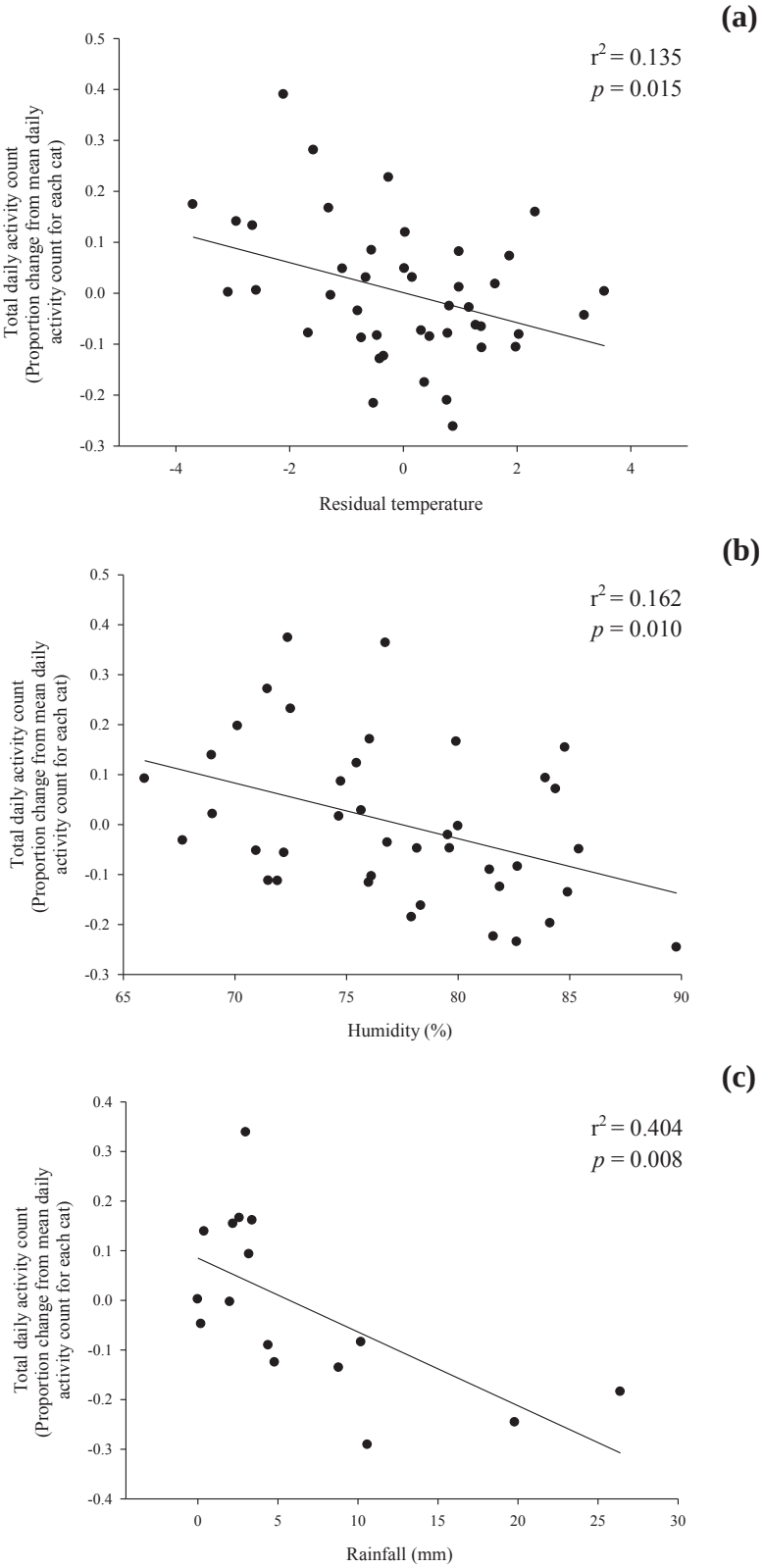

Figure 3.2 Linear regression analyses of the mean activity counts obtained from the Tukey's post hoc tests for (a) temperature, (b) humidity, and (c) rainfall.

Interestingly, a Tukey's post hoc test examining the effects of temperature on activity failed to indicate precisely where the differences occurred (Figure 3.2a), possibly because cats were inconsistent in their response to temperature ($P < 0.001$; Figure 3.3). Regression analysis of the mean values obtained from the Tukey's post hoc test indicated a significant decrease in activity with increasing temperature ($P < 0.15$; Figure 3.2a).

Analysis of individual cats, however, only showed a significant effect of temperature on the OPA of two of the 10 cats when using a Bonferroni corrected α of $P < 0.005$. Without a Bonferroni correction (i.e. α of 0.05), the activity of four of the 10 cats was affected by temperature. Three of these cats (cats 4, 9 and 10) exhibited a decrease in activity with increasing temperature ($P < 0.001$, 0.020 and 0.034 respectively; Figure 3.3), while the OPA of cat 6 increased with rising temperature ($P = 0.001$; Figure 3.3). There was also a trend, albeit non-significant, for the OPA of two (cats 7 and 8) of the remaining six cats to increase with increasing temperature, with one cat (cat 2) appearing to exhibited higher levels of activity at higher temperatures. There was no evident association between OPA and temperature for the last three cats (cats 1, 3 and 5).

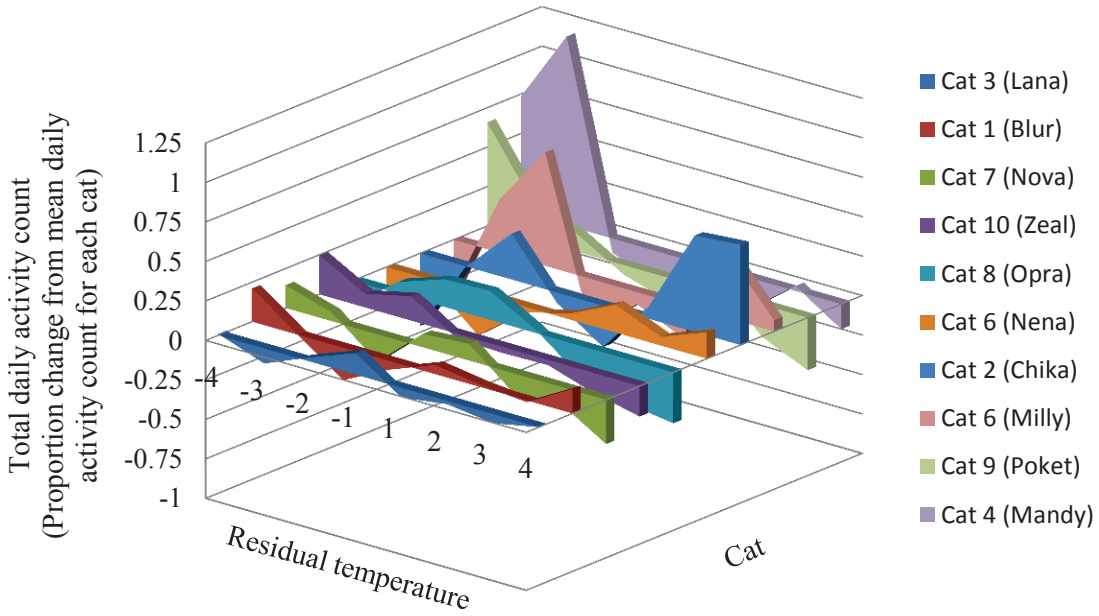

Figure 3.3 The change in activity of individual cats in response to differing average daily temperature (residual temperatures (˚C) calculated from a linear regression analysis of temperature over time). The order of cats has been arranged to provide an optimal view of each cat's activity data.

The effect of humidity on the OPA of the cats appeared to be more consistent than temperature, with higher humidity levels (≥ 85.0%) being associated with lower activity counts (Figures 3.2b and 3.4). However, the Tukey's post hoc test failed to identify how humidity affected the activity of the cats ($P > 0.05$ of all pairwise comparison), though there was a general trend for activity to be lower at higher humidity levels (Figure 3.2b). The individual assessment of each cat's activity data revealed that humidity only significantly affected the OPA of one cat (cat 4) ($P = 0.005$), and showed a strong trend for a further two cats (cats 7 and 9) ($P = 0.008$ and 0.041 respectively). For all three of these cats OPA decreased with increasing humidity (Figure 3.4). This trend also is consistent for four of the seven remaining cats (cats 1, 3, 5 and 6), although it was not significant (Figure 3.4).

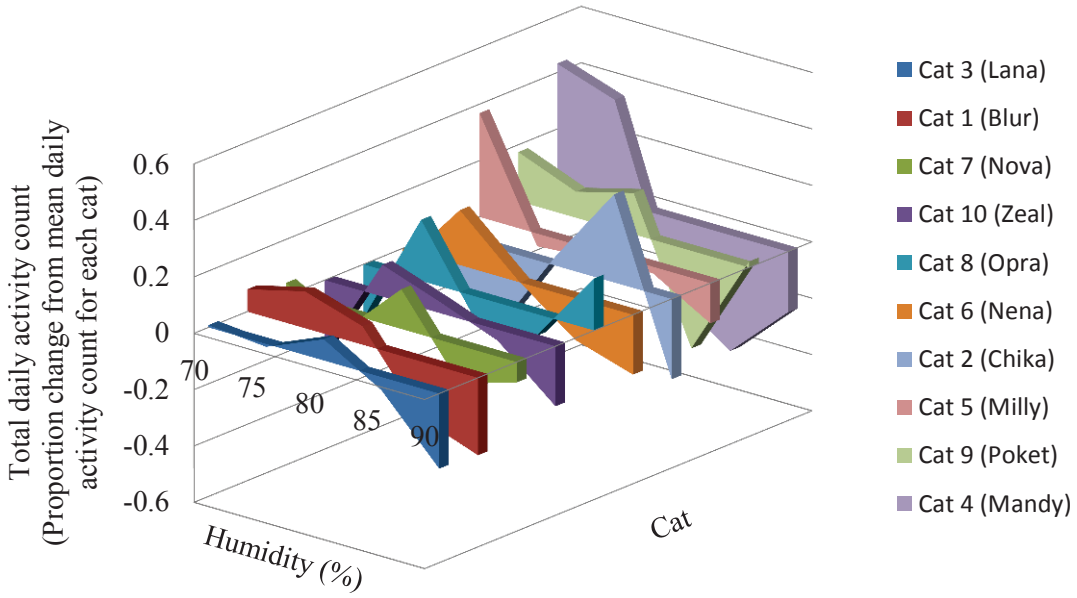

Figure 3.4 The change in activity of individual cats in response to differing average daily humidity (%). The order of cats has been arranged to provide an optimal view of each cat's activity data.

There was also a general trend for the OPA of the cats to decrease with increasing daily rainfall (Figure 3.2c), although there was considerable variation around this trend. The activity of most of the cats appeared to be lower on days when rainfall was greater than 8 to 10 mm, but the activity of the cats differed greatly when the total daily rainfall was less than this (Figures 3.2c and 3.5). Interestingly, daily rainfall only significantly

affected the activity of two cats (cats 4 and 6), suggesting that rainfall had a minimal effect on the activity of most of the cats ($P < 0.005$; Figure 3.5).

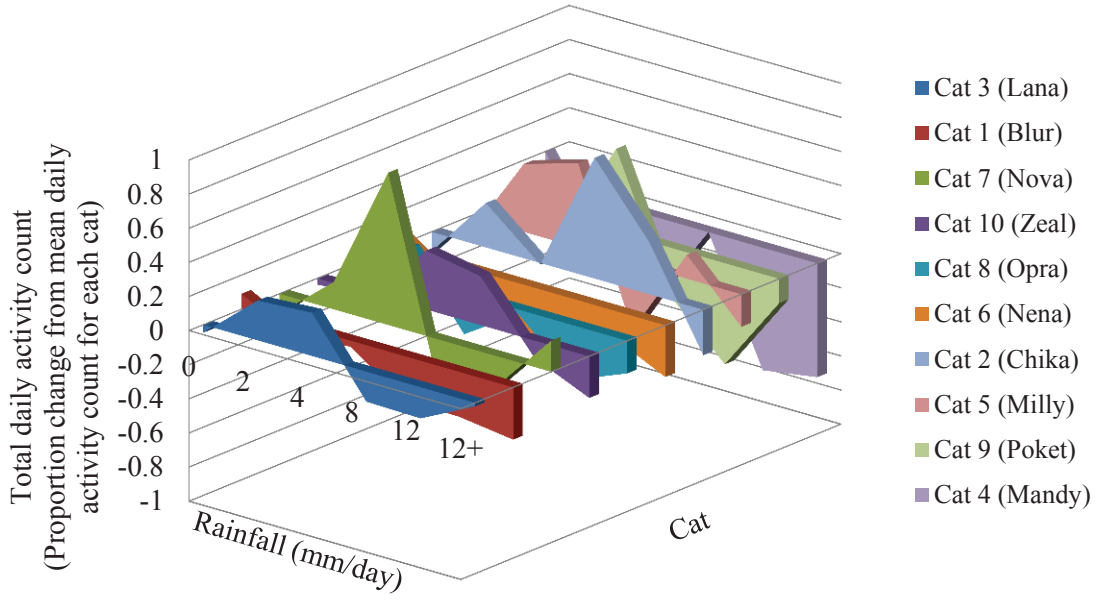

Figure 3.5 The change in activity of individual cats in response to differing total daily rainfall (mm). The order of cats has been arranged to provide an optimal view of each cat's activity data.

In contrast, reproductive state had a strong influence on the OPA of the cats ($P < 0.001$; Table 3.2), with a Tukey's post hoc test indicating increased activity during oestrus ($P < 0.001$). The individual assessment of the cat's activity data yielded consistent results, with seven of the eight cats for which both oestral and anoestral activity data were available (cats 1 and 9 appeared to be in oestrus for the entire study) exhibiting a higher activity count during oestrus (Figure 3.6). This association was only significant to the Bonferroni corrected α of 0.005 for two of these cats (Cats 2 ($P = 0.005$) and 5 ($P < 0.001$)); however, if an α of 0.05 was accepted then the relationship was significant for six of the eight cats (Figure 3.6). One cat (cat 6) exhibited a marked decrease in activity during oestrus ($P = 0.016$; Figure 3.6), which could explain why the ANOVA results suggested that the cats responded differently to oestrus ($P < 0.001$; Table 3.2). With this one exception, there was an obvious and consistent trend for activity to increase during oestrus.

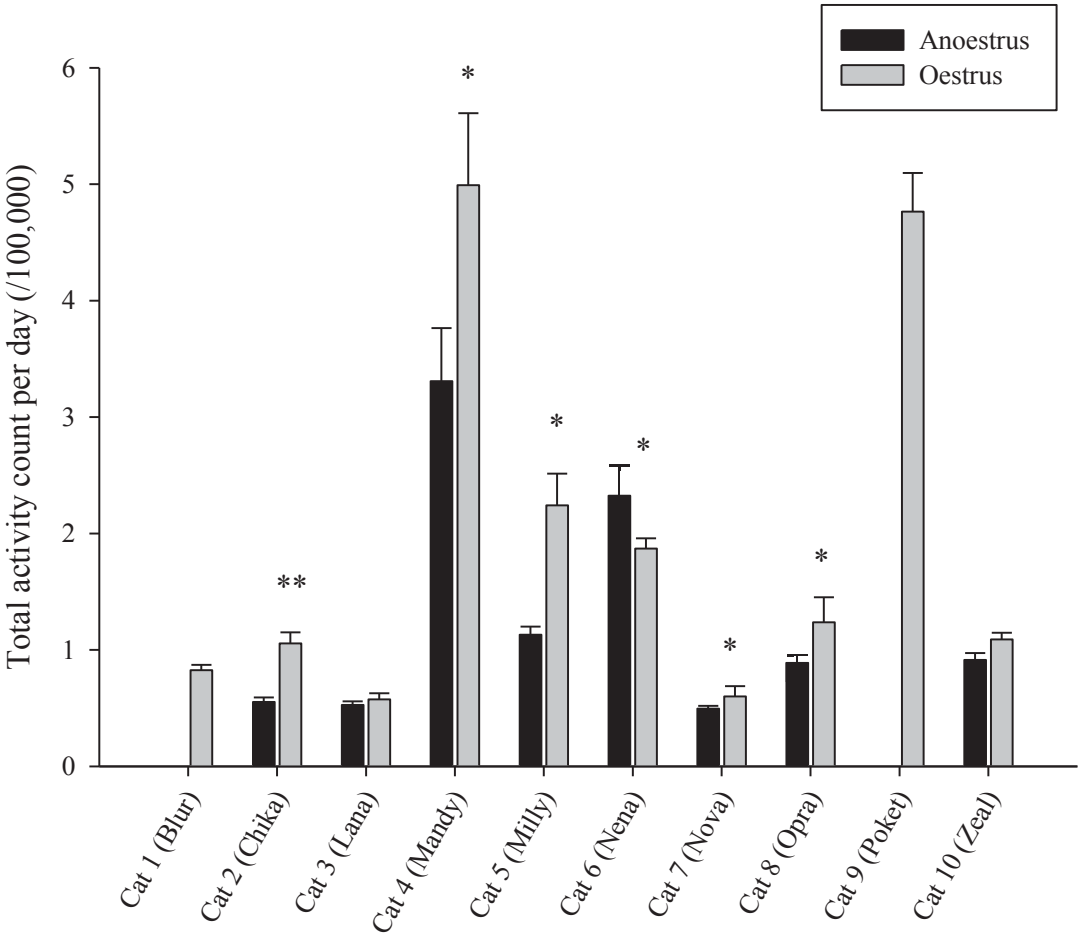

Figure 3.6 Total activity counts of each cat during anoestrus (black) and behavioural oestrus (grey) (* $P < 0.05$; ** $P < 0.005$) No anoestrus periods were detected in cats 1 and 9, thus no comparison could be made.

This association remains when the effects of environmental factors such as temperature, humidity and rainfall are considered (Figure 3.7). Indeed, the range of OPA levels associated with different temperatures, humidity levels and total daily rainfall were substantially lower during anoestrus (Figure 3.7a, c, e). There was also considerably more variability in the daily activity counts of the cats during oestrus, with the highest MMA activity counts being recorded during periods of behavioural oestrus (Figure 3.7b, d, f).

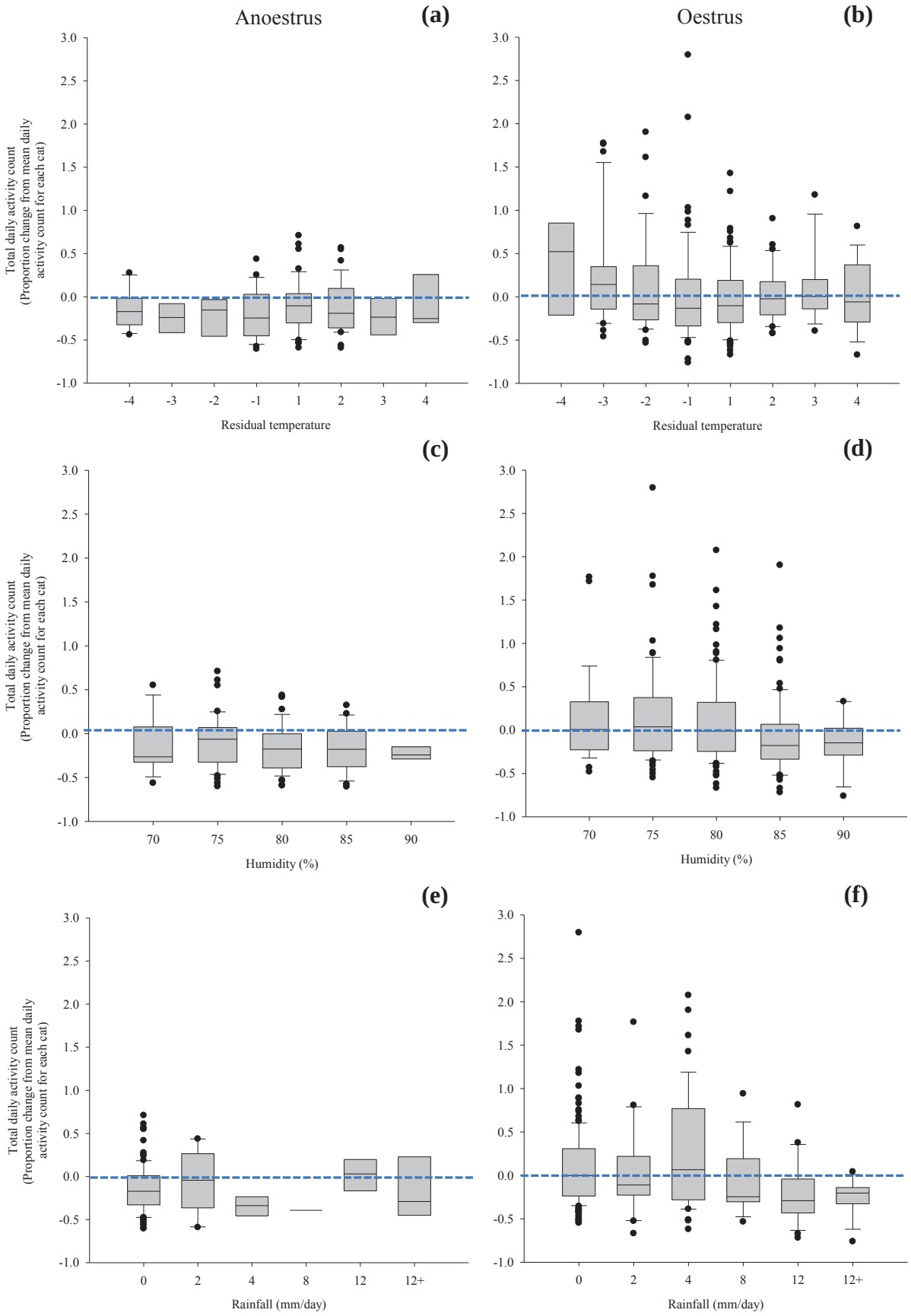

Figure 3.7 Overall physical activity (OPA) of the cats during anoestrus (left; a, c, e) and behavioural oestrus (right; b, d, f) periods with respect to temperature (a and b), humidity (c and d), and rainfall (e and f).

3.4 Discussion

This is the first study to quantitatively examine the effects of oestrus on accelerometer-based activity counts of domestic cats. It is widely accepted that cats are more active during oestrus, but until now the support for this hypothesis has been based purely on the subjective assessments of behaviour (Layhausen, 1979; Morali and Beyer, 1979). The present study provides the first quantitative support for this claim, with the accelerometer-based activity counts of the cats generally being higher during oestrus. However, it is important to consider the effects of potentially confounding variables when conducting activity-based research on cats, thus the possible effects of temperature, rainfall and humidity on the MMA activity counts of the cats were also considered.

In agreement with the previous study there was considerable variation between the overall activity counts and activity profiles of different cats (see Chapter 2 and Appendix 3). The high variability in activity between cats was likely due to behavioural differences between them (e.g. different levels of grooming, scratching or OPA) and was standardised by calculating the proportional change from the mean total daily activity count for each cat (Chapter 2; Andrews *et al.*, 2015). This enabled the activity data of multiple cats to be combined into a single dataset for analysis, a data transformation that would be recommended for future accelerometer/activity-based studies in this species.

Daily or weekly routines have been reported to influence activity and lead to inter-individual variation in the activity counts of both cats and dogs (Brown *et al.*, 2002; Lascelles *et al.*, 2008; Dow *et al.*, 2009; Brown *et al.*, 2010; Preston *et al.*, 2012), but the cats in the present study were all exposed to the same daily routine so this was an unlikely source of variability. The daily routines differed slightly between weekdays and weekends, with slight differences in feeding times and the time staff were present at the colony (see Section 1.7). It might have been reasonable to expect, then, that the OPA of the cats would have been lower during weekends. The activity of pet cats certainly appears to be greater when their owners are at home (Piccione *et al.*, 2013). Interestingly, the activity of the cats in the present study did not differ across with day of the week. Cats were housed in groups so it is possible that intragroup interactions

had a greater influence on activity than human interactions, although this was not investigated.

There is evidence to suggest that feeding time may alter the daily activity patterns of the cats, with recent publications reported a pre-feeding increase in the accelerometer-based activity counts of cats (i.e. food anticipatory activity (FAA)) (Alexander *et al.*, 2014; Deng *et al.*, 2014). The effects of FAA on the OPA of the cats in the present study were likely to be partly mitigated by the *ad libitum* feeding regime. Furthermore, any effects of FAA were unlikely to have a significant role in the context of total daily activity counts of the cats since levels of FAA would likely be similar from day to day. Nonetheless, for the purpose of future activity-based research it may be worthwhile considering the effects of FAA on the cats, especially if examining the activity of cats within a given day.

The influence of environmental factors on the OPA of cats should also be considered, since temperature, humidity and rainfall all affected the activity of the cats in the current study. The association between activity and temperature was difficult to interpret due to considerable inter-individual variation, although there was a general trend for activity to be lower at warmer temperatures. This is consistent with general observations of cats at warmer temperatures, with the cats in the present study spending the majority of their time basking in the sun when it was warmer. It might also be expected, given the thermoregulatory behaviour of cats (i.e. reduce heat loss when environmental temperature is cold), that cats would be less active at colder temperatures (Jacobson and Squires, 1970; Cabanac, 1975). However, this pattern was not observed in the present study. In fact, the highest activity counts of most cats were observed at lower relative temperatures (residual temperatures of -4 to -1).

It is possible that the cats were more active at cooler temperatures to keep warm, since physical activity is associated with greater heat production (Hilmer *et al.*, 2010). Alternatively, the minimal effect of temperature on activity is possibly due to the range of temperatures (11.5 – 20.2˚C) observed during this study period being too small to generate pronounced temperature effects on the OPA of the cats. Temperatures lower than 11.5˚C are possibly needed for the decrease in OPA that would be expected in response to colder temperatures, although further research is needed to confirm this.

Still, the variable effects of temperature on the OPA of the cats make it difficult to account for the influence of temperature on the accelerometer activity counts of cats.

Similarly, it was difficult to identify a consistent effect of humidity and rainfall on the OPA of the cats, although both high humidity (>80%) and daily rainfall (>10 mm) tended to be associated with lower activity counts. However, there was considerable variability between individual cats. The minimal effect of smaller daily rainfalls (< 10mm) could be a consequence of the colony pens being mostly sheltered from direct rain, which means that the cats could have been active without getting wet. Alternatively, the rainfall data used may not have accurately represented the rainfall at the colony, since the weather data were obtained from an off-site weather station (~7.6 km from the colony). Nonetheless, it seems that, as with temperature, both humidity and rainfall do not affect the OPA of the cats in a consistent manner, and thus it is difficult to account for these factors when conducting activity-based research in cats.

In contrast, the reproductive state of individual cats had a stronger and more consistent effect on their activity, with the majority of the cats exhibiting an increase in activity during oestrus. Interestingly, the cats that exhibited the most pronounced increases in activity during oestrus (e.g. cats 1, 4 and 5) were those individuals with the most obvious behavioural indicators of oestrus. It is therefore possible that the differences between anoestrus and oestrus activity in the other cats were misinterpreted and may have needed to be confirmed using a more accurate method for oestrus detection. The behavioural detection of oestrus can be challenging in cats due to considerable inter and intra-individual variation in the frequency and intensity at which oestrous behaviours are expressed, and no single behaviour is consistently indicative of oestrus in this species (Michael, 1961; Michael and Scott, 1964; Wildt *et al.*, 1981a; Bell, 2009).

The use of a more accurate and reliable method for assessing the reproductive status of the cats would remove the potential error associated with the behavioural detection of oestrus. This could be particularly relevant in clarifying the apparent continuous oestrus of cats 1 and 9. It is unlikely that these cats were in oestrus throughout the study period since the mean duration of oestrus in the domestic cat is approximately 7.1 days, although it is possible that they were given the extent of variation around this mean (2 - 118 days) (Verhage *et al.*, 1976; Shille *et al.*, 1979; Wildt *et al.*, 1981a; Root *et al.*, 1994; Chatdarong *et al.*, 2006).

The longitudinal assessment of plasma oestradiol or faecal oestradiol metabolites (FEM) has previously been used to accurately determine the reproductive status of domestic cats (Paape *et al.*, 1975; Verhage *et al.*, 1976; Shille *et al.*, 1979; Hendelman *et al.*, 2000; Chatdarong *et al.*, 2006), and a wide range of non-domestic felids (Wildt *et al.*, 1981b; Graham *et al.*, 1995; Henriksen *et al.*, 2005; Herrick *et al.*, 2010; Brown, 2011). However, it is not always feasible to collect plasma or faecal samples without compromising the welfare (via regular blood sampling) or influencing the OPA (solitary enclosures for faecal collection) of the cats. The author is currently investigating the potential for a highly accurate liquid chromatographic assay to rapidly quantify salivary oestradiol concentrations, though further research is needed to achieve the required sensitivity (see Appendix 2). The development of such an assay will enable analysis of the daily saliva samples that were collected from each of the cats during the present study, allowing the confirmation of their reproductive state throughout the study period (see Appendix 2).

Despite this, the results of the current study suggest that there is a strong association between OPA and behavioural oestrus in domestic cats. Thus there is potential for accelerometry and activity assessment to be used to improve the real-time detection of oestrus in cats. This would be particularly advantageous as there would be a minimal lag between the onset and identification of oestrus (c.f. monitoring plasma oestradiol, salivary oestradiol, or faecal oestradiol metabolites). While the potential application of accelerometry for the real-time detection of oestrus may seem to be of minimal importance for domestic cats, it is the first step to developing such a technique in non-domestic felids. Domestic cats often serve as a model species for endangered or threatened felids, providing opportunity to develop and improve techniques before testing them on non-domestic species (Graham *et al.*, 2004; Pukazhenthi *et al.*, 2006; Bell, 2009). Oestrous behaviours appear fairly consistent among felids, thus it would be reasonable to expect that a similar relationship between activity and oestrus may exist in other felid species, and warrants further investigation.

Many non-domestic felids including cheetah (*Acinonyx jubatus*), margay (*Leopardus weidii*), ocelot (*Leopardus pardalis*), tigrina (*Leopardus tigrinus*), Eurasian lynx (*Lynx lynx*), Pallas' cat (*Otocolobus manul*) and Geoffroy's cat (*Oncifelis geoffroyi*), do not exhibit overt behavioural indicators of oestrus (Asa *et al.*, 1992; Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002; Henriksen *et*

al., 2005). Consequently, the detection of oestrus based on behaviour is difficult at best in these species. In fact, oestrus has been referred to as "silent" in many of these felids, suggesting that the behavioural detection of oestrus is not possible (Asa *et al.*, 1992; Graham *et al.*, 1995; Wielebnowski and Brown, 1998). Strictly speaking, oestrus is not silent in these species, rather, oestrus appears to be associated with much more subtle increases in the expression of several typical behaviours (e.g. grooming/allogrooming, scent marking and increased locomotor activity) (Asa *et al.*, 1992; Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002; Henriksen *et al.*, 2005).

Despite this, it would be fair to assume that these subtle behavioural changes are functionally silent in terms of the visual detection of oestrus. The detection of these subtle behavioural changes would require continuous and labour intensive observation and possibly an intimate relationship between the animal and the observer, rendering the behavioural detection of oestrus infeasible (Michael, 1961; Michael and Scott, 1964; Wildt *et al.*, 1981a). Alternatively, it may be possible to detect these behavioural changes electronically using accelerometry. The present study certainly suggests that the behavioural changes associated with oestrus in cats correlate with an increase in OPA. Whether this increase is sufficient for the accurate and reliable detection of oestrus, however, remains to be investigated.

3.5 Conclusion

While environmental factors such as temperature, rainfall, and humidity clearly affect the OPA of colony-housed cats, they did so in a highly inconsistent manner that is difficult to account for. Some cats appeared to be more sensitive to these environmental factors than others. Further, individual cats responded differently to changing temperature, rainfall and humidity. This variability supports previous findings that suggest that the activity data of individual cats should be considered independently (Chapter 2; Andrews *et al.*, 2015). Interestingly, the cats exhibited a consistent increase in activity during oestrus, which could be particularly useful for the development of a novel and accurate accelerometer-based method for the real-time detection of oestrus in this species. While this may be of limited value for domestic cats, it is the first step in developing this methodology for use on endangered non-domestic felids in which oestrus is difficult to detect behaviourally.

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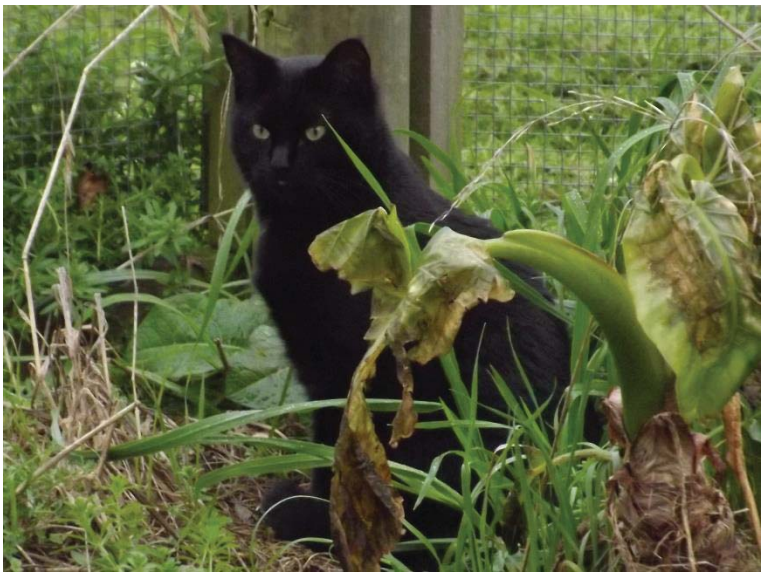
Chapter 4

General discussion and conclusions



Chapter 4: Overall discussion and future directions

4.1 Key findings of this thesis

This thesis aimed to validate the use of collar-mounted Actical® 'MiniMitter' accelerometers (MMAs) for the quantification of activity in domestic cats (*Felis catus*), and to use these devices to determine the effects of several environmental factors and reproductive state (anoestrus or behavioural oestrus) on the overall physical activity (OPA) of individual cats. The research was separated into two main research chapters that examined each objective of the thesis independently.

Chapter 2 showed that the MMA activity counts of individual cats provided an accurate representation of their observed physical activity (percentage of time spent active), thus supporting the use of MMAs for the quantification of OPA in domestic cats. However, the relationship between the MMA and the observed activity data varied considerably between cats. In addition, relatively static behaviours such as scratching and grooming were found to adversely affect the correlation between the MMA and the observed activity data of some cats. Interestingly, the effects of these relatively short-term behaviours were partly mitigated through the use of a longer sampling period or a moving average.

For the purpose of this study, observed activity was based entirely on locomotor activity, with static behaviours such as grooming and scratching being considered as inactive. This is possibly a misclassification, since these behaviours are technically a form of activity. Consequently, observed activity was potentially underestimated in cats that frequently groomed or scratched, which likely accounts for some of the disparity between the MMA and observed activity data of these cats.

In **Chapter 3,** MMAs were used to show that the OPA of colony-housed domestic cats was not influenced by day of the week, but was significantly affected by temperature, rainfall, humidity, and reproductive state (anoestrus or oestrus). The effects of temperature, rainfall and humidity on the activity of the cats as a group (with inter-individual variation accounted for by transforming the activity data into a proportion change from the mean activity for each cat) were highly variable and difficult to interpret, although some cats were certainly more sensitive to these environmental

factors than others. However, there was a general trend for activity to decrease with increased temperature, rainfall and humidity. In contrast, reproductive cyclicity had a strong and consistent effect on the OPA of the cats as a group, and individually. A comparative assessment of the activity of the cats during oestrus and anoestrus showed that the cats were more active during oestrus.

4.2 Validation and use of Actical 'MiniMitter' accelerometers

Actical® were first validated for use in cats by Lascelles *et al.* in 2008, with the authors noting a strong positive correlation between the collar-mounted MMA activity counts and observed activity (time spent above 6% mobility, movement, velocity, and distance moved) in three cats; however, the small sample size precluded investigation of inter-individual variation (Lascelles *et al.*, 2007). The authors concluded by stating that "until we have more data on populations of cats, future studies should be constructed with each individual cat acting as its own control" (Lascelles *et al.*, 2007). The research in Chapter 2 of this thesis addressed this question and is the first to investigate the magnitude of inter-individual variation between the MMA and observed activity data of domestic cats (Andrews *et al.*, 2015). The data from Chapter 2 agreed with Lascelles *et al.* (2008), and showed that the MMA activity counts and observed activity data of individual cats were highly correlated. There was, however, a high degree of inter-individual variation in the strength of the correlation (R^2: 0.65 - 1.00). The exact cause of this was unclear, although it is likely related to the frequency of specific static behaviours such as scratching and grooming. While these behaviours were defined as inactive in terms of observed activity, they activated the MMAs and increased the MMA activity counts of the cats. Consequently, these behaviours adversely affected the correlation between the MMA and observed activity of the cats, a finding which has previously been reported in cats (Lascelles et al., 2008).

A similar situation has been reported in dogs by Hansen *et al.* (2007). While the authors also focused on locomotor behaviour, they indicated that scratching and shaking led to the activation of the MMAs (Hansen *et al.*, 2007). Interestingly, the detection of these behaviours by the MMAs is probably relevant in the context for which Hansen *et al.* (2007) validated the MMAs in dogs; that is, studying the effects of non-steroidal anti-inflammatory drugs (NSAIDs) treatment on the OPA of activity of dogs with osteoarthritis (OA). Thus while categorising these behaviours as inactive may be useful

for validating MMAs, it limits the assessment of the total observed activity, which would be activity associated with both locomotory and active static behaviours (e.g. grooming, scratching and shaking).

Nevertheless, the results of Chapter 2 suggest that while MMAs can be used to monitor the OPA of individual cats, the behavioural differences among cats mean that the MMA activity counts of different cats should only be compared when the high degree of inter-cat variation is accounted for. This finding ultimately illustrates the need for treating cats independently when conducting accelerometer-based research, with each cat acting as its own control.

4.3 Sample period selection

The discrepancy between the MMA and observed activity of individual cats associated with behaviours such as grooming and scratching can be partly mitigated through the use of a longer sampler period (Chapter 2; Andrews *et al.*, 2015). However, the level of detail in the activity data (i.e. sensitivity) decreases as the sampling period increases. As a result, the selected sampling period needs to provide a balance between accuracy (minimising the variability of the activity data) and sensitivity (maintaining sufficient detail in the activity data).

A study in pet dogs (*Canis familiaris*) reported an optimal sampling period of seven days (Dow *et al.*, 2009). This was likely due to the activity of the dogs being dependent on the routines and schedules of their owners (Dow *et al.*, 2009). Unsurprisingly, dogs were substantially more activity when their owners were present, and thus, exhibited much higher activity counts in the afternoons and during weekends (Dow *et al.*, 2009). The use of a seven day sample period appeared to account for the day-to-day variation in the activity of the dogs resulting from their owners weekly schedules (Dow *et al.*, 2009). A similar situation has been reported in pet cats in the home environment (Piccione *et al.*, 2013).

Interestingly, despite a reduced staff presence at the Feline Unit on the weekends, the activity of the colony-housed domestic cats did not appear to be affected by day-of-the-week (Chapter 3). This was probably a consequence of the group housing of the cats, with intra-group interactions likely having a greater influence on the activity of the cats than human interactions. This thesis did not investigate the potential effects of

dominance hierarchy on the OPA of the cats, nor has it been investigated elsewhere. However, a study examining the dominance hierarchy and activity in cats is warranted given the potentially significant effects of intra-group interactions on the OPA of colony-housed cats. Nonetheless, the apparent lack of day to day variation in the OPA of the cats means that the seven day sampling period, recommended in dogs, is not necessary.

It is important to note that the group housing of the cats used for the research in this thesis is unusual and is not reflective of the situation in most homes or in captive breeding programs for other felid species. Piccione *et al.* (2013) observed a strong association between the activity of pet cats and the presence of their owners, suggesting that this lack of day-to-day variation in cats is limited to a colony setting, although it may also be applicable to multiple cat households. Further investigation into the day to day variation in the OPA of cats, and thus the reconsideration of sampling period, is needed if conducting research outside of a colony setting.

The activity of the cats in my study did vary considerably throughout the day (Chapter 2). Most cats exhibited a peak or peaks in activity between 1000 - 1400 h (see Appendix 1). This was likely associated with the provision of fresh food, since the cats were fed at 1000 - 1030 h. A strict feeding routine has previously been shown to induce food anticipatory activity (FAA) in cats, with individual cats exhibiting an increase in activity prior to feeding (Deng *et al.*, 2014). Ultimately, the variability in the activity of the cats throughout the day due to FAA or other daily activities means that MMA activity data would best be examined using a 24 h sampling period, at least for colony-housed cats.

Chapter 2 also showed that a moving average can be used to enhance the accuracy of the MMA activity of colony-housed cats (Andrews *et al.*, 2015). Using a moving average did not improve the accuracy of the MMAs as much as increasing the sampling interval, but sufficient information was retained for a finer scale assessment of activity (e.g. changes in activity throughout the day). Thus the use of a moving average is recommended if greater detail (e.g. sample period less than 24 h) is required from the activity data.

4.4 The influence of temperature, rainfall, and humid on the activity of cats

Chapter 3 showed that the OPA of colony housed cats was affected by temperature, rainfall, and humidity. This is the first study to examine the effects of these factors on the accelerometer activity counts of colony-housed domestic cats. While the influence of these factors on the activity of the cats was highly variable, some general trends were observed.

It was not surprising to find that the cats were less active at warmer temperatures, since the cats were observed to spend the majority of their time basking in the sun when it was warmer. Interestingly, the OPA of the cats was also significantly affected by humidity, with the cats being less activity when the average daily humidity was high (> 80%). This is likely a consequence of the strong association between humidity and rainfall, since the cats also exhibited reduced activity when rainfall exceeded approximately 5-10 mm per day. This finding is consistent with a general tendency for cats to avoid water; however, it might have been expected that the cats would be less active at even lower rainfall levels. The fact that this did not happen may be due to the semi-outdoor nature of the cats housing, with only one third of the pen being directly exposed to the rain. As a result, the cats could be active while it was raining without getting wet.

An alternative explanation for the variable responses of the cats to rainfall is that the rainfall data used did not accurately represent the rainfall at the study site. A limitation of Chapter 3 was that the weather data used were generalised for Palmerston North in New Zealand and were obtained from a weather station located some distance (~7.6 km) from the study site. Thus it is possible that there were differences in temperature, humidity and rainfall between the study site and location of the weather station, particularly with regards to rainfall. It would thus be worth investigating the effects of rainfall and humidity, as well as temperature, on the activity of colony-housed cats using an accurate on-site weather station. However, the findings from Chapter 3 suggest that while temperature, humidity and rainfall all affect the activity of the cats, they do so in an inconsistent manner that ultimately makes it difficult to account for these factors when conducting activity-based research in cats.

4.5 Oestrus and activity in domestic cats

Chapter 3 is the first study to quantitatively examine the activity of domestic cats during oestrus and anoestrus. It is widely believed that cats and other felids are more active during oestrus (Layhausen, 1979; Morali and Beyer, 1979; Asa *et al.*, 1992). However, until now the evidence for this hypothesis has been circumstantial and based on subjective observations.

Interestingly, the most pronounced increases in activity during oestrus occurred in cats in which oestrus was readily detectable from behavioural changes such as lordosis or calling. It is possible that the association between activity and oestrus in the other cats could be improved through the use of a more accurate method for monitoring ovarian cyclicity, since behavioural detection of oestrus in cats can be challenging (Michael, 1961; Michael and Scott, 1964; Wildt *et al.*, 1981). In attempt to address this, daily saliva samples were collected from the cats throughout the study (Chapter 3 and Appendix 2) with the intent of determining each cat's salivary oestradiol (E_2) concentrations over time to more accurately assess and confirm their reproductive state. Before the analysis of the collected saliva samples could occur, however, a sensitive salivary E_2 assay needed to be developed for domestic cats.

A study (Appendix 2) was designed to validate a liquid chromatographic (LC) method for quantifying salivary E_2 in cats. However, it was not possible to achieve the estimated, required assay sensitivity of less than 1 pg/ml. This estimate was derived from studies in humans where salivary E_2 concentrations are typically 0.05% to 5.00% of plasma levels (Worthman *et al.*, 1990; Shirtcliff *et al.*, 2000). If this holds true for cats, where plasma E_2 concentrations during anoestrus range from 5 - 15 pg/ml and peak oestrus plasma concentrations reach 50 - 70 pg/ml, then the salivary E_2 concentrations of cats would range from 0.06 to 0.60 pg/ml during anoestrus and 0.25 - 3.5 pg/ml during oestrus (Shille *et al.*, 1979). Thus far, a minimum assay sensitivity of 10 pg/ml for E_2-standards has been achieved using high pressure liquid chromatography (HPLC) with fluorescent detection and dansyl chloride derivatisation, a method which has been designed to improve sensitivity based on several previous publications (Schmidt *et al.*, 1978; Worthman *et al.*, 1990; Shirtcliff *et al.*, 2000; Gomez *et al.*, 2004; Nelson *et al.*, 2004). This level of sensitivity is likely to be insufficient for the accurate and reliable detection of oestrus from the saliva samples, thus further development of this or another

salivary E_2 assay is need before the collected saliva samples can be analysed and the association between activity and oestrus revisited.

Despite this, the research in Chapter 3 provides the first quantitative support for the increased activity of female domestic cats during oestrus. This observation could be extremely useful to improving the detection of oestrus in this species, offering a non-invasive and accurate method for the immediate detection of oestrus without the need for time consuming and expensive hormone analyses. While this may be of limited value for domestic cats, it could have a considerable impact on the captive management and breeding of non-domestic felids. The accurate and reliable detection of oestrus has been difficult to achieve in many endangered wild felids due to a lack of overt behavioural indictors of oestrus, which has hindered the success of captive breeding programs for these species (Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002; Henriksen *et al.*, 2005).

This study suggested that changes in OPA during oestrus could be used alongside behavioural observations to improve the real-time detection of oestrus in felids. The findings of this thesis certainly support this hypothesis, at least for domestic cats. Since the behaviours typically associated with oestrus are fairly consistent among the felid taxa, it would be worthwhile investigating the potential application of accelerometery for improving oestrus detection in captive non-domestic felids (Michael, 1961; Michael and Scott, 1964; Blomqvist and Sten, 1982; Mellen, 1993; Graham *et al.*, 1995; Umapathy *et al.*, 2007; Kinoshita *et al.*, 2009; Groot, 2013). Further research into the association between OPA and reproductive cyclicity in domestic cats is also justified, and should be conducted prior to applying this approach to other high-conservation-status felid species.

4.6 Summary of future directions

- Validation of a sensitive salivary E_2 assay. This could involve the further development of the HPLC based technique I have trialled or the development of a new salivary E_2 assay.

- Use a sensitive salivary E_2 assay to analyse saliva samples collected from each of the cats used in Chapter 3. This would enable the more accurate assessment of the cats' reproductive states throughout the study period, and thus, the

accurate examination of the effects of reproductive state on the OPA of colony-housed domestic cat.

- Investigate whether oestrous behaviour changes between individually- and group-housed cats. It is possible that the group housing of cats may have affected the expression of oestrous behaviours, potentially leading to "silent" oestrus events in more submissive cats.

- Investigate the effects of dominance hierarchy on the activity profiles of colony-housed domestic cats and the potential effects this has on the expression of oestrous behaviours in the cats. It has previously been reported that domestic cats lack an obvious dominance hierarchy as they are not adapted to social living (Van den Bos and de Cock Buning, 1994; Rochlitz, 2005). However, the cats used in this thesis provide an opportunity for investigating dominance hierarchies in cats, since the cats have been housed socially throughout their lives.

- The application of the accelerometers used in this research for on-going research in non-domestic felids is limited by the need to remove the device to extract the data, preventing real time analysis. It would be worthwhile investigating other accelerometers that offer remote access or commissioning the development of a new unit with this function. Heyrex accelerometers (Heyrex Ltd., Wellington, New Zealand) designed to assess the OPA of dogs are capable of this, but they have not yet been validated for use in cats.

- Apply this research to high-conservation-status felid species in which the behavioural detection of oestrus is difficult (e.g. cheetah (*Acinonyx jubatus*), tigrina/oncilla (*Leopardus tigrinus*), Eurasian lynx (*Lynx lynx*), margay (*Leopardus weidii*), Pallas' cat (*Otocolobus manul*) and Geoffroy's cat (*Oncifelis geoffroyi*)) (Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002; Henriksen *et al.*, 2005). An increase in OPA during oestrus has been reported in cheetah based on subjective observations of cheetah during oestrus, suggesting that this species would be a good starting point for applying the research of this thesis to non-domestic felids (Asa *et al.*,

1992). Such a study in cheetah woud be particularly useful as there is currently conflicting information regarding an oestrus-induced increase in OPA, with Wielebnowski and Brown (1998) failing to determine any change in OPA through behavioural observations.

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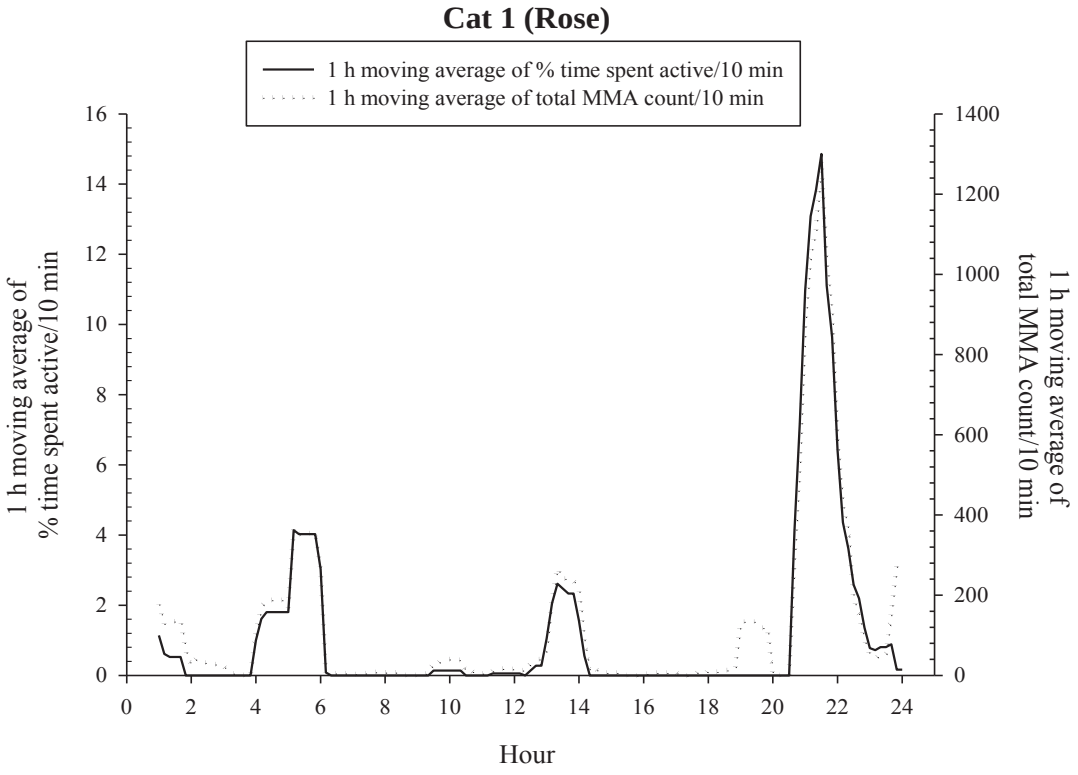
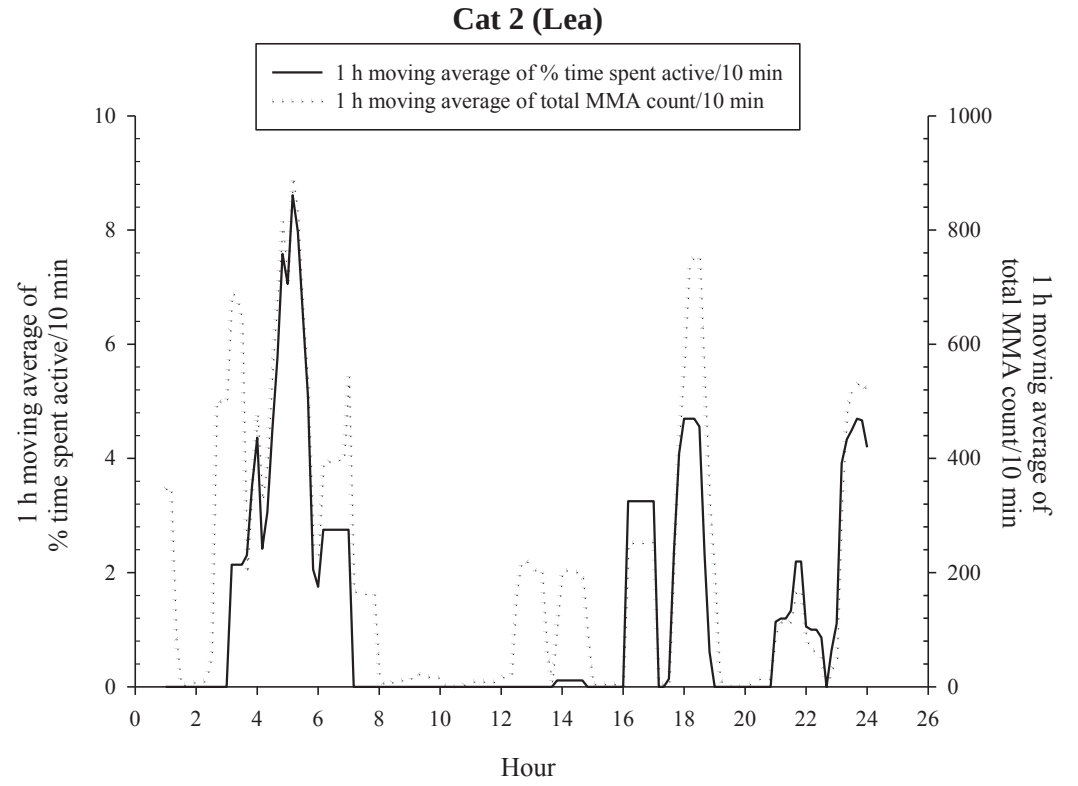
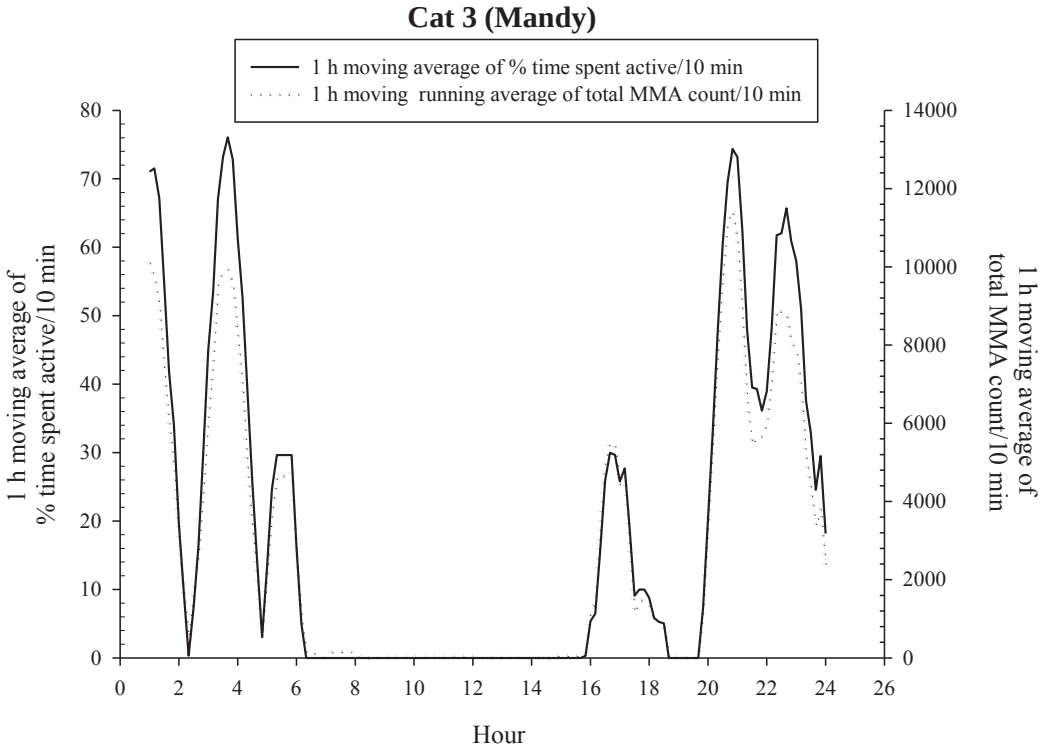
Appendix 1

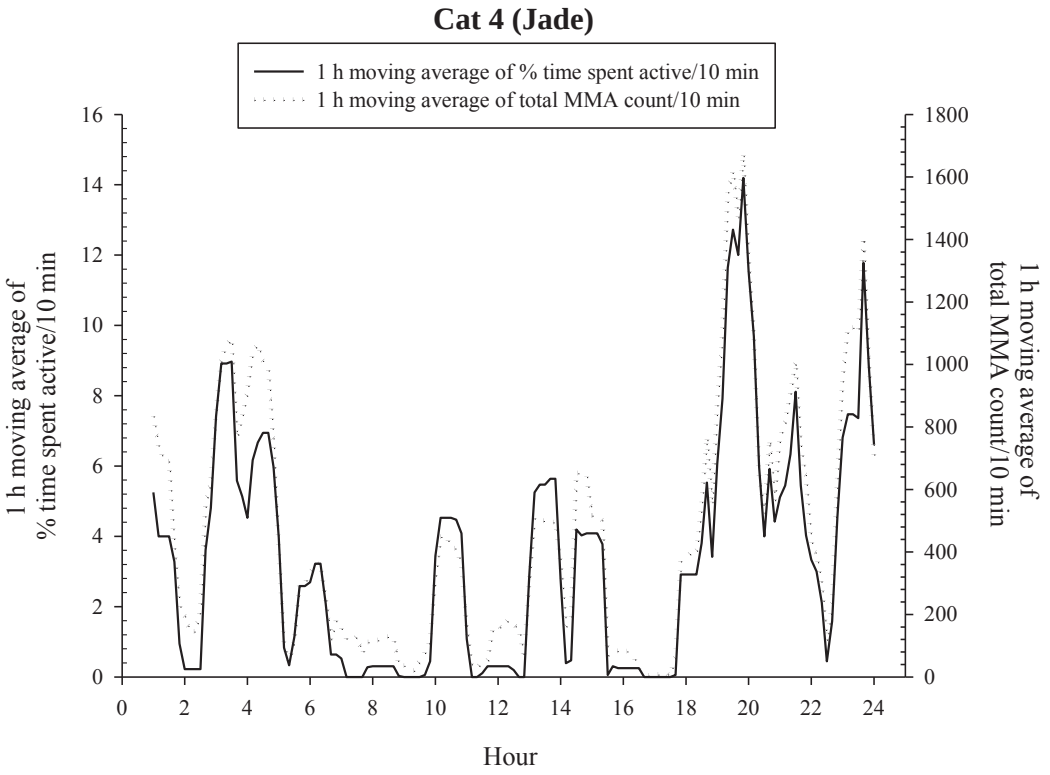
24 h MMA and observed activity profiles of the cats in Chapter 2

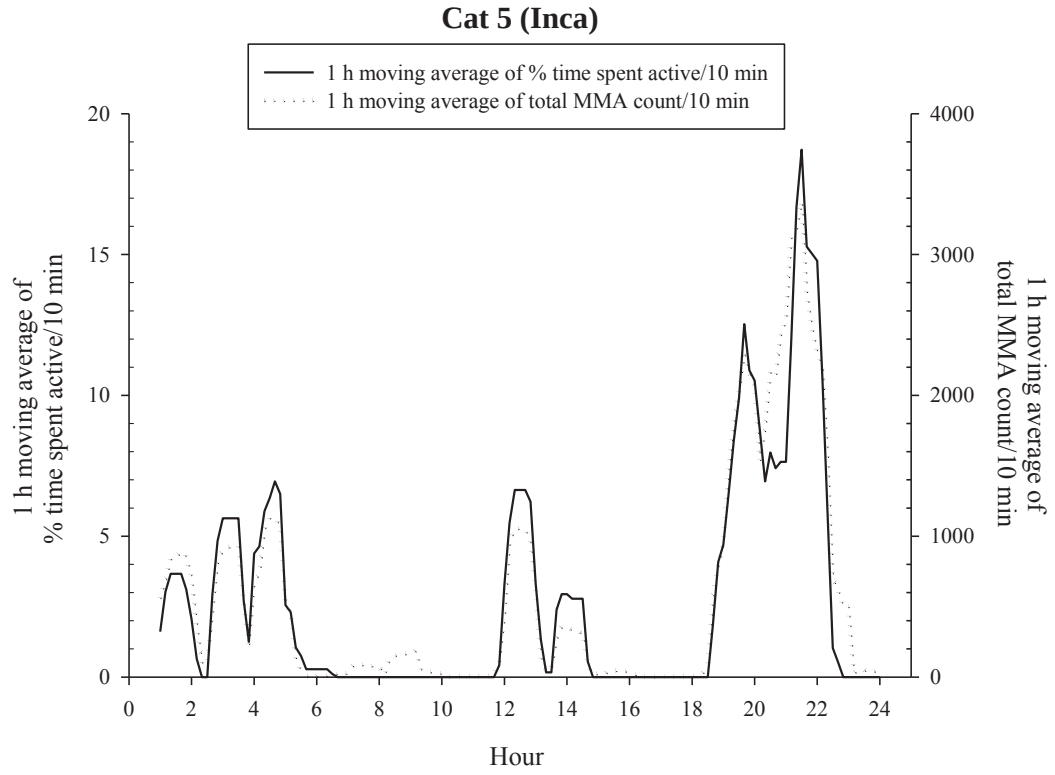
Figures A1.1 to 12 1-h moving average of the total accelerometer (MMA) counts (·····) and percentage of time spent active (—) per 10 min for each of the 12 cats studied in Chapter 2.

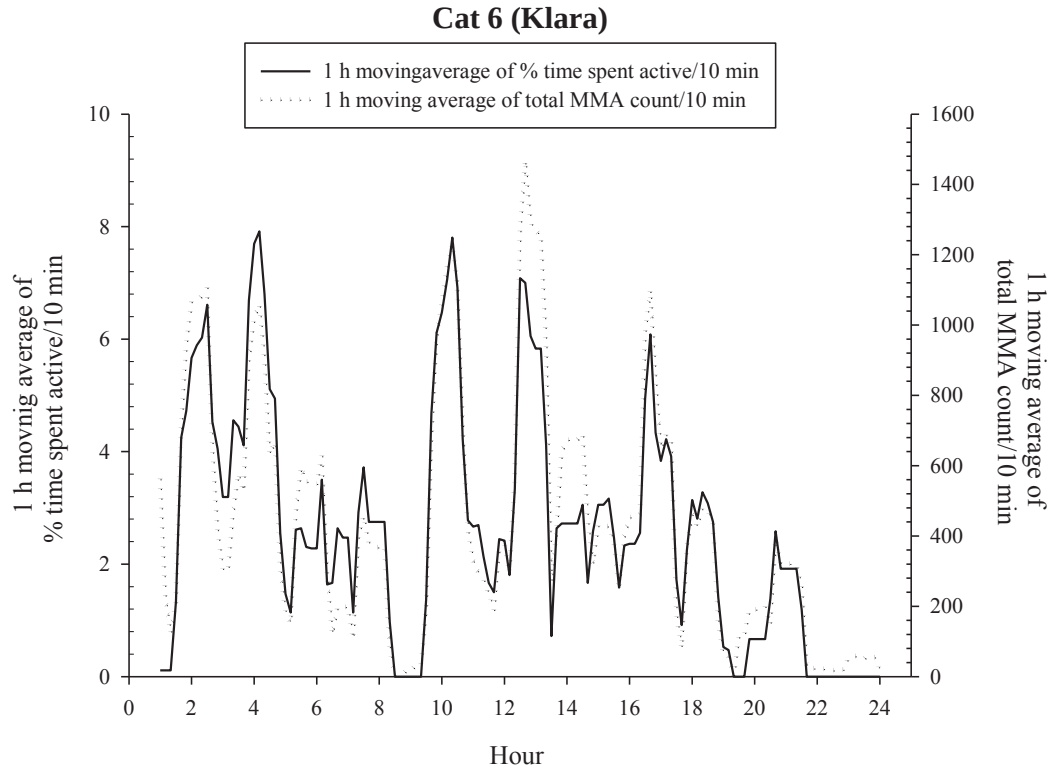


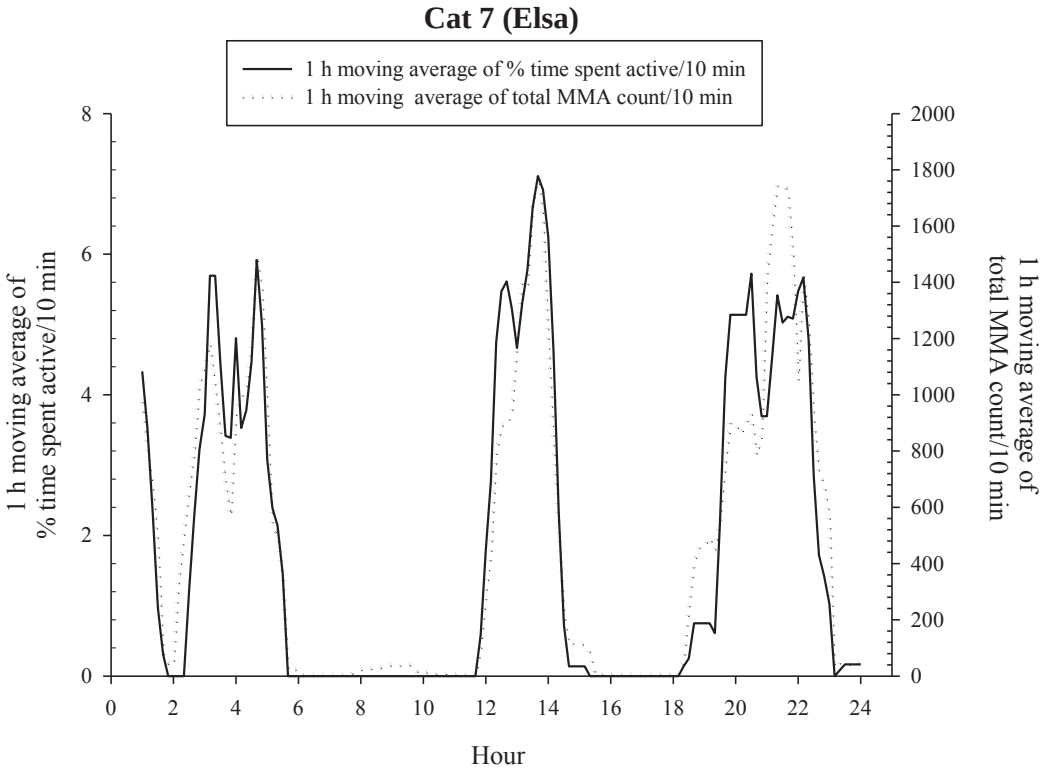


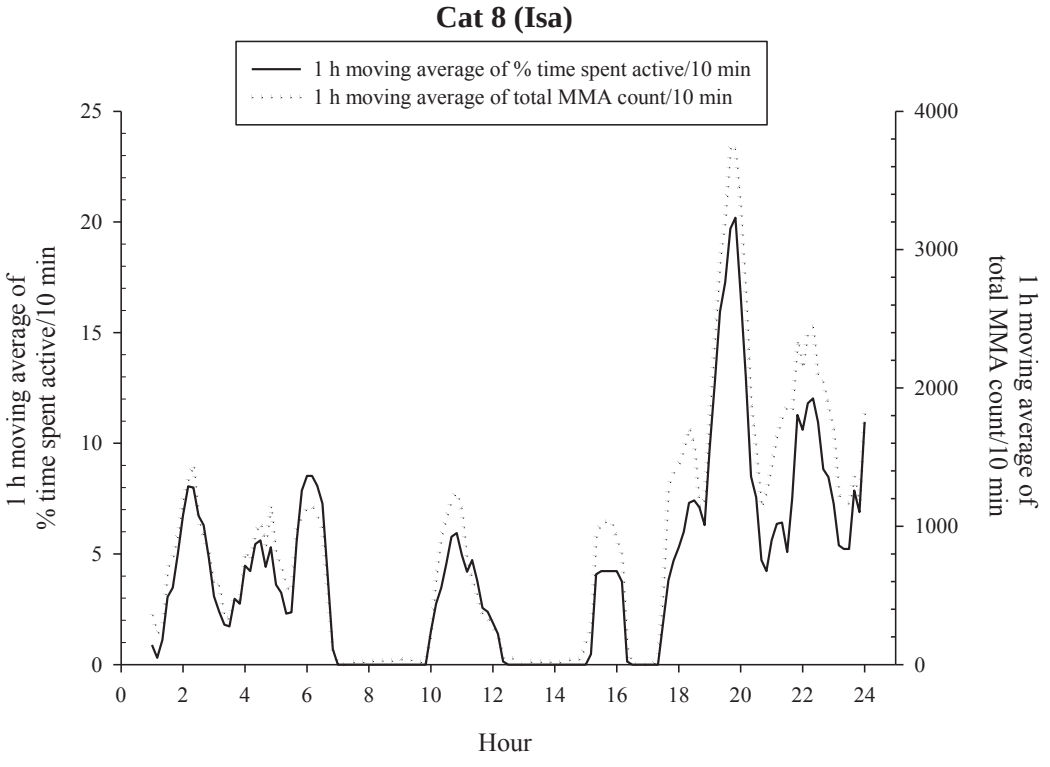

Cat 3 (Mandy)
1 h moving average of % time spent active/10 min
1 h moving running average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour

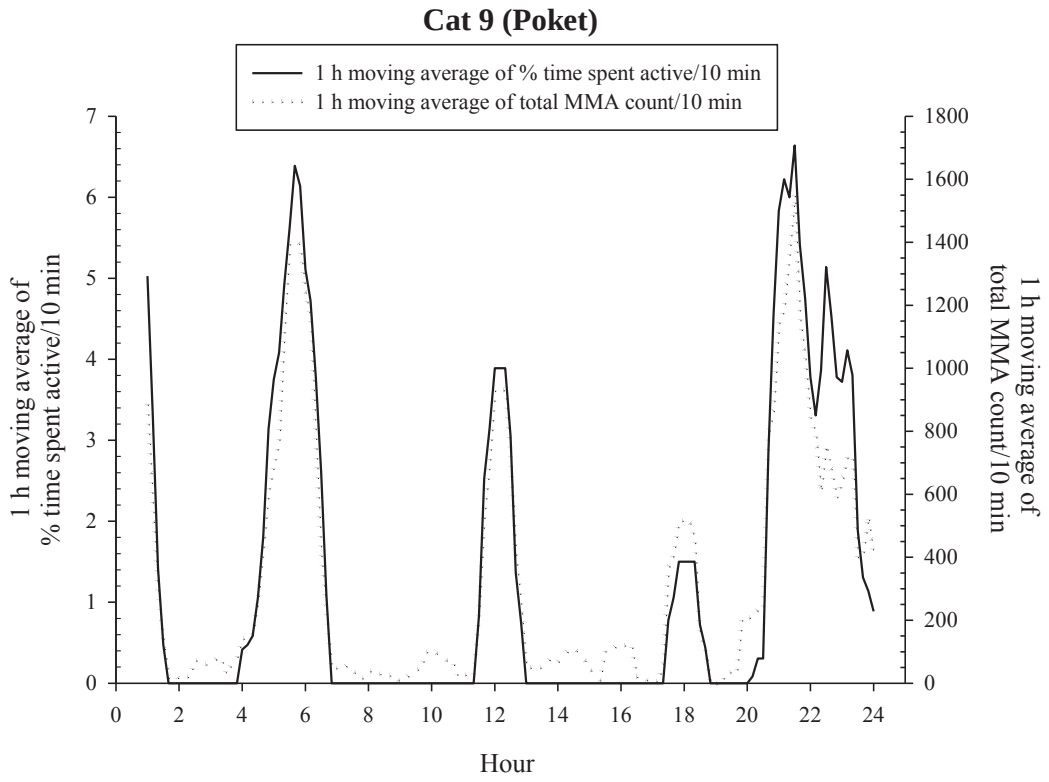

Cat 4 (Jade)
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour

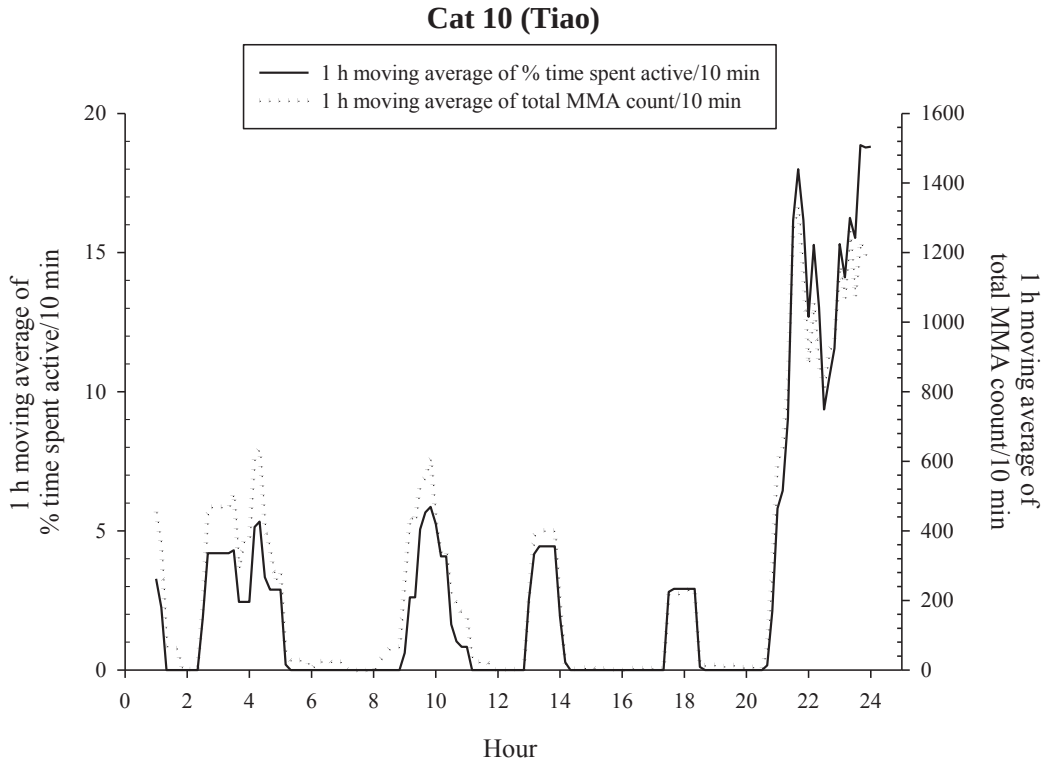

Cat 5 (Inca)
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour

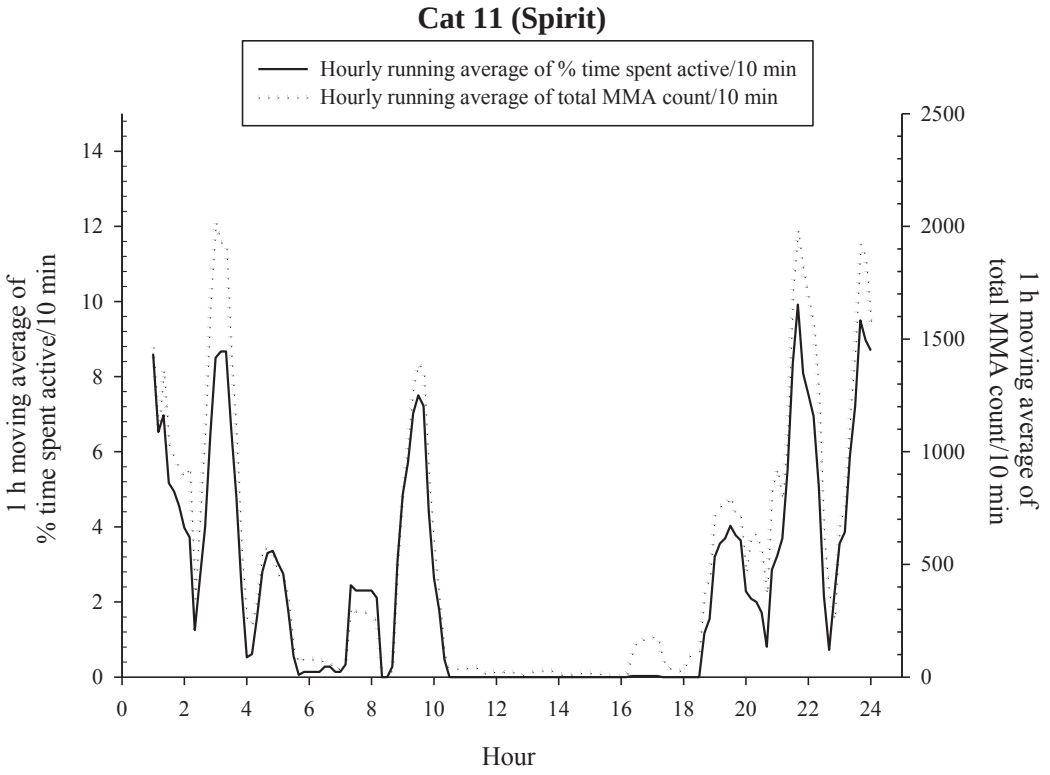

Cat 6 (Klara)
1 h movingaverage of % time spent active/10 min
1 h moving average of total MMA count/10 min
1 h movnig average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour

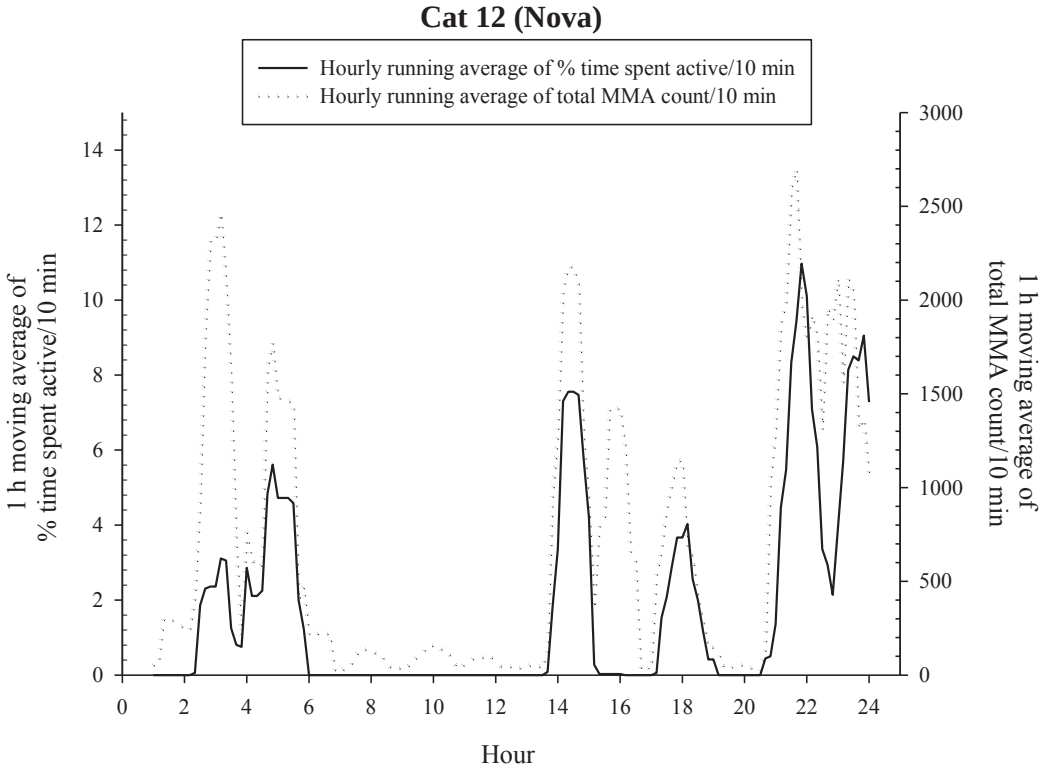

Cat 7 (Elsa)
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour


Cat 8 (Isa)
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour


Cat 9 (Poket)
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour

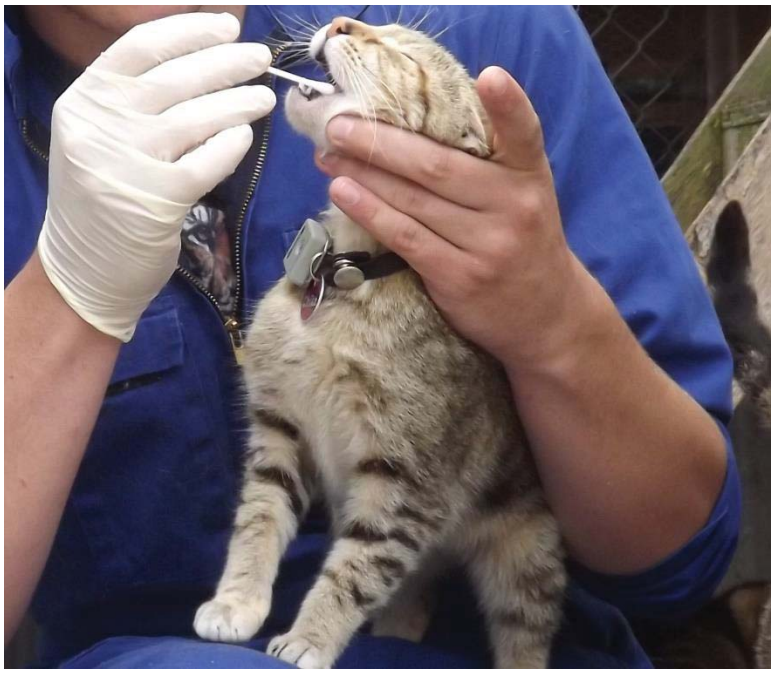

Cat 10 (Tiao)
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA coount/10 min
Hour


Cat 11 (Spirit)
Hourly running average of % time spent active/10 min
Hourly running average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour


Cat 12 (Nova)
Hourly running average of % time spent active/10 min
Hourly running average of total MMA count/10 min
1 h moving average of % time spent active/10 min
1 h moving average of total MMA count/10 min
Hour

Appendix 2

Activity as a predictor of oestrus in domestic cats (*Felis catus*)



Appendix 2: Activity as a predictor of oestrus in domestic cats (*Felis catus*)

A2.0 Abstract

*Oestrus is often difficult to detect in felids, but it can be associated with subtle increases in the expression of behaviours such as grooming, allogrooming, rubbing, rolling, scent-marking, vocalising, and locomotion. Thus it may be possible to improve oestrus detection in felids by monitoring changes in the overall physical activity (OPA) associated with oestrus which may be detected remotely via accelerometry. The present study aimed to examine the relationship between OPA, as measured by Actical® accelerometers (MMAs), and reproductive state (anoestrus or oestrus) in colony-housed domestic cats (*Felis catus*). A total of 414 days of MMA activity data were collected from 10 cats and saliva samples (minimum of 50 µL) were collected from each of the cats on a daily basis. The intention was to monitor salivary oestradiol (E_2) concentrations in the cats to identify periods of endocrine oestrus, however a technique for assessing E_2 in the saliva of cats has not yet been described. We aimed to develop a sensitive liquid chromatography assay for the quantification of salivary E_2 concentrations in cats, but this was not possible under the time constraints of this thesis. Thus the effects of reproductive state on the OPA of the cats could only be examined based on the behavioural identification of oestrus. Two of the 10 cats studied appeared to be in behavioural oestrus throughout the study period so their data were excluded from the analysis. A one-way analysis of variance (ANOVA) and Tukey's post hoc test of the data from the remaining eight cats indicated that the OPA of the cats was significantly higher during oestrus. This was further supported by the individual assessment of the cats, whereby seven of the eight cats were significantly more active during oestrus. While this finding is promising, there were problems with the reliable behavioural detection of oestrus in this context that could lead to the inaccurate categorisation of anoestrous and oestrus activity data. The cats in which the behavioural detection of oestrus was most reliable exhibited the greatest increases in OPA during oestrus. This is encouraging and suggests that the difference between anoestral and oestral activity could be clarified through the more accurate categorisation of the activity data.*

Key words

Accelerometry, Actical®, cyclicity, feline, liquid chromatography, oestradiol, ovarian, reproduction.

Abbreviations

Overall physical activity (OPA), Actical® accelerometers (MMAs), oestradiol 17-β (E_2), Massey University Animal Ethics Committee (MUAEC), ethylenediamine tetracetic acid (EDTA), analyses of variance (ANOVA), high performance liquid chromatography (HPLC), ultra performance liquid chromatography coupled with mass spectrometry (UPLC-MS).

A2.1 Introduction

The accurate and reliable detection of oestrus has been difficult to achieve in a number of felids (e.g. cheetah (*Acinonyx jubatus*), margay (*Leopardus weidii*), ocelot (*Leopardus pardalis*), tigrina (*Leopardus tigrinus*), Eurasian lynx (*Lynx lynx*), Pallas' cat (*Otocolobus manul*) and Geoffroy's cat (*Oncifelis geoffroyi*)) due a lack of overt behavioural indicators of oestrus (Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002; Henriksen *et al.*, 2005). In cheetah, while there are no obvious oestrus-specific behaviours, oestrus is associated with subtle increases in the frequencies of behaviours such as locomotion and pacing, rubbing, rolling, sniffing, vocalisation, grooming and scent-marking (Asa *et al.*, 1992; Graham *et al.*, 1995; Wielebnowski and Brown, 1998). Oestrous behaviours are fairly consistent among felid species, although there can be considerable interspecific, inter-individual- and intra-individual variation in the intensity and frequency at which these behaviours are expressed (Michael, 1961; Wildt *et al.*, 1981; Bell, 2009). This variation makes the behavioural detection of oestrus challenging, even in species that exhibit overt behavioural changes during oestrus.

Many of the behaviours that felids express more frequently during oestrus appear to correlate with an increase in overall physical activity (OPA) (Foreman, 1997; Wielebnowski and Brown, 1998; Moreira *et al.*, 2001; Brown *et al.*, 2002). Thus, it may be possible to improve oestrus detection in felids by monitoring and detecting the changes in OPA associated with oestrus. Such an increase in activity is evident in dairy

cattle (*Bos taurus*) and has been use to reliably detection more than 90% of the oestrus events in this species (At-Taras and Spahr, 2001; McGowan *et al.*, 2007). Increases in OPA associated with oestrus have also been documented in other species including mice (*Mus musculus*) (Kopp *et al.*, 2006), rats (*Rattus norvegicus*) (Gerall *et al.*, 1973), and pigs (*Sus scrofa*) (Cornou, 2006).

Until recently, the accurate quantification of activity in domestic cats and other felids has been challenging due to the need for continuous behavioural observation, which is further complicated by the relatively cryptic and crepuscular or nocturnal lifestyle of most felids. There is thus a need for a less labour intensive method for assessing activity in cats. Actical® 'MiniMitter' accelerometers (MMAs; MiniMitter., Bend, OR, USA), initially designed to assess activity in humans, have now been validated as a tool for automatically quantifying the activity of cats, with accelerometer-based activity counts providing a highly accurate representation of observed measures of OPA (e.g. time spent active or distance moved) (Watanabe *et al.*, 2005; Lascelles *et al.*, 2008; Andrews *et al.*, 2015).

This study aimed to examine the association between physical activity and reproductive cyclicity in domestic cats by comparing accelerometer-based activity counts against salivary oestradiol-17β (E_2) profiles and behavioural data from several cats over an extended period. There is currently no known technique for measuring salivary E_2 concentrations in domestic cats. An additional objective was, therefore, to develop and validate a sensitive liquid chromatography-based salivary E_2 assay for cats.

A2.2 Method

A2.2.1 Animal husbandry

The cats were housed in seven purpose-built colony cages at the Centre for Feline Nutrition, Massey University, Palmerston North, New Zealand (175°38'E, lat. 40°22'S, long.), in mixed-sex groups of seven to 10 animals, of which one to eight were studied per cage. They were fed a complete and balanced (AAFCO, 2009) commercial moist (canned) feline diet (Heinz Wattie's Ltd, Hastings, New Zealand) and had *ad libitum* access to water. Each cage of cats was allowed 1 h in a play room per week (the same time and day each week so any effects on activity could be accounted for). During this time the cats were weighed and a dental score recorded. The husbandry of the cats

complied with the Animal Welfare (Cats) Code of Welfare (Anonymous, 2007) and Massey University Animal Ethics Committee (MUAEC) protocol number 12/12, and all samples were collected in accordance with MUAEC protocol numbers 13/101 (experiment 1) and 13/44 (experiment 2).

A2.2.2 Experiment 1: Validation of a sensitive salivary oestradiol (E_2) assay

A2.2.2.1 Group 1

Fourteen healthy, intact female cats aged from 3.0 to 11.4 years (mean ± SD, 7.52 ± 2.22 years) and weighing 2.3 to 4.0 kg (mean ± SD, 3.3 ± 0.6 kg) were used for experiment one.

A2.2.2.2 Experimental design

This study was ultimately designed to obtain comparative plasma, faecal and saliva samples from 14 different cats during both periods of oestrus and anoestrus (peak and basal oestradiol concentrations respectively). Each cat was monitored daily to identify periods of behavioural oestrus. Upon entering behavioural oestrus the cats were placed in solitary enclosures so that a faecal sample could be obtained. After a faecal sample had been collected from a cat its neck was shaved to expose the jugular vein and xylocaine, a local anaesthetic, applied to the area. After 10 min, jugular venepuncture was used to collect 2 ml of blood which was then put into an ethylenediamine tetracetic acid (EDTA) coated vacutainer and mixed for 15 min. The sample was then centrifuged at 2500 rpm for 10 min and the plasma transferred to a 2 ml eppendorf tube. Finally, a saliva sample was obtained by placing a cotton bud in the sulcus between the gums and cheek in the mouth and leaving it for 5-10 s. The tip of cotton bud was removed and placed in a 1 ml eppendorf tube with a hole at the base, which was then placed inside a 2 ml eppendorf and centrifuged at 3000 rpm for 10 min to extract the saliva. In order to obtain a minimum of 50 μL two saliva samples were collected within a 20 min period and combined. All faecal, plasma and saliva samples were stored at -80˚C until analysis.

A2.2.3 Experiment 2: Overall activity and oestrus

A2.2.3.1 Group 2

Ten clinically healthy, intact female cats aged from 1.8 to 10.1 years (mean ± SD, 5.6 ± 3.0 years) and weighing 2.3 to 4.0 kg (mean ± SD, 3.2 ± 0.6 kg) were used for experiment 2. Seven of these 10 cats had also been used in experiment 1.

A2.2.3.2 Activity assessment

Activity was assessed using MMAs, which each measured 28 mm x 27 mm x 10 mm and weighed 17 g. These MMAs used an omnidirectional accelerometer to detect movement in three planes (craniocaudal, mediolateral and vertical). An acceleration force produced a voltage output that was amplified and converted into a digital value that was corrected for the effects of gravity, a constant acceleration (Lascelles *et al.*, 2008). The values were then summed for a defined period (epoch), resulting in a total activity count for that period. The maximum epoch of 1 min was selected for this study to increase the duration over which activity data could be continuously collected, which was approximately 45 days.

A2.2.3.3 Experimental design

MMAs were attached to the collars of 10 cats and position ventrally. Activity data were then collected for a total of 39 - 43 days. Cat 4 (Mandy) was removed from the study prematurely due to the need for dental surgery, and thus the activity from this cat was only assessed for a total of just 34 days. The cats were monitored daily for behavioural signs of oestrus. Typical oestrus behaviours included lordosis, calling, rubbing/rolling, increased grooming/allogrooming and an increase in social behaviour. Saliva samples were collected daily using the cotton swab technique described previously (>50 µL saliva sample per day) and stored at -80˚C until analysed using liquid chromatography.

A2.2.3.4 Data evaluation and statistical analysis

The raw activity data were downloaded from the MMAs using an Actireader® device (Mini Mitter., Bend, OR, USA), and imported into an excel spreadsheet. The data (total MMA count/1 min) were summed to prove the total MMA count per h. A moving average has previous been shown to provide more detailed activity data without

compromising accuracy (Andrews *et al.*, 2015), thus the 24 h moving average of the total activity count per h was calculated for each cat over the study period.

Previous research suggests that there is considerable inter-cat variability in the associated between MMA activity counts and OPA, indicating that care needs to be taken when comparing the activity of cats or assessing the activity data of several cats as a combined data-set (Andrews *et al.*, 2015). Accordingly, the activity data of each cat (24 h moving average of the hourly activity data) were standardised by calculating the proportion change from the mean 24 h moving average of the hourly data for the total time spent in anoestrus and oestrus. The proportional activity data of each cat were then pooled for analysis.

All statistical analyses were conducted using RStudio version 0.98.1091 (R Foundation for Statistical Computing, Vienna, Austria) and an $\alpha \leq 0.05$ unless otherwise stated. A one-way analyses of variance (ANOVA) and a Tukey's posh hoc test were used to compare the proportional activity data of the cats during anoestrus and oestrus. These analyses were then repeated for each cat individually for the 24 h moving average of the hourly activity data and an $\alpha \leq 0.005$ used as suggested by the Bonferroni correction method.

A2.3 Results and Discussion

All of the saliva, plasma and faecal samples for experiment one been were collected from each of the cats in group 1. The daily saliva samples and MMA activity data for experiment 2 were also obtained (group 1; Table. A2.1). Unlike in Chapter 3, the activity data here were analysed using a moving average, which has previously been recommended when detailed activity data are required or preferred (Chapter 2; Andrews *et al.*, 2015). However, the results of experiment 2 are currently awaiting validation of a sufficiently sensitive liquid chromatographic salivary oestradiol assay; that is, the completion of experiment 1

A study by Shille *et al*. (1979) defined oestrus in domestic cats as the period over which plasma E_2 concentrations were greater than 20 pg/ml, with E_2 concentrations peaking at approximately 50 - 70 pg/ml during mid-oestrus. In contrast, the basal E_2 concentrations associated with anoestrus ranged from 5 - 14 pg/ml (mean ± SD, 11.8 ± 4.8) (Shille *et al.*, 1979). There has been no known study to date examining the link

between salivary and plasma E_2 concentrations, thus it is difficult to determine the sensitivity required for the identification of endocrine oestrus using saliva. Reports in humans suggest that salivary E_2 concentrations are approximately 0.5 - 5% of plasma E_2 (Worthman *et al.*, 1990; Shirtcliff *et al.*, 2000). If this is the case, then the estimated salivary E_2 concentrations during anoestrus and oestrus in the cat would be between 0.06 - 0.6 pg/ml and 0.25 - 3.5 pg/ml respectively.

Table A2.3 A summary of the MMA activity data (24 h running average of the total MMA count per hour) collected from the cats in group 2 during experiment 2.

Cat No.	Cat name	Age in years at start of trial	Average weight for trial (g)	Total duration of monitoring (days)	% of time in behavioural oestrus	Average activity count/h[a] (Overall)	Average activity count/h[a] (Oestrus)	Average activity count/h[a] (Anoestrus)
1	Blur	1.84	2255	43	100.0	3466	3466	N/A
2	Chika	4.04	2680	41	73.2	3916	4466	2293
3	Lana	6.59	3970	43	34.9	2265	2362	2206
4	Mandy	6.78	3740	34	79.4	19693	21040	13750
5	Milly	6.78	2820	43	37.2	6522	9294	4835
6	Nena	8.74	3230	43	86.0	8047	9517	7847
7	Nova	1.82	3140	43	37.2	2172	2274	2111
8	Opra	7.76	3469	39	35.9	4000	4549	3684
9	Poket	1.87	2920	42	100.0	19765	19765	N/A
10	Zeal	10.10	3960	43	74.4	4355	4543	3766
Total	-	-	-	414	65.7	74201	81273	40492

[a]Average of the 24 h moving average of the total MMA count per h over the entire trial, oestrus only and anoestrus only.
Note oestrus was determined behaviourally as endocrine data were unavailable.

Initial attempts at constructing a standard curve for E_2 using high performance liquid chromatography (HPLC) yielded insufficient sensitivity. Ultra performance liquid chromatography coupled with mass spectrometry (UPLC-MS) was also inadequate, with UPLC-MS appearing to be even less sensitive to the E_2 than HPLC. A lack of sensitivity to underivatised E_2 using liquid chromatographic techniques has previously been reported, and has thus led to derivatisation with dansyl chloride or a similar compound now being widely used (Schmidt *et al.*, 1978; Read, 1993; Gomez *et al.*, 2004; Nelson *et al.*, 2004; Xiong *et al.*, 2010). We investigated whether HPLC with fluorescent detection could assess dansyl chloride derivatised E_2 standards. Preliminary investigations of this technique show promise, with a detection sensitivity of approximately 10 pg/ml.

Unfortunately a sensitivity 10 pg/ml was insufficient for the detection of the estimated salivary E_2 concentrations of cats during oestrus. In addition, an effective technique for the extraction and preparation of the saliva samples remains to be developed and has

been challenging due to the low volume and high viscosity of the saliva samples collected. Until this is achieved and an accurate salivary E_2 assay (e.g. HPLC with fluorescent detection and dansyl chloride derivatisation) has been validated, examination of the association between endocrine oestrus and OPA cannot be completed.

It was, however, possible to investigate the link between behaviourally-detected oestrus and OPA in the cats. According to the behavioural data, eight of the 10 cats exhibited one or more periods of both anoestrus and oestrus during the study. The remaining two cats (cats 1 and 9) appeared to be in behavioural oestrus throughout the study period, thus their data were excluded. A one-way analysis of variance (ANOVA) and Tukey's post hoc test showed that the OPA of the cats was considerable higher during oestrus ($P < 0.001$). This finding is also supported by individual assessment of the cats, with seven of the eight cats showing significantly ($P < 0.001$) more activity during oestrus (Table A2.1 and Figure A2.1). Cat 6 appeared to be more active during anoestrus (Figure A2.1) but this differences was not significant ($P = 0.033$) and may reflect difficulties associated with the behavioural identification of oestrus.

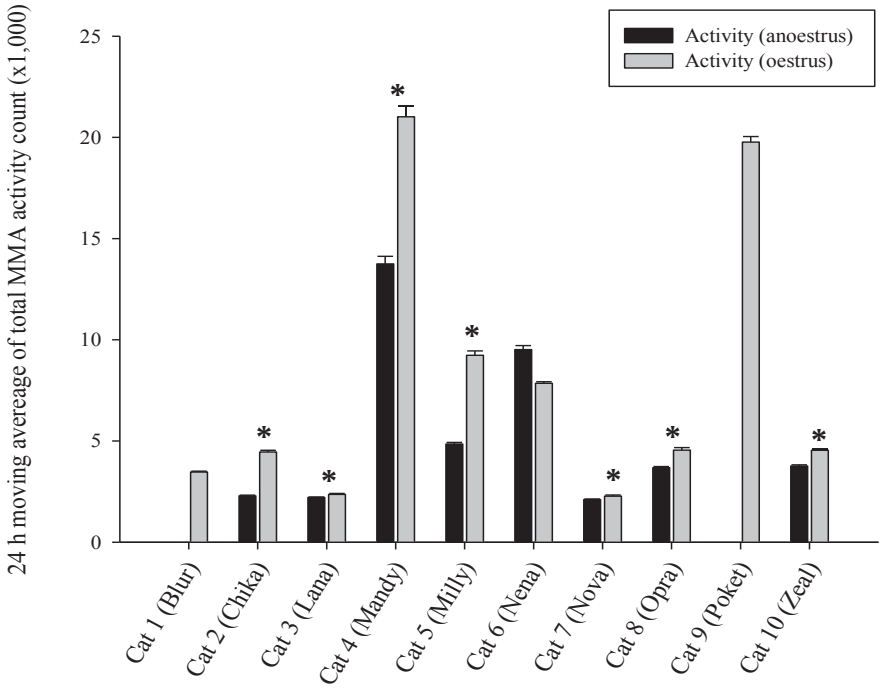


Figure A2:5 Comparison of the overall physical activity (OPA; sum of the 24 h moving averages of the total hourly MMA counts of each cat) of individual cats during anoestrus and behavioural oestrus periods. Statistical difference between the anoestral and oestral activity data of each cat (*$P < 0.005$). No anoestrus periods were detected in cats 1 and 9, thus no comparison could be made.

As mentioned previously, the behaviours associated with oestrus and the intensity at which they are displayed is highly variable between cats; in fact, there is even considerable variation between sequential periods of oestrus within the same cat (Michael, 1961; Michael and Scott, 1964; Wildt *et al.*, 1981). The behavioural detection of oestrus certainly appeared to be easier in some of the cats (e.g. cats 2, 4 and 5) in this study. Interestingly, the cats that exhibited the most pronounced increases in activity during oestrus were the individuals in which oestrus was easily detected through behavioural observation. It is possible that the difference between anoestrus and oestrus activity in the other cats could be clarified through the use of a more accurate method for detecting oestrus.

The longitudinal assessment of plasma E_2 or faecal E_2 metabolites (FEM) has been used to accurately monitor the reproductive state of cats and a wide range of non-domestic felids (Verhage *et al.*, 1976; Shille *et al.*, 1979; Wildt *et al.*, 1981; Graham *et al.*, 1995; Brown *et al.*, 2002; Henriksen *et al.*, 2005; Chatdarong *et al.*, 2006; Brown, 2011). In this study it was not feasible to collect plasma or faecal samples on a daily basis without compromising either the welfare (regular blood sampling) or the OPA (solitary enclosures for faecal collection) of the cats. These methods are similarly unsuitable for captive non-domestic felids. Blood sampling is particularly inappropriate for many non-domestic felids as it requires sedation, an undoubtedly stressful process for any animal (Schmidt *et al.*, 1979; Bonney *et al.*, 1981; Howard *et al.*, 1992; Brown and Wildt, 1997). Furthermore, common anaesthetics such as ketamine/HCL have been reported to disrupt ovarian cyclicity and prevent ovulation in felids (Howard *et al.*, 1992; Brown and Wildt, 1997). Thus it is impractical to repeatedly bloody sample cats and other felids to monitor their ovarian cyclicity, especially if monitoring for breeding purposes.

The collection of saliva from felids is likely to be possible without the need for sedation, especially if animals can be trained to chew on an absorbent object (e.g. toy) from which saliva could then be collected. So the development of a sensitive salivary oestradiol assay would benefit studies in both domestic cats and non-domestic felids.

Using the collected saliva samples to assess the reproductive status of the cats in this study would remove any error and variability associated with the behavioural detection of oestrus, and thus increase the accuracy and legitimacy of the current findings. It

would also confirm whether cats 1and 9 were in oestrus for the entire trial. This seems unlikely given that the mean duration of oestrus in the domestic cat is approximately 7.1 days; however, it is possible that they were given the extent of variation around this mean (2-118 days) (Verhage *et al.*, 1976; Shille *et al.*, 1979; Wildt *et al.*, 1981; Root *et al.*, 1994; Chatdarong *et al.*, 2006). It is evident then that the OPA of the cats in this study need to be compared during anoestrus and oestrus as described using the longitudinal assessment of salivary oestradiol.

A2.4 Conclusion

The comparison of the OPA of the cats during behaviour oestrus and anoestrus is promising and indicates an increase in activity during oestrus; however, the behavioural detection of oestrus is highly variable and difficult in some animals, potentially confounding the results of this study. It is important that the analysis of E_2 in the saliva samples collected in this study is completed so that the results can be re-evaluated in light of this data. At this stage the technique with the most promise to measure E_2 in the saliva samples appears to be HPLC with fluorescent detection and derivatisation with dansyl chloride.

A2.5 Acknowledgements

We would like to thank the staff at the Massey University Centre for Feline Nutrition for their assistance with conducting this study, with special thanks to Rachael Richardson, Margareet Hekman and Karin Weidgraaf for their assistance with blood and saliva collection. We would also like to thank Kay Rutherford (Institute of Food Science and Technology, Massey University, Albany, New Zealand), who has assisted us in attempting to develop a sensitive salivary oestradiol assay for domestic cats.

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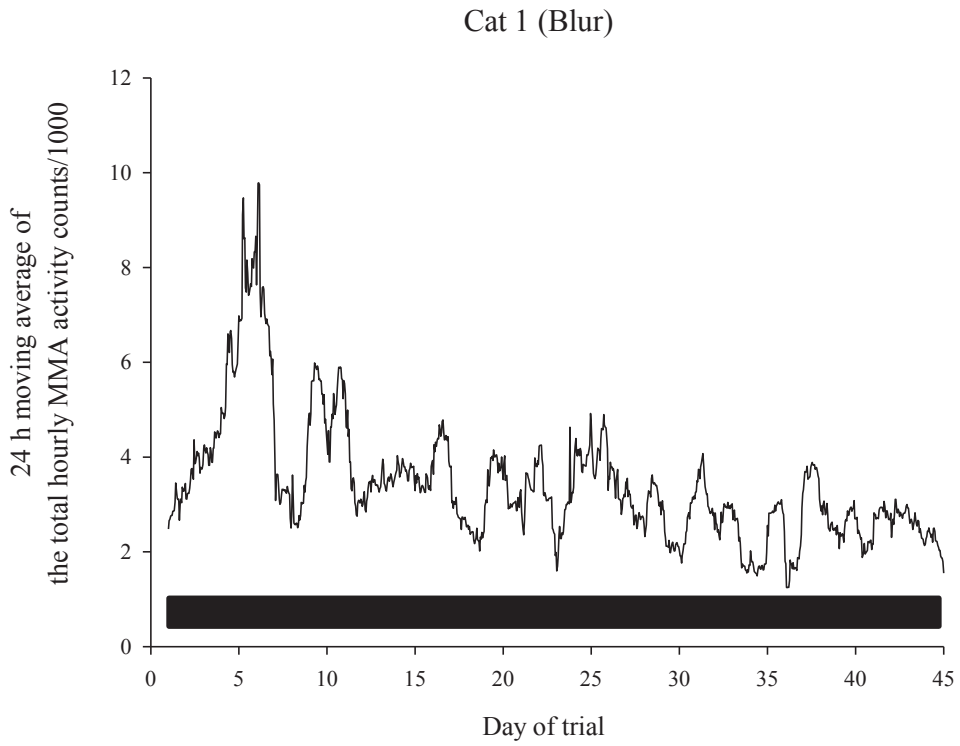
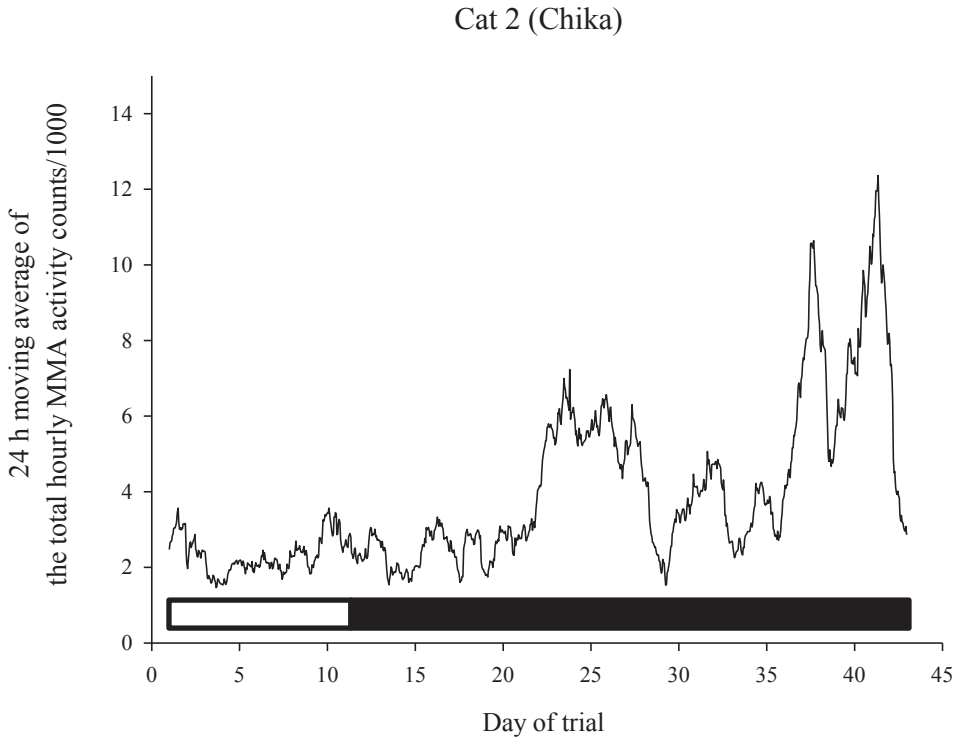
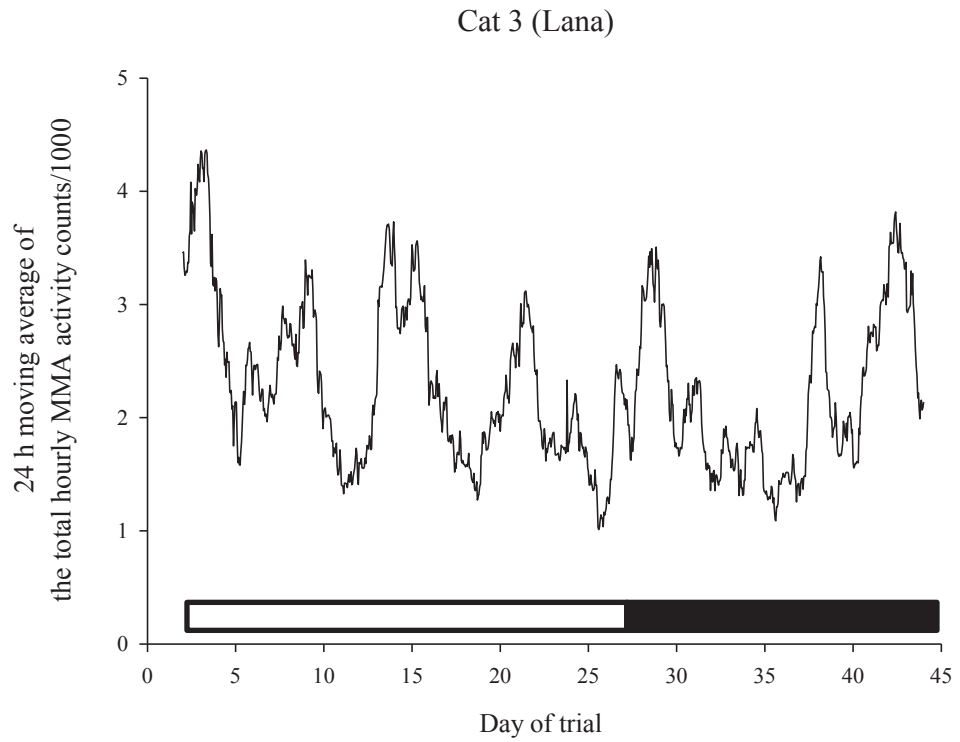
Appendix 3

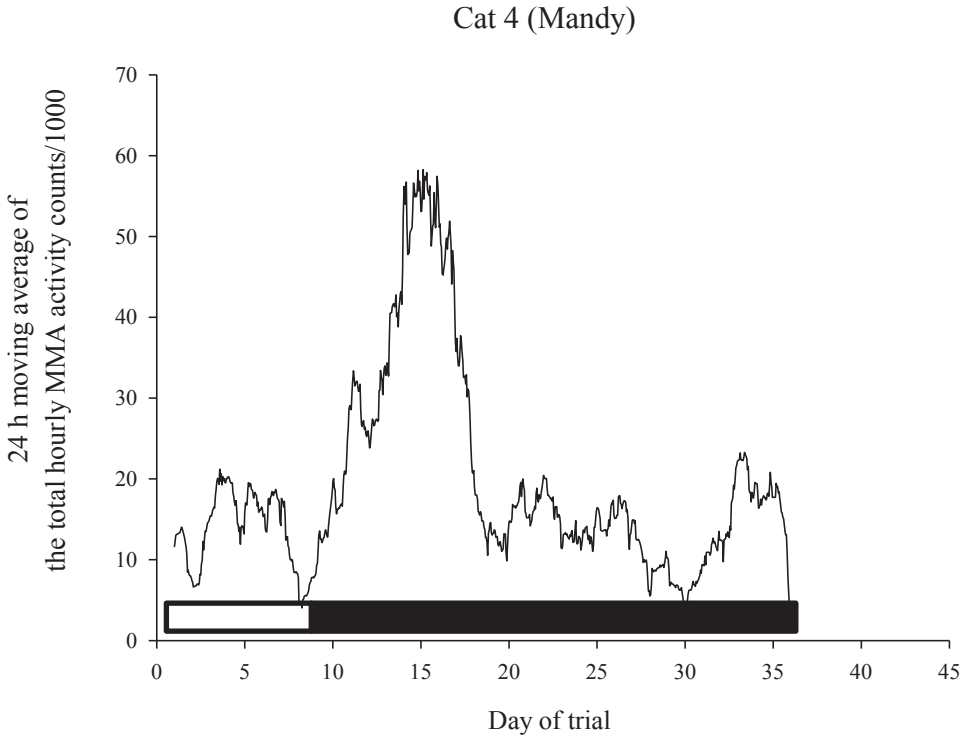
Temporal MMA activity profiles and reproductive status of the 10 cats studied in Chapter 2

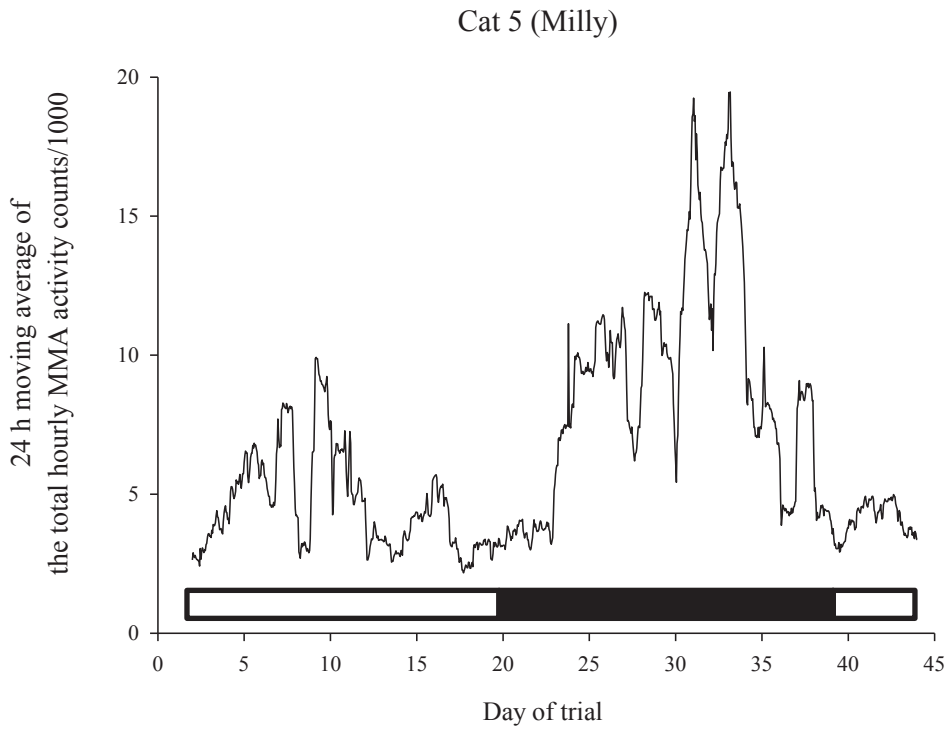
Figures A3.1 to 10 The temporal activity profiles (24 h moving average of the total hourly activity counts) and behaviourally assessed reproductive status (anoestrus (open bar) and oestrus (solid bar)) of the 10 cats studied in Chapter 3 and Appendix 2.

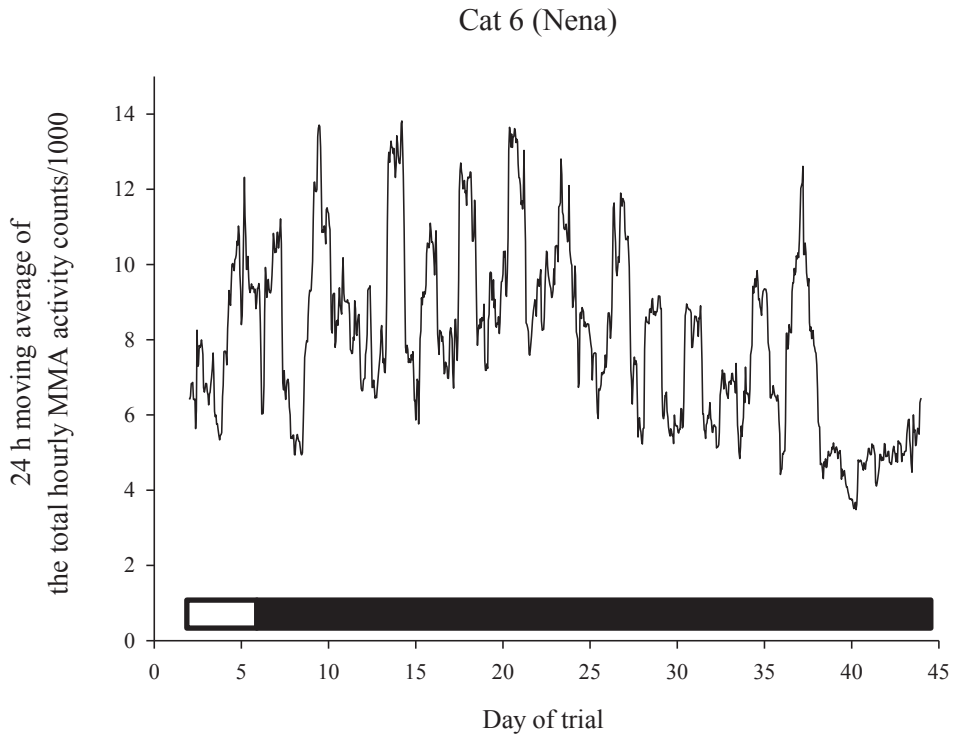




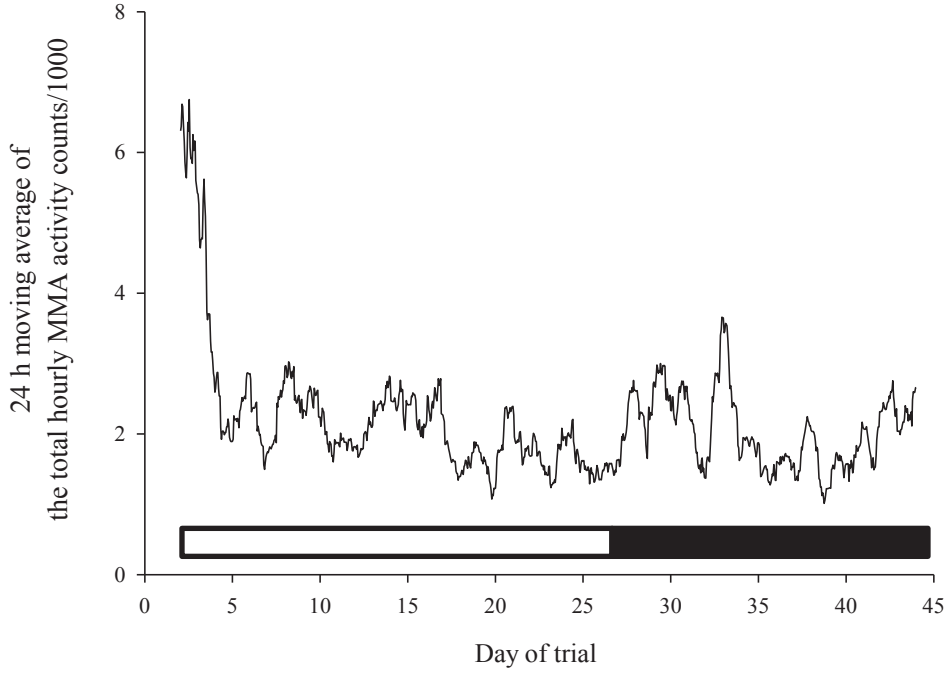

Cat 3 (Lana)
24 h moving average of
the total hourly MMA activity counts/1000
0
1
2
3
4
5
0
5
10
15
20
25
30
35
40
45
Day of trial


Cat 4 (Mandy)
24 h moving average of
the total hourly MMA activity counts/1000
0
10
20
30
40
50
60
70
0
5
10
15
20
25
30
35
40
45
Day of trial

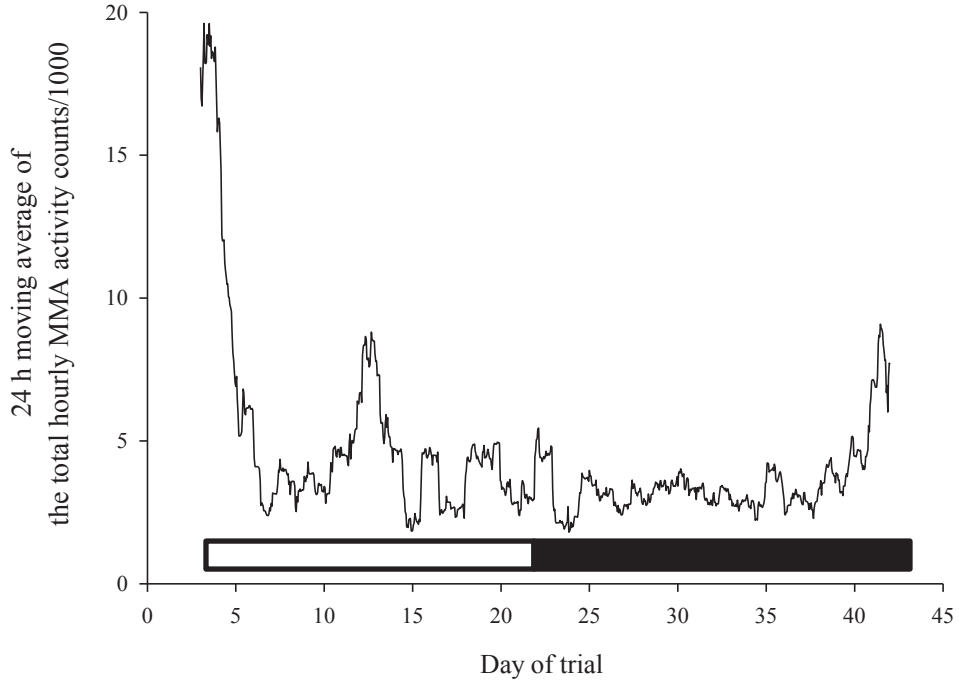

Cat 5 (Milly)
24 h moving average of
the total hourly MMA activity counts/1000
0
5
10
15
20
0
5
10
15
20
25
30
35
40
45
Day of trial

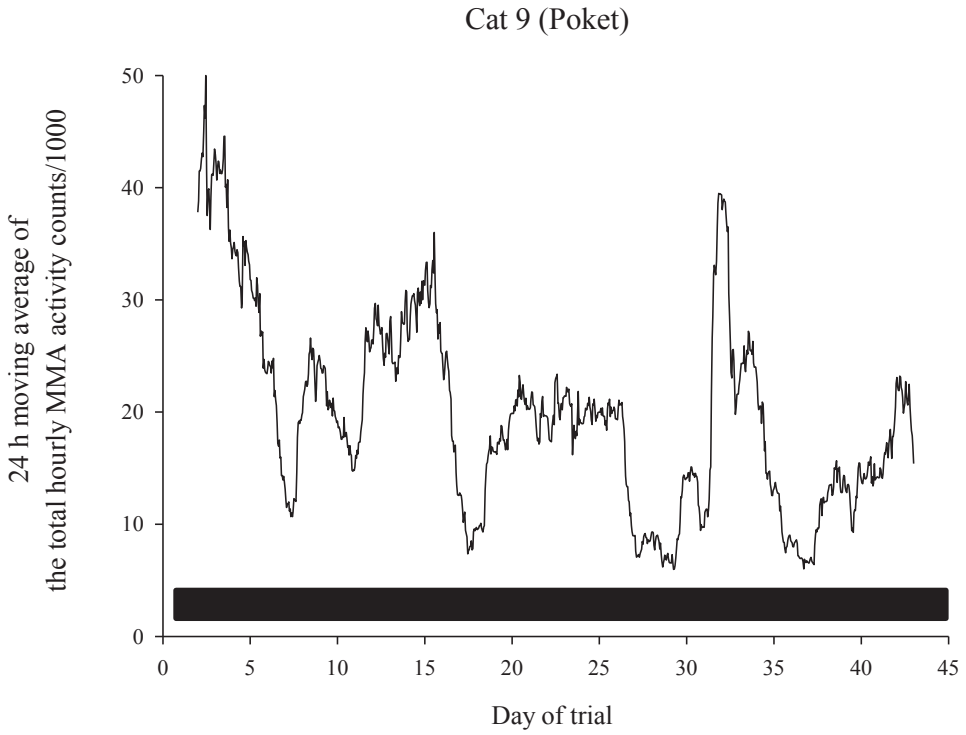

Cat 6 (Nena)
24 h moving average of
the total hourly MMA activity counts/1000
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
35
40
45
Day of trial

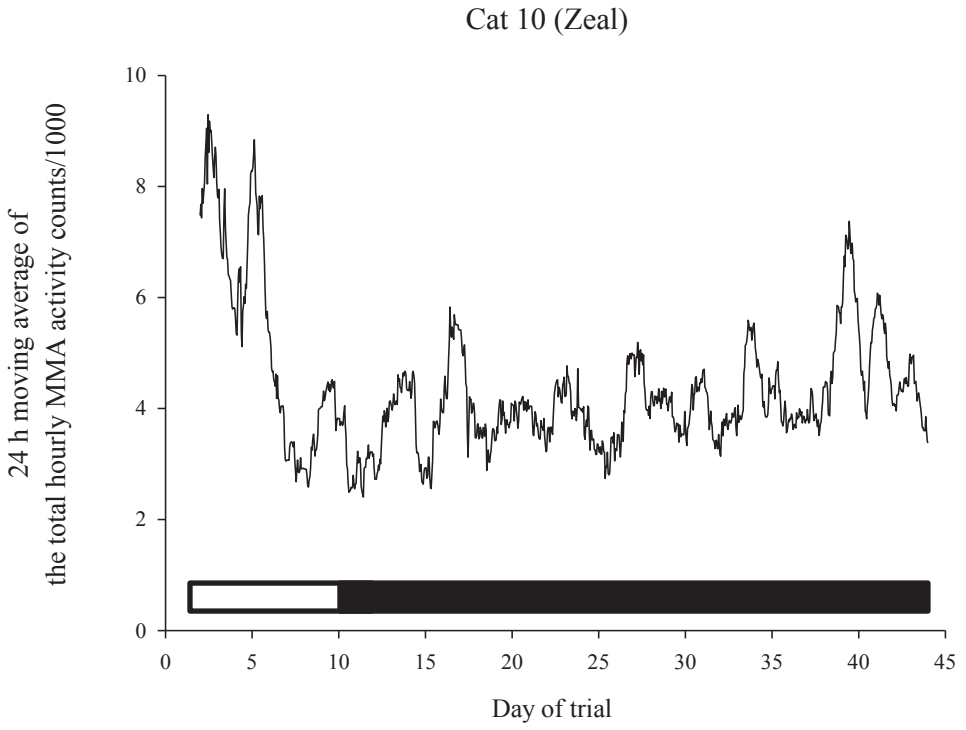
Cat 7 (Nova)



Cat 8 (Opra)




Cat 9 (Poket)
24 h moving average of
the total hourly MMA activity counts/1000
0
10
20
30
40
50
0
5
10
15
20
25
30
35
40
45
Day of trial


Cat 10 (Zeal)
24 h moving average of
the total hourly MMA activity counts/1000
0
2
4
6
8
10
0
5
10
15
20
25
30
35
40
45
Day of trial

Appendix 4

Andrews, C.J., Potter, M.A., Thomas, D.G., 2015. Quantifying activity in domestic cats (*Felis catus*) by accelerometry. *Applied Animal Behaviour Science* 173, 17-21.