# PREDICTABLE FEEDING IN ZOOS: RESEARCH METHODS AND BEHAVIOURAL EFFECTS

by

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A thesis submitted to Plymouth University
in partial fulfilment for the degree of

**DOCTOR OF PHILOSOPHY**

School of Psychology
Faculty of Science and Technology

**September 2012**

# Abstract

## Predictable feeding in zoos: research methods and behavioural effects

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The behaviour of zoo animals can come to anticipate temporally predictable feeding times. However, there is a lack of consensus over the effects of such routines on behaviour and welfare. Few studies have been published in this area, perhaps in part due to methodological challenges. The current programme of work therefore aimed to extend knowledge on the effect of predictable feeding routines, and to develop a suitable methodology to overcome challenges.

Prior to predictable feeding, tigers showed patterns of increased ‘active’ behaviours such as locomotion and pacing, and decreased inactivity. Geckos also showed increased ‘active’ behaviours such as locomotion and ‘stationary but moving head’ prior to predictable, compared to unpredictable feeding times, with significant differences between conditions (RSS = 0.059 and 0.047, $p < .05$). However, anticipatory patterns were not demonstrated for meerkats, which highlighted that other environmental factors may affect an animal’s response to temporal predictability (such as obtaining other food during the day, or signalled predictability).

Studying patterns of behavioural change over time requires long periods of observation which is often not possible for researchers. The current programme of work argues that a measure which can be reliably used by many, relatively untrained observers is necessary to study predictability. The measure of ‘busyness’, a subjective rating of animal behaviour, was tested for reliability and validity. Busyness ratings showed good inter-observer reliability (ICC > .72) and correlated with traditional measures of behaviour. Busyness ratings demonstrated clear patterns related to feeding time and gave a useful compound measure of behavioural change. The use of multiple observers was extended to a citizen science approach, where useful data on anticipation in fish were obtained from aquarium visitors using a touch screen.

The current programme of work successfully investigated the effects of predictable feeding routines on patterns of animal behaviour, alongside the development of suitable methods. The qualitative techniques developed here offer potential to increase the data obtained in future research into predictability and many other topics.

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# Acknowledgements

I am very grateful to Dr Phil Gee and Dr Vicky Melfi for their supervision throughout this programme of research, and I thank them for all of their guidance and support. I would also like to thank Dr Oliver Tucha for his advice.

Heartfelt thanks go to Paul Newbolt for his selfless support, encouragement, love and patience which has meant such a lot to me. I would also like to thank all of my family for their love and support in all of my endeavours.

I would like to thank everyone in the Field Conservation and Research Department at the Whitley Wildlife Conservation Trust for their friendship, support and cups of tea which have kept me going! In particular I would like to thank Dr Kirsten Pullen and Dr Holly Farmer for their kind support and for always listening and Dr Amy Plowman for her advice.

Thanks go to all of the students and observers who helped to collect data: Tom Adamson, Gemma Al-Jassar, Caroline Bysouth, Sonia Chapman, Douglas Clarke, Charlotte Durigan, Naomi Elder, Hayley Kendall, Kimberley Jayne, Catherine Jenkins, Sarah Johnson, Sophie Johnson, Athena Kenshole, Rhona Macbeth-Seath, Thomas Maddalena, Katie Major, Emma Nairne, Katharine Prevett, Sarah Rolfe, Laura Silvester, Charlotte Simons-Denville, Daniel Smith, Jenna Tyler, Samantha Western, Daniel Zahra, and the apprentice students.

I acknowledge the Whitley Wildlife Conservation Trust and Paignton Zoo Environmental Park for allowing this research to go ahead, and I thank all of the staff who helped with this project. In particular I would like to thank Mike Bungard for his help with the gecko study, and all of those keepers who took time out of their busy schedules to help feed animals according to a particular timetable: Andrew Fry, Paul Jacques, Nicky Jago, Rod Keen, Jill Lingard, Sam Lomax, Andy Meek, Mark Whiteside, and others. I also acknowledge the National Marine Aquarium, Plymouth for allowing this research to go ahead, and I thank Deborah Cracknell and staff for their assistance.

Thanks go to Anthony Mee for technical support and for programming the software for video recording and the touch screen programme. Thanks also go to Roger Creagh-Osborne for all of his technical support. I also thank the Maintenance Department at Paignton Zoo Environmental Park for their assistance in equipment set up.

Finally I would like to thank all of my friends and colleagues in Plymouth, Paignton and elsewhere, for their support and kind words.

# Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

The studies presented within this thesis all received ethical approval from the University of Plymouth, Paignton Zoo Environmental Park and the National Marine Aquarium (as relevant), and were carried out under full compliance with the British Psychological Society's human and animal ethical guidelines.

This study was financed with the aid of a studentship from the University of Plymouth Graduate School. Relevant scientific seminars and conferences were regularly attended at which work was often presented. Papers were prepared for publication.

## Publications:

Bishop, J.K., Gee, P., Melfi, V., Submitted. Busyness; a novel subjective measure to monitor patterns in animal behaviour over time. Journal of Applied Animal Welfare.

Bishop, J.K., Gee, P., Melfi, V. 2012. Citizen science: using aquarium visitors to record fish behaviour. BIAZA Research Newsletter. Zoo Research News. 13(3), 5.

Bishop, J.K., Gee, P., Melfi, V., 2009. How enriching are animals' routines? Introducing a new method to measure the behavioural effects of predictable husbandry routines. BIAZA Research Newsletter. Zoo Research News. 10(3), 4.

## Oral presentations:

Bishop, J.K., Gee, P., Melfi, V. (2012). Citizen Science. EAZA Annual Conference. Innsbruck, Austria. Presented by K. Pullen on behalf of the authors.

Bishop, J.K., Gee, P., Melfi, V. (2011). Recording animal behaviour using subjective measures and multiple observers. 10th International Conference on Environmental Enrichment. Portland, Oregon, USA.

Bishop, J.K., Gee, P., Melfi, V. (2011). Reliability and validity of a subjective measure to record changes in animal behaviour over time. 45th Congress of the International Society for Applied Ethology, Indianapolis, USA.

Bishop, J.K., Gee, P., Melfi, V. (2011). Reliability and validity of a subjective measure to record changes in animal behaviour over time. 13th BIAZA Research Symposium, Bristol, UK.

Bishop, J.K., Gee, P., Melfi, V. (2010). The effects of routines on the behaviour of captive animals. 2nd Annual School of Psychology Postgraduate and Staff Conference. University of Plymouth, UK.

## Poster presentations:

Bishop, J.K., Gee, P., Melfi, V. (2012). Citizen science: using aquarium visitors to record fish behaviour. 14th BIAZA Research Symposium, Newquay, UK.

Bishop, J.K., Gee, P., Melfi, V. (2012). Citizen science: using aquarium visitors to record fish behaviour. 3rd Annual School of Psychology Postgraduate and Staff Conference. Plymouth University, UK.

Bishop, J.K., Gee, P., Melfi, V. (2011). The effect of predictable routines on meerkat behaviour. The 10th International Conference on Environmental Enrichment. Portland, Oregon, USA.

Bishop, J.K., Gee, P., Melfi, V. (2010). Do meerkats anticipate feeding times? Association for the Study of Animal Behaviour Easter Meeting, Exeter, UK.

Bishop, J.K., Gee, P., Melfi, V. (2010). Do meerkats anticipate feeding times? 2nd Annual School of Psychology Postgraduate and Staff Conference. Plymouth University, UK.

Bishop, J.K., Gee, P., Melfi, V. (2009). How enriching are animals' routines? Introducing a new method to measure the behavioural effects of predictable husbandry routines. 9th International Conference on Environmental Enrichment, Torquay, UK.

Bishop, J.K., Gee, P., Melfi, V. (2009). How enriching are animals' routines? Introducing a new method to measure the behavioural effects of predictable husbandry routines. 11th BIAZA Research Symposium, Blackpool, UK.

Bishop, J.K., Gee, P., Melfi, V. (2009). How enriching are animals' routines? Introducing a new method to measure the behavioural effects of predictable husbandry routines. 1st Annual School of Psychology Postgraduate and Staff Conference. Plymouth University, UK.

## Conference awards

- 2012: Best poster of conference at the 14th BIAZA Research Symposium, Newquay.
- 2012: Second prize for best poster of conference at the 3rd Annual School of Psychology Postgraduate and Staff Conference. Plymouth University.
- 2010: Second prize for best oral presentation of conference at the 2nd Annual School of Psychology Postgraduate and Staff Conference. Plymouth University.
- 2009: Best poster of conference at the 9th International Conference on Environmental Enrichment.
- 2009: Best poster of conference at the 11th BIAZA Research Symposium, Blackpool.

Word count of main body of thesis: 74,933

Signed: ………………………………………………

Date: …………………………………………………

# Chapter 1

## *Introduction to the effect of routines on animal behaviour*

### 1.1. Introduction

The environment poses many challenges, to which animals show both physical and behavioural adaptations.  The behaviour of captive animals, can be affected by many factors, some of which are specific to the captive environment (see Hosey, 2005 and Morgan & Tromborg, 2007 for examples).  For example, behaviour might be affected by aspects of animal housing such as enclosure size, shape and furnishings (Carlstead, Fraser, et al., 1999; Hogan et al., 1988; Lyons et al., 1997; Ogden, et al., 1993), and the presence of visitors can also affect zoo animal behaviour (Fernandez et al., 2009; Hosey, 2000).  Another major influence on the behaviour of captive animals is their husbandry; the events imposed upon them by their keepers.  The keepers themselves and their actions can also affect animal behaviour (e.g., Hosey, 2008; Mellen, 1991).

Whilst it is obvious that environments have a physical structure, it could be argued that they can also have a temporal structure.  That is to say, changes in the environment may occur regularly, and that regularity might be seasonal, monthly, daily or according to some other cycle.  Behavioural adaptation to the temporal structure of zoo environments is the focus of the current programme of research, although literature from other captive environments (such as agricultural and laboratory environments) will be included as relevant.

Many ecologically significant events occur with a temporally predictable pattern, hence temporal behavioural adaptation is important. Synchronisation of behaviour with events in the environment is evident in many organisms (Mistlberger & Rusak, 2005) and research on such synchronisation will be discussed in the following review.

Captive animals, in their human-managed environments, experience many timed events to which they might behaviourally adapt. These include natural events such as the 24-hour light/dark cycle, and human-imposed events such as the arrival of zoo visitors or staff. Captive animals rely on their keepers to provide for their needs such as feeding and cleaning, and in the daily lives of the keeper these events can form a routine, and become fixed at certain times of the day. However, the timing of these events is often not considered in terms of how it will affect animal behaviour, despite evidence that it can influence behaviour and welfare (Berger, 2011; Waitt & Buchanan-Smith, 2001). Events may also be set purposefully at certain times, for example, feeding times might be set to allow viewing by zoo visitors. Relative to wild conspecifics, captive animals live in predictable and structured environments (Wemelsfelder & Birke, 1997). When events become temporally structured in this way, there is potential for the behaviour of the animals to show adaptations to their regular occurrence, such that it anticipates them.

The predictability of an event is the degree to which the occurrence of that event can be forecast. Events that can be predicted can potentially lead to a change in behaviour prior to the onset of the event, in anticipation of the event or reinforcement offered. Bassett and Buchanan-Smith (2007) identified two main types of predictability: temporal and signalled. Temporal predictability refers to the regularity with which an event occurs at a particular time of day, or according to a particular time schedule, whereas signalled predictability refers to the reliability with which a particular event is preceded by a signal or cue, which can occur irrespective of the time of day. Signalled predictability can be a confounding variable in studies of temporal predictability, as discussed further in Section 1.4.3, and there is not always a clear separation between the different types of predictability in the literature. The work presented in this thesis will focus on temporal predictability (henceforth referred to as predictability or temporal predictability); however reference will be made to signalled predictability where relevant (henceforth specifically indicated as signalled predictability), and in reviewing the literature, both types of predictability will be included due to the above-mentioned lack of separation.

The following is a review of some of the literature on timing in animals, and of some of the issues surrounding predictability and anticipation, and corresponding behavioural changes. It will focus on the behaviour of captive animals, as this is where most of the experimental studies have taken place.

## 1.2. Animal Timing

In order to investigate the effects of temporal predictability, it is first necessary to give a brief review of the mechanisms of timing in animals. There are different traditions in timing research. One tradition is that of chronobiology, which involves the study of synchronisation of behaviour with environmental rhythms. Research in chronobiology has shown that animals display behavioural rhythms with different periods. Examples include circadian rhythms that are based around a daily light-dark cycle, and circatidal rhythms that are based around the timing of tidal systems. These rhythms can persist in the absence of time cues (free-run), and so appear to be endogenously generated and self sustaining (Mistlberger & Rusak, 2005).

Biological rhythms are repeating patterns which occur within a set time period. For example, circadian rhythms have a period of one cycle per day that differs slightly from 24 hours in constant conditions. This rhythm is thought to be produced by an endogenous oscillator which measures duration or elapsed time against the progression of the cycle, and can be synchronised to an environmental cycle such as day length. This environmental cycle is known as a zeitgeber, and the process of synchronisation is known as entrainment (Mistlberger & Rusak, 2005). The phase and period of the internal clock of diurnal animals becomes entrained to the light cycle, and is thought to involve a light entrainable oscillator (LEO) located in the suprachiasmic nucleus of the hypothalamus (Mistlberger, 2011). This LEO controls the synchronisation of daily patterns of behaviour such as waking up as it gets light and going to sleep as it gets dark in diurnal animals. In addition to the LEO, it has been hypothesised that a food entrainable oscillator (FEO) also exists (Mistlberger, 2011), as patterns of behaviour can also be synchronised around feeding times (e.g., Herrero et al., 2005; Piccione et al., 2007; Sulzman et al., 1977). However the FEO system is not as clearly understood as the LEO (Challet, 2010; Mistlberger, 2011).

Another tradition in timing research is the study of short intervals, often as brief as a few seconds. There are a variety of procedures used to investigate timing in this way, and these studies are generally carried out in laboratories with animals such as rats or pigeons. The animals are usually required to perform a response such as pushing a lever or pecking a key in order to obtain reinforcement (such as food), and various interval schedules, such as Fixed Interval (FI) procedures, can be used (Lejeune & Wearden, 2006). In Fixed Interval (FI) procedures, there is a set length of time (t) between reinforcements, and a lever press will not result in reinforcement until that time has elapsed (Lejeune & Wearden, 1991). After training on an FI schedule, animals can soon show temporal regularities in their lever pushing response. The pattern shown is of few or no responses early in the interval, which increase in frequency to a peak at or near to the fixed interval value (which show a scalloped curve when plotted on a graph) (Dews, 1978; Lejeune & Wearden, 2006).

A number of theories have been put forward to explain animals' abilities to discriminate time on these short interval studies, and these are explained in detail in other literature. For example, the Scalar Expectancy Theory (SET) (Gibbon, 1977), the Behavioral Theory of Timing (BeT) (Killeen & Fetterman, 1988) and the Learning to Time Theory (LeT) (Machado, 1997). The various theories and traditions of timing are not necessarily mutually exclusive, and may all contribute to timing mechanisms (Mistlberger, 2009). It may not necessarily be clear which mechanisms are relevant to the study of predictable husbandry events, however these theories serve to demonstrate that predictable events can influence patterns of animal behaviour.

Whilst circadian timing concerns periods of around 24 hours, and short interval timing concerns intervals of seconds or minutes, there is little research into intermediate time intervals. One exception is a study by Crystal (2001) where rats (*Rattus norvegicus*) were given access to a meal at intervals of 14, 22, 22.5, 24, 25.5, 26 and 34 hours. The rats were observed to inspect their food dispenser with increasing frequency prior to, and in anticipation of the feeding time, however this anticipatory behaviour was less accurate for those feeding intervals less than, and greater than 24 hours, with greater variability in timing ability demonstrated at these times. Such variability would not be expected under the principles of short interval timing (scalar timing, as described by Gibbon [1977]), suggesting that short interval mechanisms were not used at these intervals. The greater accuracy around 24 hours demonstrates the strength of circadian timing. Whilst it is possible that that a greater number of exogenous cues (such as day length and daily patterns of human activity) were available to entrain timing of 24 hour intervals, this study serves to demonstrate a difference between the mechanisms of short interval and circadian timing. However, whilst more variable, the rats did show timing ability to these non-circadian times, and in a later study (Crystal, 2006) timing ability was demonstrated at 16 and 21 hours. It was therefore suggested that there may be another, long interval oscillatory mechanism used to time long intervals outside of the circadian range (Crystal, 2006).

In the captive environment, there are many events such as feeding or husbandry, which occur at intervals that are much longer than a few seconds. For example, these events may only occur once or a few times each day, or once every couple of days (for example large felids on ‘feed and starve day’ timetables as mentioned in Chapter 3). There is much evidence, particularly from laboratory studies, to suggest that events occurring at predictable times, such as a fixed feeding routine, can affect patterns of behaviour. For example, Gee et al. (1994) found that goldfish (*Carassius auratus*) showed increased lever pressing in the time leading up to a predictable feeding time on a 24 hour cycle, and Aschoff and von Goetz (1986) showed increased tugging at a food cup by squirrel monkeys (*Saimiri sciureus*) to obtain food in the time leading up to a predictable daily feed time.

Animals have also been observed to show changes in behaviour prior to predictable events that do not involve an operant response (such as lever pressing). For example, animals may show Food Anticipatory Activity (FAA) prior to a predictable feeding time. This activity has typically been described as increased wheel running or other forms of locomotion or movement, often recorded by movement past an infrared motion detector. Food anticipatory activity has largely been studied in small mammals, including rats (Mistlberger & Rusak, 1987), mice (*Mus musculus*) (de Groot & Rusak, 2004) and Syrian hamsters (*Mesocricetus auratus*) (Abe & Rusak, 1992), or in fish, including tench (*Tinca tinca*) (Herrero, et al., 2005), trout (*Oncorhynchus mykiss*) (Chen & Tabata, 2002), golden shiners (*Notemigonus crysoleucas*) (Reebs & Lague, 2000), greenback flounder (*Rhombosolea tapirina*) (Chen & Purser, 2001), Japanese sea catfish (*Plotosus japonicus*) (Kasai et al., 2009), Arctic charr (*Salvelinus alpinus*) (Brännäs et al., 2005) and sea bass (*Dicentrarchus labrax*) (Azzaydi et al., 2007). Increased activity in anticipation of food has also been demonstrated in other animals such as mink (*Mustela vison*) (Hansen & Møller, 2008) and in horses (*Equus caballus*) (Peters et al., 2012), where locomotion increased prior to feeding, alongside other measures such as heart rate and investigating behaviours.

Other behaviours have also been shown to change prior to predictable feeds, for example Salazar-Juarez et al. (2003) demonstrated increased drinking behaviour in rats in anticipation of a restricted feed. The range of anticipatory behaviours, and the effects of predictable events will be discussed in Section 1.4.

## 1.3. A note on animal welfare

It should be noted that the predictability of certain events for captive animals, may have effects that are relevant to the welfare of those animals. The concept of animal welfare has been extensively researched, and hence is not repeated here. However, it is important to recognise that there are certain behavioural and physiological indicators which have, under certain circumstances, been used as indicators of poor welfare state. Example welfare indicators are listed here, and will be used, in the current thesis, under the conditions and definitions set out by the stated authors. For example, abnormal behaviours can be used as welfare indicators (Mason et al., 2007; Mench & Mason, 1997), and can include behaviours such as self-directed behaviours (Baker & Aureli, 1997; Castles et al., 1999; Maestripieri et al., 1992) and stereotypic behaviour, such as pacing (Mason, 1991; Mason, et al., 2007). Inactivity can, in certain circumstances, have welfare implications in captivity, due to its potential association with obesity (Bloomsmith & Lambeth, 1995). In addition to behavioural indicators of welfare, physiological indicators such as gastric ulcers or high levels of 'stress' hormones such as glucocorticoids (e.g., Lane, 2006) have been taken to indicate poor welfare. All of these indicators can be indicative of differing levels of stress (as defined by e.g., Broom & Johnson, 1993) and can hence indicate welfare state.

It is not the aim of this programme of work to provide a detailed evaluation of the effects of predictability on animal welfare. There are a range of effects of predictability (as discussed in Section 1.4), which occur in different situations, and an overall effect on welfare cannot yet be determined based on the available literature. However, it is evident that predictability has important effects on animal behaviour, and welfare implications for such effects should be kept in mind when reviewing the literature.

## 1.4. Effects of predictability

As discussed in Section 1.2, animals have been demonstrated to show behavioural changes in response to predictably occurring events. However there is a lack of consensus in the literature over the effects of such predictability, in terms of the types of behaviour affected by predictability (temporal and/or signalled), the anticipatory response of the animals, and the benefits (or otherwise) of predictable and unpredictable routines. The following section reviews some of the literature regarding the effects of predictable and unpredictable routines, which is also shown in Table 1.1. As much of the previous literature has examined the effects of predictability of aversive and appetitive events separately, this structure will also be followed below.

*Table 1.1: Summary of the literature concerning the effects of predictable and/or unpredictable routines, categorised by preference for, or benefit of routines as either suggested by the authors or inferred from the results. N.B. this is a simplified version of these findings and aversive event examples cover only a sample of the literature, as this was not the main focus of the current thesis. *Denotes study of signalled predictability.*

| **TEMPORALLY PREDICTABLE / UNPREDICTABLE EVENTS** | **Predictable Preferred/Better** | | | **Unpredictable Preferred/Better** | | |
|---|---|---|---|---|---|---|
| | **Reference** | **Species** | **Reason for categorisation** | **Reference** | **Species** | **Reason for categorisation** |
| **Aversive events** | Badia et al. (1975) | Rats | Preference for predictable shocks | Lawler et al. (1984)* | Rats | Predictable shocks → greater elevations in blood pressure |
| | Badia et al. (1973)* | Rats | Preference for predictable shocks | Miller et al. (1983)* | Rats | Rats perceived predictable shock as highly aversive |
| | Gliner (1972)* | Rats | Preference for predictable shocks, and fewer stomach ulcers than unpredictable. | Pitman et al. (1995)* | Rats | Predictable shock → higher plasma corticosterone and norepinephrine levels |
| | Price (1972)* | Rats | Unpredictable shocks → more ulcers and greater weight loss than predictable | | | |
| | Weiss (1972)* | Rats | Unpredictable shocks → more gastric ulcers than predictable | | | |
| | Galhardo et al. (2011)* | Cichild | Predictable confinement → lower cortisol | | | |
| **Appetitive events** | Pérez -Padilla et al. (2010) | Rats | Unpredictable feeds → body weight loss, low food intake & excessive wheel running activity | Altman et al. (2005) | Lions | Predictable feeds → pacing prior to feed |
| | Prokasy (1956) | Rats | Preference for predictable food delivery | Carlstead (1998) | Felids | Predictable feeds → pacing prior to feed |
| | Steinhauer (1984) | Rats | Preference for predictable small rewards over unpredictable lager rewards | Jenny & Schmid (2002) | Tigers | Unpredictable feeds → decreased pacing |

| **Table1.1 continued** | **Predictable Preferred/Better** | | | **Unpredictable Preferred/Better** | | |
|---|---|---|---|---|---|---|
| | **Reference** | **Species** | **Reason for categorisation** | **Reference** | **Species** | **Reason for categorisation** |
| **Appetitive events** | Ulm et al. (1987) | Rats | Unpredictable feeds → less weight gained & disruption of food utilization. | Lyons et al. (1997) | Felids | Predictable feeds → pacing prior to feed |
| | Carlstead (1986)* | Pigs | Unpredictable signals → increased competition, aggression and variation in weight gain. | Mallapur & Chellam (2002) | Leopards | Predictable feeds → FAA, restless and anxious behaviour prior to feed |
| | | | | Shepherdson et al. (1993) | Fishing cats | Unpredictable feeds → decreased pacing & increased behavioural diversity |
| | | | | Weller & Bennett (2001) | Ocelots | Predictable feeds → pacing prior to feed |
| | Ulyan et al. (2006) | Brown capuchins | Predictable feeds → more time in proximity, activity & social behaviour and lower cortisol | Bloomsmith & Lambeth (1995) | Chimpanzees | Unpredictable feeds → decreased inactivity and abnormal behaviour |
| | | | | Fritz & Fritz (1979) | Chimpanzees | Unpredictable feeds used to prevent aggression caused by predictable feeds |
| | Cañon-Jones et al. (2012) | Atlantic salmon | Unpredictable feeds → higher level of more severe fin damage | Krishnamurthy (1994) | Francois Langurs | Predictable feeds → stress vocalisations prior to feed |
| | Sanchez et al. (2009) | Sea bream | Predictable feeds → lower cortisol and preparatory response | Wasserman & Cruikshank (1983) | Hamadryas baboons | Predictable feeds → aggression prior to feed |
| | | | | Wilson & Wilson (1968) | Chimpanzees | Predictable feeds → aggression prior to feed |
| | | | | Galhardo (2011) et al.* | Cichild | Predictable feeds → anticipatory behaviour and higher cortisol |
| | | | | Brännäs et al. (2005) | Arctic charr | Predictable feeds → increased aggression prior to feed |

### 1.4.1. Aversive events

There have been several studies carried out on the behaviour of animals in relation to predictable and unpredictable aversive events (such as electric shocks); however the majority of these have been conducted in laboratory settings and have produced some mixed conclusions in terms of the benefits of either schedule (see Table 1.1).

In preference test studies, where animals are given a choice between unpredictable and predictable shocks, preference has been shown for predictable shocks. For example, Badia et al. (1975) tested rats in an operant conditioning chamber. Shock was delivered according to fixed-time (predictable) (60 seconds) or variable-time (unpredictable) (8-230 seconds) intervals and the rats were able to switch from one shock schedule to the other for 3 minutes by pushing the lever. A higher rate of lever pressing was shown in the rats able to change from a variable to a fixed time schedule, than in the rats able to change from a fixed to a variable schedule. This has led to the theory that an element of predictability makes the shock less aversive (Bassett & Buchanan-Smith, 2007).

In addition to animal preferences for predictable aversive events, predictable aversive events may compromise welfare less than unpredictable aversive events, as symptoms of distress and compromised health have been associated with unpredictable shock. For example, Gliner (1972) and Price (1972) found increased occurrence of stomach ulcers in rats exposed to unpredictable, rather than predictable (signalled) shock. Price (1972) also noted increased weight loss in the unpredictable shock conditions.

In contrast, other studies have shown negative effects to be associated with predictable, rather than unpredictable routines. For example, Pitman et al. (1995) found that rats exposed to predictable (signalled) shocks had higher plasma corticosterone and norepinephrine levels than those exposed to unpredictable (signalled) shock. Lawler et al. (1984) found that, contrary to predictions, predictable shock (signalled) resulted in greater elevations in blood pressure in rats than unpredictable shock. It has also been suggested (Abbott et al., 1984) that over long periods, predictable, rather than unpredictable shocks may be more stressful.

Whilst some studies of rats have demonstrated a preference for predictable shock (as described above), it has been suggested that rats actually ‘perceive’ predictable shocks to be more aversive than unpredictable shocks. For example, Miller, Greco, Vigorito & Marlin (1983) trained water-deprived rats to discriminate between high-intensity and low-intensity electric shocks by pressing ‘high aversive’ or ‘low aversive’ levers to receive a reward of water. Results showed that when the shocks were made predictable or unpredictable (signalled), more ‘high aversive’ lever pressing was shown when shocks were predictable, indicating higher perceived intensity of the predictable condition. The authors suggest that in this case, the signal may have acquired aversive properties.

Studies of predictable and unpredictable aversive events have therefore shown mixed results, with neither routine clearly emerging as either preferred by the animals or causing fewer physiological symptoms of stress. The results found by Miller et al. (as described above), regarding higher perceived aversiveness of predictable (signalled) shocks raises an important point over the different types of predictability. Many studies of predictable aversive events use signalled predictability. It is possible that animals may respond differently to signalled rather than temporally predictable aversive events, which creates further difficulties in drawing conclusions from the already mixed literature.

Whilst electric shocks have been the focus of the literature reviewed above, shocks would not be expected to be routine events in the lives of most captive animals. Research that may be more directly relevant has considered confinement as an aversive event, for example Galhardo et al. (2011) found the predictable confinement of cichlids (*Oreochromis mossambicus*) resulted in lower plasma cortisol than unpredictable confinement. The authors therefore recommended that husbandry procedures should involve reliable signalling of confinement. Other events in the lives of captive animals also have the potential to be aversive, such as cleaning, locking away at night, veterinary checks and visits by crowds of zoo visitors (e.g., Hosey, 2000). It seems reasonable to assume that the timing of these potentially aversive events could affect animal behaviour and welfare. However, as yet there is little research to inform decisions on their timing

### 1.4.2. Appetitive events

There are many potentially appetitive events in the lives of zoo animals, including feeding and the provision of environmental enrichment. As described in Section 1.2, there is evidence, mainly from laboratory studies, to suggest that predictable feeding times can affect animal behaviour, such that behaviours change in anticipation of the feed. However, as for studies of aversive events, there is a lack of consensus over the effects of predictable and unpredictable routines.

In some early laboratory-based preference tests, animals were shown to have a preference for predictable appetitive events. For example, rats were found to show a preference for the side of a maze in which food was consistently provided after a delay of 30 s, compared to the side that offered food on only half of the trials (Prokasy, 1956). Rats have also been shown to demonstrate a preference for small rewards when given consistently after a 15 s delay (predictable), over larger rewards given on only 50% of occurrences after the same delay (unpredictable) (Steinhauer, 1984). However, preferences between different types of schedule are not always demonstrated; de Jonge, Ooms, et al. (2008) gave pigs (*Sus scrofa*) the choice between levers offering food rewards according to fixed or variable intervals of 8 seconds, to test the hypothesis that the pigs would prefer an unpredictable schedule that more closely simulated wild conditions. However, the pigs did not show such a preference. It is worth noting that in many of the studies reviewed, the animals were domesticated, and therefore may have behaved differently to wild or zoo animals. For example in some studies of contrafreeloading, domestic animals such as hens have been shown to choose the freely available food resource rather than working for reinforcement as would be expected by contrafreeloading theory and as shown by their wild counterparts (Schütz & Jensen, 1999). If domestic animals show these differences in behaviour then any possible effect of domestication on preferences for predictability should also be considered.

It has been argued that predictable feeding schedules are better for animals since predictability enables animals to make a suitable preparatory response such as salivation (Prokasy, 1956) or insulin release (Ulm et al., 1987). Prokasy (1956) theorised that the rats' preference for the predictable side of the maze (as described above) was to enable a suitable preparatory response to the food reinforcer (salivation), and that through this preparatory response, the food provided a maximum reinforcement.

Whilst some preference tests have revealed a preference for predictable feeding, in many captive environments, animals are not able to choose their feeding routine and are restricted to the feeding routine provided by their keepers. Hence the animals have little control over the timing of food presentation (see Section 1.4.3 for further details regarding control). Studies of the effects of such routines must be made by observing changes in behaviour related to the routine, and as will now be reviewed, a range of behavioural changes have been reported, with uncertainty over which type of routine is better for animal welfare.

Some studies of predictability have found predictable feeding routines to be associated with stereotypic behaviour such as pacing. For example, Carlstead (1998) reported pacing in four felid species prior to feeding time. In another study, Weller and Bennett (2001) examined the activity budgets of captive ocelots (*Leopardus pardalis*) and found that pacing behaviour peaked in the morning, prior to predictable feeding times, indicating anticipation of the feeding event. However, it should be noted that this pre-feed peak in pacing was only found in four of the six ocelots observed. Lyons et al. (1997) also reported greater levels of pacing by various felid species in the hour before feeding, than in the hour after feeding; anticipatory behaviour or frustration of appetitive behaviour were suggested as reasons for this. However, this result was not statistically significant (potentially an effect of the low sample size or variation between individuals or species), and it could be expected that behaviour would differ before and after feeding, therefore care should be taken in interpretation of such a result, as discussed further in Chapter 2.

Abnormal and self-directed behaviours have been associated with predictable feeding routines in stump-tailed macaques (*Macaca arctoides*) (Waitt & Buchanan-Smith, 2001). The macaques' rates of self-directed behaviour, inactivity, vocalisation and abnormal behaviours increased significantly in the hour prior to a predictable feeding time. Further, when feeding times were delayed (and hence considered by the authors to be more unpredictable), these behavioural patterns were prolonged, suggesting that delays to a predictable feeding schedule can increase the stress caused by ‘predictable’ routines. However, this study used only normally occurring deviations to feeding time, rather than a purposely designed schedule. As such, few repeat observations were made of each feeding condition (early, on time, or late feeds) also, only 10 min observation periods within the hour prior to feeding were reported, which limits the strength of subsequent conclusions, as is further discussed in Chapter 2. In addition, it could be questioned whether deviation from a predictable feeding routine would cause the same behavioural response as a truly unpredictable routine, which raises issues over what degree of variability in timing is required to make a routine ‘unpredictable’ (as described further in Section 2.2).

Other behaviours associated with predictable feeding times include possible stress vocalisations (PSV) in François’ langurs (*Trachypithecus francoisi*) (Krishnamurthy, 1994). These were vocalisations which were described as “vocalisations related to stereotypic behaviour and possible stress under captive conditions” (p.91), and were found to occur prior to feeding, associated with stereotypic behaviours such as circling the enclosure. These vocalisations have not been reported in the wild, and ceased soon after feeding.

Predictable feeding regimes have also been associated with aggression in some primates prior to set feeding times, for example in Hamadryas baboons (*Papio hamadryas*) (Wasserman & Cruikshank, 1983) and chimpanzees (*Pan troglodytes*) (Wilson & Wilson, 1968). However, in contrast, Cañon Jones et al. (2012) found that unpredictable feeding led to more severe aggression in Atlantic salmon parr (*Salmo salar*). Cañon Jones et al. used fin damage as an indicator of poor welfare, and found unpredictable daily feeding routines to result in a higher frequency of, and more severe fin damage compared to those on predictable feeding routines. It was suggested that this higher rate of fin damage was due to a change in the social dynamics of the fish fed unpredictably, which did not happen in the predictably fed fish. Overall levels of aggression were higher in predictably fed fish, but unpredictably fed fish experienced poorer welfare as they experienced more severe aggression.

Some studies have succeeded in reducing behaviours associated with poorer welfare, that have been observed under predictable feeding times, by reducing the regularity of feeding regimes, thus creating unpredictable regimes. Jenny and Schmid (2002) studied two Amur tigers (*Panthera tigris altaica*) who were traditionally fed at the same place and time of day, hence feeding was temporally predictable. Prior to the study, these tigers were reported to show stereotypic pacing behaviour (up to 1.5 hr during 6 hr of observations). The authors suggested that the pacing was due to permanently frustrated foraging behaviour caused by the set feeding time, as any foraging attempts by the tiger throughout the rest of the day would be unsuccessful, yet the motivation to forage would remain, leading to pacing behaviour. Jenny and Schmid compared pacing behaviour during traditional feeding (predictable) to an unpredictable condition in which food was provided in electronically controlled feeding boxes, which made food accessible only at unpredictable times. The provision of the feeding boxes resulted in a significant reduction in daily time spent pacing, which suggests a benefit of the unpredictable routine.

Whilst Jenny and Schmid's study was successful in demonstrating a reduction in pacing as a result of the feeding boxes, there are several extensions to this research which may provide more informative results in terms of the tigers' response to temporal predictability. Jenny and Schmid provided the different feeding conditions for only 3 days at a time, and randomised the order of condition presentation. Whilst differences were demonstrated between conditions, the variability in the tigers' routine caused by the changing conditions may have affected the results, and it could be questioned whether the same results, or any habituation effect would be seen if a longer period of exposure to each condition was used. The authors do not report any data pertaining specifically to pre-feeding times, or at times throughout the day. Such data would be beneficial in order to determine the precise effect of the traditional, predictable feeding time on patterns of tiger pacing. In addition, this study includes several potential confounding variables; the feeding boxes required the tigers to actively open them in order to obtain food, thus incorporating an element of control into the tigers' feeding behaviour (see Section 1.4.3 for further discussion of control), the boxes themselves were novel objects and hence may have had enriching effects and they also resulted in the presentation of multiple feeds, placed in different locations in the enclosure, all of which may have affected behaviour. The authors themselves recommended further study in which food is provided at unpredictable times by keepers, rather than by the feeding boxes, and this would go some way to addressing the effects of these other variables, thus isolating the effect of temporally unpredictable feeding.

Unpredictable routines have also been shown to be beneficial to primates. For example, Bloomsmith and Lambeth (1995) studied 4 groups of chimpanzees that had historically been fed on a predictable routine. Six months prior to the start of the study, the predictability of the feed time was reduced for 2 of the 4 groups, resulting in 2 predictably fed groups (fed at a mean time of 08:16 with a standard deviation of 10 min) and 2 unpredictably fed groups (fed at a mean time of 09:08 with a standard deviation of 57.2 min). Data were collected using instantaneous scan sampling every 30 seconds during 30 minute observation periods immediately prior to the feed and during a comparison 30 minute observation in the morning, taken at a time which did not conflict with other pre-feed times. Higher levels of inactivity and abnormal behaviours were observed in the period prior to the predictable feeding time, compared to the time prior to the unpredictable feed and in the comparison observation session, and it was suggested that feeding on an unpredictable schedule was associated with increased species-appropriate behaviours.

In contrast to the studies described above, some researchers have found predictable feeding routines to be more beneficial. For example, Ulyan et al. (2006) studied brown capuchins (*Cebus apella*) on a predictable and unpredictable feeding routine; in the latter, feeds were provided up to 2 hours earlier or later than the set predictable feed times, on a random schedule. The capuchins showed more time inactive and less time in proximity and engaged in social behaviour in the hour before the feed in the unpredictable condition, compared to the predictable condition. Higher cortisol concentrations were also found in the unpredictable condition. The authors suggested that predictable feeding schedules were therefore more beneficial to the capuchins. Ulyan et al. exposed the capuchins to each condition for a long period (6 weeks), therefore increasing the strength of the conclusions on the effect of the conditions (further described in Section 2.2). However, the unpredictable condition involved deviations from a predictable routine, rather than a purposefully designed random schedule, and it could be questioned whether results would be different using a truly ‘unpredictable’ feeding schedule (as also mentioned above regarding Waitt and Buchanan-Smith [2001], and further discussed in Section 2.2).

Pérez-Padilla et al. (2010) also reported negative effects of unpredictable feeding, whereby rats showed excessive wheel-running, lower food intake and greater weight loss, resulting in greater activity-based anorexia when fed on an unpredictable schedule, compared to those on a predictable schedule. Similarly, Ulm et al. (1987) found unpredictable feeding to result in less weight gain than predictable feeding, which was interpreted as a negative effect within the conditions of the study. Sánchez et al. (2009) reported that unpredictable feeding of sea bream (*Sparus aurata*) resulted in higher cortisol than predictable feeding and as a result recommended predictable feeding times as beneficial for fish welfare.

The above review of the literature reveals a lack of consensus on the effects of predictable and unpredictable routines. Even within studies there may be conflicting conclusions. For example, Gilbert-Norton et al. (2009) compared coyote (*Canis latrans*) behaviour on predictable and unpredictable feeding routines, and found greater levels of scent marking and howling associated with unpredictable routines which they concluded were at more species-typical levels, thus positive welfare indicators, compared to predictable routines. However the authors also suggested that increased howling and marking may have been due to food-related frustration on the unpredictable routine.

### 1.4.3. Reasons for variation in the effects of predictability

A variety of behavioural and physiological effects have been demonstrated in association with predictable and unpredictable routines. Reasons for this variety may include differences between studies such as the experimental design, and the subjects and their environment; indeed, an animal’s response to predictability may be specific to a particular situation.

There are many, often co-varying factors that could potentially affect the predictability of events, and the animal's response to that predictability. Such factors include the type of predictable event such as aversive or appetitive, or the type of reinforcement. For example, timing experiments have found that the animal's response differs with differing reinforcements, in terms of reinforcement type (e.g., liquid or solid food) or magnitude (amount or duration of reinforcer) (Lejeune, 1990). In studies involving an operant response from the animal, the type of operant response can also be relevant. For example, pigeons show more accurate timing with a perching response than with a key pecking response (as described in Lejeune, 1990).

Other factors influencing an animal's response to predictability may include its internal state and motivation, previous experiences, and the species and feeding ecology. For example, predictable feeding routines as reviewed in the current chapter have been shown to cause, amongst other behaviours: aggression in pigs and primates, pacing in carnivores, self-directed behaviours in stump-tailed macaques, food anticipatory activity in a range of species and inactivity in chimpanzees. Table 1.1 details some of these differing results. So far no particular behaviour has clearly emerged as specifically constituting an anticipatory response. Different species may differ in their responses to predictability, and in their timing abilities and accuracy. Lejeune and Wearden (1991) reviewed the literature on Fixed-Interval responding in different species and found different responses. For example, "higher" animals such as cats and monkeys showed good temporal regulation, rats and mice showed poorer regulation at long intervals, and fish, turtle doves and turtles showed poorer temporal regulation. It may therefore be necessary to consider each case individually and determine what anticipatory behaviours are shown for the particular species and circumstances under investigation.

Studies of predictability have differed in their methodologies. For example different feeding schedules, observation lengths, study lengths, feeding times and interpretations of 'unpredictable' feeding have been used. Such methodological differences may be another reason for differences in the data. For example whilst evidence exists for animal timing ability at short intervals (e.g., Lejeune & Wearden, 1991) and for intervals close to 24 hours (e.g., Mistlberger, 1994) there has only been limited research into timing ability at other intervals such as a few hours (e.g., Crystal, 2001). Hence there is currently a gap in the knowledge regarding the effects of different routines, and the extent to which patterns of animal behaviour can be affected by scheduled events at different intervals. Issues of methodology are an important factor in the interpretation of studies of predictability and will be further reviewed in Chapter 2.

There are also several potential confounding variables in the study of temporal predictability which may affect the results. These include the amount of control that an animal has over its feeding time, or its access to food, and also signalled predictability, as described below.

### *Control*

Studies into predictability are often confounded by aspects of control, for example, Weinberg & Levine (1980) suggest that in many studies of predictable aversive events, the variable of control is also introduced, and may itself affect the response of the animal to the aversive event. If, in the process of providing predictability (or unpredictability) to the animal, they gain greater control over the event, such as the feeding boxes provided by Jenny and Schmid (2002) (as described in Section 1.4.2), then it may be this, rather than predictability *per se*, that accounts for the effects attributed to predictability. Relevant aspects of control may include control over the event itself (e.g., feeding), or control over elements of the animal’s environment, and the two may not be mutually exclusive. Such concepts of control are developed below.

Bassett and Buchanan-Smith (2007) argue that the concepts of predictability and control are closely related, and control is thought to be important to the welfare of animals, (Wemelsfelder & Birke, 1997), with detrimental effects caused by a lack of control (Mineka & Kihlstrom, 1978; Waitt & Buchanan-Smith, 2001). Bassett and Buchanan-Smith (2007) reviewed the relationship between predictability and control and formed the following hypotheses: 1) the effects of predictability and control can be additive; 2) control can provide predictability, 3) a predictable situation provides an animal with control. They suggest that animals which live without predictability or control will show behavioural problems.

Animals in captivity have reduced control over their environment, for example if they cannot find food in their enclosure, or access to food is determined by the husbandry regime and feeding times. Similarly, they have no control over events such as the arrival times of zoo visitors. It should be noted that many studies of predictability in laboratory conditions differ from zoo-based studies in that the animals have some degree of control over their food delivery. Whilst the food availability is still restricted to certain time periods, within those time periods the retrieval of food is contingent on the animal's own lever-pushing behaviour. Zoo-animals are often restricted to their keepers putting the food into the enclosure.

It has been suggested that when control is absent, making events as predictable as possible may aid animals in coping (Waitt & Buchanan-Smith, 2001) because it gives them information about when the events will and, perhaps more importantly, will not occur. This may enable the animals to make a preparatory response (Prokasy, 1956), or give them information about a 'safe period' when they know not to expect an event (Abbott, et al., 1984). The importance of such a 'safe period' was demonstrated for an aversive event by Weiss (1972); rats were given electric shocks via an electrode attached to their tails, which meant that a preparatory postural change to avoid the shock would be unsuccessful, and the rat would still receive the full shock. The rats were exposed to either unpredictable or predictable shock, and those exposed to the unpredictable shock suffered from more gastric ulcers than those exposed to the predictable shock. In the absence of a postural preparatory response, Weiss suggested that it is likely that there was another reason for the predictable shock being less stressful than the unpredictable shock, which could be timing of the safe period.

In contrast, it has also been suggested that in the absence of control, predictability may be more aversive than unpredictability (Bloomsmith & Lambeth, 1995; Weinberg & Levine, 1980). In predictable environments (studies reviewed in Section 1.4.2) animals can become locked into cycles of anticipating events, but in unpredictable environments there is more potential and motivation for animals to investigate and forage in their environment (Johannesson & Ladewig, 2000). However, investigation and foraging are only possible when the captive environment provides for these behaviours (Bassett & Buchanan-Smith, 2007), and therefore allows the animal to afford some control over its environment. In this respect, animals in zoos may be expected to respond differently to animals in laboratories, as their semi-natural environment often allows for increased, although not complete control, for example through opportunities for foraging, or environmental enrichment (e.g., Sambrook & Buchanan-Smith, 1997; Young, 2003). One function of environmental enrichment objects can be to increase the complexity of the environment and enable events such as feeding to be contingent on the animals' own behaviour (Carlstead, 1998). If factors such as enrichment, and environmental complexity can increase control, and predictability can affect behaviour in the absence of control, then it seems reasonable to suggest that predictability may be less important in environments where the animals have increased control, thus affecting an animal's behavioural response and hence contributing to some of the differing results found in the literature.

The relationship between predictability and control is complicated. However, control is an important factor which should be considered in interpretation of studies of predictability.

### *Signals and signalled predictability*

The signalled predictability of events can affect animal behaviour, and can become an extraneous variable in studies of temporal predictability. Animals can use many different signals or cues to control behaviour, including endogenous (internal) cues (such as metabolic processes, or timing mechanisms) or exogenous (external) cues (such as light signals or keeper presence). Exogenous cues will be the focus of the following section. There is substantial literature which reports that animals can respond to events that occur after predictable signals. For example, Düpjan et al. (2008) found that signals indicating a forthcoming electric shock elicited vocalisations in pigs, in anticipation of the shock. Signalled predictability may have effects over and above those of temporal predictability. For example, Badia et al's (1975) study (as described in Section 1.4.1) where rats were found to prefer temporally predictable shocks over temporally unpredictable shocks, was extended to introduce a condition where a signal (tone) was introduced to the temporally unpredictable shock only. This led to a reversal of the preference, and the rats preferred the signalled condition. This was taken to suggest that signalled predictability is more important to animals than temporal predictability.

Animals often receive signals for events, and determining which cues are used by animals can be challenging, especially outside of the laboratory. There are many non-intentional factors that could become cues. For example, the sight of keepers, noises from other enclosures, the sound or smell of food preparation, particular behaviours by visitors or an increase in crowd sizes at feeding times, as well as intentional factors such as keepers calling the animals or shaking their keys, or specific sounds or lights. It is possible that the animals use a number of different signals as cues to events. Studies intending to use a signal to cue an event therefore need to take this in to account.

As mentioned above, it is possible that in studies on temporal predictability the results may actually be confounded by predictable signals. Bassett and Buchanan-Smith (2007) argue that this is the case in the study by Bloomsmith and Lambeth (1995). In their study, chimpanzees fed on a temporally predictable schedule showed higher levels of inactivity and abnormal behaviours in the period prior to feeding than chimpanzees fed on a more unpredictable feeding schedule. However, all of the chimpanzees could hear the vocalisations of the other chimpanzees when receiving their food. This could have acted as a cue, and in the case of the chimpanzees fed on an unpredictable schedule, this cue was unreliable in that it was rarely followed by food. The behaviour of the chimpanzees could therefore have been affected by the unreliable signal rather than by the unpredictable time. Alternatively, temporal predictability may confound studies of signalled predictability. For example, Polizzi di Sorrentino et al. (2010) suggested that the feeding cues of keepers preparing food at temporally predictable feeding times on weekdays led to an increase in grooming behaviour in capuchins which was suggested to have conflict prevention purposes. However, their control comparison time was around the same time of day to the normal feeding time but on weekends instead, (when the capuchins were fed at a different time). The authors suggest that this removed the element of feeding cues but controlled for the circadian variability of behaviour. However it is possible that these results were confounded by temporal predictability, and the capuchins were expecting feeding cues at this time. Therefore the reduction in grooming at the control time may have been due to the performance of other anticipatory behaviours (not reported) caused by the lack of arrival of an expected feeding time.

Studies therefore run a risk of confounding temporal and signalled predictability. In fact, as stated by Bassett and Buchanan-Smith (2007): “as yet, no studies have separated the effects of signalled and temporal predictability of appetitive events in an attempt to tease apart their relative importance to animals.” (p237). Hence signalled predictability is another important consideration in interpretation of studies into the effects of temporal predictability, and will be revisited in Section 2.2.

## 1.5. Gaps in the literature, and rationale for the current thesis

This review of the literature revealed a range of behaviours associated with predictable routines, with no clear consensus as to the effects or welfare benefits of a particular routine. Regardless of the conclusions drawn in the literature over the positive or negative effects of predictability, it seems clear that predictability can have important effects on animal behaviour. Those effects may vary and also be dependent on individual circumstances and situations, therefore more research is needed in order to understand how predictability can affect behaviour.

Detailed research on the effects of predictability is limited, and often the results are confounded by variables other than the temporal predictability of an event. Most studies of animal timing have been carried out using rodents, fish or sometimes birds in laboratory conditions. There are only a limited number of studies looking at animal timing in semi-natural environments such as zoos or aquaria, and of these, there are few that specifically look at timing ability or anticipation as the main focus of the study. One possible reason for this may be due to methodological difficulties in obtaining sufficient data, as is the focus of Chapter 2. Indeed, an original aim of the current thesis was to investigate the effects of temporally predictable feeding on zoo animals, but during this work it became apparent that it was necessary to develop the methodology required for such study. The current thesis therefore had two main aims: to investigate the impact of temporally predictable feeding routines on zoo animals and, alongside this, to develop a novel, qualitative methodology that would allow achievement of that aim.

The first aim of the current programme of work was, therefore, to investigate the impact of temporally predictable feeding routines, and the behavioural response of animals to such predictability; looking at anticipatory behaviours, patterns of behaviour over time, and the effects of temporally predictable and unpredictable routines. The temporal predictability of feeding times was the focus of this thesis, as feeding is the most commonly studied appetitive event, and an event which can readily become fixed into a daily routine in captive environments. The current thesis will add to the literature on timing abilities in animals living in semi-natural (zoo or aquarium) environments. This will further our understanding of anticipatory behaviour and timing capacity in different species, and will contribute to knowledge of how housing and husbandry affects the behaviour of captive animals.

It is evident that there are methodological problems when studying the effect of predictability, as demonstrated by the differing studies reviewed in the current chapter. These methodological problems are reviewed in Chapter 2. The second main aim of the current thesis was therefore to develop a suitable method for the study of predictability, to avoid some of the problems found in previous research (this rationale is further developed in Section 2.3). Such methodology was developed throughout this thesis, but with a particular focus in Chapters 4 and 7. Additional challenges to the study of predictability were identified during the course of this programme of work and were incorporated into the development of suitable methodology.

To outline the experimental chapters of the current thesis: Chapter 3 presents an investigation of the effects of a temporally predictable feeding routine on the behaviour of zoo-housed tigers. Methods were employed in an attempt to mitigate some of the methodological short-comings of the previous literature (as detailed in Chapter 2) and to trial a novel, qualitative method of data collection (see Section 2.9) which was designed for use by multiple observers. Chapter 4 reports how this novel method was further developed and tested for reliability and validity in a controlled, laboratory situation. This method was then employed and tested in Chapter 5, alongside traditional measures, in a field study of the effect of a predictable feeding routine on zoo-housed meerkats. Chapter 5 also reports on improvements to the traditional methodology (as detailed in Chapter 2) and on an investigation into the effects of a predictable feeding routine on meerkat behaviour, whilst expanding on Chapter 3 through the use of an experimental design. Following on from the results of Chapter 5, in which certain environmental conditions were found to be more variable than expected, Chapter 6 reports an investigation on the effect of predictable feeding on captive gecko behaviour using an experimental design in controlled conditions. Finally, Chapter 7 reports how the qualitative methodology developed throughout this thesis was trialled in a ‘citizen science’ study to test whether the method was suitable for use by zoo and aquarium visitors to collect data on animal behaviour.

# Chapter 2

## A discussion of methodology

### 2.1. Review of previous methodology

A review of the literature (Chapter 1) found that studies of predictability fall into one of three main categories. Firstly, there are those that specifically test an animal's ability to time using different time intervals and procedures, these include tests of circadian timing, or fixed or peak interval procedures. Most of these studies have taken place in laboratory settings using an operant task. For example Lejeune (1971, as cited in Lejeune & Wearden, 1991), tested the timing ability of domestic cats (*Felis catus*), where the cats had to press a lever for a reinforcement of milk on fixed interval schedules of 120, 300, 600 and 900 s. Data showed an increase in the frequency of lever pressing leading up to the reinforcement time, suggesting an ability to time the interval and anticipate the reinforcement.

In many captive environments, such as zoos, animals do not receive feeds as a result of an operant response such as lever pressing; hence data cannot always be collected using the methods employed in laboratory studies. The second category of studies are therefore those which study the behaviour of animals on a predictable feeding schedule and note changes in their behaviour prior to the feed (sometimes as a side to the main aim of the study). These behavioural changes are then interpreted as anticipation of the feed. These studies include a greater number of zoo-based studies and are usually observational, with no intervention. For example, Mallapur and Chellam (2002) found that leopards were significantly more active and showed restless and anxious behaviours prior to set feeds.

The third category includes studies which compare the effect of predictable and unpredictable routines on animal behaviour, some of which look at the general activity budget, and others are more focused on the behavioural expression directly before the feeds. These studies have been carried out in a variety of locations such as farms, laboratories and zoos. For example, Johannesson and Ladewig (2000) studied the behaviour of dairy calves subject to predictable and unpredictable feeding times. Results showed little difference in calf behaviour between predictable and unpredictable routines, however it was found that for those calves that were accustomed to a predictable routine, delays to feeding and hence deviations from the predictable schedule, were associated with increased ‘comfort’ (licking or scratching self) and eating behaviour.

For the purposes of this methodology review, a formal literature search was carried out using the Web of Science search engine (Thomson Reuters, 2011), with search terms related to predictability and feeding regimes (as detailed in Appendix 2.1). It was necessary to carry out multiple searches due to the high number of key words which could be associated with this research, and these searches were carried out on 24/09/2011, for the time span “All Years”. Search results were filtered manually to extract all experimental (i.e. excluding review or theoretical) papers that were clearly relevant to the topic of predictability of appetitive events in captive animals. Papers with a neuroscience, nutritional or biochemical theme were excluded, as were papers that solely focussed on spatial predictability.

A total of 52 papers were found to be relevant according to the above criteria, and of those, only 17 were found that compared predictable and unpredictable appetitive events, four of which focused on signalled predictability not temporal predictability. These 17 studies used different techniques to compare predictable and unpredictable routines. One of these comparison studies compared the results of only physiological measures (weight gain) and three studies used preference tests or operant tasks to study animals’ choices of unpredictable or predictable conditions.  Thirteen studies (of the 17 comparison studies) recorded behaviour in response to unpredictable and predictable routines, and four of these 13 studies also used physiological measures such as growth, weight and cortisol levels in addition to behavioural observations.

In terms of the techniques used, of the 13 studies which included behavioural data, three collected indirect data on locomotion by counting turns of a running wheel, or breaks of an infra-red photocell beam.  The remaining 10 studies used more traditional measures of behavioural sampling: scan (n=4), continuous (n=4), all occurrences (n=1), and a combination of methods (n=1).  Therefore of the original 52 papers retrieved in this literature search, only 10 compared predictable and unpredictable appetitive events using types of behavioural observation methods that are commonly used in zoo environments.  These papers are summarised in Table 2.1.

*Table 2.1: Summary of the behavioural observation methods used in the literature which compares predictable and unpredictable routines.*

| Study duration category | Length of observation per day | Reference | Species | Sampling method | Study length | Observations | Within/between subjects design |
|---|---|---|---|---|---|---|---|
| **Short duration of animal experience of routine (≤ 15 days)** | **≤ 2 hrs** | Galhardo et al. (2011) | Cichlid | Scan sampling | 2 days training on feeding condition, followed by 1 day of test trials | 10 sampling points at 5 min intervals per fish. | Within |
| | | Gilbert-Norton et al. (2009) | Coyote | Continuous | 12 treatment days | 2 hour daily observations on 5 days per coyote | Between |
| | | Noguiera et al. (2011) | Collared peccary | Continuous | 15 days per feeding condition | 5 mins per individual | Within |
| | **> 2 hrs** | n/a | n/a | n/a | n/a | n/a | n/a |
| **Long duration of animal experience of routine duration (> 15 days)** | **≤ 2 hrs** | Bloomsmith & Lambeth (1995) | Chimpanzees | Scan sampling | Feeding condition changed 6 months before study, followed by 25 days of observation | 30 mins prior to feed and 30 mins at another time of day | Between |
| | | Carlstead (1986) | Pigs | All occurrences | 13 weeks | 15 mins before feed | Between |
| | | Ulyan et al. (2006) | Brown capuchins | Scan sampling | 6 weeks per condition | 1 hour pre-feed and 1 hour post-feed on 29 days (predictable condition) and 33 days (unpredictable condition) | Within |
| | **> 2 hrs** | Johannesson & Ladewig (2000) | Dairy calves | Various | 58 days | 48 hours of observation when the calves were 5 weeks and 8 weeks old. | Between |
| | | Shepherdson et al. (1993) | Leopard cats | Continuous | 1 month per experimental condition (plus 8 days baseline) | 24 hours of daily observation | Within |
| **Mixed conditions (days of unpredictable and predictable feeding interspersed)** | **≤ 2 hrs** | Waitt and Buchanan-Smith (2001) | Stump-tailed macaques | Scan sampling | 20 sessions on schedule feeding, 4 sessions early feeding, 5 sessions delayed feeding | 10 mins per group of macaques in hour before feeding | Within |
| | **> 2 hrs** | Jenny & Schmid (2002) | Amur tigers | Continuous | Study a) 72 days: 3 days per condition (repeated). Study b) 48 days: 1 day per condition (repeated) | Study a) 360 min per day on 3rd day of feeding regime. Study b) 330 min per day on 3rd day of feeding regime. | Within |

## 2.2. Limitations of previous methods

The following section gives details of some of the limitations and methodological challenges that are apparent in the studies of predictability outlined above, including those noted whilst reviewing the literature, and those noted by the authors themselves. The focus of this section will be on those 17 papers found to compare predictable and unpredictable routines; however other relevant papers from the above literature search on predictability and anticipation will also be included, where relevant.

The extent to which studies can elucidate patterns of behaviour associated with predictable routines is determined by the sampling period used, and in many cases it does not seem adequate. Predictable feeding is likely to affect patterns of behaviour over an extended period of time, yet of the 10 studies using behavioural observation detailed above (Section 2.1 and Table 2.1), only three report more than 2 hours (total) of behavioural observations per day. For example Waitt and Buchanan-Smith (2001) reported behavioural data for only 10 minutes in the hour before feeding time. Bloomsmith and Lambeth (1995) studied chimpanzee behaviour on predictable and unpredictable routines by observing behaviour in 30 minute, pre-feed observation sessions and in comparison 30 minute, non-feeding sessions. The latter were taken at a time which best matched the general morning activity levels of the chimpanzees whilst not conflicting with another pre-feed time. This enabled comparison of the different sessions; however Bloomsmith and Lambeth's study was not designed to investigate behavioural changes over the time leading up to predictable events. It may be advantageous, in future work, to consider behaviour over more extended periods of time, so that the full ramifications of a predictable routine can be appreciated. Care must be taken if comparing behaviour in pre-feeding time observation sessions, to another observation session, that any differences in behaviour are not just time of day effects.

Some studies have compared the behaviour of animals directly before feeding and directly after feeding, for example, Weller and Bennett (2001) compared pacing behaviour in ocelots in the 30 mins prior to and following the feed. Care must be taken when interpreting results such as these, since it would be expected that animals would behave differently just after being fed. The difference between the two samples therefore may not be due to anticipation prior to the feed, but due to post-feeding satiety. Results of an unpublished thesis (hence not found in the above Web of Science search) by Sandhaus (2004) on the feeding regimes of giant pandas, found that behaviours (e.g., inactivity) during pre-feed and post-feed observation sessions differed significantly from each other, but they also differed from behaviour during non-feed observations taken at a different time of day. This highlights the importance of including observation sessions at a different time of day to the feeds, to provide a control. Post-feed data can however, add to a study when combined with a comparison of predictable and unpredictable feeding times. Ulyan et al. (2006) compared the pre and post feeding behaviour of brown capuchins on predictable and unpredictable feeding routines (see Section 1.4.2). Results showed differences in behaviours such as inactivity and social behaviour in pre-feeding sessions, between the routines, hence the effect of the routine was demonstrated. It was also found that when the capuchins were fed on an unpredictable schedule, they showed increased ‘tension’ behaviours in the post feed session when fed early compared to when fed late or on time, thus the post feeding data was of benefit to the study.

Predictability studies can be negatively impacted by small sample sizes and a low number of repeat observation sessions. For example in their study of stump-tailed macaques, Waitt and Buchanan-Smith (2001) collected data during only 4 sessions of early feeds and 5 sessions of late feeds, compared to 20 sessions of on-time feeds; thus data on early/late feeds may be less reliable.

Johannesson and Ladewig (2000) observed calf behaviour in only two observation sessions during the course of their study; once when the calves were 5 weeks old, and again when they were 8 weeks old. Whilst these observation sessions lasted for 48 hours and, as discussed above, the longer observation period is associated with advantages; greater numbers of repeat observations would be beneficial in revealing further patterns of developing anticipatory behaviour.

In the literature found comparing behaviour in unpredictable and predictable conditions, the length of time animals were exposed to each condition varied from, for example, 1 day (Jenny & Schmid, 2002) to 13 weeks (Carlstead, 1986). It is unlikely that short exposure to a feeding condition would be enough to establish its effects in terms of development of patterns of behaviour, hence longer exposure is necessary.

The shorter the study, the more likely that results are not due to the type of routine but rather a consequence of the change in routine.  For example, Gilbert-Norton, et al. (2009) studied the behaviour of coyotes on either predictable or unpredictable feeding routines.  However, the coyotes were accustomed to being kept on a predictable routine, and during the experiment were exposed to only 16 consecutive days of unpredictable feeding.  It is therefore possible that the results reflected the effect of changing to a less predictable routine, and not the effect of the unpredictable routine itself.  Indeed, Sandhaus (2004) suggests that animals should be observed over a longer period of time, in case the behaviour observed initially is a learning period, and adaptation to the routine is still developing. However, as studies generally do not report the development of patterns of behaviour, there is, as yet, no indication of how long such a period of observation should be.  Some studies that have opted for a between subjects design have taken the effect of changing the routine into account by exposing the animals to the feeding routine for a period of time prior to the start of data collection.  For example, Bloomsmith & Lambeth (1995) changed the feeding regime for groups of chimpanzees 6 months before the study.  Other authors have used young animals that are naive to any particular feeding routine (e.g., Johannesson & Ladewig, 2000).  It could be argued that responses to predictability or unpredictability may be slow to develop, or be subject to habituation.  If so, studies which extend for long periods may be beneficial in determining the effects of predictability.  For example, Ulyan et al. (2006) observed the behaviour of brown capuchins over 6 weeks after a change to an unpredictable feeding routine.  Such a time period should give more opportunity for any effects of habituation to be observed than in shorter studies, (however it should be noted that the capuchins in Ulyan et al’s study were not reported to habituate to the new routine).  In similar studies using a within subjects design, whereby the individuals experience a change of routine, it is important to consider the order of experimental conditions, since changing from a predictable routine, and losing predictability may have different behavioural effects to switching from an unpredictable routine to a predictable routine. However previous research has not specifically manipulated the order of such experimental conditions.

Another methodological point that could affect studies of anticipation is the question of what constitutes unpredictability. Different authors have used different feeding schedules which they have classed as ‘unpredictable’ and there is no clear definition as to the limits of predictability. In their study of tigers, Jenny and Schmid (2002) created an ‘unpredictable’ routine whereby feeding boxes would open twice, at times that the authors described as semi-random, between the hours of 0900 and 1730. Sánchez-Vazquez et al. (1997) were able to use a greater range of feeding times, whereby goldfish were fed in either the dark or the light period, with inter-feed intervals varying from 12 to 36 hours. In contrast, Ulyan et al. (2006) used a more restricted ‘unpredictable’ schedule involving variation around a set time, by giving early feeds (between 13:00 and 14:00), on-time feeds (between 14:30 and 15:30), and late feeds (between 16:00 and 17:00). In a study on giant panda behaviour, Sandhaus (2004) was only able to provide unpredictable feeding times at: 1 hr earlier, at the same time, or 1 hr later than a baseline feeding time, due to management practicalities. It could be questioned whether such routines which involve only a deviation from the normal feeding time can be classified as ‘unpredictable’, indeed the amount of variation in feeding times required for unpredictability is yet to be established. Johannesson and Ladewig (2000) avoided the confusion of unpredictable routines *versus* deviations from predictable routines by studying 3 conditions: the ‘control’ group received a predictable milk feed at 07:00 and 14:00 every day, the ‘irregular schedule’ group received 2 milk feeds at unpredictable times of the day, between 06:00 and 13:00 and then again between 13:00 and 21:00. A third group, the ‘occasional deviation’ group, received milk at the same time each day, except on a couple of experimental days when they were fed 3 hours late. This study found no significant differences in calf behaviour at 8 weeks old in the ‘control’ and ‘irregular schedule’ groups (predictable and unpredictable feeding), however they found significant differences in behaviours in the ‘occasional deviation' group, such as increased 'comfort' behaviours, suggesting that the type of routine may not affect the behaviour of calves, but deviations from a predictable routine can cause frustration.

Other challenges to studies of predictability include potential confounds. The results of Sandhaus's (2004) study on giant pandas were confounded by animal moves between enclosures which likely affected behaviour. For example, the authors suggest that this may have increased stereotypic behaviour, thus preventing levels from returning to baseline levels in a reversal design (with the feeding conditions: predictable/unpredictable/predictable), and reducing confidence in the conclusions. Cues and signals can also confound studies on anticipation. For example, in Bloomsmith and Lambeth's (1995) study, the chimpanzees received unreliable feeding cues from other chimpanzees, as described in Section 1.4.3. In addition, in the study by Gilbert Norton et al. (2009) as described in Section 1.4.2 and above), the coyotes on the unpredictable schedule (previously accustomed to a predictable schedule), were able to hear the sounds associated with the predictably fed coyotes receiving their food, which may have affected their behaviour. In the study of stump-tailed macaques (Waitt & Buchanan-Smith, 2001), the macaques received feeding cues when staff members returned to the laboratory. However these cues were also sometimes unreliable. It is important to note that both signalled and temporal predictability can affect animal behaviour, and animals that receive predictable feeding times with associated reliable (predictable) signals may behave differently to animals receiving predictable feeding times but with unreliable signals.

In addition to studies where signalled and temporal predictability are confounds, spatial predictability can also be a problem. For example, in their study of leopard cat (*Prionailurus bengalensis*) behaviour, Shepherdson et al. (1993) changed both the temporal and spatial predictability of the feeding regime by adding multiple feeds, hidden in the enclosure, which resulted in decreased pacing, and increased behavioural diversity compared to daily feeding. This study also did not clearly fit the category of comparing predictable and unpredictable feeds and it would be more accurate to describe this study as comparing different types of unpredictability due to the provision of different numbers of feeds at different times of the day. In a study by Nogueira et al. (2011) on the effect of feeding regimes on the behaviour of collared peccaries (*Pecari tajacu*), temporal predictability was confounded, not only by spatial predictability but also by the presence of a food enrichment device. These confounds and the fact that observations only took place at feeding time, with no observation of any potential anticipatory behaviour prior to feeds, mean that this study is difficult to interpret in terms of the effects of predictability.

Another problem encountered when studying predictability is that a range of behaviours may change in anticipation of a predictable event, and it may not be possible to identify a specific anticipatory behaviour. It is likely that anticipation is a quality of behaviour, and any behaviour might anticipate predictable events. Different species may show different responses, as described in Section 1.4, for example felids may pace (Carlstead, 1998) and chimpanzees may become more inactive (Bloomsmith & Lambeth, 1995). In addition, individuals of the same species may vary in their anticipatory response (Sandhaus, 2004). Also, behaviours that could be considered to be anticipatory may occur in situations where anticipation would not be expected and hence, in these circumstances, do not suggest anticipation. For example Jenny and Schmid (2002) found that changing the feeding routine of a male tiger when housed singly did not affect his stereotypic behaviour, suggesting that there was another cause of the pacing, perhaps one related to his being housed singly. Other occurrences of behaviours therefore make it difficult to interpret behaviour related to anticipation. In addition to changes in specific behaviours indicating anticipation of an event, enclosure use can also change, for example pacing in leopards was shown to take place in an area from which the leopards could see the approach of their keepers (Mallapur & Chellam, 2002). Hence there are a range of aspects of behaviour that can be affected by predictable routines.

Housing conditions and domestication may also affect the results of studies of predictability. de Jonge et al. (2008) found that, contrary to their predictions, based upon pig natural foraging strategies, pigs showed preferences for fixed (predictable) intervals of food reinforcement over variable (unpredictable) intervals. They suggested that this is a result of the test environment not reflecting natural foraging conditions, and of the domestication of the pigs. The authors suggested that this may have created a situation where it may have been necessary for these pigs that were reared in the standard 'barren' environment, to 'learn' to appreciate the benefits of unpredictability. This therefore raises questions over the applicability of such farm or laboratory studies to advising the husbandry of captive but non-domesticated animals in semi-natural environments (i.e. zoo animals). Housing conditions have also been shown to affect the response of mice to predictable feeding times. For example, de Groot and Rusak (2004) demonstrated that mice housed on open shelves showed food anticipatory activity (wheel running) whereas mice housed in isolation boxes did not.

## 2.3. Summary and approach for thesis

The main methodological problems identified in this review are that there are few published papers that investigate the effects of predictability in semi-natural environments such as zoos, and few investigate these effects by observation of behavioural parameters. Predictable (or unpredictable) routines may potentially affect behavioural patterns over an extended period of time, however many studies are restricted to short sampling periods over a day, sometimes with only limited numbers of repeat observations. The length of exposure to the routine that the animals receive can also be limited, and therefore results may not take into account factors such as: any immediate effects caused by the experimental change in routine, the potential for the slow development of behaviours associated with the routine, or any habituation of the animals to the routine, that may be reflected in their behaviour. There is no consensus over the definition of an ‘unpredictable’ routine, and previous research has often been confounded by signalled or spatial predictability, or other environmental factors.

The research conducted for this thesis therefore aimed to address some of these issues. Research was carried out on animals in zoo environments to test the effects of predictability in semi-natural environments, and behavioural responses were observed on predictable and (where relevant) unpredictable feeding routines. All predictability studies in this thesis were carried out over a minimum of 3 weeks per condition to allow, as relevant to the particular study: a large number of repeat observations, long exposure of the animals to the feeding routine, data collection on the overall effect of the routine and observation of developing patterns of behaviour as the routines became established. Where possible, data were also collected over most of the daylight period of the day in order to obtain detailed results on daily behavioural patterns. Unpredictable routines were made as unpredictable as practicalities allowed, using purposely designed feeding schedules with randomly generated feeding times, rather than utilising naturally occurring delayed or early feeds. This aimed to create a clear distinction between predictable and unpredictable feeding conditions. Efforts were also made to avoid potential confounds, and only temporal predictability was studied. In this thesis, predictability studies were carried out using traditional methods of data collection. However, a novel methodology, designed for use in such studies was also developed and will be discussed later in this chapter (Section 2.9) and in Chapters 3, 4, 5 and 7.

## 2.4. Objective (quantitative) behavioural data collection techniques in this thesis

The objective (quantitative) behavioural data collection techniques used in this thesis were instantaneous sampling of state behaviours, all occurrence recording of event behaviours, and one-zero sampling.

Instantaneous sampling (Altmann, 1974; Martin & Bateson, 1993) involves dividing the length of the observation session into sample intervals. On the instant of each sample interval, e.g., on each minute, the observer records the state behaviour of the animal or animals using an ethogram of behaviours for reference. Sampling intervals used in this thesis were every 1 minute during 30 minute observation sessions. Instantaneous sampling was used in Chapters 5 and 6, due to its suitability for calculating a proportion of the sampling points in which a particular behaviour was observed. This was therefore suitable for comparing proportions of behaviour between feeding conditions (e.g., in pre-feed observation sessions). Instantaneous sampling is a standard technique in animal behaviour research (Hawkes et al., 1987) and hence it was also used in Chapter 4 to validate the qualitative measure described later in this chapter. Validation requires comparison against a standard measure and hence instantaneous scan sampling was considered appropriate for use.

All occurrence sampling involves recording each occurrence of a particular behaviour, and in Chapters 5 and 6, this technique was used to record the occurrence of event behaviours. As all occurrence sampling can provide information on the rate of occurrence and can be used to investigate temporal changes in the rate of particular behaviours (Altmann, 1974) it was considered suitable to determine temporal patterns of behaviours.

One-zero sampling (Altmann, 1974; Martin & Bateson, 1993) involves recording whether or not a particular behaviour occurred during a sampling interval. One-zero sampling is sometimes criticised as a sampling method as it can over-estimate durations of behaviour, and under-estimate the number of bouts performed (Dunbar, 1976; Martin & Bateson, 1993). However, one-zero sampling has also received support (Martin & Bateson, 1993; Rhine & Linville, 1980; Smith, 1985) in that it is a reliable measure and provides data that closely correlate with frequency and duration of behaviour, and give a suitable index of the 'amount' of behaviour. However it has also been argued that regardless of the data obtained on frequency and duration, these are not the only valid components of behaviour, and one-zero sampling produces an important measure of the occurrence of a behaviour (Smith, 1985). Indeed one-zero sampling was considered suitable for use in this thesis, as for the current research it was sufficient to know whether or not a particular behaviour occurred, without detail on the exact frequency or duration. These data on occurrence of behaviours gave patterns over time, for example, in the time leading up to a predictable feed. Hence one-zero sampling was used in Chapter 3 to study patterns of tiger behaviour over time. One-zero sampling also enabled the monitoring of more than one behaviour per study interval, which was necessary as the exact behavioural response to predictable events is not known. The ability to record more than one behaviour was also important in the validation of the qualitative method ('Busyness': Chapter 4) where, in addition to comparing to a known standard (i.e. instantaneous sampling) it was important to determine exactly which behaviours corresponded to the qualitative measure. One-zero sampling enabled all relevant behaviours to be recorded, and analysed for their association with the values reported on the qualitative measure (see Chapter 4).

It is important to note that this research was carried out in a zoo environment, where many captive environmental variables influence animal behaviour that may not occur in wild or even laboratory or farm situations. For example, visitors to the zoo can affect animal behaviour (e.g., Chamove et al., 1988; Davey, 2007; Hosey, 2000), and there may be maintenance work or animal moves which are beyond experimental control. However, an aim of this work was to understand the effects of predictability specifically in zoos, where the issue of predictability related to keeper routines or public feeding times is of particular interest. The specific captive variables can therefore be key to an animal's response to predictability, within this environment.

### *Statistical Analysis used in this thesis*

Statistical analysis was carried out on the data, as appropriate, taking into account suitable statistical methods for the sample size. Hence, randomisation analysis (Todman & Dugard, 2001) was used in Chapters 5 and 6 to compare periods of observation in different feeding conditions. However, whilst use of inferential statistics provided a useful quantification of comparisons between samples, in the current thesis, such statistical analysis is largely used to provide support to descriptive analysis and indeed, in places, inferential statistics were not appropriate. The nature of recording changes in behaviour over time (as required for Chapters 3, 5 and 7) required sequential data collection; hence graphical and descriptive analysis was more appropriate in these circumstances. Indeed, it is the pattern of behaviour over time and details such as start and end times of behavioural changes, which are the important considerations in studies of anticipatory behaviour. As discussed in Section 2.2, the study of predictability and anticipation would benefit from observation over a greater period of time in order to determine detailed patterns of behaviour. Statistical techniques that compare grouped data at two or more specific points would not be sufficient to reveal these patterns. Such grouping and sampling of data would restrict the interpretation of what may, in actuality be a progressive change in behaviour, and such sampling of behaviour in studies of predictability is criticised in Chapter 2. Indeed, reliance on sampling and inferential statistics would introduce an additional problem of determining the size of samples to compare. Therefore, where possible in the current thesis, data were collected over an extended period of time, and plotted such as to allow visual and descriptive analysis of patterns of behaviour.

In addition to the benefits provided by descriptive analysis of behavioural patterns, many inferential statistical tests are not suited for use with small-*n* study designs (as used in the current thesis); hence descriptive analysis is the necessary approach. Whilst there are some inferential statistical tests that are suitable for use with small-*n* designs (such as randomisation analysis [Todman & Dugard, 2001] as used in Chapters 5 and 6 of the current thesis), many inferential statistical tests are not suited to deal with small-*n* designs. However, there is a substantial tradition of small-*n* or single-subject research in the literature. For example, much of the work of B.F. Skinner used single-subject designs and did not advocate the use of statistical analysis (e.g., Skinner, 1966). Instead small-*n* designs are best-suited to graphical and descriptive presentation of data which is advantageous “if one is interested in the development of behaviour in a single organism over time” (Morgan & Morgan, 2001, p. 122) as in the current research. Such a graphical approach to data analysis has widespread application (Johnston & Pennypacker, 2009), indeed it has been stated that “scholars argue that the data from an individual participant behaving under well-specified conditions should provide unequivocal evidence of an independent variable’s effect and that such an effect should be visible to the naked eye” (Morgan & Morgan, 2001, p. 121).

A descriptive approach has also been taken in previous literature related to patterns of behaviour over time, for example Gee et al. (1994), Sánchez-Vázquez et al. (1997) and Weller and Bennett (2001) include descriptive analysis of temporal patterns of behaviour. Therefore descriptive analysis of patterns of behaviour was deemed to be a suitable method of analysis in much of the current research.

## 2.5. Methodological difficulties

Sections 2.1-2.3 described some of the limitations of previous behavioural research on predictability. To mitigate some of these issues, a large amount of time needs to be allocated to observing animals and collecting data. Behavioural data collection can be an intensive process, for example with samples taken every few seconds or minutes, over observation periods. Temporal changes in behaviour obviously need to be observed in real time, and improved data collection methods would require observations over the course of as much of a day as possible, and over many days, in order to determine patterns of behaviour over extended periods. Whilst the present programme of research aimed to overcome the problems of limited observations by increasing the duration of observations, outside of this study, it is not always possible for observers to commit such large amounts of time. One solution is to use many observers; however the use of traditional measures of behavioural data collection depend on extensive training, and it is not always possible to have a sufficient number of trained personnel available to carry out such observation. For example, training would be required to develop knowledge and understanding of sampling techniques and rules, and awareness of issues such as observer drift and sampling accuracy. Observers would then each need to be able to identify individual animals and carry out preliminary observations, the importance of which is stressed by Martin and Bateson (1993) (p.56). Also a detailed ethogram of behaviours would need to be learnt, and data collection techniques tested for inter-observer reliability.

Computer software is available to aid data collection, but this is often expensive and it requires training to use. For example, Noldus Information Technology produce software such as EthoVision® which allows automated data collection on behaviour and movement. However, this involves expense and substantial training for use, and such software is often not practical for research in large, semi-natural environments, as found in zoos. For example it may not be possible to obtain quality video footage of a sufficient amount of the enclosure for such computer analysis. Alternative automated methods include measures of wheel-running activity (e.g., Pérez-Padilla, et al., 2010; White & Timberlake, 1995) or detection of locomotor activity using photocells (e.g., Sánchez-Vásquez, et al., 1997; Sánchez, et al., 2009). However, these methods are only really suitable for use in a laboratory situation.

It therefore seems that there is a need for a method of observation that would allow data collection by a number of different observers, thus reducing the length of time that any one person needs to commit, but without the need for extensive training. Such a method would need to be easily accessible to and importantly, reliable with relatively untrained observers. The rest of this chapter will discuss the use of simple and subjective (or qualitative) methods with multiple observers, as possible data collection techniques to resolve this issue. This chapter will also suggest a novel method for use in studies of predictability, the development of which will form a second study area for this thesis.

## 2.6. Subjective (Qualitative) methods of behavioural data collection

Subjective methods of data collection are also known as qualitative methods, or observer ratings (Meagher, 2009), and involve measurement which requires some form of interpretation of the observed concept of interest by the observer. Such measures often take the form of ratings, which require an observer to give scores for a variable using units of measurement that are defined by the observer, rather than using standardised units as found in more objective research (Meagher, 2009). These ratings can be for variables such as behavioural traits or aspects of personality, and usually involve integration of a number of pieces of information, in a holistic approach, to evaluate aspects of animal behaviour (Wemelsfelder et al., 2001; Whitham & Wielebnowski, 2009). This is in contrast to the more traditional approaches of animal behaviour data collection, where individual components are recorded separately and objectively, for example using counts or durations (e.g., Martin & Bateson, 1993).

Subjective scales are regularly used in studies of psychology or social sciences (Meagher, 2009; Wemelsfelder, 2007) for example the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) is a self-report scale designed to measure human mood and emotion, which has been tested for, and found to be reliable and valid (e.g., Crawford & Henry, 2004). Also, the Trait Meta-Mood Scale (TMMS) (Salovey et al., 1995) is a rating scale commonly used to monitor feelings and emotions in humans. However such measures are rarely used in studies of animal behaviour, and traditional ethogram-based methods using frequencies or durations of specific behaviours are much more commonly used (Meagher, 2009) (such measures include e.g., instantaneous scan sampling and will henceforth be referred to as traditional measures). There are, however, some examples of the use of subjective measures in ethological research. Early examples include the work of Stevenson-Hinde and Zunz (1978) who asked observers to rate behaviourally defined adjectives related to personality of rhesus macaques, and work by Feaver et al. (1986) in which ratings were given for aspects of the ‘behavioural style’ of domestic cats. Other work on domestic cat behaviour involved the development of a Cat-Stress-Score (Kessler & Turner, 1997, 1999). This score describes seven possible stress levels of a cat from ‘fully relaxed’ to ‘extremely stressed’, based on posture and behavioural elements (Kessler & Turner, 1997). Using this measure, the authors were able to monitor the stress levels of cats in a boarding cattery (Kessler & Turner, 1997), and correlate cat stress levels with group density (Kessler & Turner, 1999). Whilst other authors (e.g., Wemelsfelder, 2007) have classed the Cat-Stress-Score as a qualitative measure, it is perhaps a measure that is not entirely qualitative, and combines aspects of both subjective and objective studies. Each level of the cat stress score is precisely defined in terms of the behavioural and postural elements from which it is composed (Kessler & Turner, 1997), as is the case for descriptions of behaviour in a traditional ethogram. However it is then up to the observer to combine these elements into a single rating of the whole animal, based on their subjective interpretation of their observations.

Other behavioural work using qualitative measures has included ratings of specific behavioural traits. Wielebnowski (1999) used ratings of behavioural attributes in order to develop predictors of breeding success in captive cheetahs (*Acinonyx jubatus*). Behavioural traits of 44 cheetahs were rated by the experimenter and two keepers at each of four breeding facilities. Ratings were made for each cheetah on 18 behavioural adjectives (e.g., calm, excitable and friendly) using a visual analogue scale (where observers marked their rating on a scale line from minimum to maximum, which could be measured to give a quantitative rating). These ratings were analysed using a Principle Components Analysis (PCA) in order to determine the major components of cheetah behaviour. These components were compared with variables related to breeding success, and it was determined that non-breeding cheetahs scored significantly higher on the component ‘tense-fearful’ than breeding cheetahs. Thus, the use of subjective measures resulted in data that could have future applied benefits for the captive breeding management of cheetahs. In a similar study on black rhinoceros *(Diceros bicornis),* Carlstead, Mellen et al. (1999) asked keepers at 19 different zoos to rate their black rhinoceros on 52 different behavioural elements using a rating scale of 1-5, based on frequency of performance or applicability of an attribute. Results were grouped into 14 behavioural traits to give a behaviour profile for each individual. Comparisons with variables such as breeding success revealed that results for the traits ‘dominant’ and ‘olfactory’ behaviours (males) and ‘chasing/stereotypy/mouthing’ (females) were negatively correlated with reproductive success, once again providing useful, applied results for captive management.

Animal personality can be assessed by observers who integrate aspects of an animal’s behaviour to give subjective ratings on particular personality traits. For example, Gosling et al. (2003), asked 78 dog owners to rate the personality of their dogs, by recording their agreement with particular statements such as “[my dog] is curious about many different things”, on a scale of 1 (disagree strongly) to 5 (agree strongly). Results demonstrated that personality in dogs could be judged as accurately as for personality studies of humans. Pederson et al. (2005) asked 38 observers to rate personality adjectives for 47 chimpanzees (*Pan troglodytes*) (mean of 4.7 raters per chimpanzee) on a seven point scale.

Forty three personality descriptive adjectives were rated by the observers and a PCA was used to identify six main personality factors in chimpanzees: Extraversion, Dependability, Agreeableness, Emotionality, Openness and Dominance. These factors (with the exception of Dominance) were similar to the five factors of personality that are commonly found in studies of human personality.

In recent years, a qualitative method termed 'free choice profiling' (FCP) has been developed for use in animal behaviour studies (Wemelsfelder et al., 2000; Wemelsfelder, et al., 2001) whereby observers are free to choose their own descriptive terms for animal behaviour. They then rate the observed animal with regards to these terms, using a visual analogue scale. Rating scores are then analysed using the Generalized Procrustes Analysis (GPA); the multivariate statistical technique associated with FCP. This method can account for the lack of fixed reference points caused by the observers creating their own descriptive terms, and instead matches patterns between the animals. Free Choice Profiling has since been used successfully in a number of studies e.g., Minero et al. (2009), Rousing and Wemelsfelder (2006) and Wemelsfelder et al. (2009).

Despite the success of qualitative studies such as those previously mentioned, it has been suggested that the use of subjective measures may be open to certain criticisms such as anthropomorphism, bias, or lack of reliability or validity (Meagher, 2009; Rousing & Wemelsfelder, 2006; Wemelsfelder, 2007). However there are a number of benefits to using qualitative measures. For example, (as discussed in Section 2.5) if qualitative measures are relatively simple to use, then they have the potential to be used by many observers, hence resulting in the collection of a large volume of data whilst reducing the length of observation required by a single observer. Also, as measures which utilise human interpretation, qualitative measures involve integrating many pieces of information, such as the animal's behaviour and posture, and the context of the behaviour, and consequently can provide a richness of information which is not available from objective (traditional) measures (Wemelsfelder & Birke, 1997), since such traditional approaches usually record these elements of behaviour separately, or do not record them at all (Wemelsfelder, et al., 2000). In this respect, qualitative methods can take “into account various aspects of behaviour and its context that are difficult to quantify” (Rousing & Wemelsfelder, 2006, p. 51). This also allows direct assessment of the animal’s behaviour rather than retrospective inference as is achieved from more traditional data (Rousing & Wemelsfelder, 2006). In studies of animal welfare, this direct and integrated assessment or ‘whole animal’ approach has been argued to be important to determine what an animal is doing, how it does it and the context, in order to judge the quality of an animal’s experience directly, and hence assist in welfare assessment (Wemelsfelder, 2007).

Qualitative measures can therefore be useful for certain types of data collection. It should be noted that, whilst commonly accepted for use in behavioural studies, even traditional, quantitative methods are not completely free from those problems which may be associated with qualitative measures. For example elements of traditional measures rely on human judgement, such as deciding when a behaviour starts and stops. Also, the initial definition of a behavioural category involves an element of subjectivity, e.g., determining what constitutes stereotypic behaviour (Meagher, 2009).

In order to address these criticisms, formal methodologies are required, which need to be tested for both reliability and validity of results. With careful design and testing, subjective methods can produce useful results and this has been established for many of the studies discussed so far, hence their confident conclusions. The issue of reliability and validity of subjective measures will be further reviewed in Chapter 4.

## 2.7. Observer Experience

When designing a study with multiple observers, one consideration should be that the previous animal behaviour research experience of the observers may potentially influence the results. Wemelsfelder (2007) states that for both qualitative and quantitative methods, experienced and skilled judgement is important for effective use. However, Wemelsfelder et al. (2000) demonstrated that untrained observers, with no previous experience of observing pigs, successfully used the FCP method to record the behavioural expressions of pigs. A further study (Wemelsfelder, et al., 2001) found that observers with previous experience of observing animals, but not pigs, were also able to use the FCP method successfully on pigs. Tami and Gallagher (2009) specifically tested the ability of people with different levels of experience with dogs (dog owners, vets, dog trainers and non-dog owners) to describe dog behaviour. Participants were asked to classify the behaviour of dogs from video footage, using adjectives such as ‘friendly’ and ‘aggressive’. Overall, the results showed that there was no significant difference in the participants’ abilities to label dog behaviour, and that differences in rating ability occurred irrespective of experience. However non-dog owners recognised play solicitation significantly less than the other categories of observers, indicating that there may be some influence of experience. Renner and Renner (1993) tested the ability of expert and novice observers to determine whether rats, as shown on video clips in a neutral context, had come from enriched or impoverished environments, based on their behaviour. Results showed that the novices were as accurate as the experts in determining the environmental history of the rats, although the experts used broader vocabulary for describing the reasons for their judgements.

The above findings suggest that inexperienced observers are able to accurately use qualitative measures of animal behaviour. This provides reassurance that it might be possible to develop a subjective measure that would require little training, in order to investigate behaviour related to the predictability of routines in zoo animals. Obviously care must be taken in the design for such a method to ensure that it is suitable for novice observers. However there are elements of research that can be carried out without the need for extensive training. This concept is utilised to take advantage of volunteer researchers in “Citizen Science” projects, whereby members of the public, with relatively little training, contribute data to a study.

## 2.8. Citizen Science

Citizen Science is increasingly used to obtain large volumes of data for research projects; allowing the study of topics that would be impossible with only a single observer or a small group. The process of citizen science involves volunteers using a simple and straight forward method to collect data which contributes to a study, in order to both increase the volume of data that can be collected and potentially benefit the volunteers with an educational experience (Bonney et al., 2009). For example, the Galaxy Zoo project is an international project in which anybody with an internet connection is invited to classify galaxies from the Sloan Digital Sky Survey. Instructions are given on a website and participants are asked to look at photographs of galaxies and determine, for example, whether they are spiral or elliptical, and their rotation direction. As of April 2009, over 200,000 people had been involved with this project, resulting in over 100,000,000 classifications of galaxies (Raddick et al., 2010). The sheer number of participants classifying each galaxy can allow exclusion of classifier error, and the percentage agreement between participants can aid decisions on reliability (Lintott et al., 2008). Such has been the success of this project that, as of April 2009, it has enabled 50 research projects and 16 peer-reviewed articles (accepted or submitted) (Raddick, et al., 2010). Other examples of citizen science include monitoring bird populations (e.g., Bonney, et al., 2009; Lepczyk, 2005; McCaffrey, 2005). Some of these studies require some training, for example some studies at the Cornell Lab of Ornithology provide a large training pack for volunteers including instructions, identification posters and bird sounds (Bonney, et al., 2009), however other projects require only limited training on identifying common birds. For example the Royal Society for the Protection of Birds (RSPB) runs “The Big Garden Birdwatch” project (RSPB, 2011) which requires residents to watch and record birds in their garden for just one hour, with the aid of a simple ID sheet of common birds.

There are many other examples of citizen science projects ranging from monitoring human-coyote interactions (Weckel et al., 2010) to searching for possible important archaeological dig sites using satellite images (National Geographic, 2011). All of these projects demonstrate that it is possible to use many people, from all backgrounds and levels of training, to collectively gather large volumes of data. However, such citizen science-type research does require specifically designed techniques in order for it to be accessible to volunteers and produce reliable results. Special protocols need to be designed and then the reliability of the results must be tested (Cohn, 2008). An aim of this programme of research was to develop such a method of data collection which was suitable for use with multiple volunteers.

## 2.9. Introduction to 'Busyness'

Zoos and aquariums often benefit from a supply of students and volunteers willing to dedicate some time to animal observation. Following the example set by citizen science, in the current programme of work, a qualitative method was developed which could be used by these volunteers or by zoo keepers. As argued in Section 2.5, there is a need for a method that is simple and straightforward, requires little training, and allows observers to each contribute only a small amount of observation time. The measure of 'Busyness' (as described later in this section) was previously designed to be a simple method of collecting behavioural data, but had only been trialled in a few undergraduate projects (P. Gee, personal communication, 2008). The concept of busyness showed great potential as a solution to those challenges identified in the study of predictability, as it was a simple method that was potentially usable by many observers to record data on patterns of behaviour over time. Busyness also showed potential as a 'real time' measure of behaviour. Those qualitative measures that already exist in the study of animal behaviour record overarching attributes such as personality, and there is a need for a qualitative measure that can capture ongoing patterns of behaviour. Hence busyness was chosen as a focus of this programme of research, to be developed as a measure of dynamic behaviour, and as one possible example of a simple, subjective measure.

Busyness is a measure of how 'busy' an enclosure of animals appears, so it is a measure of 'how much is going on' in an enclosure. Busyness is rated on a scale of 1-5 (quiet-busy) for all of the animals in an enclosure; hence individual recognition is not required, thus reducing training time. This rating scale technique is similar to that of rating a particular behavioural trait, as used in the studies previously mentioned (e.g., Carlstead, Mellen, et al., 1999). A scale of 1-5 was chosen as a simple and easy to use scale rather than a visual analogue scale, which was considered to be too time consuming for data collection at regular sampling intervals. Busyness is a subjective measure and as such, detailed descriptions are not provided for each level of the scale. Instead, ratings are based on the observer's opinion of how 'busy' the animals are. Just how much description is required of the busyness levels is addressed in Chapter 4.

A rating of busyness can be determined at regular sampling intervals, and as such the measure provides a means of collecting data on patterns of behaviour over time, hence the current programme of research sought to develop busyness in order to determine patterns of behaviour associated with predictable feeding routines. Busyness has potential in other applications, such as the study of behavioural responses to environmental enrichment, however the current focus of development will be with regards to studies of predictability.

As a straightforward rating scale, busyness is a simple concept, and should require little training for use; therefore it should address some of the problems found in previous studies of predictability, allowing the collection of large volumes of data, over long observation periods, with multiple observers. The concept of busyness therefore seems of great potential use to future research; therefore an aim of the current research was to test the value of busyness, as determined by its correspondence to traditional measures of animal behaviour and by the inter-observer reliability of the data collected. Busyness was trialled both in the field, and in a controlled laboratory environment, where detailed tests of validity, reliability and applicability were possible, in order to develop the measure as a suitable tool for use in the study of predictability. This development of busyness will be further described in Chapter 4.

# Chapter 3

## *Daily behavioural patterns of a pair of Sumatran tigers (Panthera tigris sumatrae) under a predictable husbandry routine*

## 3.1. Introduction

### 3.1.1. Introduction to the tiger, including behaviour and feeding ecology, with focus on the Sumatran tiger

Tigers (*Panthera tigris*) are the largest members of the Family Felidae (Mazák, 1981; Nowell & Jackson, 1996) and as a species they are classified as endangered by the International Union for Conservation of Nature (IUCN) (Chundawat et al., 2011). Six subspecies of extant tiger are recognised by the IUCN: the Amur (*P.t.altaica*), Bengal (*P.t.tigris*), Indochinese (*P.t.corbetti*), South China (*P.t.amoyensis*), and Sumatran (*P.t.sumatrae*) tigers (Chundawat, et al., 2011; Mazák, 1981; Nowell & Jackson, 1996), and the recently recognised Malayan tiger (*P.t.jacksoni*) (Kawanishi & Lynam, 2008; Luo et al., 2004). Sumatran tigers are endemic to the Indonesian island of Sumatra and are the smallest extant subspecies of tiger, with a male body length range of 2.2-2.55m (Nowell & Jackson, 1996). Sumatran tigers are classified as critically endangered by the IUCN (Linkie et al., 2008) with fewer than 400 individuals remaining in-situ in Sumatra (Seidensticker, 2010).

The majority of the non-conservation-specific literature on tigers considers the tiger species (*Panthera tigris*) as a whole, therefore unless specifically mentioned otherwise, the following section refers to *Panthera tigris*.

Tigers are traditionally considered to be a solitary species, with the exception of mothers with cubs up to the age of 18-28 months (Nowell & Jackson, 1996). However many reports now exist, of sightings of family groups including adult males, or other groupings of adult tigers (e.g., Nowell & Jackson, 1996; Schaller, 1967; Thapar, 2004) suggesting that tigers are not completely solitary. Tigers are opportunistic predators (Chundawat, et al., 2011) feeding on a range of large, mainly ungulate prey such as wild pigs and deer. Sumatran tigers, with their smaller body size are more suited to taking the small prey that is present in their rainforest habitat, but they are still capable of taking large prey such as wild cattle (*Bos*) species (Seidensticker & McDougal, 1993).

Tigers are traditionally reported to be a nocturnal or crepuscular species (e.g., Mazák, 1981; Nowell & Jackson, 1996; Shankhala, 1978). However they are reported to also hunt during the day (Nowell & Jackson, 1996; Shankhala, 1978) and Sunquist (1981) stated that tiger behaviour is not closely related to the light/dark cycle, since some daytime activity and movement occurs. Indeed some of the main prey species of the Sumatran tiger such as wild pig (*Sus scrofa*) and Argus pheasant (*Argusianus argus*), are reported to be diurnal (O'Brien et al., 2003) which, it seems sensible to suggest, would necessitate diurnal hunting from the tigers. Camera trapping evidence supports this assumption and Tilson and Nyhus (2010) reported Sumatran tiger activity in Way Kambas National Park at times throughout the day as well as at night. Tigers vary their hunting times, for example, during observations of tigers in Chitwan, Sunquist (1981) reported that fresh tiger kills were found in the late afternoon, at night and in early to mid morning. Indeed, Seidensticker and McDougal (1993) demonstrated that even when tethered prey was available, tigers varied in the times of day at which they took prey (both within and between individual tigers) with some tigers taking prey prior to 1600 and others between 1600-1900, or after 2000. Tigers have been reported to hunt approximately once per week (Nowell & Jackson, 1996), and Sunquist (1981) estimated that lone female tigers in Chitwan would make a kill once every 8-8.5 days. The prey is then eaten, in decreasing amounts over the next few days, and there may then be days with little or no food consumption (Dierenfeld et al., 1994; Sunquist, 1981).

### 3.1.2. Tiger captive management

There are currently 250 Sumatran tigers held in captivity in ISIS (International Species Information System) member institutions (ISIS, 2011). Zoo-housed tigers are usually fed a diet of beef or horse products, either in the form of commercially prepared feline diets or as carcass meat with supplements. Bones are recommended for oral health and to promote natural feeding behaviours, and the diet may be supplemented with whole animal carcasses, such as rabbits or fowl, for variety (Dierenfeld, et al., 1994; Shoemaker et al., 1997). As wild tigers do not hunt or feed on every day of the week, zoo-housed tigers are often not fed every day. Dierenfeld et al. (1994) report that tigers' appetites and body conditions improve if they are fasted for one or two days per week, and they recommend one fast day per week. Shoemaker et al. (1997) recommend one or two fast days per week in order to prevent problems of obesity. On fast days it is recommended to give either no food or a small amount of food such as bones (Dierenfeld, et al., 1994; Shoemaker, et al., 1997).

#### *Captive influences on tiger behavioural patterns*

Like other zoo animals, daily patterns of tiger behaviour can be affected by a number of captive environmental variables. For example the arrival and departure of visitors, or routines of enclosure cleaning and maintenance (see Section 1.1). Environmental enrichment can affect behaviour, indeed specific goals of enrichment may include decreasing stereotypic behaviour, increasing activity or increasing behavioural diversity (for examples of tiger enrichment see e.g., Bashaw et al., 2003; Law et al., 1997; Hare, 1998; Van Metter et al., 2008). Given the evidence on the effect of environmental enrichment on behaviour, it seems reasonable to suggest that the timing of the provision of enrichment might itself influence patterns of behaviour. Routines of moving animals between enclosures can also influence behaviour. For example, many zoos will house tigers in their inside quarters over night, and food may be used to encourage the animals inside. This is often done for security or practical reasons, although Law et al. (1997) argue that this may not be necessary.

A major determinant of behavioural patterns in captivity is feeding routine (see Section 1.4.2). Captive tigers may be fed at a set time of day. This may be for the purposes of a public feeding event or talk, or to fit in with the husbandry routines of the keepers, or (as mentioned above), to entice the tigers into their night quarters at the end of the working day. For example, in an opportunity sample of 13 zoos in the United Kingdom, 10 zoos fed their tigers at a fixed (as opposed to random) time, 6 of which involved a public talk and 3 of which were specifically timed to aid husbandry (personal communication, April 2012). This predictable feeding has the potential to influence patterns of tiger behaviour and will be investigated in the current chapter.

### 3.1.3. Effects of predictable feeding on felid behaviour

As discussed in Chapter 1, temporally predictable feeding can have an effect on animal behaviour, and debate exists as to the benefits or otherwise of predictable or unpredictable routines. The behaviour of carnivores, particularly felids, has been reported to be affected by predictable routines. For example, domestic cats have shown patterns of lever pressing prior to a milk feed that was provided at set intervals (see Section 2.1).

In zoo environments the effects of predictable routines can manifest in felids as observable changes in patterns of behaviour that anticipate predictable feeds. For example captive leopards (n=14) were observed to show significantly lower levels of resting prior to their usual feeding times (Mallapur & Chellam, 2002). Consequently, activity (a measure combining running, climbing, jumping, standing, walking and rubbing/rolling behaviours) increased (although not significantly) prior to feeding, and the leopards were noted to be ‘restless’ and ‘anxious’ prior to the feeding time.

Predictable feeding times have also been associated with stereotypic behaviours in captive felids as described in Section 1.4.2. Stereotypy prior to feeding was also observed in African lions (*Panthera leo*) by Altman et al. (2005), who found that 93% of all pacing (from observations between 9:00 and 17:00) occurred prior to feeding. However, this effect may have been a result of signalled predictability rather than anticipation of the time of day, as the authors suggest that the lions may have been cued by the smell of thawing meat or the activities of keepers. Many such factors can become feeding cues for zoo animals, and (as described in Section 1.4.3) animals can anticipate events based on temporal or signalled predictability. Signalled predictability may confound studies of temporal predictability; Altman et al. (2005) hypothesised that when the lions were switched from a daily feeding schedule to a random, 3 day feeding schedule, they would no longer be able to anticipate when they would be fed. However, the lions were twice as likely to pace on feeding, than on starve days, potentially due to the aforementioned cues. Feeding cues may impact studies of animal timing, but are not always considered or reported.

A reduction in the predictability of feed times (i.e. creating unpredictable routines) can affect behaviour in some felids. Shepherdson et al. (1993) reduced the predictability of feeds to captive leopard cats (*Prionailurus bengalensis*) by providing the cats with multiple feeds at irregular intervals instead of a single, daily feed. This resulted in increased exploratory behaviours and increased overall diversity of behaviours, as well as a reduction in stereotypic pacing.

Predictable feeding routines have also been reported, albeit anecdotally, to affect the enclosure use of captive felids. For example, in their study of leopards, Mallapur and Chellam (2002) observed that prior to feeding, the leopards used the areas of their enclosure from which they could see the keepers approaching, and also the area of the enclosure leading to the off-exhibit feeding area. Similarly, Altman et al. (2005) observed that the lions increased pacing prior to feeding, and this pacing behaviour was directed at the entrance to the night quarters in which they were fed.

Tigers, specifically, have been shown to be affected by the predictability of routines, as demonstrated by Jenny and Schmidt (2002) (described in Sections

1.4.2 and 2.2). Whilst not specifically testing anticipation, this study demonstrated that predictability can affect tiger behaviour, with decreased pacing on an unpredictable routine.

### 3.1.4. Busyness

As discussed in Chapter 2, one reason for the low number of published papers on anticipation of temporally predictable events in zoo environments may be the challenge of applying a suitable methodology. The use of multiple observers and a simple, subjective method of observation was suggested as a possible solution to this problem, with the measure 'busyness' proposed as a subjective measure that might be suitable (Section 2.9). However, an assessment of the validity and reliability of this measure is required in order to establish its utility.

As behavioural effects of predictable routines have previously been reported in felids, the current study was used as an opportunity to test the measure of busyness. Tiger busyness was recorded alongside traditional measures of behaviour, in order to obtain an initial assessment of the suitability of busyness for tracking behavioural changes associated with predictable routines.

### 3.1.5. Rationale and aims

In Chapter 1 it was argued that there is need for further research into the effect of predictability on zoo animals due to a lack of consensus on the benefits, or otherwise, of predictability. The present study was designed to add to the literature on the behavioural effect of predictability using Sumatran tigers as a case study. As explained in Section 3.1.1, tiger natural history shows tigers to be opportunistic hunters (Chundawat, et al., 2011) that are active and able to feed at any time of the day or night, depending on prey availability and satiation (e.g., Nowell & Jackson, 1996; O'Brien, et al., 2003; Seidensticker & McDougal, 1993; Sunquist, 1981). Despite this, many zoo-housed tigers are restricted to feeding at a set time of day, and in accordance with the literature on the effect of predictable feeding on felid species, it seems reasonable to suggest that these restrictions on feeding might have effects on the pattern of tiger behaviour.

Whilst the studies reviewed in this chapter have shown that predictable routines can affect the behaviour of felids, many of these studies were designed to investigate topics such as general activity budgets or stereotypic behaviour, rather than intentionally setting out to assess the presence of anticipation in itself. Therefore one aim of the current study was to specifically determine whether a pair of captive tigers on a predictable feeding routine showed particular patterns of behaviour throughout the day and prior to feeding, that could be related to anticipation of the predictable feeding time. Details of any such patterns were investigated, including start time, duration and behaviours which anticipate predictable feeding. An aim of this study was therefore to investigate detailed patterns of tiger behaviour, over an extended period of time, avoiding the problems of limited observation lengths as found in previous literature (Section 2.2)

Another aim of the current research was to investigate any patterns of enclosure use of the tigers, throughout the day and particularly as feeding time approached.

A final aim of the current research was to investigate whether busyness (as suggested as a potentially suitable measure in Section 2.9) could be used to record patterns over the day, which would both reflect the patterns of behaviour obtained using traditional measures, and be related to the regular feeding event. This study therefore aimed to make an initial validation of busyness against traditional measures of data collection, and also test the inter-observer reliability of the measure.

## 3.2. Methods

### 3.2.1. Housing and Husbandry

The subjects were one male and one female Sumatran tiger (*Panthera tigris sumatrae*) housed at Paignton Zoo Environmental Park, UK since 2002 (male) and 2004 (female). Their housing included a 2248m$^2$ outdoor, on-show (to the public) area of grass and trees in addition to indoor, off-show dens. The outdoor enclosure contained two slightly raised platforms which acted as both vantage points and shelters for the tigers. The enclosure perimeter was enclosed with a wooden fence on two sides (which were adjacent to the indoor quarters and to the neighbouring lion enclosure), and a chain-link fence on the other two sides (which were adjacent to a lake with a variety of wildfowl species, and to a path with observation areas for visitors to the enclosure) (see Appendix 3.1 for an enclosure map).

The tigers' husbandry routine, which they had experienced for at least one month prior to the start of the study, was as follows: the tigers were given access to the outdoor area in the morning before zoo opening at 10:00, where they remained during the day. The tigers were routinely let back into their indoor dens, one tiger at a time, at approximately 16:40 each day, where they stayed for the night. The tigers were fed at 16:40 once indoors, although they received only a small amount of food on starve days. For the purposes of this study, the 16:40 event will be referred to as the feed time. The feed time remained relatively constant each day within the constraints of the keepers' daily routines, with the earliest observed occurrence at 16:32, and the latest at 16:49 (Mean = 16:40, Standard Deviation = 4.97).

### 3.2.2. Traditional behavioural observations

*Observers*

Data were collected by the experimenter and seven observers recruited from the final year of an undergraduate psychology degree course at the University of Plymouth. These students received several training sessions, and practised using the methods for at least two days prior to the start of the study to ensure that all were consistently discerning the correct behaviours. The degree of this consistency was determined by comparisons with other observers and with the experimenter. Multiple observers were used to increase the number of observations possible, and to allow busyness to be tested for inter-observer reliability (see Section 3.2.4.).

### *Procedure*

One-zero sampling was used to collect behavioural data at one-minute intervals during 30 minute observation periods; each tiger was observed every other minute (*n*= 15 mins for each tiger) from 10:00 until feed time. Data were collected on 34 observation days (on week days over a 75 day period between 17/11/08 – 30/01/09), and over the course of the study each minute in the observation day was observed at least 12 times (with a maximum of 20 repeats, and mean and standard deviation of 16 and 2.62 respectively). A total of 55 hrs of data were recorded for the male tiger, and 55 hrs and 6 mins for the female tiger. Minutes after 16:40 were not regularly repeated since the tigers were usually inside by this time (minimum *n* for a minute after 16:40 = 1 at 16:48).

Behaviour categories for one-zero sampling were ‘not visible’, ‘sleeping’, ‘inactive alert’, ‘locomotion’, ‘pacing’, ‘investigating’, ‘marking’, ‘feeding’, ‘self maintenance’, ‘social activity’ and ‘agonistic behaviour’ (for definitions, as based on previous work by the author, and also Weller and Bennett (2001) see Table 3.1). A value of 1 was recorded for any of these behaviours that occurred during the one-minute interval, and a value of 0 was given to behaviours that did not occur during the one-minute interval. In addition, the category of ‘inactive alert’ was given levels of low, medium or high, for separate analysis. The presence or absence of keeping staff was also recorded every minute. One-zero sampling was chosen for this study as it was sufficient to know whether or not a particular behaviour occurred during a minute, in order to determine the pattern of occurrence of that behaviour over time. One-zero sampling also enabled more than one behaviour to be recorded each minute if necessary, in order to determine the pattern of occurrence of each behaviour.

### *Enclosure Use*

Instantaneous sampling was used at alternate minute intervals to record the location of the focal tiger in the enclosure in terms of the enclosure quarter, and resource area in which the tiger was located (see Appendix 3.1 for an enclosure map).

*Table 3.1: List and descriptions of tiger behaviours recorded using one-zero sampling.*

| Behaviour | Definition |
|---|---|
| Not visible | Tiger is obstructed from view |
| Sleeping | Tiger is lying sternally, laterally or on back. Eyes are closed and the animal's body is relaxed showing little or no response to stimuli. |
| Inactive alert (low, medium, high) | Tiger is not moving, remaining in one location, not engaging in any other activity, but eyes are open. Different levels of inactive alert are as follows:<br>Low = tiger is lying down sternally, laterally or on back and is inactive and not asleep but shows minimal responses to external stimuli, e.g., looks around with eyes, and ears may move but otherwise little movement.<br>Medium = tiger is either lying down sternally or sitting up. Tiger is more responsive to external stimuli and shows steady movements of eyes, ears or head towards certain stimuli.<br>High = tiger is sitting up, standing, or possibly lying down sternally. Tiger is very responsive to external stimuli and turns head quickly towards the source of stimuli. Ear position, eyes and head show that the animal is very alert. May involve a change in position, e.g., from lying down to sitting up. May be followed by a change in behavioural state, e.g., locomoting to investigate a stimulus. |
| Locomotion | Activity involving movement of the whole body from one spot to another that is greater than one body length of the animal. This may include running, trotting, walking, jumping and climbing. |
| Pacing | Repetitive and unvarying locomotion with the same route taken at least three times. Animal may walk in a figure of eight and turn the same direction. |
| Investigating | Exploration of environment. May involve sniffing objects or touching / scratching them (different to scratch marking). May walk with nose to the ground. |
| Marking | Including spray marking, scratching objects (e.g., trees) with front claws, rubbing objects with head or body, scraping ground with hind legs – may or may not be followed by urination or defecation. |
| Feeding | Ingesting food or water into the body from the environment, or food source. |
| Self-maintenance | Any single action towards themselves, e.g., scratching, grooming, licking. Animal may use its tongue, its paw or its mouth to clean itself. |
| Social activity | Animal interacts in a positive way with another animal of the same species. May include head rubs, body rubs, mating, sniffing the other animal, playing, or grooming the other animal. |
| Agonistic behaviour | Visual or vocal aggressive or threatening behaviour directed at another animal or human. May include fighting, full or mock charges, lunges, bites, baring teeth (excluding flehmen), pouncing (not play) and scratching. May or may not be returned by another animal. Body may tense and ears flatten. |

### 3.2.3. Busyness

Busyness data were collected simultaneously with traditional behavioural observations. Observers recorded busyness scores for the whole enclosure (both tigers combined) every minute during the 30-minute observation periods; from 10:00 until feed time. Data were collected on the same observation days and times as traditional behavioural data (as described above) by the same observers, covering every minute of the observation day at least 12 times (maximum 20 repeats) to give a total of 110 hours and 28 minutes of observations. As stated earlier, minutes after 16:40 were not regularly repeated since the tigers were usually inside by this time (minimum *n* after 16:40 = 3, at 16:49). It should be noted for clarity that the total minutes of busyness observation are not equal to the sum of the total minutes of male and female observation as, on occasions, once a single tiger was let inside at the end of the day busyness recording continued on the enclosure that now contained the remaining tiger, even though traditional behavioural data observations had stopped for the tiger that had gone inside.

The busyness of the tiger enclosure was rated on a scale from 1 to 5 (low to high), reflecting the behaviour of both tigers over the preceding minute. A rating of 1 was used to indicate that both of the tigers in the enclosure were very quiet, for example, sleeping or inactive and hardly alert, and level 5 indicated that both tigers were very busy, at the highest level of arousal and activity. For this study, aimed at initially developing the method of busyness, behaviours at the different busyness levels were suggested to the observers (as shown in Table 3.2), however, the observers were aware that this was a subjective measure and gave their scores based on their own opinions of the tigers' behaviour.

*Table 3.2. Break-down of behaviours suggested to be seen at each busyness level, as suggested to the observers.*

| Busyness | Definition | Behavioural example | Density example |
|---|---|---|---|
| 1 | Very quiet | Sleeping or inactive, hardly alert | Almost all animals in enclosure |
| 2 | Quiet | Inactive but alert, self maintenance, slow movement | Equal numbers of animals inactive to slow active |
| 3 | Moderate | Locomotion or pacing of moderate speed, social activity | Almost all moderately active |
| 4 | Busy | Higher levels of locomotion or pacing | Majority of animals in enclosure |
| 5 | Very busy | Highest level of arousal and activity | All animals in enclosure |

### 3.2.4. Data analysis

For each behaviour and each tiger, results were calculated as proportions of the total number of observations for each minute of the observation day in which the behaviour was recorded. This was achieved by dividing the total number of times a particular behaviour was observed for the same minute of the day by the total number of observations taken at that minute over all of the repeats of observations. The proportions of each behaviour were plotted and examined for daily patterns. As an aim of this study was to investigate the detailed patterns of tiger behaviour over time (indeed, Chapter 2 showed the need for detailed patterns of behaviour in the study of predictability), proportions of each behaviour were plotted for each minute of the observation day. As such, descriptive analysis of the results was considered to be the most appropriate analysis (see also Section 2.4), rather than grouping the data for inferential analysis, which would lose detail of the behavioural pattern and potentially cause different results depending on the groupings that were used. The current study did not involve changing experimental conditions, therefore there was no need to group data for comparison and it was more important to investigate the pattern of behavioural change over time.

Analysis of enclosure use was carried out by calculating the proportion of observations at each minute of the day in which each tiger was observed in each quarter of the enclosure (areas A, B, C and D). After initial inspection of the data on resource area use, it became apparent that a particular area of the enclosure was used by the tigers following a temporal pattern, therefore enclosure use data were combined for areas down the side of the enclosure adjacent to the visitor/keeper pathway and the indoor quarters (as highlighted in Appendix 3.1). The proportion of observations at each minute of the day in which each tiger was observed in this new enclosure area was then calculated and analysed descriptively.

The mean busyness score was calculated from all of the observations conducted at each minute of the day, and the pattern of mean busyness levels over the course of the observation period was plotted. Inter-observer reliability was calculated using Cohen’s Kappa (Cohen, 1960), for observations of busyness made by three pairs of observers who were able to observe simultaneously (but independently), for up to 90 minutes during the course of the study. These data were also tested for correlations between different observer’s rankings using a Spearman’s rank correlation analysis. This was done in addition to the Kappa analysis because it was possible that observers could give different absolute values but still show the same patterns and trends. Proportions of behaviours were also correlated (Spearman rank) against the mean busyness score.

## 3.3. Results

### 3.3.1. Traditional measures of behaviour

Visual inspection of the results of the traditional behavioural observations revealed that the occurrence of some behaviours changed throughout the day, such that they formed patterns over the course of the observation day, whilst other behaviours were more variable, and did not show a particular trend with the time of day.

Behaviours showing a daily temporal pattern included locomotion, pacing, sleeping and inactive alert. Locomotion for the male tiger (Figure 3.1) was observed to peak at proportions (of observations at each minute) of over .80 at the start of the day, the middle of the day and the end of the day, with the lowest proportions (below .60) shown between 11:14-12:36, and 14:00-15:12. After 15:12, the proportion of observations showing locomotion behaviour rose to a peak of 1 at 15:52 and remained high until the tigers' feed time. The female showed no clear pattern in locomotion behaviour between 10:01 and 12:41 (Figure 3.2) however, a decrease in locomotion behaviour was shown from a proportion of .57 at 12:41 to a proportion of .08 at 13:21, after which time locomotion increased to a peak of 1 at 16:09 (with an additional peak of 1 at 14:55), where it mostly remained until the feed time.

The male tiger showed higher levels of pacing than the female (Figures 3.1 and 3.2). The proportions of observations in which pacing was observed for the male, were variable throughout the day, but generally decreased during the morning from a proportion of .57 at 10:10, to .14 at 12:42, followed by an increase to a peak of .63 at 13:30. Proportions then decreased in the early afternoon to .16 at 14:52, followed by an increase to .45 at 15:14, around which level the proportion fluctuated until the feed time. A decrease in proportions was observed at the end of the day, reaching .00 at 16:42. The female tiger showed low levels of pacing throughout the day, with no particular trends until 15:43, after which time a trend is shown for an increase in the proportion of observations in which pacing was recorded, leading up to the feed time, with a maximum proportion of .50. However, the proportion does still vary around this trend.

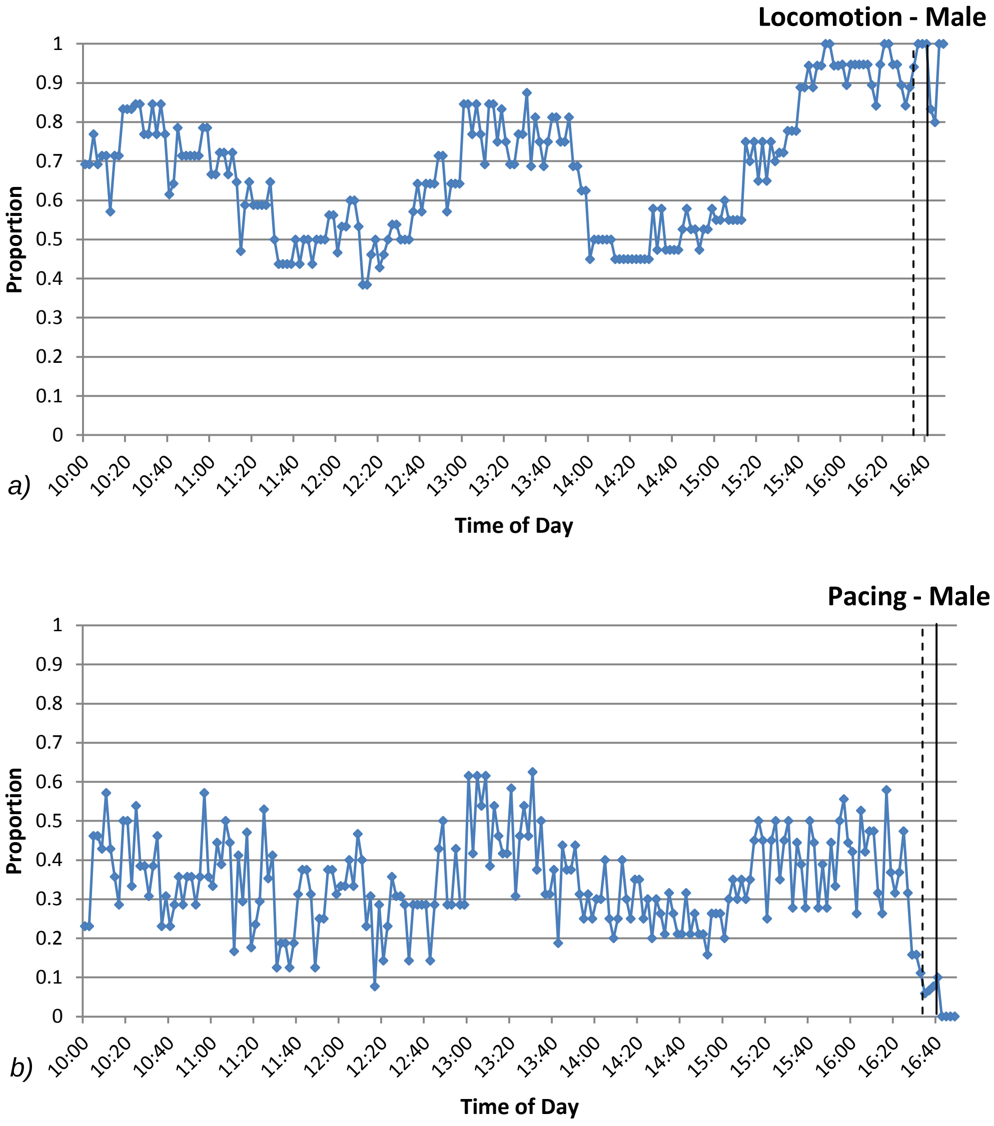


*Figure 3.1: Male Tiger. Proportion of all repeats of each minute of the day in which a) locomotion and b) pacing was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

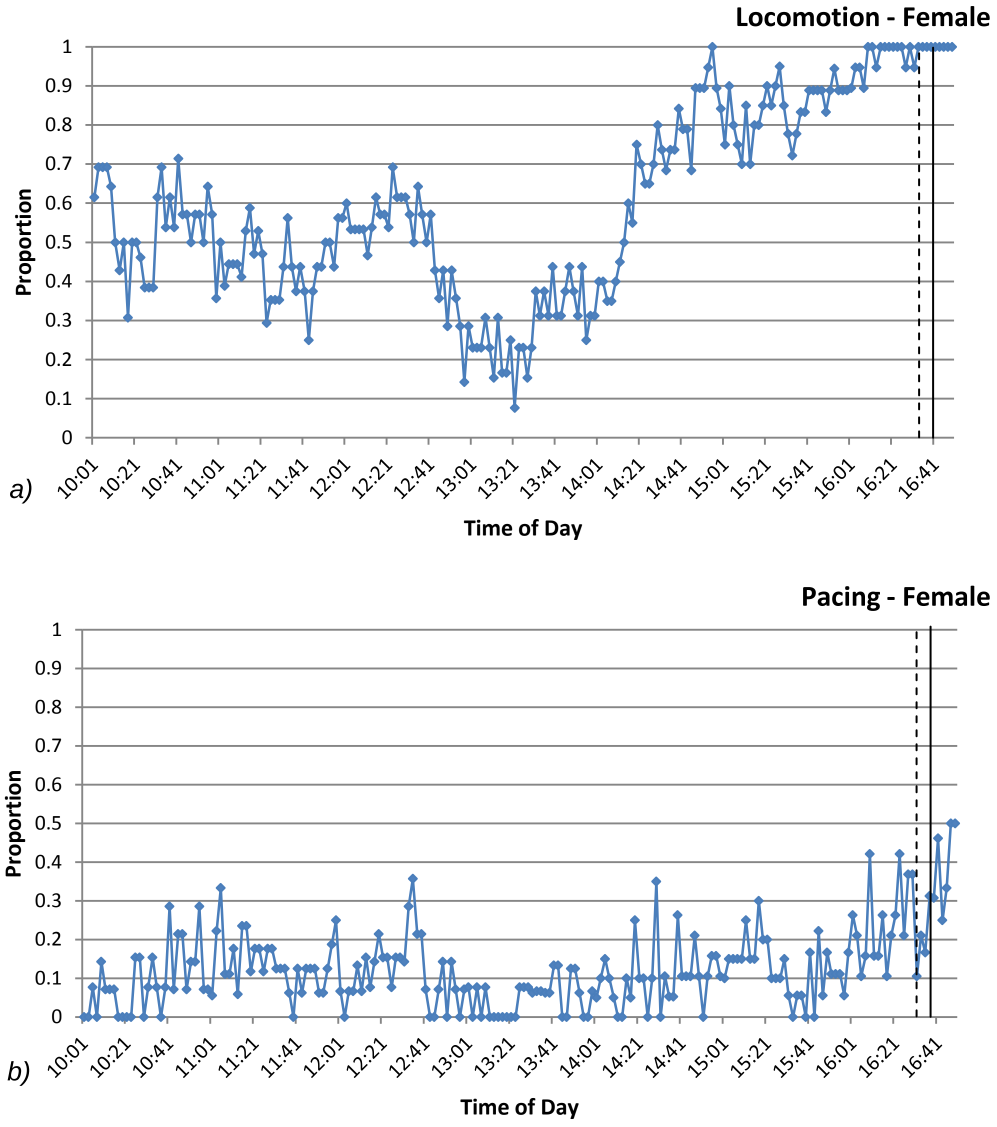


*Figure 3.2: Female Tiger. Proportion of all repeats of each minute of the day in which a) locomotion and b) pacing was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

The male tiger showed little or no sleeping at the end of the observation day. This is particularly evident between 15:34 and the tigers' feed time, between which, sleeping behaviour was hardly ever observed. Some (variable) sleeping was shown in the middle of the day, although the male tiger slept less than the female. Proportions of observations in which sleeping was recorded for the female tiger varied throughout the morning and early afternoon, with a slight peak of .29 at 11:17 and another of .42 at 13:17. Proportions of sleeping then decreased during the early afternoon until 14:37, after which time sleeping was almost never recorded (Figures 3.3-3.4).

Separation of low, medium and high levels of inactive alert behaviour revealed that medium and high levels contributed little to the overall proportions of inactive alert and therefore did not merit separate analysis. The pattern of inactive alert behaviour for the male tiger (Figure 3.3) shows an inverse trend compared to the male tiger's locomotion behaviour. Two peaks in inactive alert were shown, the first at a proportion of .38 at around 11:38 and the second at a proportion of .47 at 14:42. Inactive alert reached very low levels in the middle of the day in between these two peaks, and also decreased from a peak of .47 at 14:44 to .00 at 15:48, after which time, little or no inactive alert behaviour was shown until the feed time. The female tiger showed an initial increase in inactive alert at the beginning of the day (Figure 3.4), when proportions increased to .31 at 10:17. After this time, no obvious pattern in inactive alert behaviour was shown until 13:43, after which time the proportion of observations gradually decreased from .50 to .00 at 16:05. Little or no inactive alert behaviour was then shown until the feed time.

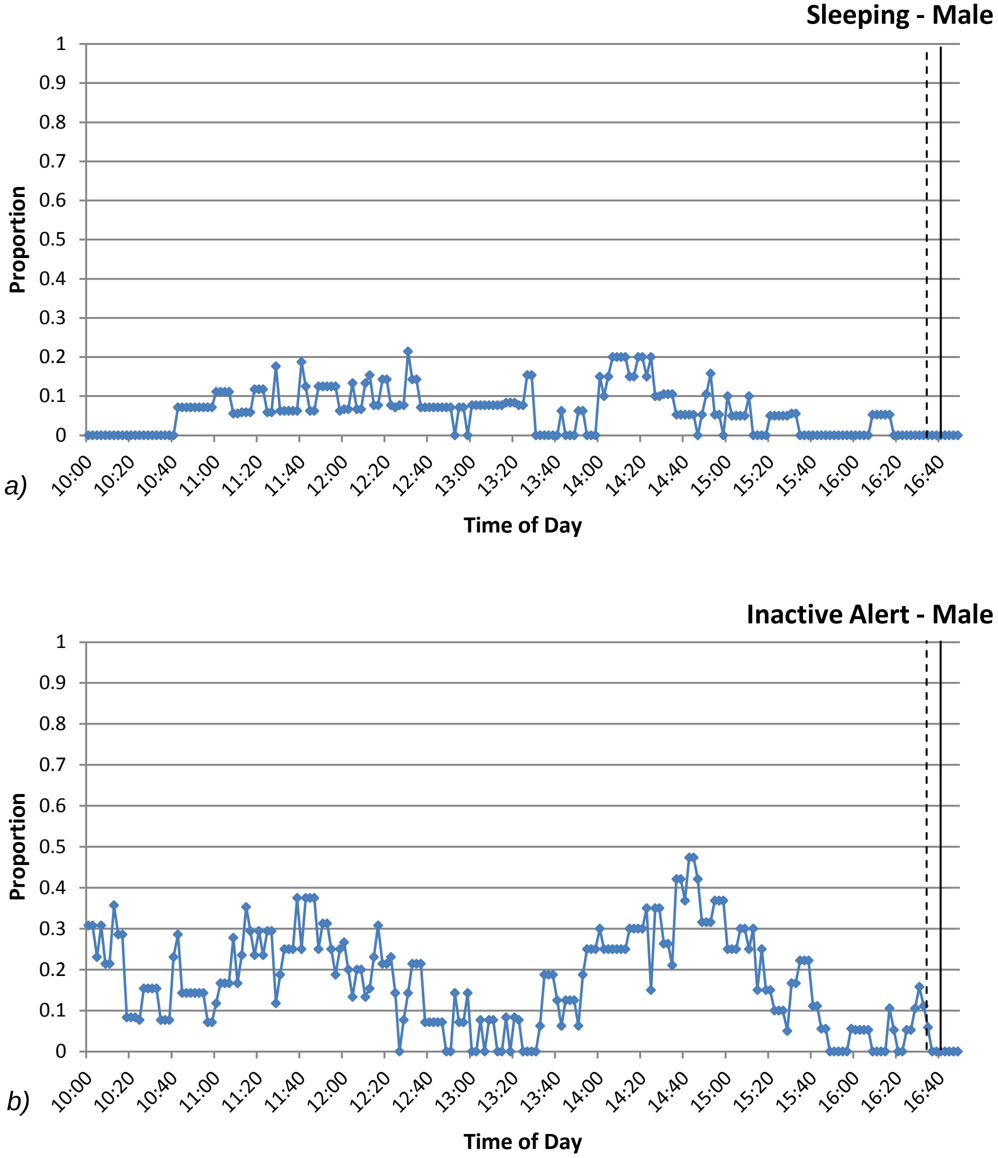


*Figure 3.3: Male Tiger. Proportion of all repeats of each minute of the day in which a) sleeping and b) inactive alert behaviour was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

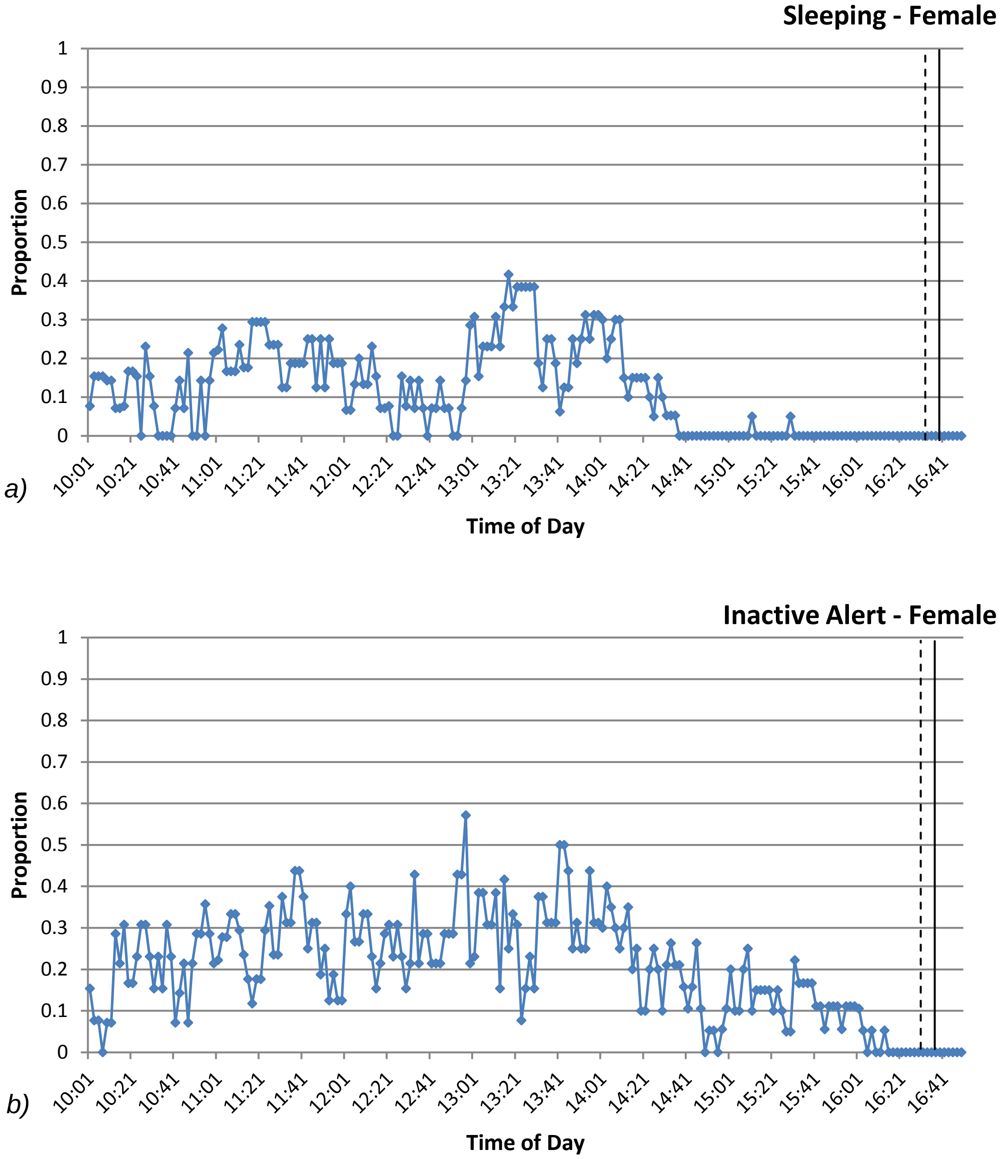


*Figure 3.4: Female Tiger. Proportion of all repeats of each minute of the day in which a) sleeping and b) inactive alert behaviour was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

Behaviours that did not seem to show large variations or clear patterns over the course of the day included investigating, marking, self-maintenance, social behaviour, agonistic behaviour and not visible (Figures 3.5-3.10). Investigating behaviours occurred at relatively low levels. Both tigers showed slightly more investigating at the beginning of the observation day (maximum proportion: .31 for either tiger), and also in the middle of the day for the male tiger (peak proportion of .31 at 13:22), however there were no other obvious patterns in investigating behaviour throughout the day for either tiger (Figures 3.5 and 3.6). Self maintenance behaviour was also performed only at low levels, particularly for the male tiger who showed no particular pattern of self maintenance behaviour throughout the day (Figures 3.5). Proportions of self maintenance behaviour for the female tiger varied throughout the morning and then decreased to a low level (mostly fluctuating around proportions of .00 or .05) from 14:01pm onwards (Figure 3.6). Marking behaviour was also shown at low levels, particularly for the female tiger, and showed no particular patterns throughout the day (Figures 3.7 and 3.8). Similarly, social behaviour showed no particular patterns in proportions throughout the day (Figures 3.7 and 3.8). Proportions of observations in which the tigers were reported to be 'not visible' were also low (Figures 3.9-3.10). Both tigers showed a sudden peak in 'not visible' at the very end of the day, and the male tiger showed slightly higher proportions of 'not visible' between 12:00 and 12:58 (maximum proportion of .26) (Figure 3.9). There were no other obvious patterns in 'not visible' throughout the day. Feeding and agonistic behaviours were shown at very low frequencies and hence are not presented here.

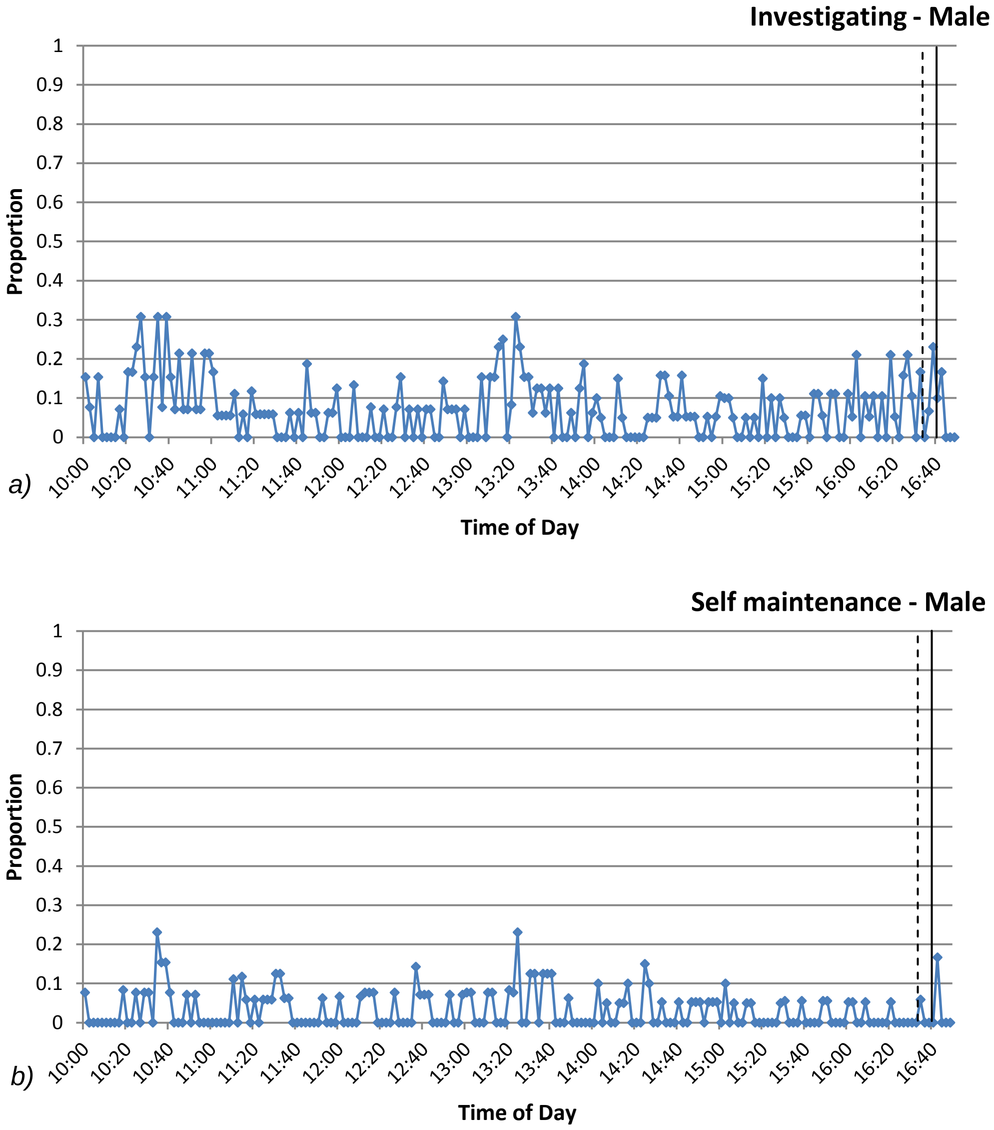


*Figure 3.5: Male Tiger. Proportion of all repeats of each minute of the day in which a) investigating and b) self maintenance behaviour was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

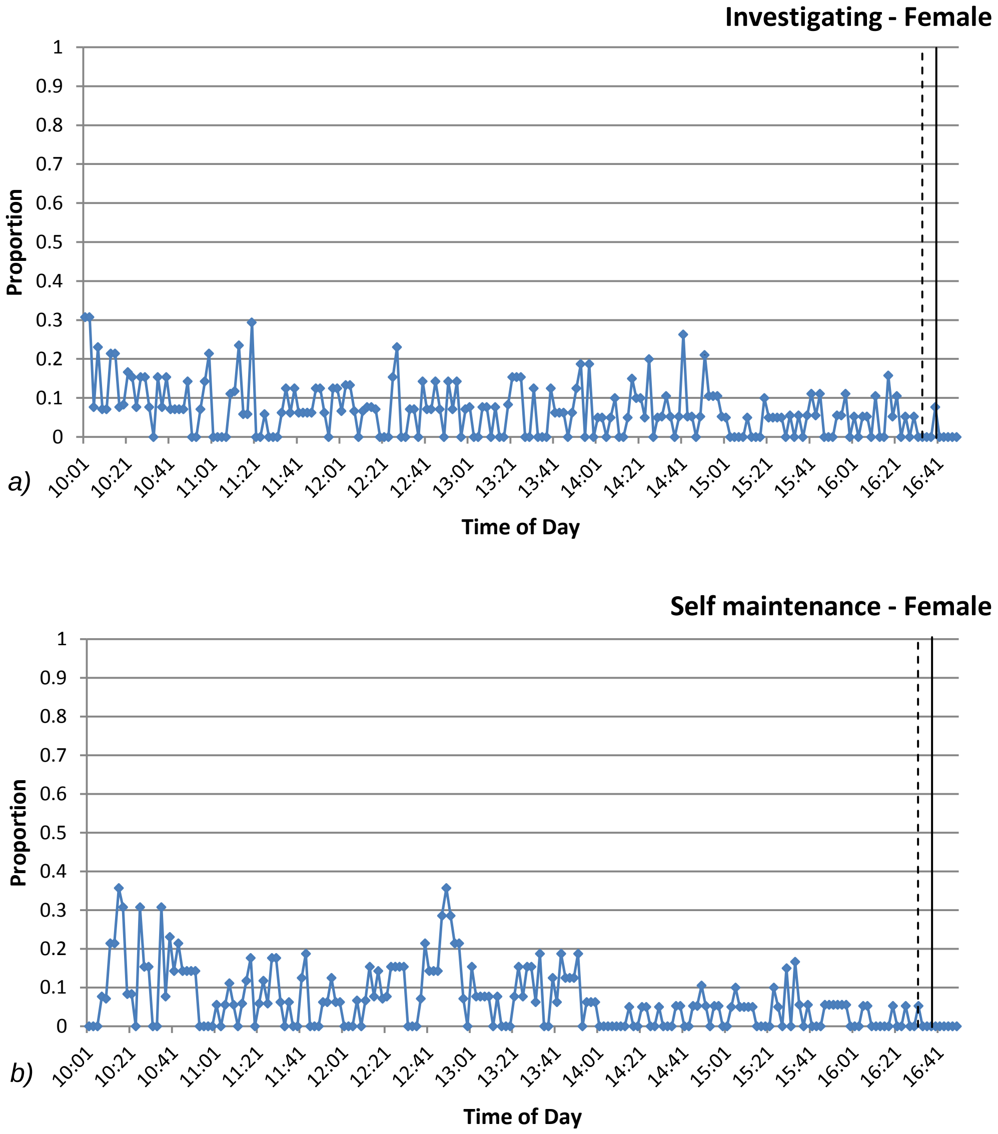


*Figure 3.6: Female Tiger. Proportion of all repeats of each minute of the day in which a) investigating and b) self maintenance behaviour was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

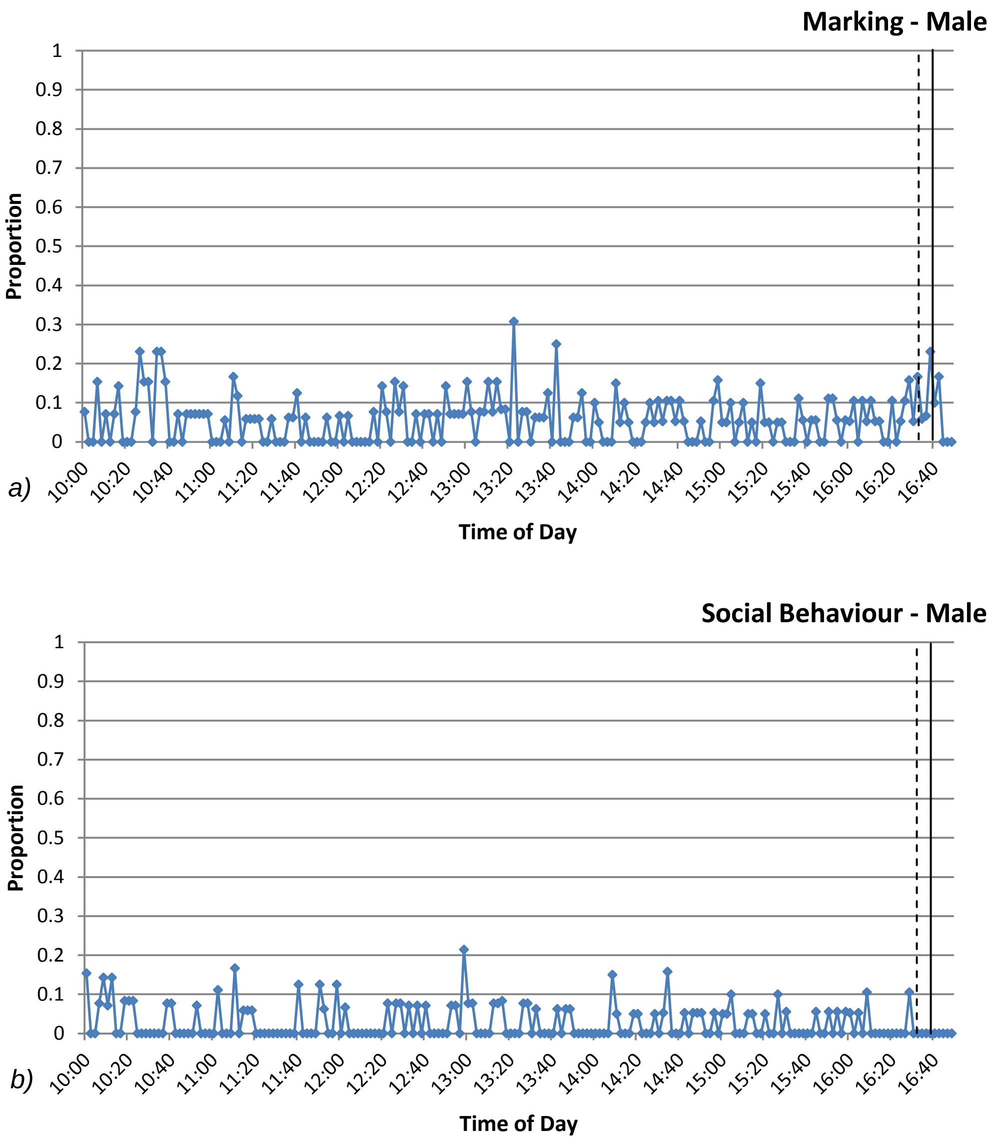


*Figure 3.7: Male Tiger. Proportion of all repeats of each minute of the day in which a) marking and b) social behaviour was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

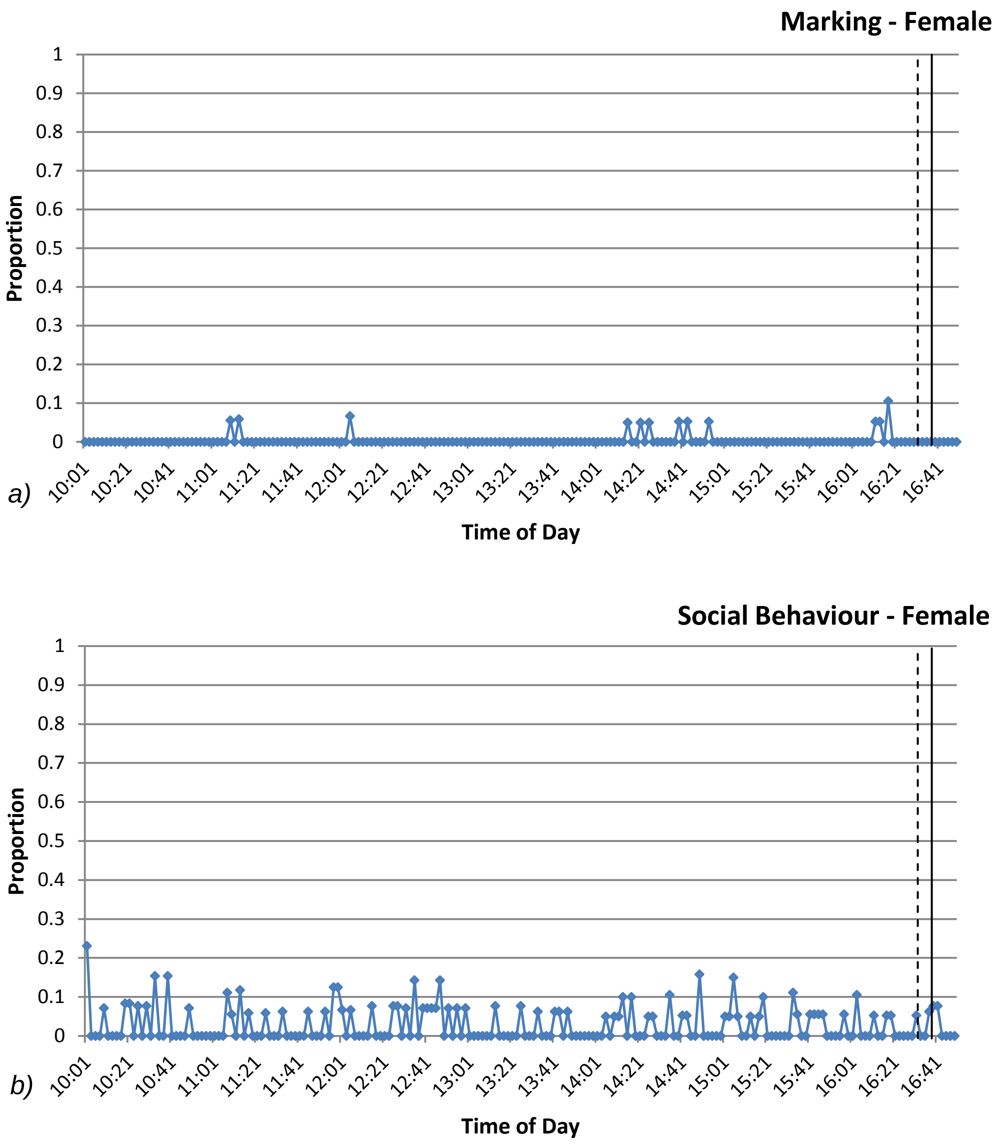


*Figure 3.8: Female Tiger. Proportion of all repeats of each minute of the day in which a) marking and b) social behaviour was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

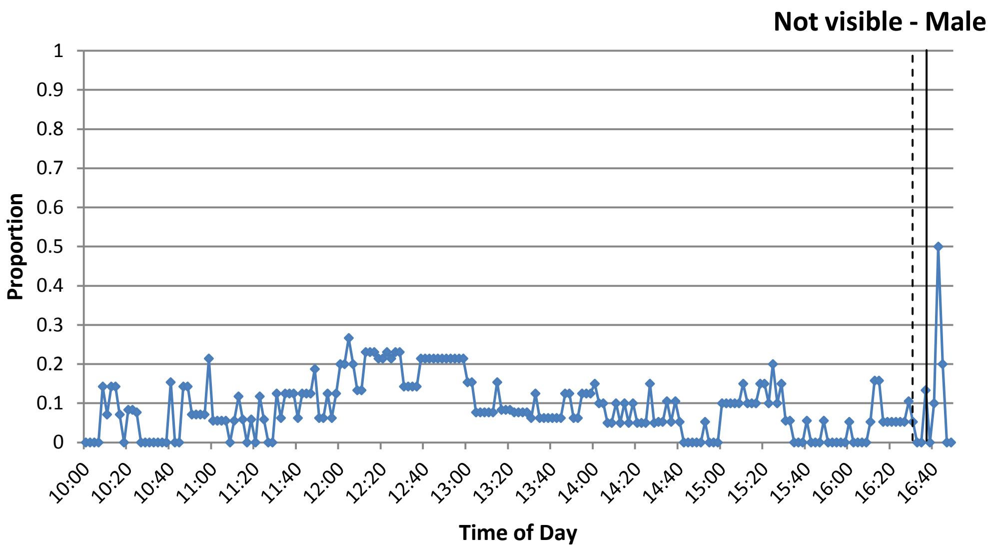


*Figure 3.9: Male Tiger. Proportion of all repeats of each minute of the day in which the tiger was recorded as not visible. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

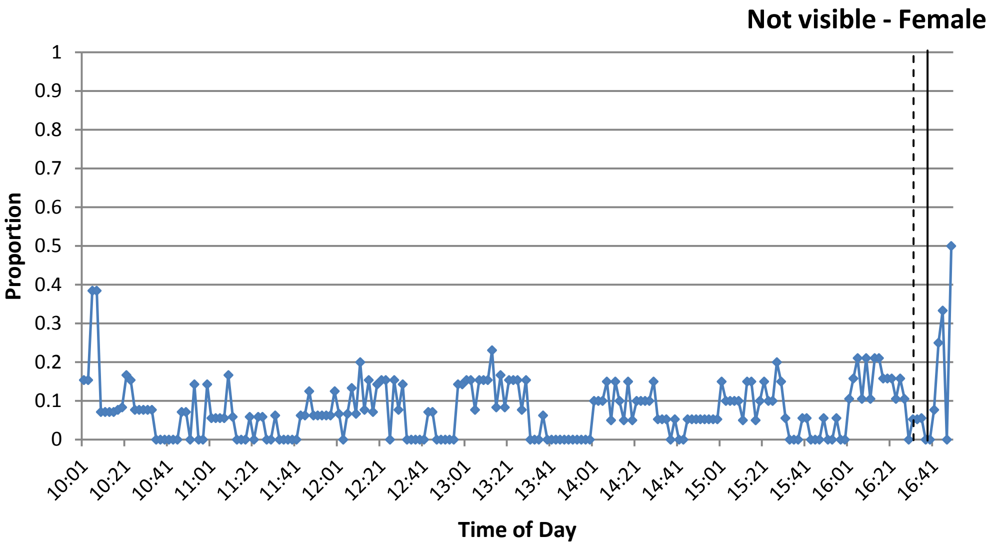


*Figure 3.10: Female Tiger. Proportion of all repeats of each minute of the day in which the tiger was recorded as not visible. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

### *Enclosure use results*

The male tiger was observed in section B in a greater proportion of observations than any other section (Figure 3.11). The trends in use of section B followed a similar pattern to that of pacing for the male (Figure 3.12). Proportions of observations in which the male tiger was in area B showed a peak of .92 at 13:04, decreasing to .40 at 14:08. Proportions then increased throughout the afternoon to 1.00 at 16:46. The male's use of sections A, C and D varied, but were relatively similar in the morning, with the exception of a peak in the proportion of area C at 12:30 (.50). Proportions of areas C and D decreased in the afternoon and were very low (<.10) from approximately 15:50 until the feed time. During this time the male was mostly recorded in either area A or area B. The male tiger's use of area A increased in the afternoon, particularly prior to the feed time with a peak proportion of .58 at 16:30.

The female tiger was also observed in area B in a greater proportion of observations than in the other areas in the morning (Figure 3.11), and until approximately 12:29, proportions of observations in areas A and D were similar. Proportions of observations in area C peaked at a value of .36 at 12:31, which corresponded with a trough in the proportion of scans in area A. Proportions of areas C and D remained at fairly similar, low levels throughout the afternoon and both decreased in the late afternoon before the feed time (≤ .11 after 16:01). After 13:31, the proportion of observations in which the female was recorded in area B decreased and the proportion in area A increased. From this time until approximately 16:27 the female was mostly observed in areas A and B with a gradual increase in the use of area A and a gradual decrease in the use of area B, until 16:27, at which point the use of area B increased to a peak proportion of 1.00, and use of area A decreased to a .00, at 16:43.

A closer look at the tigers' use of areas A and B revealed a pattern in the use of a specific area that comprised a 4m wide strip next to the fence along the entire length of one side of the enclosure and in front of the entrance to the tiger house. The definition of the enclosure areas meant that this also included the area within 1m of the perimeter fence on the other sides of the enclosure, except beside the lion enclosure (see Appendix 3.1). Both tigers showed an increase in the use of this area throughout the afternoon as feed time approached (Figure 3.13).

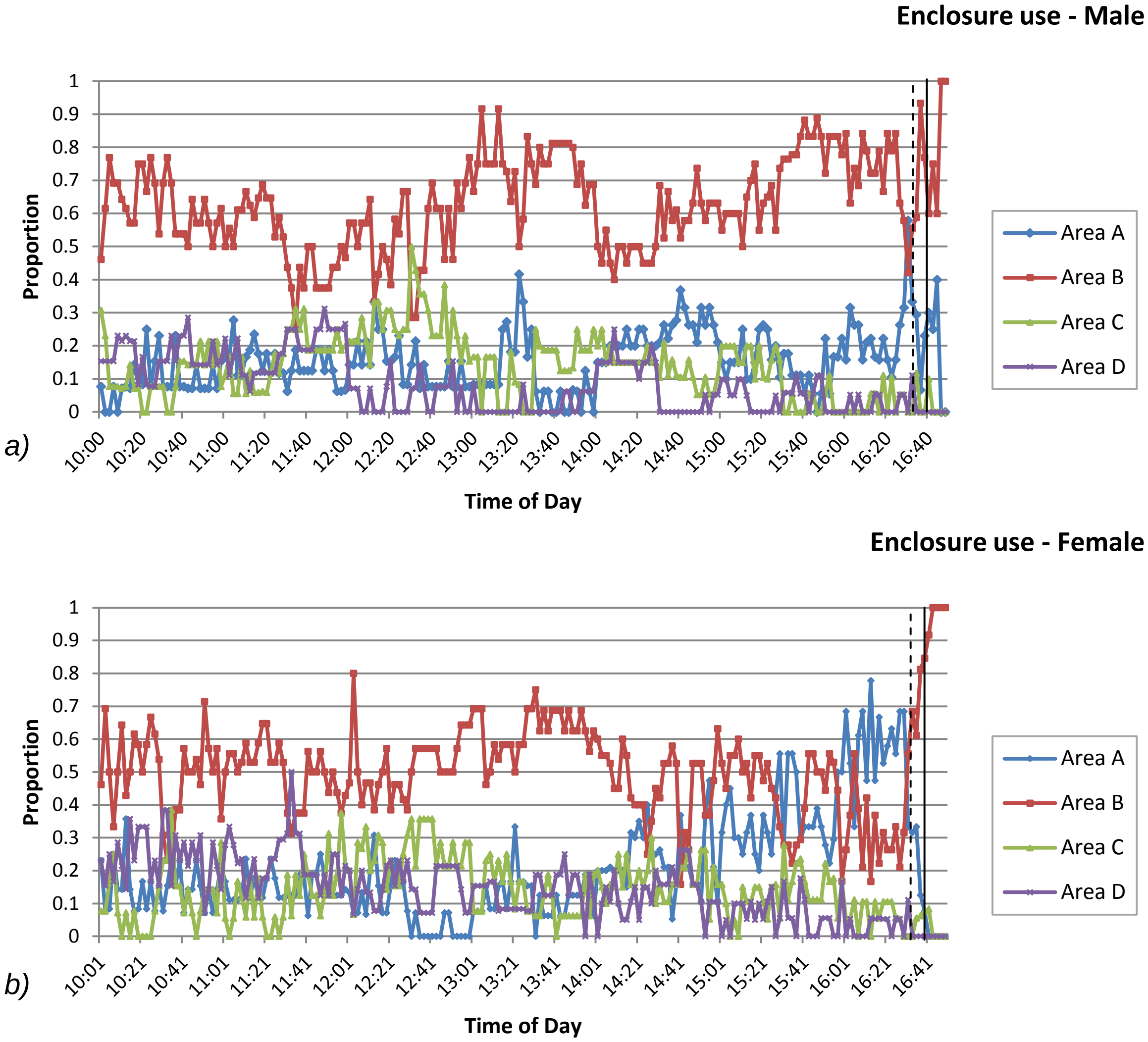


*Figure 3.11: Proportion of all repeats of each minute of the day in which the a) male and b) female tigers were observed in each enclosure quarter. Lines show use of each enclosure quarter (A,B,C or D) The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

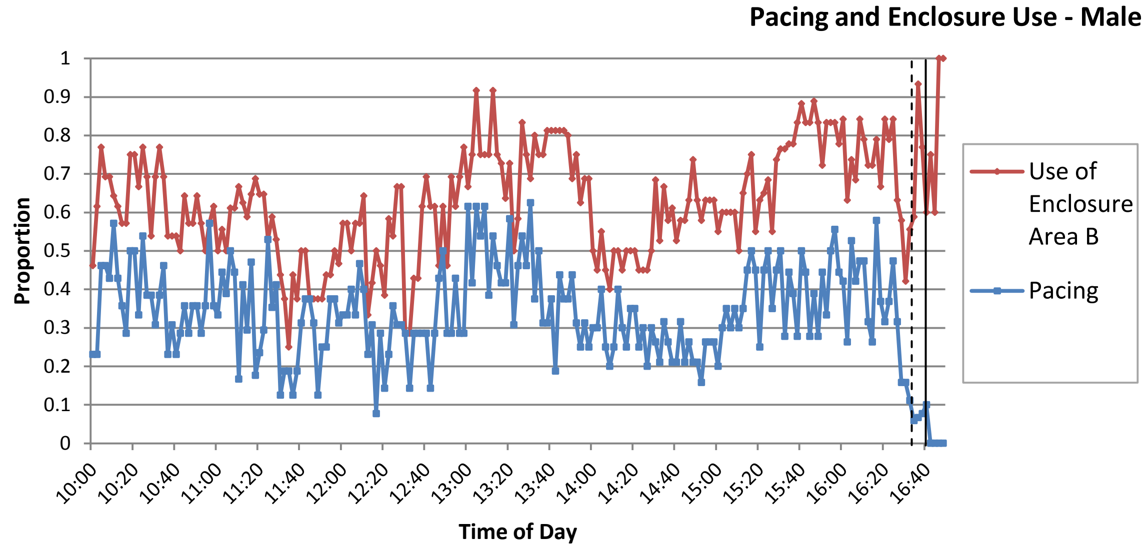
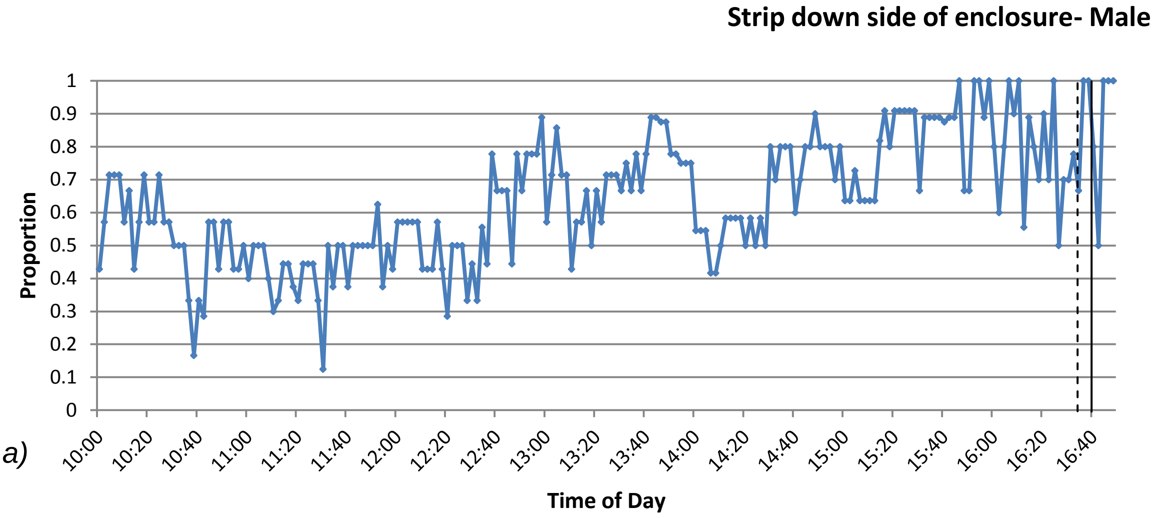
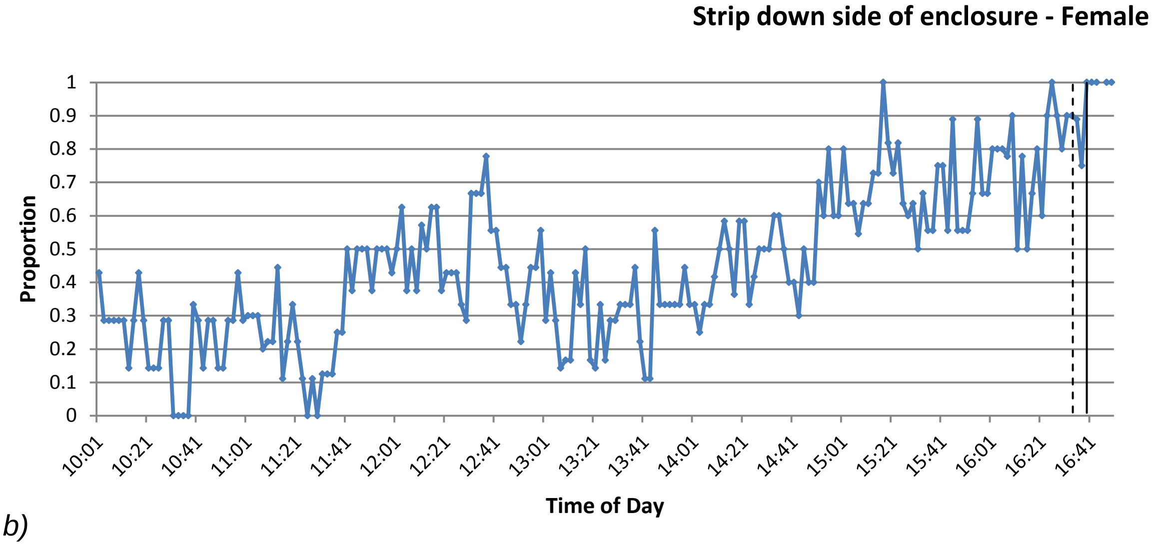


*Figure 3.12: Male Tiger. Proportion of all repeats of each minute of the day in which male tiger was observed in enclosure area B, plotted against the proportion of all repeats of each minute of the day in which pacing was observed. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time.*



a)



b)

*Figure 3.13: Proportion of all repeats of each minute of the day in which the a) male and b) female tigers were observed in 4m wide strip next to the fence along the entire length of sections A and B and in front of the entrance to the tiger house (Maximum n for a data point = 12, minimum = 6. n for minutes after 16:40 is lower, as tigers were usually inside by this time). The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time.*

### 3.3.2. Busyness results

*Busyness scores throughout the day*

Mean busyness scores showed a slight decrease from 2.31 at 10:00 to 1.69 at 11:43, they then showed a plateau during the middle of the day and then increased from 1.75 at 14:00, throughout the rest of the afternoon to a peak of 3.56 at 16:37 (Figure 3.14). A decrease in mean busyness was shown at the very end of the day to 2.67 at 16:49.

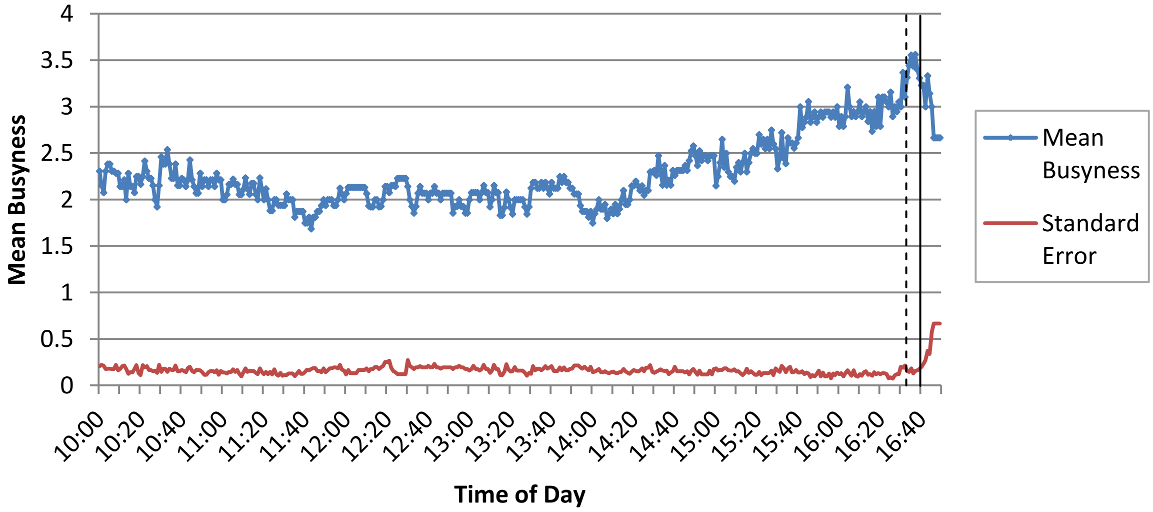


*Figure 3.14: Mean busyness throughout the day (over all days of observation). The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20).*

*Inter-observer reliability*

There was a high degree of agreement between the three pairs of students that collected data for inter-observer reliability tests, with significant concordance between their results (Kappa ≥ .78 for all three pairs, *p* < .01; Table 3.3). Spearman's rank correlations between the students' data were all positive with values ≥ .86, and significant at *p* < .01 (Table 3.3).

*Table 3.3: Cohen's Kappa and Spearman's correlations of busyness scores for sessions in which two observers simultaneously collected data.*

| **Inter-observer reliability test** | **Kappa value** | ***p*** | ***N*** | **Spearman's rho** | ***p*** | ***N*** |
|---|---|---|---|---|---|---|
| Observer 1 & Observer 2 | .84 | *p* < .01 | 74 | .94 | *p* < .01 | 69 |
| Observer 3 & Observer 4 | .89 | *p* < .01 | 95 | .90 | *p* < .01 | 90 |
| Observer 4 & Observer 5 | .78 | *p* < .01 | 65 | .86 | *p* < .01 | 60 |

### *Correlation of busyness with conventional measures of behaviour*

Busyness showed statistically significant, positive correlations with locomotion for the male tiger, and with locomotion and pacing for the female tiger, and showed significant, negative correlations with inactive alert, sleeping and self maintenance for the male tiger, and with inactive alert, sleeping, investigating and self maintenance for the female tiger (Table 3.4).

*Table 3.4: Spearman's correlation coefficients calculated for mean busyness (over all days of observation), for each minute of the study day, with proportions of behaviours, observed for each minute of the study day (N for correlations = 250).*

| | **Locomotion** | **Pacing** | **Inactive alert** | **Sleeping** | **Investigating** | **Self Maintenance** | **Social** |
|---|---|---|---|---|---|---|---|
| Male | .59** | .01 | -.33** | -.61** | .04 | -.14* | -.01 |
| Female | .85** | .36** | -.73** | -.77** | -.16* | -.29** | -.03 |

Note. *p <.05, **p <.01

## 3.4. Discussion

### 3.4.1. Daily patterns of behaviour

*Behavioural patterns prior to feeding*

Both tigers showed patterns in the distribution of certain behaviours over the course of the day. In particular, increases in locomotion and, to some extent, pacing were demonstrated for both tigers in the lead up to the feeding time, with corresponding decreases in inactive alert and sleeping behaviour. Increases in locomotion in the lead up to feeding were particularly apparent, and the afternoon increase in locomotion was the greatest increase in this behaviour over the day, with proportions remaining high and stable prior to the feed.

These patterns of behaviour suggest that the tigers were becoming more active, in anticipation of the feed. This may be considered to be a form of Food Anticipatory Behaviour (FAA), as previously described in felids and many other species (see Sections 1.2 and 1.4.2). For example, Mallapur and Chellam (2002) found that captive leopards showed higher activity, and significantly lower inactivity in the hour immediately prior to set feeding times, compared to a randomly chosen observation hour after the feed. Similarly, Peters et al. (2012) observed significantly higher activity in horses immediately prior to feeding, compared to a baseline time of day (although pre-feeding data were only reported from the time after feeding cues had commenced, which may have influenced behaviour).

It was noted anecdotally in the current study that the tigers became more active and animated as the feeding time approached and that, not only did locomotion increase prior to the feed, but that locomotion became faster, with higher occurrences of trotting and running (Bishop, pers. obs.). This is in agreement with Mallapur and Chellam (2002) once again, who noted that as well as increasing activity, the leopards became more ‘restless’ and ‘anxious’ prior to feeding.

Female pacing levels were lower than those for the male tiger, nevertheless both tigers showed an increase in pacing in the afternoon, leading up to the feeding time (with the exception of the drop in male pacing immediately prior to the feed time). This is in agreement with previous literature which has reported an increase in felid pacing prior to predictable feeding times. For example, Altman et al. (2005) and Carlstead, (1998) reported, for a range of felid species, that pacing mostly occurred in the time prior to feeding, and Weller & Bennett (2001) found significantly higher pacing prior to, than after feeding times. The relatively high pre-feed levels of pacing in the current study suggest an influence of the feeding time on pacing behaviour.

Both tigers showed patterns of enclosure use prior to the feeding time. During the afternoon, and prior to the feed, the male tiger showed high use of area B and the female tiger showed high use of areas A and B. More specifically, afternoon use of areas A and B was largely restricted to a strip of the enclosure up to 4m away from the fence and the indoor quarters (see Appendix 3.1), and use of this area increased in the lead up to feeding time. This was the area along the enclosure fence, from the area beside the indoor quarters (where the tigers would later be fed), along the fence where the keepers would walk, to the fence at the bottom of the enclosure from which the tigers could see the keepers approaching from a distance. This is consistent with the findings of Mallapur and Chellam (2002) who reported that, prior to feeding, the leopards used the area from which they could see the approach of the keepers. Specifically, the leopards alternately covered the area near to the off-show area where they were fed, and the fence area from which they could see the keepers approaching with the feed. The same is true in the current study, as the tigers were observed to walk along the above mentioned strip of the enclosure, at varying speeds in the time prior to the feeding time.

Altman (2005) reported that increased pacing in lions was directed at the night quarters in which the lions would be fed, and was likely cued by the keepers preparing the food. This was also observed in the current study upon arrival of the keepers where the tigers increased their use of the area in front of their night time quarters (Appendix 3.2), and female pacing was at its highest at this time.

It is possible that the arrival of the keepers to the enclosure could have acted as a cue to feeding and hence feeding would then have occurred with signalled predictability (as discussed in Bassett & Buchanan-Smith, 2007 and in Sections 1.4.3 and 3.1.3). However, it is important to note that changes in behaviours and enclosure use prior to the feeding time, began, and reached a relatively constant high (or low) level, in advance of the arrival of the keepers to the enclosure (mean observed keeper arrival time = 16:33). Thus, it appears that the tigers were responding to the temporal predictability of the feeding time even before the occurrence of a signal. This does not exclude the possibility that the tigers also responded to the keeper arrival cue, indeed both the tigers were observed to further increase their use of the area up to 4m in front of their night time quarters upon the arrival of the keepers (see Appendix 3.2), and the male tiger decreased pacing around the keeper arrival time. But prior to this, the tiger's behavioural patterns were already changing in a manner consistent with anticipation of a temporally predictable event.

This study has therefore demonstrated that a pair of tigers fed according to a predictable feeding time, showed patterns in particular behaviours and in enclosure use, in the time leading up to the feeding time. Thus it seems likely that the tigers' behaviour was affected by the feeding time, such that the behaviour anticipated the feed. It should, however be noted that not all recorded behaviours showed feed anticipatory patterns, and there were also patterns of behaviour at other times of the day (as described below). Consideration of other behavioural patterns should be made, in order to fully appreciate the extent of those patterns related to the feeding time.

### *Other daily behavioural patterns*

In addition to the pre-feeding peak in locomotion, the male tiger showed another, albeit lower, peak in locomotion in the middle of the observation day. This peak was not shown by the female tiger, who instead showed increased sleeping and inactive alert behaviours at this time. Daily behaviour patterns can be affected by many factors such as the light cycle, patterns of zoo visitor attendance, and other cues to behaviour that vary systematically across the day (see Sections 1.1 and 1.2). Such may have been the case here, although particular cues could not be identified at this time of day.

The male tiger also showed a peak in pacing in the middle of the day, which was, in fact greater than that shown prior to feeding. This suggests that, whilst it is still possible that male pacing increased in anticipation of the feeding time, there may be alternative or additional causes of the male's pacing behaviour. Indeed there can be many causes of pacing (see Mason, 1991). Additional causes for pacing were also concluded in the study by Jenny and Schmid (2002) (as described in Sections 1.4.2 and 2.2); stereotypic pacing for the male tiger was reduced with the use of an automated box feeder when the pair of tigers were housed together, but not when the male was housed individually. It was concluded that when housed together pacing was a result of frustrated appetitive behaviour (potentially akin to the pre-feeding pacing in the current study). However, Jenny and Schmid suggested that when housed individually, pacing was due to another factor such as separation from previously experienced social contact with conspecifics. Further research would be necessary to determine the cues to pacing in the middle of the day in the current study. Considering that the male's pacing followed the same patterns as his use of area B, an area which was close to the visitor path, the relationship between visitor-related cues and pacing would be worthy of further research, although there is only limited evidence of a visitor effect on felid behaviour Hosey (2008).

Certain behaviours, including investigating, self maintenance, marking, social behaviour, and not visible did not show a particular pattern across the day, or even in the time immediately preceding the feed. Indeed, these behaviours are rarely reported in the literature related to predictability. However, it is perhaps surprising that investigating did not show a pattern related to feeding time, as it is an appetitive behaviour which might be expected to change in the approach to feeding. Shepherdson et al. (1993) found a connection between feed predictability and investigation behaviour, as a reduction in the predictability of leopard cat feeding resulted in greater 'exploratory' behaviours. However, Shepherdson et al. provided multiple opportunities for feeding in order to reduce the predictability, hence increased exploration would be necessary to retrieve these feeds. In the current study, feeding opportunities were constant over the day, and were never available in the outdoor enclosure, so exploration for food was not necessary.

As proportions of ‘not visible’ were low and relatively constant over the day, it is possible to have confidence that the data were not distorted by periods of ‘unknown’ behaviour. The peak in not visible at the end of the day is due to the tigers being let inside the indoor quarters during some observation minutes at this time, an area which was not visible to the observers.

### 3.4.2. Evaluation of traditional behavioural results

The method employed in this study enabled the recording of behaviour throughout the day. Descriptive analysis of the data determined patterns of increase or decrease in the lead up to the feeding time. However, it should be noted that the current study used only descriptive analysis, and without inferential statistics to indicate the likelihood that these patterns would be present in a population, and without systematic replication to indicate their generality, any inferences from these data should be made with caution. However, the behavioural patterns described, were achieved over a substantial number of repeat observations. It therefore seems reasonable to suggest that these patterns can be held with a degree of certainty.

The increases in locomotion and pacing behaviour prior to feed time follow similar general patterns to those seen in the scalloped curves created in laboratory studies of fixed interval timing, where an animal’s operant responses increase during the time preceding the fixed interval time (e.g., Dews, 1978; Lejeune & Wearden, 2006 and as described in Section 1.2). The method employed in the current study therefore enabled detailed interpretation of patterns of behaviour, allowing identification of: precise times of low and high proportions of behaviour, the time at which the behaviours started to increase (akin to the ‘break-point’ in studies of short interval timing, as described by Lejeune and Wearden [2006]) and the length of time that a behaviour was sustained prior to the feeding time. In a zoo environment, and in the absence of automated recording of operants, this seems a useful method for identifying the effects of predictable routines.

An aim of this thesis was to explore the effect of predictable routines on zoo animal behaviour through description of behavioural patterns over extended periods. This was achieved in the current study. For example, increases in locomotion were found to begin approximately 1.5 hours before the feed for the male tiger, and even earlier for the female tiger, and high levels of locomotion were sustained for over 30 minutes prior to the feeding time. Had the current study sampled only the 30 minutes or 1 hour prior to the feed (as discussed in Section 2.2), then these details would have been lost from the data. The extended time period of observation in the current study also allowed for determination of behavioural patterns before the arrival of the keepers, and associated feeding cues. Such feeding cues are often either not reported or are a potential confound in timing studies, for example Altman et al. (2005) suggested that feeding cues may have influenced lion behaviour in their study, and Peters et al. (2012) only reported horse behaviour after feeding cues had commenced, despite collecting data prior to these cues. Information on behaviour prior to the feeding cues would have given more detail on the effect of temporal predictability. A strength of the current study was that the analysis of extended periods allowed for complete interpretation of behavioural patterns related to the timing of feeding.

It must be taken into consideration that whilst a possible association has been identified between the set feeding time and behavioural patterns, without manipulation of the feeding time, this conclusion cannot be confirmed. It is possible that these behaviour patterns are not pre-emptive of the forthcoming feed and instead may be caused by some other environmental variable (such as changes in light or temperature levels, or visitor numbers, or being let inside the sleeping den, which happened at the same time as feeding). Whilst it seems reasonable to use the current data in association with previous literature to suggest an effect of the feeding time on tiger behaviour, further study would be required to firmly support to this conclusion. Specifically, feeding times should be manipulated (and separated from other husbandry events) to determine whether behavioural patterns are shifted according to the new feeding routine. If increases or decreases in behaviours could be demonstrated prior to the new feeding time, this would provide evidence that these behaviours were indicative of anticipation. This would help to establish an anticipatory role for those behaviours which also peak at other times of the day, when anticipation would not be expected.

It should also be taken into consideration that this study used only two individuals, with institution-specific husbandry and enclosure influences. It would be unwise to generalise the findings to other settings without caution. However, the results of the current research were consistent with previous literature and extend knowledge on patterns of anticipatory behaviour. In order to replicate this study and increase the sample size, a great deal of observation time would need to be committed. Section 3.4.4 (below) will discuss a suitable method for such observation.

It should be noted that during the first half of this study a visitor viewing platform was in the process of construction close to area A, and was opened for visitor use approximately half way through the study period. It is therefore possible that this influenced the behaviour of the tigers. However if this were the case, any such effects would have been experienced throughout the day, and daily variation in its influence on behaviour would not be expected. It therefore seems unlikely that this viewing platform affected the tigers' patterns of behaviour overall. Nevertheless, further study once building work had ceased and the tigers had become habituated to the platform would be recommended for comparison.

During the course of this study, certain behaviours were observed which may be worthy of inclusion in future research. For example, increased speed of locomotion was noted prior to feeding time. Running, trotting, and walking were not separated in the current definition of locomotion, however, future work should observe the various speeds of locomotion in order to determine whether these different types of locomotion vary systematically in the approach to feeding time. Indeed, this was incorporated in the study to be described in Chapter 4 (which explored the behaviours associated with changes in assessments of busyness).

The tigers were also observed to walk up and down the 4m strip of the enclosure near the path and indoor quarters. This behaviour was repetitive in nature but did not fit the precise definition of pacing used in the current study as identical routes were not taken on each pass. A new category of 'repetitive walk' was therefore added in to Chapter 4, to account for this behaviour.

Finally, it was also noted that as the feeding time approached, the tigers became more alert (as discussed in Section 3.4.1) and frequently stopped to look in the direction from which the keepers would approach. Part way through the data collection period it was attempted to incorporate this ‘stop and gaze’ behaviour into the method. However as the observers found it difficult to accurately record gaze direction, this behaviour was not included in further analysis. In future work it may prove useful to develop a reliable measure of gaze direction in order to determine whether the tigers increase their lookout for the keepers, prior to feeding.

### 3.4.3. Busyness results

Busyness data showed distinct patterns over the day and in particular during the afternoon, where mean busyness increased in the lead up to feed time. This pattern of increasing busyness started over 2 hours before the mean keeper arrival time, and coincided with an increase in locomotion prior to the feed. Indeed, busyness was significantly and positively correlated with locomotion for both tigers. Busyness was also positively correlated with pacing for the female tiger, and was significantly negatively correlated with inactive alert and sleeping for both tigers. These correlations with inactive alert and sleeping seem logical in light of the afternoon decreases in these behaviours. The increase in busyness leading up to the feeding time (and correlated increase in locomotion and decrease in inactivity) could be considered to indicate food anticipatory activity, suggesting that busyness was successful in capturing anticipatory behaviours.

### 3.4.4. Evaluation of busyness

As described above, busyness was successful in capturing anticipatory behaviours, and showed patterns in the time leading up to feeding. Busyness results were also achieved with good inter-observer reliability, thus providing support for the use of this measure. Landis and Koch (1977) categorised Kappa scores of 0.61-0.80 as substantial agreement between observers, and 0.81-1 as almost perfect agreement. Therefore the values achieved here of 0.78, 0.84 and

0.89 are evidence of good reliability of busyness scores between observers. Correlation analyses also revealed good inter-observer reliability, with significant correlations between busyness scores from different observers. These correlations demonstrate that the observers agreed in terms of the trends in busyness that they recorded, therefore even if the different observers did not always record exactly the same numerical scores, they agreed that the busyness of the tigers increased or decreased at the same time. This inter-observer reliability implies that the subjective ratings were usefully related to actual changes in the scene observed.

Busyness results correlated with a number of behaviours related to anticipation, suggesting that it is a useful composite measure of behaviour and anticipation. Previous research on many species has revealed changes in a number of behaviours in relation to predictability, for example pacing in felids (Carlstead, 1998), vocalisation in François' langurs (Krishnamurthy, 1994) and activity/inactivity in chimpanzees and felids (Bloomsmith & Lambeth, 1995; Mallapur & Chellam, 2002), and no single behaviour is apparent as constituting anticipation of a predictable event (Reebs & Gallant, 1997). Indeed, it may not be possible to specify in advance the changes in behaviour that anticipate a predictable event. Busyness could therefore be a useful combined measure of behaviour for such studies, since this measure does not focus on one, specific behaviour, but encompasses all behaviours of the animals in the enclosure.

As described in Section 3.4.1, it was observed that the tigers showed descriptive or qualitative changes in behaviours prior to the feeding time, such as increased animation and speed of locomotion, and increased levels of alertness. This was also observed by Mallapur and Chellam (2002) who described the leopards in their study as 'restless' and 'anxious' prior to feeding. Such descriptive qualities of the behaviour were not accounted for in the one-zero, ethogram-based method of data collection, but could be taken into consideration when assigning a busyness score. This quality may be useful in future research as an alternative to analysis of individual behaviours, where small changes may not show a clear effect. This has been cited as an advantage of other qualitative measures whereby, as a result of their integrative nature, the observer is able to combine many pieces of information which would normally be recorded separately, thus offering improved clarity (through ease of interpretation) of the results (Meagher, 2009; Wemelsfelder, et al., 2000). It is important to evaluate such advantages of qualitative measures alongside the potential disadvantages of, for example, unreliability and bias (as described in Section 2.6). However, these disadvantages can be overcome with suitable testing of the reliability and validity of the measure. Good inter-observer reliability and validity were demonstrated in the current study, and will be further explored in Chapter 4.

Busyness scores showed a decrease at the very end of the observation day. At that time one tiger had been let into the house and the other tiger was waiting to be allowed inside. This drop of busyness may be a genuine reflection of the remaining tiger's behaviour at this time, or it may be an effect of the fact that the observers were now only watching one tiger and hence dropped their busyness ratings. In further research, it should be emphasised to observers that busyness is a measure of all animals in the area of observation, in order to reduce any potential confusion. However, it should be noted that the data for times after 16:40 are based on only a few repeat observations as the tigers were usually inside by this time.

The observers in the current study required substantial training in order to collect data using the traditional observation methods. However this was a relatively straightforward study with only two animals. Future work, with a more complicated design may require even more training, in addition to the time required to collect data. In contrast, training for busyness was only a small component of the training time; hence this measure may prove useful in future work. The extent of training required for the application of busyness will be further investigated in Chapter 4.

In the current study, definitions were provided for each of the five busyness ratings. It was decided that for the rest of this thesis, busyness would be investigated using definitions for only the anchor points of the scale (the extreme ratings of 1 and 5). The more defined this measure becomes, potentially the more reliable it will become, but it will also become less subjective and will require more training and experience to use. Whilst the training requirement in the current study was relatively short, if busyness can be shown to be reliable and valid when training is only necessary for the anchor points, then training and preparation time would be further decreased, thus making busyness a quick and easily accessible method, and potentially useful for further study to increase the sample size. Therefore this revised version of the scale was used in Chapter 4.

### 3.4.5. Wider implications and further thoughts

Whilst this study was not designed to assess the welfare implications of predictable routines, it should be noted that the tigers' daily routines involved well defined patterns of pacing (as discussed in Section 1.3) and restricted enclosure use for a large period of the afternoon. Thus, this study has demonstrated how detail on patterns of behaviour can be ascertained, providing scope for further research into the welfare effects of such patterns. Further work is recommended to compare the effects of using an unpredictable feeding schedule, or provision of multiple daily feeds, as these methods have previously been found to reduce stereotypies and increase behavioural diversity in felids (e.g., Jenny & Schmid, 2002; Shepherdson, et al., 1993). Feeds during the day in the outdoor enclosure could stimulate appetitive foraging behaviour during the day. An increase in opportunities for appetitive foraging behaviours with the provision of feeding boxes led to reduced stereotypic behaviour in tigers (Jenny & Schmid, 2002), and a similar conclusion could be drawn from the work of Shepherdson et al. (1993) where an increased number of daily feeds led to increased exploratory behaviour and decreased stereotypy in leopard cats.

The effect of environmental enrichment on patterns of behaviour, and the effect of the timing of enrichment provision, in relation to food anticipatory behaviour is also a route for further research. Such research would open a new dimension in the study of environmental enrichment, as few studies investigate the effect of enrichment timing on its 'effectiveness'. Environmental enrichment can be used to give control to the animals (Sambrook & Buchanan-Smith, 1997). Predictability and control are both important to animal welfare and can be linked (as discussed by Bassett & Buchanan-Smith 2007; Waitt & Buchanan-Smith, 2001, and in Section 1.4.3). Whilst the tigers' feeding time was predictable in the current study, the tigers had no control over its occurrence, and this may be a reason for the pre-feed patterns of behaviour. It should therefore be investigated whether environmental enrichment could offer the tigers more control over their environment, and hence influence patterns of behaviour. For example, Jenny and Schmid (2002) reported that the introduction of feeding boxes which tigers had to check and open, gave the tigers control over their feeding (in addition to creating an unpredictable feeding routine), which resulted in a decrease in stereotypic behaviours.

Further research could also investigate the effect of providing a reliable signal to feeding. Bassett and Buchanan-Smith (2007) recommend using a unique, reliable signal to announce feeding, with the aim that the animals would associate this signal with feeding, and reduce associations with other unreliable signals (which could lead to frustration). However, in the current study the tigers already received a signal for feeding; the arrival of the keepers. Whilst this could be argued to be an unreliable signal, as the keepers also visited the enclosure at other times of the day, such visits were infrequent, and their arrival at the end of the day clearly announced the feeding time. Bassett and Buchanan-Smith (2007) suggest that providing a predictable signal would facilitate feeding at unpredictable times, however in the current situation, temporally predictable feeding was used for management reasons and (as established in Section 3.4.1) the tigers responded to the temporal predictability of that feeding time, and changed their behavioural patterns long before a signal would become useful. Therefore, in this situation, Bassett and Buchanan-Smith’s recommendation of signals is unlikely to be relevant. The detailed patterns and durations of behaviours provided in the current results were therefore beneficial to this interpretation.

### 3.4.6. Summary and conclusions

The current study has been successful in identifying some patterns of behaviour which anticipate a predictable feeding time. As predicted, locomotion and, to some extent pacing behaviours showed increases prior to feeding, and sleeping and inactive alert behaviours decreased. These results demonstrate that, despite the fact that tigers are opportunistic hunters (Chundawat, et al., 2011), that adapt their behaviour to feed at potentially any time of the day or night, (e.g., Nowell & Jackson, 1996; O'Brien, et al., 2003; Seidensticker & McDougal, 1993; Sunquist, 1981), when fed according to a predictable routine in captivity, behaviour can become fixed into a regular pattern that commences well before the feeding time. These data further support the existing literature on felid behaviour in relation to predictable routines, and suggest that animal managers should take the timing of feeds into consideration when organising their husbandry routines. Further investigation would be necessary to determine precise welfare implications of such behaviour; however this study has demonstrated both the existence, and extent of behavioural patterns that can be caused by predictable routines. Manipulation of the tiger feeding regime was not possible for management reasons and also due to the birth of tiger cubs at the end of this study, therefore further work in this thesis (e.g., Chapters 5, 6 and 7), involving direct experimentation of the effects of predictable and unpredictable routines, was carried out with other species.

Busyness results demonstrated a pattern of increase leading up to feeding time, with significant correlations with traditional measures of behaviour, and hence was successful in capturing anticipatory behaviours. Busyness was determined to be a composite measure of behaviours incorporating descriptors of those behaviours such as increased alertness and speed of movement. Thus busyness has provided an overall interpretation of the tigers' anticipatory behaviour. That the mean busyness data contained patterns and correlated with specific behaviours suggests that busyness scores were not randomly assigned, and instead corresponded to actual trends in tiger behaviour over the day, thus providing initial validation of the measure. Inter-observer reliability was also found to be good, providing support for the future use of this measure in the study of anticipation. Despite this, in Chapter 4 it will be necessary to further explore the reliability and validity of this measure, and its relationship to more traditional measures of behaviour.

# Chapter 4

## *Development of busyness; a subjective measure of animal behaviour*

### 4.1. Introduction

Chapter 2 argued the case for the development of a subjective measure that can be used with multiple observers, in the study of predictability (and potentially in other research). ‘Busyness’ was suggested as one such measure and this chapter describes a series of studies that were carried out to develop and test this measure. Subjective, or qualitative measures, must be tested for both reliability and validity in order to demonstrate their value (Meagher, 2009), and busyness must undergo such testing in order to assess its value as a measure of behaviour.

The current chapter describes four studies that were carried out in order to test the utility of busyness as a suitable measure in the study of predictability.

#### 4.1.1. Reliability

Subjective measures must be tested to ensure that they can be used reliably, and therefore provide consistent and repeatable results. This is particularly important for measures such as busyness, which are designed to be used with multiple observers, hence a high level of inter-observer reliability (also called inter-rater reliability) is important. The intra-observer reliability; the consistency within ratings of a single observer, can also be tested. However, Meagher (2009) has noted that this form of reliability has only been reported in a few studies of behavioural ratings.

Reliability of subjective measures is usually established prior to testing for validity, since a measure is of no use if it is not reliable (Meagher, 2009). Demonstrating reliability can be a first step in showing that, whilst subjective, a measure does not produce arbitrary results, rather there is consistency in the data. According to Wemelsfelder (1997) "If satisfactory reliability can be established, it follows that the qualitative assessment of behaviour can be submitted to systematic and repeatable procedures of measurement. This should be sufficient to endow a subject-based approach of animal behaviour, despite its non-mechanistic character, with objective, scientific status." (p. 85).

Reliability has been demonstrated in several subjective measures (albeit with differing definitions of what constitutes 'good' reliability). For example, in a study by Gosling (1998), personality ratings of 34 spotted hyenas (*Crocuta crocuta*) were made by 4 observers who knew the hyenas well. Reliability was tested using what the author described as coefficient alpha reliabilities (presumably Cronbach's alpha), and a mean alpha reliability of .71 was calculated. Reliability levels of over .70 have been recommended as satisfactory (Nunnally, 1978) (although other levels are often reported as acceptable), and Gosling concluded that the observers were able to consensually apply the personality traits to hyenas. It should be noted that this mean was calculated from a range of reliability values from .05 to .90, therefore some traits were rated with low reliability. However, Gosling stated that these scores were at least as high as those found in single-item scores in similar studies carried out on humans. As mentioned in Section 2.6, since personality measures take the form of observer ratings, they are subjective or qualitative and hence open to the criticisms often afforded to subjective measures in general. Nevertheless, if in cases such as Gosling (1998), the reliability of such measures can be established, one can have more confidence in their value. The range of reliability scores achieved by Gosling also emphasises the importance of testing and reporting reliability for all aspects of subjective measures.

In a later review of the animal personality literature, Gosling (2001) found good reliability of trait ratings across a range of personality studies, and calculated a weighted grand mean correlation of .52, across 375 separate reliability estimates. This was made up of a wide range of reliability estimates, ranging from .08 to .95. However the author considered the overall value to be substantial, and to compare favourably with equivalent correlations in the human personality literature. These examples demonstrate how reliability testing can support the use of a measure, but they also highlight inconsistencies in the acceptable reliability level.

In studies of black rhinoceros and cheetah behavioural traits (Carlstead, Mellen, et al., 1999; Wielebnowski, 1999), inter-observer reliability of the trait ratings was tested using Kendall's coefficient of concordance between the trait rating scores for individual animals that were given by the observer and/or by keepers. Most traits obtained good levels of inter-observer reliability. Those, few traits for which low levels of concordance were obtained, for example, traits with a reliability (*W*) value of less than 0.50 and associated *p* values greater than .05 (Wielebnowski, 1999) were excluded from further analysis, thus demonstrating that testing for reliability can ensure that those subjective measures which go forward for further use, are robust. Similarly, Freeman et al. (2010) compared ratings for elements of elephant social behaviour, given by keepers of 33 female elephants at 14 facilities. Behavioural elements for which the reliability of ratings had *p*-values above .05 on a given elephant (Kendall's coefficient of concordance), were removed from the rest of the analysis. Lloyd et al. (2007) also used a similar technique for developing the Horse Personality Survey (HPS). Sixty one horses were each rated by at least two raters, on a series of 30 behaviourally defined adjectives such as 'active' and 'curious'. Reliability of the scores for each horse was tested using Kendall's coefficient of concordance, and the reliability of scores for each adjective were correlated between observers using Spearman's rank correlation. Those horses and adjectives for which reliability was not statistically significant ($p > .05$) were removed from further analysis. This allowed for reliable data on 25 behavioural adjectives, from 44 remaining horses, to be used in Principal Components Analysis, to extract the components of horse personality. After removal of the unreliably scored horses, reliability between raters was high, with $W$ and $r_s$ values of .61 and .51 respectively, values which the authors claim are comparable to other studies on animal and also human personality.

Inter-observer reliability of behavioural traits has been demonstrated in studies of chimpanzees and orang-utans using intraclass correlations (ICC) (e.g., King & Figueredo, 1997; King & Landau, 2003; Weiss, et al., 2006). In each of these studies, several observers at different zoos rated a number of apes on different personality traits. Intraclass correlation coefficients were calculated using the ICC (3,1) and (3,k) models (Shrout & Fleiss, 1979), which are based on a two-way ANOVA, and treat the observers as fixed effects, and calculate reliability for a single observer and for the mean of multiple observers respectively. Values of ICC varied between the different traits that were rated, with minimum values of .20 and .40, and maximum values of .79 and .91 (ICC (3,1) and (3,k) respectively) across the three studies cited above. Values such as these are often considered acceptable, comparing favourably to similar reliability scores for human personality traits. For example, McCrae and Costa (1987), who assessed the five-factor model of personality in humans and reported ICC values between raters of .30-.65. However Portney and Watkins (2000) suggest that ICC values over .75 are required for good reliability, and Chang (n.d.) suggests that ICC values of .70-.80 indicate strong agreement with values of >0.8 indicating almost perfect agreement (with 0-0.2, 0.3-0.4 and 0.5-0.6 indicating poor, fair and moderate agreement respectively).

The Free Choice Profiling (FCP) method (as described in Section 2.6) has also been shown to be reliable. In early tests of this method (Wemelsfelder, et al., 2000) observers independently decided upon descriptive terms for the behavioural traits of pigs. Each pig was then given scores for this term of either a 1 (trait present) or a 0 (trait absent). When tested for reliability using GPA analysis, significant agreement was found between observers in their assessment of pig behaviour. When the FCP method was expanded and observers were asked to give trait ratings using a visual analogue scale instead of the 1 and 0 scoring system, observers still showed good agreement in their assessments of pig behaviour (Wemelsfelder, et al., 2001). Indeed, Wemelsfelder (2007) stated that, in 10 years of research using the FCP method, over 60 trials have demonstrated significant agreement between observers.

### 4.1.2. Validity

After demonstrating the reliability of observer ratings, the next step is to test for validity (Whitham & Wielebnowski, 2009). A review of the use of observer ratings and qualitative methods by Meagher (2009), states that validity of such methods needs to be tested “to ensure what is being measured is actually the phenomenon of interest” (p4). Whilst reliability demonstrates the agreement between observers, it does not demonstrate the ‘relevance’ of the measure, hence validity testing is required. Therefore, it was important to test busyness for validity in addition to reliability testing, in the present programme of research.

A number of different types of validity are recognised, as discussed by Cronbach and Meehl (1955) and more recently, in the context of animal behaviour data collection methods, by Meagher (2009) and Whitham and Wielebnowski (2009). Firstly, content validity considers the extent to which the items in the method being validated actually represent the construct for which the method is intended. This is usually established by a number of experts who assess the appropriateness of the items. Secondly, criterion validity involves comparing the results achieved by the method to a “gold standard” of measurement. For example results could be tested against known clinical or laboratory data such as blood counts. However studies involving animal welfare rarely use this type of validity due to the lack of a “gold standard” for comparison (Whitham & Wielebnowski, 2009). A third type of validity is more commonly used when testing methods of animal behaviour, that of construct validity. Construct validity is achieved by testing the method to be validated against the closest other measure, for example behavioural observations or physiological measures.

Existing subjective measures have undergone tests for validity, including tests for construct validity. For example, Jones and Gosling (2005) reviewed studies of behavioural traits and temperament in domestic dogs and found evidence of validity by comparison with behavioural observations. Of the studies reviewed, in which subjectively rated behavioural traits were validated against specific behavioural measures such as play, locomotion or flight behaviour, results generally supported the validity of the qualitative measures of temperament. Validity coefficients in the studies reviewed (with the strongest interpretable mean validity coefficient reported as .48) compared well against benchmark values found in similar studies on humans, where such correlations are typically in the range of .20 - .30 (Jones & Gosling, 2005).

The FCP method has also undergone tests for validity. For example, Rousing and Wemelsfelder (2006) carried out a study in which 12 observers used the FCP technique to rate the behaviour of dairy cows in 25 video clips of cow behaviour. Quantitative data were also collected from the video by measuring the frequency and duration of certain behaviours from an ethogram. Qualitative data were analysed using the GPA (as explained in detail in Wemelsfelder et al. [2000] and Wemelsfelder et al. [2001]) to produce ‘consensus profiles’ which were then correlated (Spearman rank) with the behavioural frequency and duration data observed. Results showed significant correlations between qualitative scores and quantitative measurements for certain behaviours such as social licking and head butting. In a similar test on the FCP method, this time on pig behaviour, the results of the GPA analysis showed significant Pearson correlation with quantitative data on behaviour (Wemelsfelder et al., 2003). Wemelsfelder (2007) stated that in 10 years of research on the FCP technique, tests of validity have shown meaningful correlations between qualitative and quantitative measures of behaviour, demonstrating that through suitable validity tests, subjective methods can provide useful data on the topic of investigation.

Correlations of qualitative data with more traditional measures of behaviour have been used to validate personality measures. For example, Pederson et al. (2005) rated personality factors of chimpanzees, using terms such as extraversion and agreeableness. Frequencies of 25 behaviours were also recorded and results showed significant correlations of the personality factors with certain behaviours, which was interpreted as showing good construct validity for the personality factors. In their study to develop the Horse Personality Survey (HPS) (as described in Section 4.1.1), Lloyd et al. (2007) also validated the measure against behavioural observations. Horses were observed by continuous observation for eight, 15 minute observation periods over a period of several days. Spearman's rank correlations were then carried out on the durations of observed behaviours, with the components of horse personality. It was found that 20 of the observed behaviours were significantly correlated with the components of personality, for example the component 'dominance' was significantly, positively correlated with behaviours such as 'herding', and 'head threat given'.

Other methods used for validating qualitative measures have included comparing ratings of behavioural traits to objective measures of a specific behavioural response. For example, in their study of black rhinoceros, Carlstead, Mellen et al. (1999) compared behavioural trait ratings with results from a novel object/scent test. Significant correlations were found between particular behaviours and behavioural trait ratings. For example, the trait 'fear' was correlated with a long latency to approach the novel scent. To validate measures of behavioural traits in cheetahs, Wielebnowski (1999) recorded the behaviour and approach time of cheetahs to their mirror image and these were correlated (Spearman rank) with specific, relevant behaviours. Significant correlations were found. For example, the trait 'curious' was correlated with approach time, where more 'curious' individuals took less time to approach the mirror image, and the trait 'aggressive' showed a positive correlation with agonistic vocalisations. In both of these studies, validation was achieved with evidence of correlation between at least some aspects of traditional measures of behavioural observation. It is important that traits are correlated with appropriate behaviours, to demonstrate an appropriate link between the trait and the behaviours that it measures.

### 4.1.3. Application

As an extension to testing validity, it is important that a measure can be practically applied to a study. Whilst tests of the construct validity of subjective measures, such as those previously described, typically focus on the detail of correlations with particular behaviours, it seems sensible to suggest that the applicability or utility of a measure, and the degree to which it reflects behavioural changes with changing experimental conditions, is also an important aspect of validity. Whilst this is also an element of construct validity, it takes testing of the measure a step further towards suitability for general use, than the literature described so far.

Establishing validity and applicability should mean that results will be useful and meaningful when the measure is applied to a scientific question. For example, Minero et al. (2009) were able to validate a method of behavioural assessment of horse behaviour, and apply it to the study of the effect of handling, on foals' responses to an unfamiliar human. Instantaneous scan sampling of behaviours (quantitative) and FCP descriptions of behaviour (qualitative) were derived from video clips of foals before and after a period of daily handling. Results indicated that elements of each method were highly correlated with each other, and each method captured a difference in the behaviour of the horses before and after handling. Interestingly, this difference was found using both methods but was only statistically significant for the qualitative (FCP) results. The authors suggested that the study showed a meaningful relationship between the qualitative and quantitative measures and both are useful measures of foal behaviour. Further, they suggested that the qualitative measure can add to the data obtained by quantitative methods, and can provide an interpretive element to quantitative data.

The success of Carlstead, Mellen et al.'s (1999) validation of measures of behavioural traits in black rhinoceros allowed them to use these measures in a study on the effects of the zoo environment on black rhinoceros reproductive success (Carlstead, Fraser, et al., 1999). Findings showed that two particular behaviour traits ('dominant' and 'chasing/stereotypy/mouthing') were affected by the enclosure type and were negatively correlated with breeding success in males and females. Also, it was found that behaviour traits affected the success of rhinoceros pairings for breeding, whereby assertive females and submissive males made compatible pairs. Thus the qualitative measures revealed important results for the captive management of black rhinoceros.

As a result of testing the reliability and validity of the HPS (Lloyd, et al., 2007), it was possible to use this survey in a further study to determine differences in horse personality between breeds (Lloyd et al., 2008). The HPS was used to rate personality traits of 1223 horses from eight different horse breeds, and it was determined that there were significant differences between the personality of horses from the different breeds, with the ‘Anxiousness’ and ‘Excitability’ trait. Thus validation in the above examples, determined the subjective measures to be useful tools in studies of animal behaviour.

### 4.1.4. Instructions and training

Few studies using subjective measures of animal behaviour offer details on the instructions that are given to observers about using the rating scales; such as details on the anchor points (the points at the extremes of the scale), for example Carlstead, Mellen et al. (1999) and Wielebnowski (1999) list only the categories rated, and how the ratings were made e.g., on a 1-5 scale, or by marking on a visual analogue scale. Also, few studies have investigated the effect that instructions can have on the results. Perhaps the most detailed instructions are published for FCP studies (e.g., Wemelsfelder, et al., 2000; Wemelsfelder, et al., 2001), in the sense that the observers generate their own descriptive terms to rate. This is, in effect, the lowest level of instruction that the observers could receive, as by defining their own traits, they have set their own anchor points for the rating scale. The FCP method has been tested, and found to be reliable and valid, even with this low level of instruction (e.g., Wemelsfelder, et al., 2000; Wemelsfelder, et al., 2001).

In a meta-analysis of scales used in marketing research, Gilbert et al. (1984) stated that a problem of the marketing and social science literature is “the relative absence of information about the effects of research design choices on reliability and validity estimates” (p 360). The authors posed a number of questions relating to the sampling, and measure characteristics of rating scales, including the effect of labelling the points on a scale, on the reliability of a scale. Gilbert et al. (1984) compared 144 coefficients of reliability of rating scales used in marketing and social science journals and hypothesised that those scales for which all of the points of the scale were labelled, would have higher reliability scores than for those scales for which only the polar points of the scale were labelled. However, their results showed similar reliability scores for rating scales with these different levels of description, and the hypothesis was not supported. Whilst Gilbert et al.’s (1984) results suggest that there may be no difference in the reliability of scales using different levels of detail in the descriptions of the scale points, their study compared reliability scores obtained for different rating scales rather than comparing the same scale with different labelling. There has been little work done, investigating the reliability of the same scale, when used with differing levels of scale description and instruction. Gilbert et al. (1984) were unable to investigate the effect of different wordings provided for ratings scales as “most reports failed to include the actual scale employed” (p 370). Whilst Gilbert et al’s (1984) study is relatively old, there is little other literature that addresses this aspect of rating scale design, particularly in the animal behaviour literature. An aim of the present chapter is to explore the effect of the level of instruction detail on scale reliability, in a study of zoo animal behaviour.

### 4.1.5. Introduction to the busyness studies

The studies cited in this chapter demonstrate that, whilst subjective measures may, at first glance be open to criticisms of inaccuracy, bias, or anthropomorphism (as mentioned in Section 2.6), such measures can be shown to be reliable and valid. Busyness is a subjective measure in the sense that it requires observers to assess their perception of how ‘busy’ they think animals are in a given space (e.g., an enclosure), and report this as a rating. This could be likened to a rating of a single personality or behavioural trait in the studies previously described. The aim of this chapter was to assess the value of busyness as a measure, by testing aspects of its inter-observer reliability and validity.

The following sections describe four studies that were carried out in order to develop the measure of busyness and to test its utility as a simple method to record change in behaviour over time using multiple observers. Each study was carried out using short video clips of tiger behaviour which were shown to undergraduate psychology students, who rated the busyness of animals in each video clip. Using this approach, a number of aspects of busyness could be tested. In Study 1, the inter-observer reliability of busyness was tested using intra-class correlations (ICC). This method was chosen as a suitable method for calculating the reliability of multiple observers. It considers the relationships between variables within a class of measurement, unlike interclass measures such as the Pearson *r* (McGraw & Wong, 1996). The ICC also considers the variance between and within the raters, the variance between the objects being rated and the error variability (Field, 2005) and hence was considered to be an all-encompassing, and relevant test of inter-observer reliability.

In Study 2, busyness was compared against traditional measures of animal behaviour to ensure that busyness was sensitive to relevant aspects of behaviour. In other words, the construct validity of busyness was tested. This was required as there is no ‘gold standard’ to compare this novel measure against, so traditional measures of data collection were the closest comparison. As the application of observers’ own subjective ratings of traits has previously been demonstrated to reflect observable aspects of animal behaviour (see Section 4.1.2 and Chapter 3), it was predicted that the degree of busyness would correlate with the occurrence of certain behaviours, and depending on the level of busyness, different behaviours would be observed. In particular, active behaviours were predicted to be positively correlated with busyness score.

Study 3 was formulated as a consequence of Study 2, in order to study the effect of instruction (anchor point) detail on the mean busyness scores given by the participants, and on the inter-observer reliability of the measure. It was deemed necessary to investigate the required level of instruction detail, as it was noted in Study 2 that the student participants generally gave lower busyness ratings than the experimenter, and that participants gave few ratings at the maximum busyness score, which raised questions regarding the required detail on how the anchor points of the scale should be described. There is also a lack of previous research investigating the effect of different levels of instruction for a single rating scale (as described in Section 4.1.4). In Study 3, students all watched the same video clips but were given one of three different levels of instruction detail: low, medium and high. It was predicted that greater detail and descriptions for the two anchor points of the busyness scale (1 and 5) would result in higher mean busyness ratings for those participants in the high detail instruction group, as they had a clearer conception of tiger behaviour at the top of the busyness scale. It was also predicted that the high detail instructions would result in higher values for inter-observer reliability as busyness was more clearly defined and hence less open to individual interpretation.

Busyness was designed as a measure to record change in animal behaviour over time. This was studied in Chapter 3, where busyness was successfully found to show a pattern over the course of the day, leading up to the predictable event, which was also reflected in patterns of more traditional measures of tiger behaviour. However, it could be argued that as the observers were aware of the predictable event, they were expecting this pattern of busyness. This expectation might then be reflected in their subjective busyness scores. Therefore, in order to address this issue, and ensure that busyness is applicable to studies of behaviour change over time, Study 4 investigated busyness ratings for clips of tiger behaviour throughout the day, under conditions where participants were blind to any developing temporal patterns of behaviour. Video footage was filmed, throughout the day, of tigers in the same enclosure, and under the same husbandry routine as in Chapter 3, and this footage was shown to the participants in a random, non-chronological order. It was predicted that when the means of the participants’ scores were returned to chronological order, they would reflect a pattern in the busyness of the tigers, over the course of the day and in the lead up to the feeding time. It was also predicted that behavioural data collected from this video would reflect a related temporal pattern in those behaviours that had previously been found to correlate with busyness in Study 2 (Section 4.3).

## 4.2. Study 1: Testing the inter-observer reliability of busyness

### 4.2.1. Methods

*Participants*

The participants were 48 undergraduate psychology students from the University of Plymouth who received course credit for participation in the study. Participants attended the study in groups of up to 11 participants at a time.

*Materials*

The video used in this study was a collection of 20, minute long clips of tigers, filmed at Paignton Zoo Environmental Park, UK. The tigers were two male and two female Sumatran tigers (*Panthera tigris sumatrae*): a mother and three, 1 year old cubs. These tigers were filmed in their outdoor enclosure at the zoo; as a zoo visitor would view the enclosure. Video footage was filmed over several days, at different times of the day. The experimenter rated the busyness of each minute of video; the experimenter had previous experience of observing tiger behaviour, using both traditional and busyness measures (see Chapter 3). Twenty, one-minute clips were selected, incorporating video that was considered to demonstrate a range of behaviours and busyness levels. It was not always possible to keep all four tigers in camera shot, however, all video clips showed a minimum of 2 tigers.

Video clips were played with the corresponding background sound of the enclosure, unless this sound included the voices of visitors to the zoo enclosure. If voices were heard, the sound file for that minute clip was muted and overlaid with sound from another clip which consisted of purely background noise, with no distinguishing sound features. This was to avoid the use of human voices and to avoid any distracting comments, or any comments which may have influenced the participants' opinions of the tigers' behaviour.

Three different 20 minute video files were produced (videos 1, 2 and 3), each with a combination of the 1 minute video clips in a different random order, as determined using a random number generator in Microsoft Excel. Use of the three videos was to control for any potential order effect. A checksheet was used to record participants' responses.

### *Procedure*

Participants were told that they would be watching a video of tigers in a zoo, and that they would be trialling a new rating scale for use in research in animal behaviour. All participants had the right to withdraw from the study and were reassured of confidentiality, but were also told that the study was designed to test the method itself and not their individual performance. Participants were then given an instruction sheet which explained: the format of the study and the video, what they would have to do, and how to determine the busyness of the animals/enclosure. As a subjective measure, busyness was not defined in detail. However, the concept of busyness was explained as "your subjective opinion of how much you think is going on in the enclosure. For example, you might like to think about what the tigers are doing and how many of them are doing it", and behavioural examples for the anchor points of busyness levels 1 and 5 were given: "where 1 is the lowest level of busyness that the enclosure could be (for example with all tigers asleep), and where 5 is the highest level of busyness that the enclosure could be (for example with all tigers very animated and active)". A full copy of the instruction sheet is given in Appendix 4.1. Participants were debriefed after each session and reminded of the purpose of the study and how their data would be used.

After briefing, the participants watched one of the 3 video files ($N$ = 15 for video 1, $N$ = 17 for video 2, $N$ = 16 for video 3) and recorded busyness scores for each of the minute clips.

### *Data Analysis*

Participants' scores for each video clip were compared using descriptive analysis, and inter-observer reliability was calculated using intraclass correlations (ICC)

(Field, 2005; Shrout & Fleiss, 1979). The ICC model used for this study was ICC (2,1), the two-way random effects model (Shrout & Fleiss, 1979). This particular model was chosen as it is based on a two-way analysis of variance, and considers the effect of both the observers (raters) and the video clips (objects). In this model the raters are considered to be random effects (i.e. sampled from a larger population of potential raters) and the reliability is calculated considering single measures i.e. the expected reliability of a single rater's scores, rather than the mean of several observers. Whilst reliability scores were likely to be lower when calculated for single measures than for means (i.e. the ICC [2,k] model), it was considered important to demonstrate a substantial level of reliability for single measures. This was because busyness is not designed to be used by a group of observers at the same time, but rather by individual observers at different times. A result of substantial ICC (2,1) reliability would therefore be strong evidence for the suitability of busyness for further use. The ICC calculation was run twice; firstly testing for consistency, and secondly testing for agreement (McGraw & Wong, 1996). In this way it was possible to test whether the participants agreed about the relative increases and decreases in busyness between clips (consistency), and whether they agreed on the absolute values of busyness (agreement) (Field, 2005).

Data from two of the participants (both from video 3) had to be removed from the ICC calculations as these participants did not give a rating for all of the video clips, and the ICC calculation requires complete data sets. Therefore the total *N* for the ICC calculations was 46. These participants were not removed from the descriptive analysis.

A further test of reliability was carried out using Spearman's rank correlations of the participants' scores ($N = 46$, as above) with the busyness scores given by the experimenter, in order to test for correlation and agreement between the experimenter and the participants.

Modal values have been presented in the following section (Section 4.4.2) when comparing participants' scores for the different video orders. Modal ratings were deemed appropriate in this case, as the participants gave a single rating score from a discrete category scale of 1-5 and agreement could be demonstrated if the modal score was the same for each video order.

### 4.2.2. Results

*Effect of video order*

The data were initially analysed according to the video watched (1, 2 or 3), and the mode of the participants’ busyness ratings for each video clip, for each video order are shown in Figure 4.1. For most of the video clips there was exact agreement on the modal value of busyness, regardless of the order in which those clips were presented. Disagreement on the modal busyness rating occurred for only 6 of the video clips (clips 1, 2, 6, 9, 12 and 17) and in these cases, modal values never varied by more than +/- one busyness rating. Intraclass correlation (ICC) scores for all participants within each video order showed either strong or near perfect agreement (Table 4.1). When these data were combined, the calculated ICC score for the whole data set, for all video orders, still showed strong agreement. Whilst there is some variation in the busyness ratings given for each video clip in the different video orders (as demonstrated in Appendix 4.4 which gives the results of the participants’ busyness ratings for each minute clip, for each video order), the agreement of modal busyness ratings, and the strong ICC values suggests that there was no major effect of the order of the video clips on the participants’ busyness ratings. Therefore all of the data from each video order were pooled for further analysis.

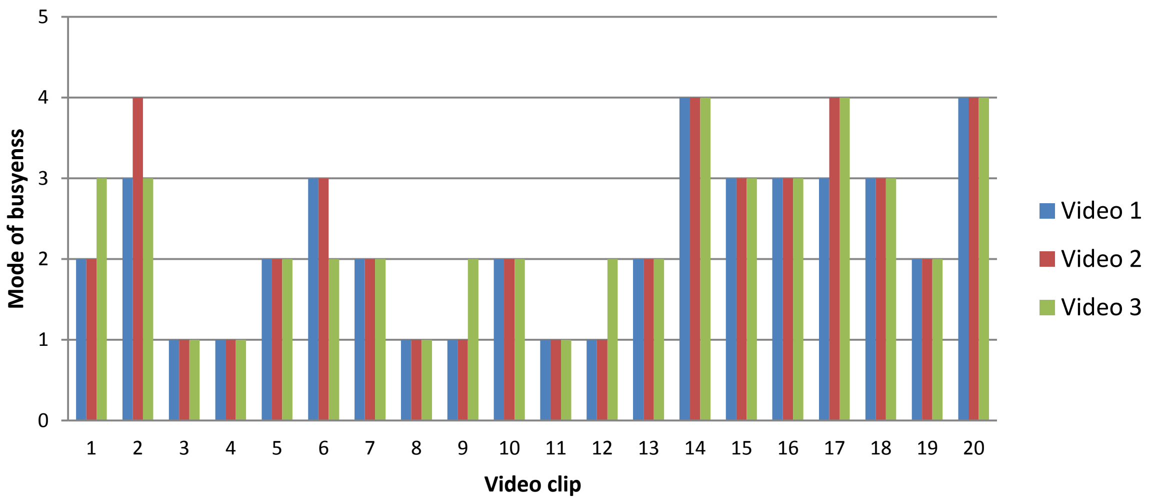


*Figure 4.1: Mode of participants' busyness ratings for each video clip, for each video order.*

*Table 4.1: Intraclass correlation results for participants' busyness scores, for each video and for all video orders combined.*

| Video Order | N | ICC model | ICC value | Confidence intervals | | F-test (with true value 0) | *p*-value | Degrees of freedom |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | | |
| 1 | 15 | (2,1) consistency | .81 | .71 | .91 | 67.51 | <.001 | 19 |
| | | (2,1) absolute | .77 | .65 | .88 | 67.51 | <.001 | 19 |
| 2 | 17 | (2,1) consistency | .80 | .68 | .89 | 66.84 | <.001 | 19 |
| | | (2,1) absolute | .76 | .63 | .87 | 66.84 | <.001 | 19 |
| 3 | 14 | (2,1) consistency | .80 | .69 | .90 | 57.65 | <.001 | 19 |
| | | (2,1) absolute | .72 | .57 | .85 | 57.65 | <.001 | 19 |
| All (combined data) | 46 | (2,1) consistency | .80 | .69 | .89 | 179.33 | <.001 | 19 |
| | | (2,1) absolute | .74 | .61 | .86 | 179.33 | <.001 | 19 |

### *Comparisons of participants' busyness ratings*

Figure 4.2 and Table 4.2 show the percentage of participant responses of each busyness rating, for each video clip (data from all participants). There was very high agreement between participants for some of the clips. For example, a high percentage of participants rated clips 1 to 4 with a busyness score of 1, with a maximum of 97.92% of the participants rating clip 1 with a busyness score of 1.

For other clips (n=5), a high percentage of participants rated busyness at a particular modal score, with other participants only differing by only +/- one busyness score. This includes clips 7 to 9 where the modal busyness rating was 2, with some participants (maximum of 25%) giving a rating score of 1, and some participants (maximum of 16.67%) giving a rating score of 3; and clips 19 and 20 where the modal busyness rating was 4, with some participants (maximum of 27.08%) giving a rating score of 3 and some participants (maximum of 27.08%) giving a rating score of 5.

For some of the clips (n=6), the participants' ratings were mostly split between two busyness scores. For example: participants' ratings for clips 5 and 6 were mostly split between busyness scores 1 and 2, ratings for clips 11 to 13 were between busyness scores 2 and 3, and ratings for clip 18 were between busyness scores 3 and 4.

For some clips (n=5), for example clips 10 and 14 to 17, there was slightly more variation in the participants' responses, with some participants giving a rating of greater than +/- one from the modal busyness score. Participants' scores also deviated from the mode by more than +/- one busyness rating for clips 4, 11, 12, 13 and 18 (Table 4.2) although this selection of clips demonstrate greater agreement between participants and have already been included in the categories of agreement described above. Where participants' ratings varied by more than +/- 1 busyness rating away from the modal rating for that clip, this was generally for only a low percentage of participants (maximum 14.58%), as shown in the final column in Table 4.2.

### *Intraclass correlation results*

Table 4.1 gives the ICC values for all of the participants ($N$ = 46). Values of .80 and .74 were achieved for ICC when tested for consistency and absolute agreement, respectively. Confidence intervals indicate lower reliability limits of no less than .69 and .61 (consistency and absolute, respectively), and $F$ and $p$ values indicate that ICC values were significantly greater than zero.

### *Correlations*

Spearman's rank correlations between the busyness ratings given by the experimenter and by each of the participants were all highly and significantly correlated (Appendix 4.5). However, the mean busyness scores given by the participants over all of the clips were generally lower than that of the experimenter.

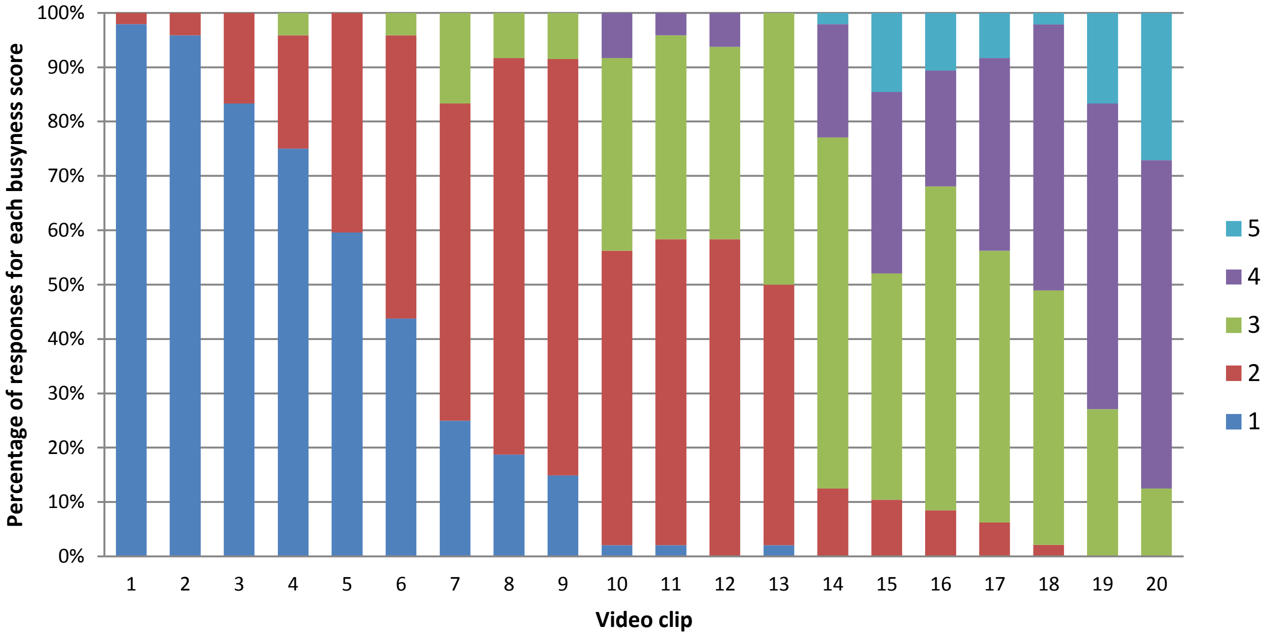


*Figure 4.2: Percentage of participants rating each video clip at each level of busyness. Colours represent the different busyness levels. Busyness scores run from 1 (low) to 5 (high).*

*Table 4.2: Percentage of participants' ratings for each video clip showing the variation around the modal busyness rating (shaded cells indicate the modal busyness score for each clip)*

| Video Clip | Number of ratings | Percentage of ratings at each busyness level | | | | | Percentage of ratings that differ from the modal rating by more than +/- 1 busyness level |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | |
| 1 | 48 | 97.92 | 2.08 | 0 | 0 | 0 | 0 |
| 2 | 48 | 95.83 | 4.17 | 0 | 0 | 0 | 0 |
| 3 | 48 | 83.33 | 16.67 | 0 | 0 | 0 | 0 |
| 4 | 48 | 75.00 | 20.83 | 4.17 | 0 | 0 | 4.17 |
| 5 | 47 | 59.57 | 40.43 | 0 | 0 | 0 | 0 |
| 6 | 48 | 43.75 | 52.08 | 4.17 | 0 | 0 | 0 |
| 7 | 48 | 25.00 | 58.33 | 16.67 | 0 | 0 | 0 |
| 8 | 48 | 18.75 | 72.92 | 8.33 | 0 | 0 | 0 |
| 9 | 47 | 14.89 | 76.60 | 8.51 | 0 | 0 | 0 |
| 10 | 48 | 2.08 | 54.17 | 35.42 | 8.33 | 0 | 8.33 |
| 11 | 48 | 2.08 | 56.25 | 37.50 | 4.17 | 0 | 4.17 |
| 12 | 48 | 0 | 58.33 | 35.42 | 6.25 | 0 | 6.25 |
| 13 | 48 | 2.08 | 47.92 | 50.00 | 0.00 | 0 | 2.08 |
| 14 | 48 | 0 | 12.50 | 64.58 | 20.83 | 2.08 | 2.08 |
| 15 | 48 | 0 | 10.42 | 41.67 | 33.33 | 14.58 | 14.58 |
| 16 | 47 | 0 | 8.51 | 59.57 | 21.28 | 10.64 | 10.64 |
| 17 | 48 | 0 | 6.25 | 50.00 | 35.42 | 8.33 | 8.33 |
| 18 | 47 | 0 | 2.13 | 46.81 | 48.94 | 2.13 | 2.13 |
| 19 | 48 | 0 | 0 | 27.08 | 56.25 | 16.67 | 0 |
| 20 | 48 | 0 | 0 | 12.50 | 60.42 | 27.08 | 0 |

### 4.2.3. Discussion

There was no major effect of the order in which the video clips were shown to the participants. Three different orders of video clip presentation were used to control for any order effect (i.e. participants ratings for a particular clip being influenced by the preceding clips). Modal busyness scores showed good agreement for each clip across the video orders, and inter-observer reliability (ICC) scores showed strong agreement for each video order and for all participants combined. Results for all videos were therefore pooled. When given the opportunity to comment on the method, some participants noted that the rating scale became easier to use as they progressed through the study. It was therefore decided that, in future studies, a number of example clips would be given during the study briefing, to allow the participants to become accustomed to some examples of the behaviour that they might expect to see.

Overall, the participants showed good inter-observer reliability in their busyness ratings, as demonstrated by the ICC results and by visual inspection of the agreement between participant's scores. For most of the clips either a high percentage (up to 97.92%) of the participants agreed on the busyness rating for that clip, or participants agreed on a modal rating, with some participants then giving a rating of +/- one busyness level from that modal score. For some clips the participant's scores were mostly spilt between 2 busyness ratings. Split scores like this were perhaps to be expected for some clips, as the busyness method required participants to select ratings from a discrete category scale in order to record what was continuous behaviour. Behaviour will not always fit exactly into discrete busyness categories, and there will be transitions between categories. However, it is important to note that on these occasions, the participants' scores only differed between two adjacent busyness levels so there was no major disagreement between participants. Only a small proportion of the participants ever recorded a busyness score that was more than +/- one busyness level away from the mode.

Results of the intraclass correlation tests showed high ICC values. There is little peer-reviewed literature regarding the interpretation of ICC scores, and a range of ICC values have previously been accepted to indicate good reliability. For example values of ICC ranging from .20 to .91 have been reported in studies of great ape personality (e.g., King & Figueredo, 1997; King & Landau, 2003; Weiss, et al., 2006). As discussed in Section 4.1.1, such scores are often deemed acceptable in comparison to similar reliability scores for human personality traits. However, requirements for good reliability have been set at over .75 (Portney & Watkins, 2000), or .70-.80 for strong agreement and >.80 for almost perfect agreement (Chang, n.d.). In accordance with these guidelines, the ICC results from the current study are at the high end of the ICC range, and indicate strong to almost perfect agreement between participants. Confidence intervals for ICC were also high, with F-tests indicating intraclass correlations significantly greater than zero, thus supporting the strong ICC agreement.

Correlations of participants' scores with those of the experimenter demonstrated strong correlations, indicating that not only was there a strong agreement between the participants, but the participants also agreed with the experimenter on the relative busyness of the video clips. Participants did however seem to anchor their ratings around a lower mean busyness score than the experimenter. This will be explored further in Studies 2 and 3 (Sections 4.3 and 4.4).

In conclusion, and considering the results for individual video clips and overall ICC values, the method of busyness showed substantial inter-observer reliability, both in terms of agreement on the relative increases and decreases in busyness across the clips, and also in terms of absolute agreement of busyness rating scores. Participants' busyness ratings were reliable with each other, and with the experimenter. It therefore seems that busyness can be used with good reliability with student observers. Busyness therefore shows potential as a measure for use with multiple observers, but further testing is now required in order to validate busyness against more traditional measures of data collection. Study 2 was designed to address this issue.

## 4.3. Study 2: Validation of busyness against traditional measures of animal behaviour

In the following study, busyness was recorded alongside more traditional measures of data collection, in order to validate busyness.

### 4.3.1. Methods

*Participants*

The participants were 39 undergraduate psychology students from the University of Plymouth, who received course credit for participation. Participants had not previously taken part in Study 1.

*Materials*

The video used in this study was 100 minutes of video of tiger behaviour, filmed in the same enclosure as Study 1. The subjects were two Sumatran tigers (*Panthera tigris sumatrae*); a mother and her 22 month old male cub. As in Study 1, video footage was filmed over several days, at different times of the day, and each minute was rated for busyness by the experimenter. One hundred minutes of video were collated, that were considered to demonstrate a range of behaviours and busyness levels.

The 100 minute-long video was made up of 5-minute sections of video. Each 5-minute section was broken down into one-minute clips, considered by the experimenter to be of a similar level of busyness, and together they represented a constant busyness level for that 5-minute section (see Appendix 4.6). Most 5-minute sections were made up of 5, continuously-filmed minutes of video, however it was necessary in some cases to edit together different minute clips of video of a similar busyness level in order to produce 5 minutes of suitable video. Video sound was edited as for Study 1.

Three different, 100 minute, video files were produced (videos 1, 2 and 3), with the 5-minute video sections in a different random order in each, as determined using a random number generator in Microsoft Excel, with the proviso that 5 minute sections of the same busyness level would not be shown consecutively. Use of the three video orders was to control for any potential order effect. Each 100 minute video was split into two, smaller videos of 45 and 55 minutes respectively, to be shown to the participants in two experimental sessions on different days (as participants were only able to take part for 1 hour at a time).

### *Procedure*

#### *Busyness data collection*

In each session participants were briefed and debriefed as in Study 1 (Section 4.2.1), and given an instruction sheet (Appendix 4.2). Participants also received these instructions in the second study session to ensure consistency in their use of the method. In the first session participants were initially shown 3 minutes of the video which they could use to practise the busyness method and to get an initial idea of the tiger behaviour they would see. After briefing and practice, participants then watched one of the 3 video files ($N$ = 14 for video 1, $N$ = 14 for video 2, $N$ = 11 for video 3) and recorded busyness scores for each minute of the video. Participants were given a 3 minute break after 30 minutes.

#### *Traditional behavioural data collection*

Data were collected by the experimenter, who was an experienced observer of tiger behaviour, using instantaneous scan sampling and one zero sampling every minute; taken at the start of each one-minute clip. Behavioural categories were generated based on ethograms developed over previous studies by the experimenter on large felids (e.g., Chapter 3 and also Bishop et al. [2007]), and also based on Weller and Bennett (2001), and included locomotion (walking, trotting, running), inactive alert, sleeping pacing and repetitive walking (see Appendix 4.7.). If the tiger was not in camera shot at the start of the minute, ‘not visible' was recorded. State and event behaviours (see Appendix 4.7.) were also recorded using one-zero sampling, if they occurred during each minute. These two measures of time sampling were used as comparison measures. Instantaneous scan sampling was used as it is one of the most traditional and often preferred methods of time sampling (Martin & Bateson, 2007), and was therefore considered to be suitable as a standard measure for validation of busyness. One-zero sampling was also used as it allowed more than one behaviour to be recorded per minute of observation, which was important in order to study which behaviours constituted the different busyness levels (see Section 2.4. for further discussion of time sampling methods).

### *Data analysis*

In the following data analysis, mean and modal busyness scores were calculated from the participants' data. Mean scores enabled a detailed comparison of busyness scores for each video clip for correlation analysis and modal scores gave a measure of the most frequent participant responses, whilst still keeping the results within the categories of busyness.

#### *Busyness: reliability and order effects*

To test for reliability and order effects, participants' results were initially analysed according to the video order in which they appeared and were watched by the participants. Mean and modal busyness scores were calculated from the participants' results for each one-minute video clip, for videos 1, 2 and 3, and these results were compared descriptively. Intraclass correlations were carried out using the ICC (2,1) model as in Study 1. After checking for any order effect, the results given by all participants were combined and overall mean and modal busyness values were calculated for each one-minute video clip.

### *Behaviours associated with busyness*

All of the behaviours that were observed in each of the one-minute clips, at each modal busyness level, were listed (for each tiger), and the percentages of clips at each modal busyness level, in which these behaviours occurred, were calculated. The number of observations (one-zero) of each behaviour associated with each modal busyness level was graphically displayed, in order to show those behaviours which composed each modal busyness level, and their relative representation at that busyness level. One-zero results were used for the above analyses in order to include all of the behaviours shown in each video clip.

### *Busyness comparisons with behaviour results*

The mean and modal busyness scores were calculated from the results given by all participants for each group of 5 minute video sections. The proportion of instantaneous scans of each behaviour were calculated for each tiger, for each 5-minute section, and these values were correlated (Spearman's rank) against mean and modal busyness scores. In addition, proportions of each 5 minute section in which one zero behaviours were observed for each tiger were also correlated against mean and modal busyness scores. For the instantaneous scan data only, prior to calculating proportions, scans in which the focal tiger was not visible at the start of the minute were removed from the analysis (for that tiger). Therefore, in these cases, the proportions of behaviours were calculated over the reduced number of minutes. These proportions were also correlated with corrected mean and modal busyness scores, which were calculated using busyness values for only those clips in which the tiger was visible on the scan. Bonferroni corrections were not used in the current analysis as the current analysis did not fit the criteria often used to justify Bonferroni corrections (e.g. Cabin & Mitchell, 2000; Rice, 1989). The current analysis did not require a single significant result to test a null hypothesis, and each correlation was an independent test to determine how each behaviour related to busyness (whether significant or not). Hence, it was decided that Bonferroni corrections were unnecessary and would, in fact, lead to a risk of Type II errors if used in the current analysis.

### 4.3.2. Results

*Busyness: reliability and order effects*

Figure 4.3 shows the mean and modal busyness ratings recorded by the participants for each video clip, for each of the 3 video orders. Agreement between busyness scores for each video clip, for each video order was good, with only clip 25 showing a difference in mean busyness score of greater than 1 busyness level between videos 2 and 3. Clips 10, 23, 25 and 53 showed a difference of >1 modal busyness level between videos 2 and 3, and clips 62 and 64 showed a difference of >1 modal busyness level between videos 1 and 2. Overall, modal busyness scores from each video order were exactly the same for 46 (out of 100) of the minute video clips, with a further 48 differing by only 1 busyness level between video orders. Generally video 2 gave lower busyness scores, with video 3 producing higher busyness scores. Intraclass correlation scores (Table 4.3) were high for each video order (minimum ICC score of .75), and for all of the participants' data combined. Due to the low variation between busyness scores for each minute clip in each video order, and the good inter-observer reliability, all data were pooled for further analysis.

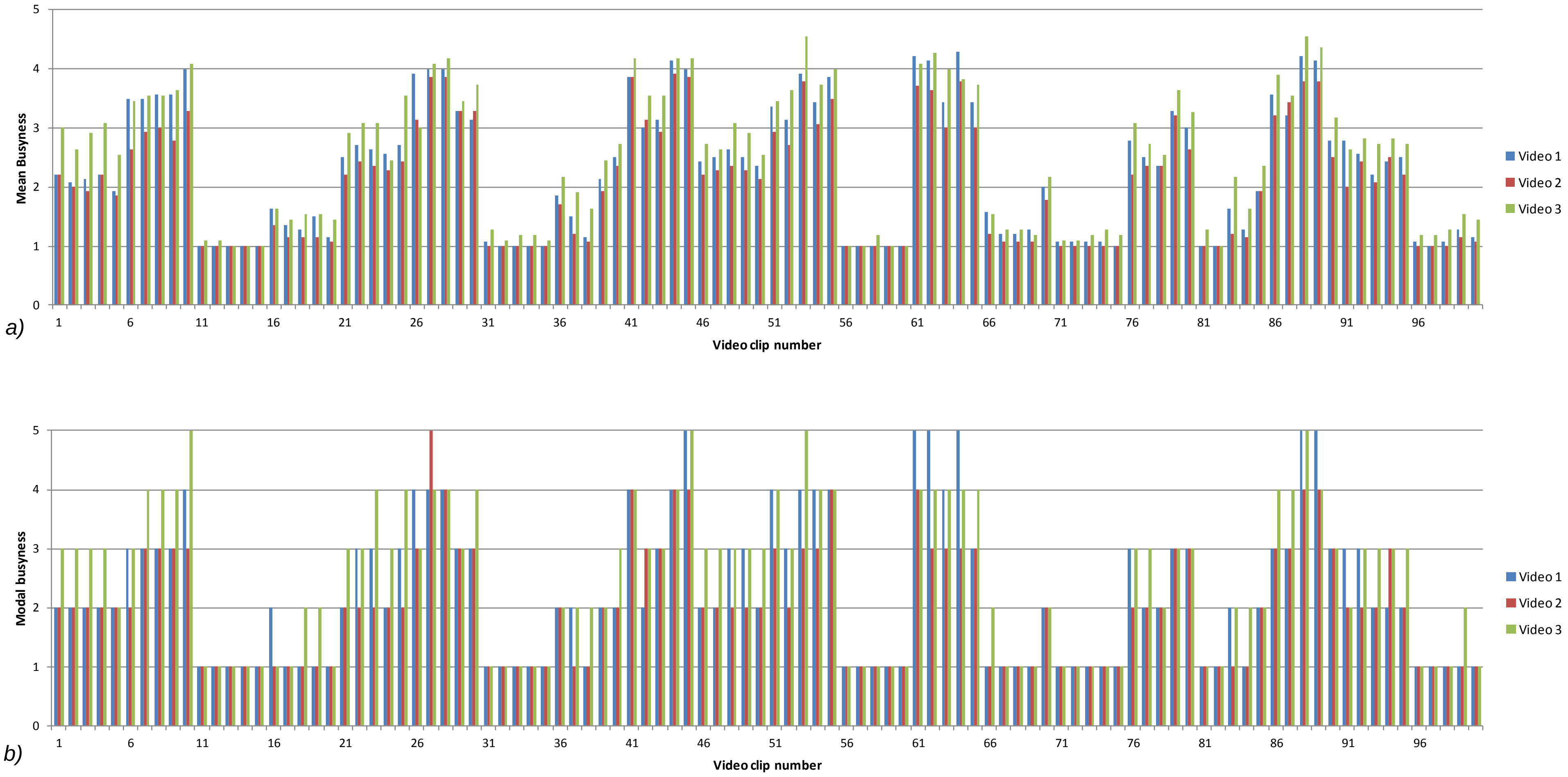


*Figure 4.3: Mean (a) and Mode (b) of participants' scores of busyness for each video clip, grouped by the video order that the participants were shown. Results for each video order have been rearranged to one, standard order.*

*Table 4.3: Intraclass correlation results for participants' busyness scores, for each video and for all video orders combined.*

| Video Order | N | ICC model | ICC value | Confidence intervals Lower | Confidence intervals Upper | F-test (with true value 0) | *p*-value | Degrees of freedom |
|---|---|---|---|---|---|---|---|---|
| 1 | 14 | (2,1) consistency | .81 | .77 | .86 | 61.78 | <.01 | 99 |
|  |  | (2,1) absolute | .75 | .68 | .82 | 61.78 | <.01 | 99 |
| 2 | 14 | (2,1) consistency | .83 | .79 | .87 | 71.58 | <.01 | 99 |
|  |  | (2,1) absolute | .79 | .72 | .84 | 71.58 | <.01 | 99 |
| 3 | 11 | (2,1) consistency | .82 | .78 | .86 | 51.99 | <.01 | 99 |
|  |  | (2,1) absolute | .78 | .71 | .84 | 51.99 | <.01 | 99 |
| All (combined data) |  | (2,1) consistency | .82 | .77 | .86 | 174.45 | <.01 | 99 |
|  |  | (2,1) absolute | .75 | .69 | .81 | 174.45 | <.01 | 99 |

Fewer clips were rated with a modal busyness score of 5 (*n* = 3) compared to scores of 1, 2, 3 and 4 (*n* = 38, 24, 24 and 11 respectively). This was despite the design of the experiment in which all busyness levels were considered to be represented evenly. Overall, the participants rated the busyness of the clips lower on the busyness scale than the experimenter, with an overall mean for all participants and all clips of 2.28, compared to the experimenter mean over all clips of 2.97.

### *Behaviours associated with busyness*

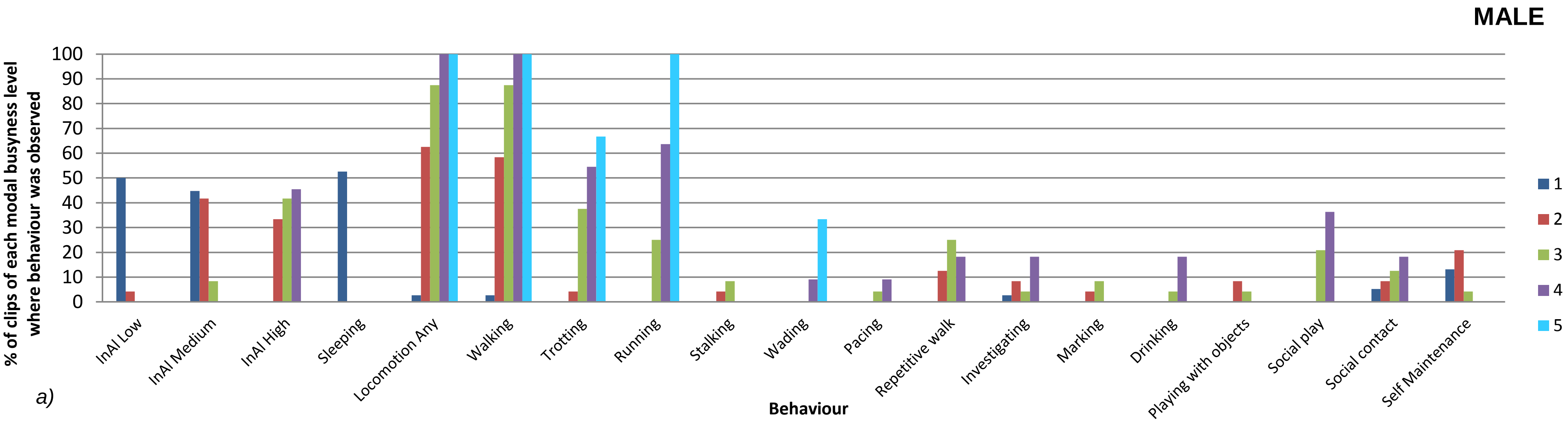
Table 4.4 shows a list of behaviours that were observed in video clips of each modal busyness rating and demonstrates that there were differences in the behaviours observed at different busyness ratings. Behaviours such as inactive alert and sleeping were observed at the lower busyness levels, with sleeping only ever observed in clips with a busyness score of 1. A greater number of behaviours were observed in clips of busyness scores 2, 3 and 4, with behaviours such as pacing and repetitive walking, drinking, play behaviours and marking only observed in these, mid-range busyness scores. Walking was the only locomotion behaviour observed in clips of busyness level 1, with walking and trotting shown in clips of busyness level 2, and walking, trotting and running shown for busyness levels of 3, 4 and 5. Such patterns of locomotion were also shown in the combined percentages of all types of locomotion (Figure 4.4) which demonstrated that locomotion of any type was observed in a low percentage (2.6% male, 0% female) of clips considered to represent busyness scores of 1, but was observed with increasing frequency as the busyness level increased; 100% of the clips at busyness level 5 showed some form of locomotion. The same trend was shown for the faster locomotion types, with an increasing percentage of the clips of each busyness level showing the behaviour as the busyness score increased. Faster locomotion was observed more as busyness level increased (although lower percentages of clips showed walking and trotting at busyness level 5 for the female tiger), with 100% of the clips of busyness 5 showing running behaviours for both tigers, and only walking behaviour shown in clips of busyness level 1.

In contrast, a negative trend was shown for the stationary and less ‘alert’ behaviours. Inactive alert (low) and (medium) occurred in a decreasing percentage of the clips as busyness level increased. Inactive alert (low) was observed in 50% (male tiger) and 57.9% (female tiger) of the clips of busyness 1, compared to only 4.2% (male) and 0% (female) of the clips of busyness 2, with no occurrences in clips of busyness 3 to 5. Inactive alert (medium) showed a similar trend and was not observed in clips of busyness 4 to 5 for the male tiger or 3 to 5 for the female tiger. Inactive alert (high) was mostly observed for only the middle values of busyness and percentages increased for higher busyness levels, but this behaviour was not shown in clips of busyness 1 or 5 (except at a low percentage of busyness 1 for the female tiger). Other mid-range behaviours were repetitive walk which was shown at its highest percentage at busyness 3, and pacing, which increased in percentage in mid-range levels of increasing busyness but was not shown at the extremes of the busyness scale. Social play was also shown at increasing percentages with increasing busyness level for mid-range busyness scores of 3 and 4; and social contact was shown at increasing (albeit low) percentages in clips of increasing busyness. Other, animated behaviours such as wading occurred in clips of higher busyness levels (4 or 5) only. Certain behaviours such as investigating, drinking and marking occurred at percentages that were too low to be sure of their trends related to busyness, however they appeared to occur in clips of mid-range busyness. It is important to note that the differences in bar height between the behaviour categories in Figure 4.4 is due to some behaviours being observed in the video clips at different frequencies to others, therefore whilst busyness levels can be compared for a particular behaviour, comparisons of different behaviours in these figures should be interpreted with caution.

Figure 4.5 shows similar patterns of results as those described above, and shows the occurrence of behaviours within each busyness level. For both tigers, observed behaviours from video clips of busyness level 1 showed the greatest proportion of inactive behaviours, and as busyness increased, inactive behaviours took up a smaller proportion, and locomotory behaviours took up a greater proportion until, at busyness 5, only walking, trotting, and running (and wading for the male tiger) behaviours were shown. Locomotory behaviours included more animated behaviours (such as running and trotting) at the higher end of the busyness scale. Busyness levels 2 to 4 show a greater behavioural diversity, with the male tiger showing a greater range of behaviours than the female.

*Table 4.4: List of behaviours observed using one-zero sampling for both tigers, in any and all video clips of each modal level of busyness. Green text indicates that behaviour was shown by both tigers, red text indicates that behaviour was shown by female tiger only and blue text indicates that behaviour was shown by male tiger only at that busyness level.*

| | Modal busyness level | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| Behaviours shown in clips of each busyness level | Inactive alert low | Inactive alert low | | | |
| | Inactive alert medium | Inactive alert medium | Inactive alert medium | | |
| | Inactive alert high | Inactive alert high | Inactive alert high | Inactive alert high | |
| | Sleeping | | | | |
| | Walking | Walking | Walking | Walking | Walking |
| | | Trotting | Trotting | Trotting | Trotting |
| | | | Running | Running | Running |
| | | Stalking | Stalking | | |
| | | | | Wading | Wading |
| | | Pacing | Pacing | Pacing | |
| | | Repetitive walk | Repetitive walk | Repetitive walk | |
| | Investigating | Investigating | Investigating | Investigating | |
| | | | Drinking | Drinking | |
| | Self maintenance | Self maintenance | Self maintenance | | |
| | | Playing with objects | Playing with objects | | |
| | | | Social play | Social play | |
| | Social contact | Social contact | Social contact | Social contact | |
| | | Marking | Marking | | |



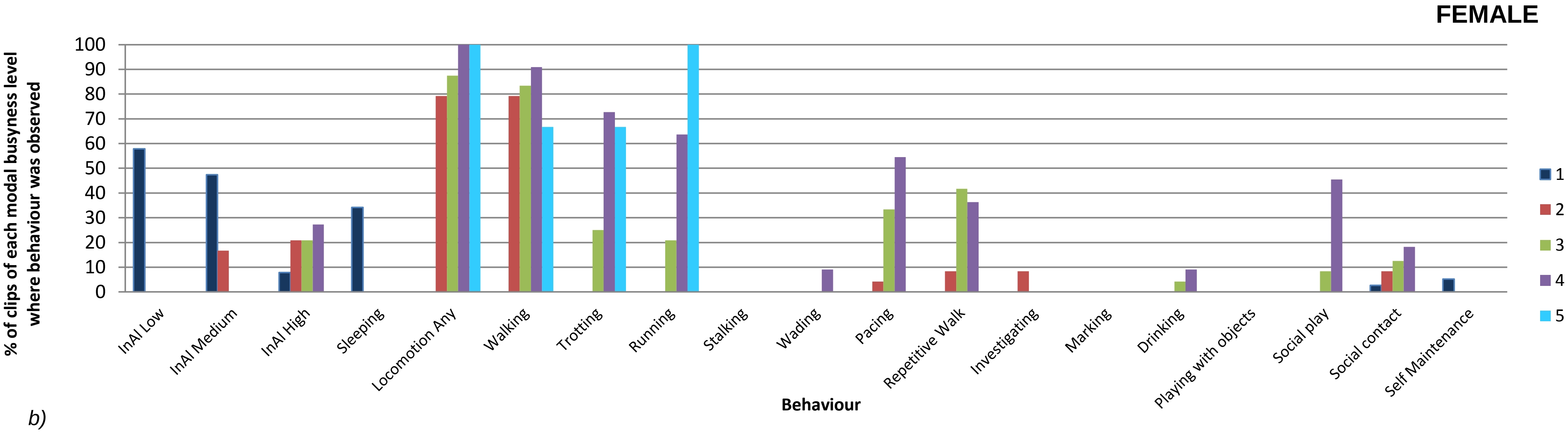


*Figure 4.4: Percentage of the total number of clips of each modal busyness level in which each behaviour was observed for the a) male and b) female tigers. (Locomotion any is the sum of walking, trotting and running behaviours).*

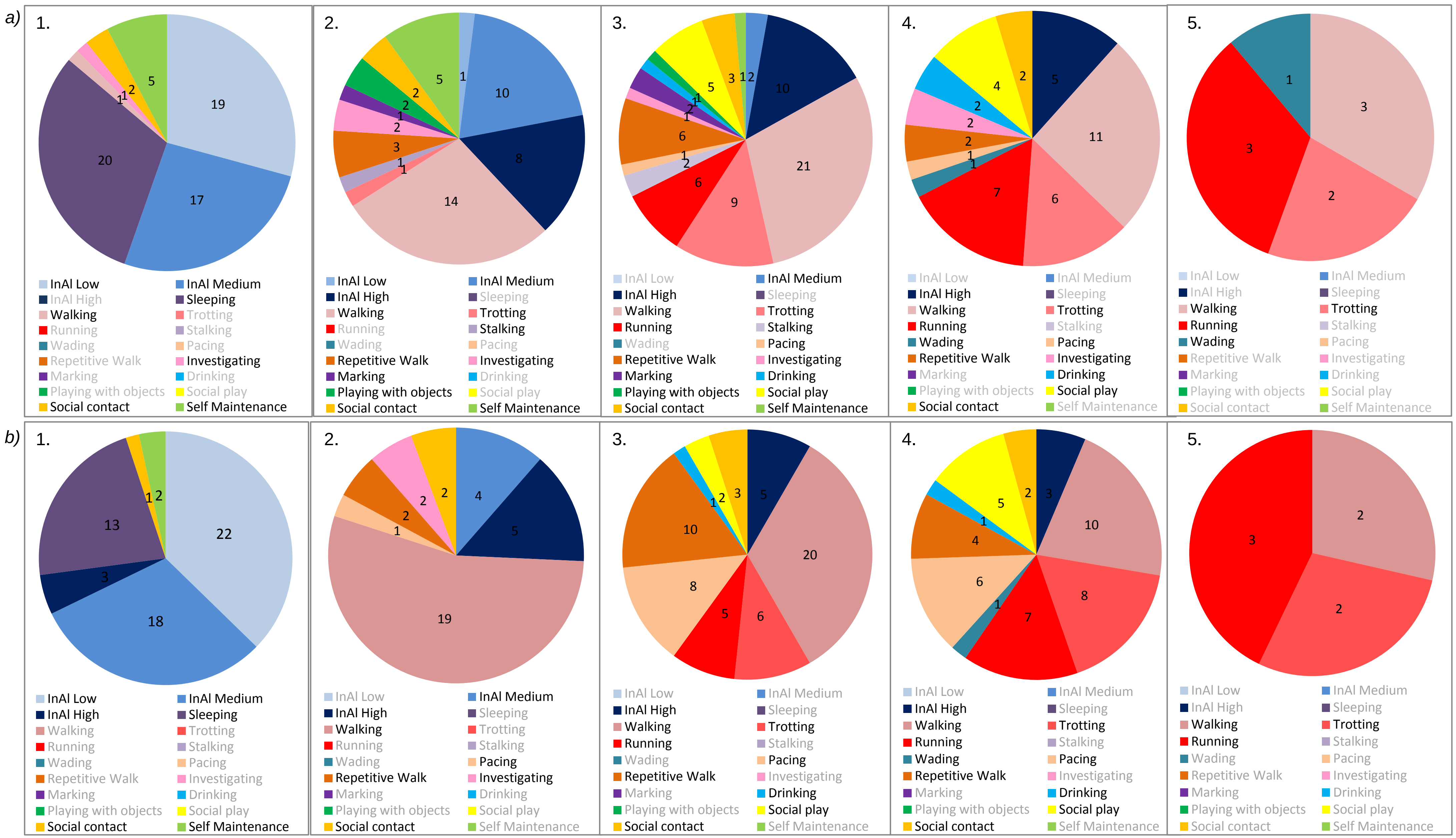


*Figure 4.5: Pie charts showing the number of observations of each type of behaviour for the a) male and b) female tiger within clips of each modal busyness level (numbers displayed inside segments). Chart numbers 1-5 represent modal busyness levels of 1-5 respectively. N.B. results did not produce an equal number of clips of each busyness level therefore numbers of observations of behaviours should not be directly compared across busyness levels and are just provided as a reference.*

*Comparisons of busyness with traditional measures of animal behaviour*

Spearman's rank correlations of the instantaneous scan data with busyness (as listed in Tables 4.5-4.6 and displayed in Figures 4.6-4.7) showed significant, positive correlations (at the .01 and .05 significance levels) between mean and modal busyness and the behaviours: locomotion (any)$^{m,f}$, walking$^{m,f}$, trotting$^{m}$, pacing$^{f}$ and repetitive walk$^{f}$ (correlation with modal busyness only). Correlations of the one-zero data with busyness (as listed in Tables 4.7-4.8 and displayed in Figures 4.8-4.9) also showed significant, positive correlations with: locomotion (any)$^{m,f}$, walking$^{m,f}$, trotting$^{m,f}$, pacing$^{m,f}$ and repetitive walk$^{m,f}$, but the list of significantly correlated behaviours additionally included: running$^{m,f}$, social play$^{m,f}$, inactive alert (high)$^{m}$ and drinking$^{m}$. Overall, analysis of one-zero data revealed significant correlations of busyness with a greater number of behaviours, and at a higher level of significance than did the instantaneous scan data.

Significant negative correlations were shown in the results of the instantaneous scan data between mean and modal busyness and the behaviours: inactive alert (low)$^{m,f}$, sleeping$^{m,f}$ (except for modal scores for the female tiger), inactive alert (any)$^{f}$ and inactive alert (medium)$^{f}$ (correlation with mean busyness only). Similar results were shown for the one-zero data collection with the addition that inactive alert (medium) showed significant correlations for both mean and mode, for both tigers. These significant correlations are summarised in Table 4.9. Investigating, marking, social contact and self maintenance behaviours were not significantly correlated to busyness and, for the male tiger were shown across the range of busyness levels. For the female tiger, these behaviours were observed at low proportions so did not show clear patterns.

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$^{m}$ indicates that results were significant for male tiger, $^{f}$ indicates that results were significant for female tiger

*Table 4.5: Spearman's correlations of proportions of scans of each behaviour (within 5 minute sections of video, or as adjusted where tiger was not visible on the scan) with mean and modal busyness scores for each 5 minute section of video (busyness scores also adjusted where tiger was not visible on the scan) for the male tiger. N=20.*

| | Inactive alert (any) | Inactive alert (low) | Inactive alert (medium) | Inactive alert (high) | Sleeping | Locomotion (any) | Walking | Trotting | Running | Wading | Pacing | Repetitive walk | Investigating | Play (object) | Play (social) | Self maintenance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean Busyness | -0.20 | -0.59** | -0.26 | -0.39 | -0.73** | 0.87** | 0.81** | 0.52* | 0.41 | 0.30 | 0.18 | 0.23 | 0.02 | 0.02 | 0.35 | -0.14 |
| Mode Busyness | -0.30 | -0.63 | -0.35 | -0.39 | -0.62** | 0.86** | 0.79** | 0.52* | 0.43 | 0.36 | 0.23 | 0.19 | 0.04 | 0.04 | 0.43 | -0.14 |

*Note*. *p<0.05, **p<0.01

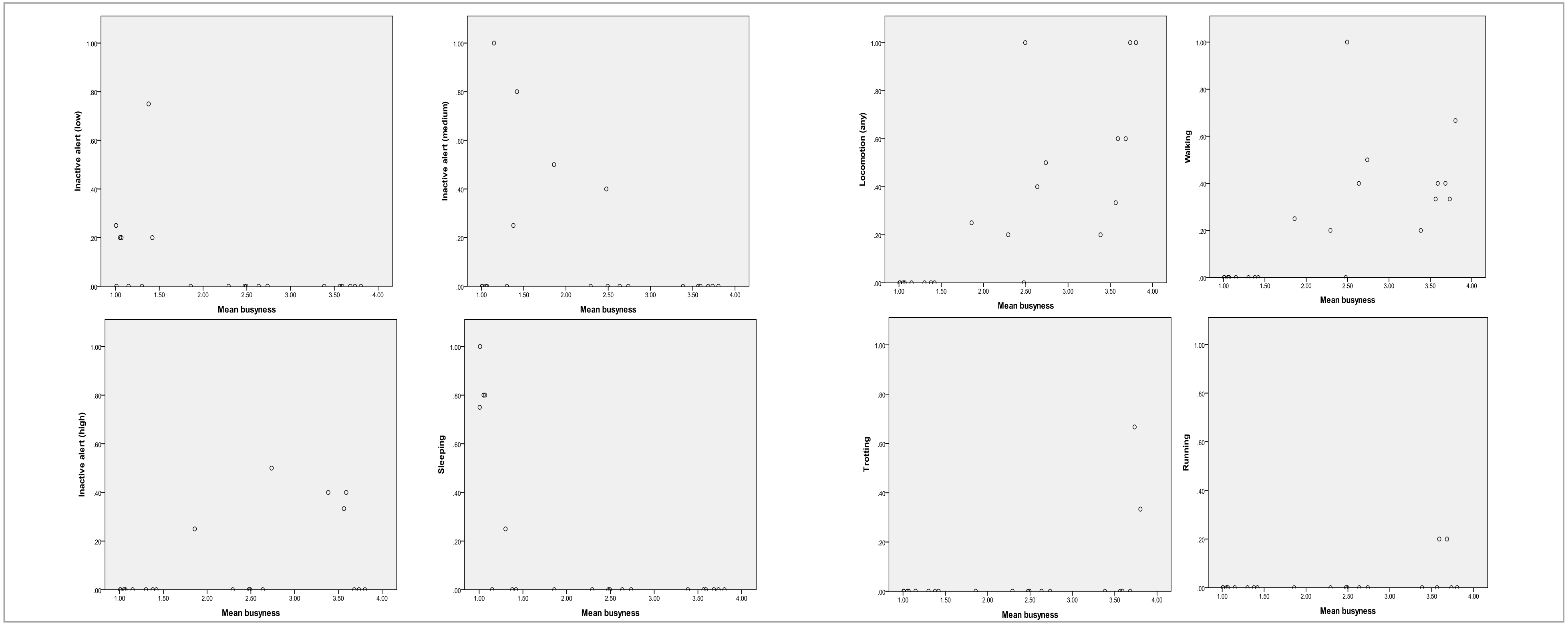


*Figure 4.6. The proportion of scans of 5 minute sections of video showing inactive/sleeping & locomotory behaviours for the male tiger against mean busyness level for those 5 minutes (adjusted for not visible data), as correlated in table 4.5. The mean results (rather than modes) are plotted in order to spread the points along the axis and allow easier visualisation of the data. Graphs are not shown for investigating, pacing, repetitive walking, play, self maintenance or wading behaviours due to the low number of points above zero that were plotted. Trotting and running are presented here for interest.*

*Table 4.6: Spearman's correlations of proportions of scans of each behaviour (within 5 minute sections of video, or as adjusted where tiger was not visible on the scan) with mean and modal busyness scores for each 5 minute section of video (busyness scores also adjusted where tiger was not visible on the scan) for the female tiger. N=20 for mean results and N=16 for modes (Correction of proportions for minutes in which tiger was not visible resulted in too few data points to calculate modes for some behaviours, hence their lower N of 16).*

| | Inactive alert (any) | Inactive alert (low) | Inactive alert (medium) | Inactive alert (high) | Sleeping | Locomotion (any) | Walking | Trotting | Running | Pacing | Repetitive walk | Self maintenance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean Busyness | -0.74** | -0.71** | -0.45* | -0.01 | -0.60** | 0.67** | 0.53* | 0.35 | 0.26 | 0.67** | 0.40 | -0.14 |
| Mode Busyness | -0.82** | -0.70** | -0.46 | -0.06 | -0.45 | 0.73** | 0.51* | 0.45 | 0.39 | 0.85** | 0.66** | -0.24 |

*Note*. *p<0.05, **p<0.01

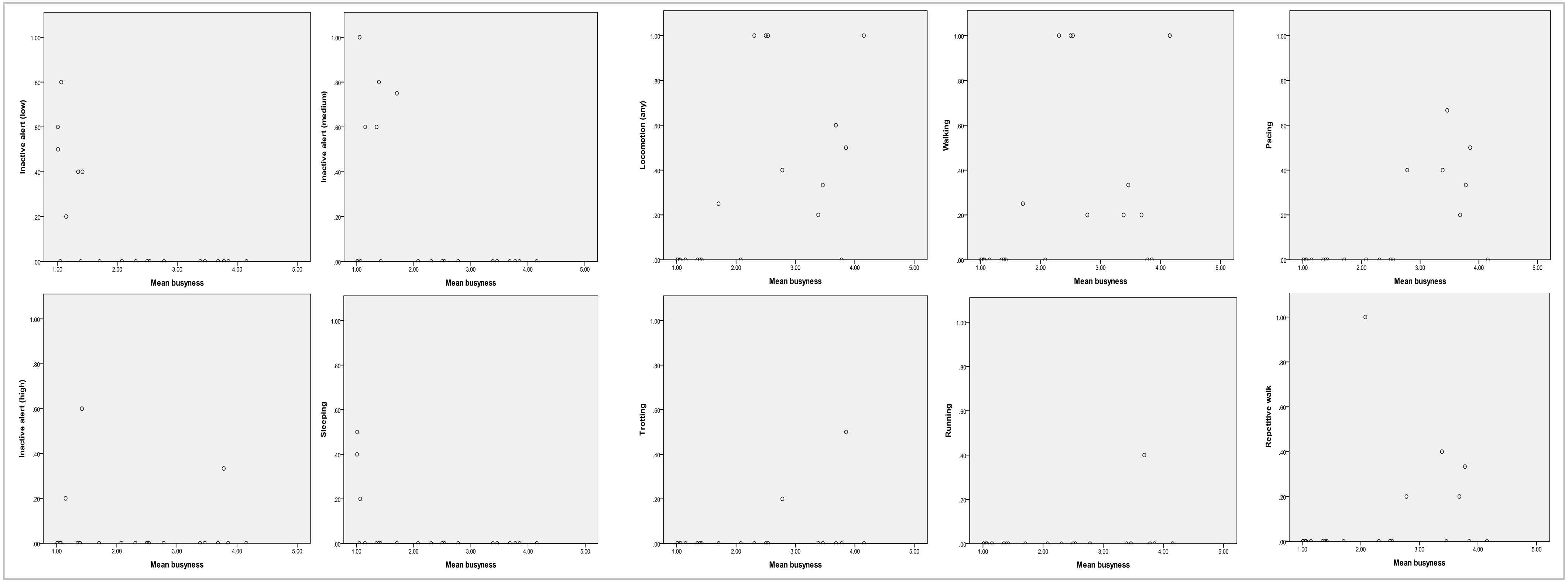


*Figure 4.7: The proportion of scans of 5 minute sections of video showing inactive/sleeping, locomotory and repetitive behaviours for the female tiger against mean busyness for those 5 minutes (adjusted for not visible data), as correlated in Table 4.6. The mean results (rather than modes) are plotted in order to spread the points along the axis and allow easier visualisation of the data. A graph is not shown for self maintenance behaviour due to the low number of points above zero that were plotted. Trotting and running are presented here for interest. Due to the correction for not visible data from scans for each tiger, mean busyness results for this graph are slightly different to Figure 4.6 (different scan results for each video clip resulted in omission of different results thus affecting the averages slightly). Busyness results would normally be the same for both tigers but an exception has been made for the purpose of this analysis.*

*Table 4.7: Spearman's correlations of proportions of the clips in the 5 minute video groups, in which behaviours were observed by one-zero sampling for the male tiger, with mean and modal busyness scores for the video groups. N=20.*

| | Inactive alert (low) | Inactive alert (medium) | Inactive alert (high) | Sleeping | Locomotion (any) | Walking | Trotting | Running | Stalking | Wading | Pacing | Repetitive walk | Investigating | Marking | Drinking | Play (object) | Play (social) | Social contact | Self maintenance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean Busyness | -0.739** | -0.458* | 0.602** | -0.733** | 0.893** | 0.898** | 0.798** | 0.717** | 0.284 | 0.338 | 0.347 | 0.614** | 0.231 | 0.061 | 0.449* | 0.020 | 0.745** | 0.350 | -0.017 |
| Mode Busyness | -0.783** | -0.561* | 0.652** | -0.625** | 0.921** | 0.909** | 0.855** | 0.720** | 0.334 | 0.376 | 0.334 | 0.570** | 0.217 | 0.038 | 0.510* | 0.021 | 0.794** | 0.182 | -0.007 |

*Note*. *p<0.05, **p<0.01

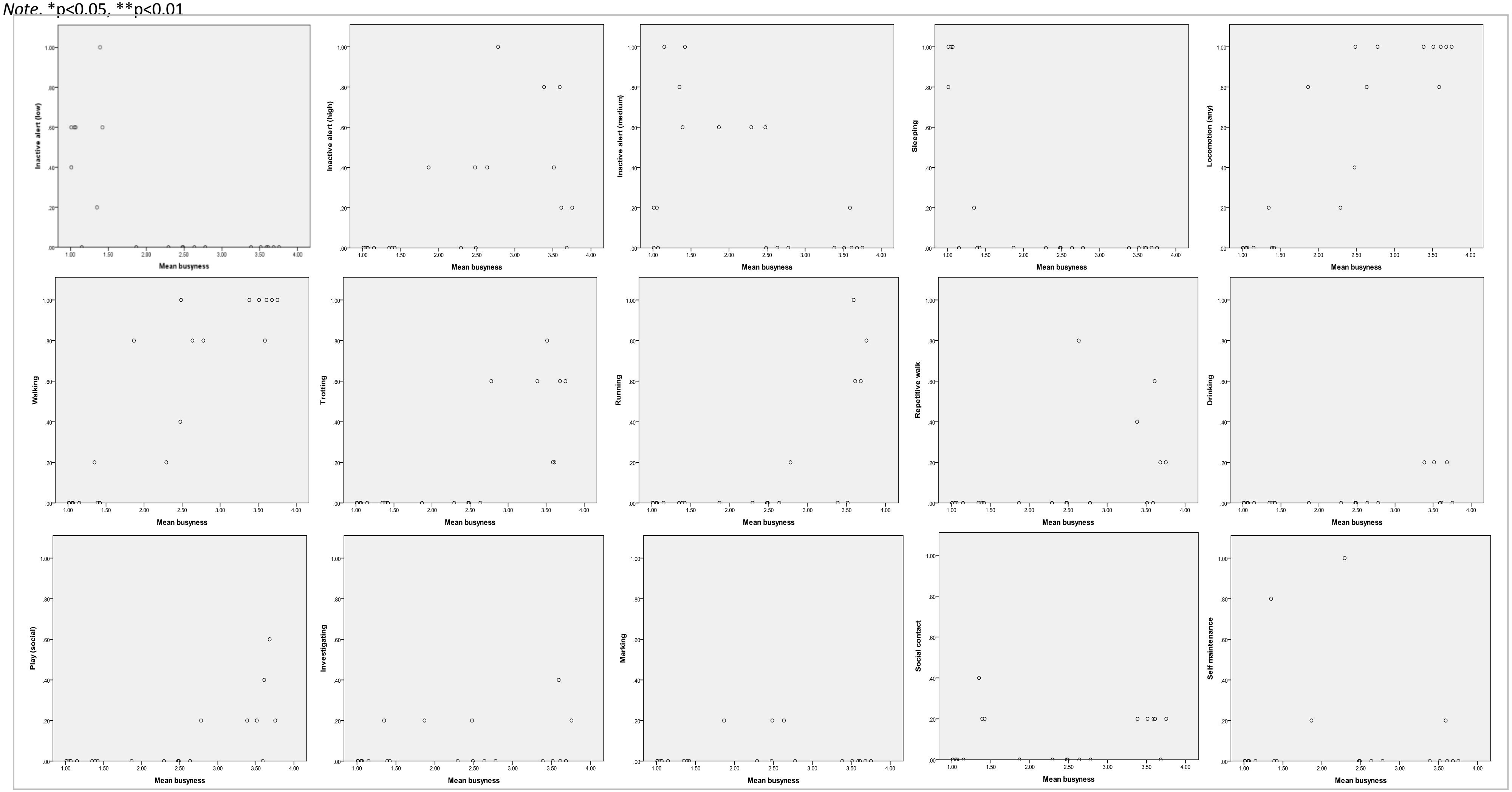


*Figure 4.8: The proportions of the clips in the 5 minute video groups, in which behaviours were observed by one-zero sampling for the male tiger, against mean busyness scores for the video groups. Graphs of stalking, wading, pacing and object play behaviours are not shown due to the low number of points above zero that were plotted.*

*Table 4.8: Spearman's correlations of proportions of the clips in the 5 minute video groups, in which behaviours were observed by one-zero sampling for the female tiger, with mean and modal busyness scores for the video groups. N=20.*

| | Inactive alert (low) | Inactive alert (medium) | Inactive alert (high) | Sleeping | Locomotion (any) | Walking | Trotting | Running | Stalking | Wading | Pacing | Repetitive walk | Investigating | Marking | Drinking | Play (object) | Play (social) | Social contact | Self maintenance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean Busyness | -0.857** | -0.530* | 0.328 | -0.594** | 0.846** | 0.823** | 0.812** | 0.721** | n/a | 0.338 | 0.712** | 0.768** | -0.087 | n/a | 0.376 | n/a | 0.737** | 0.407 | -0.231 |
| Mode Busyness | -0.858** | -0.575** | 0.286 | -0.542* | 0.780** | 0.758** | 0.861** | 0.725** | n/a | 0.376 | 0.780** | 0.825** | -0.167 | n/a | 0.440 | n/a | 0.781** | 0.242 | -0.364 |

*Note*. *p<0.05, **p<0.01

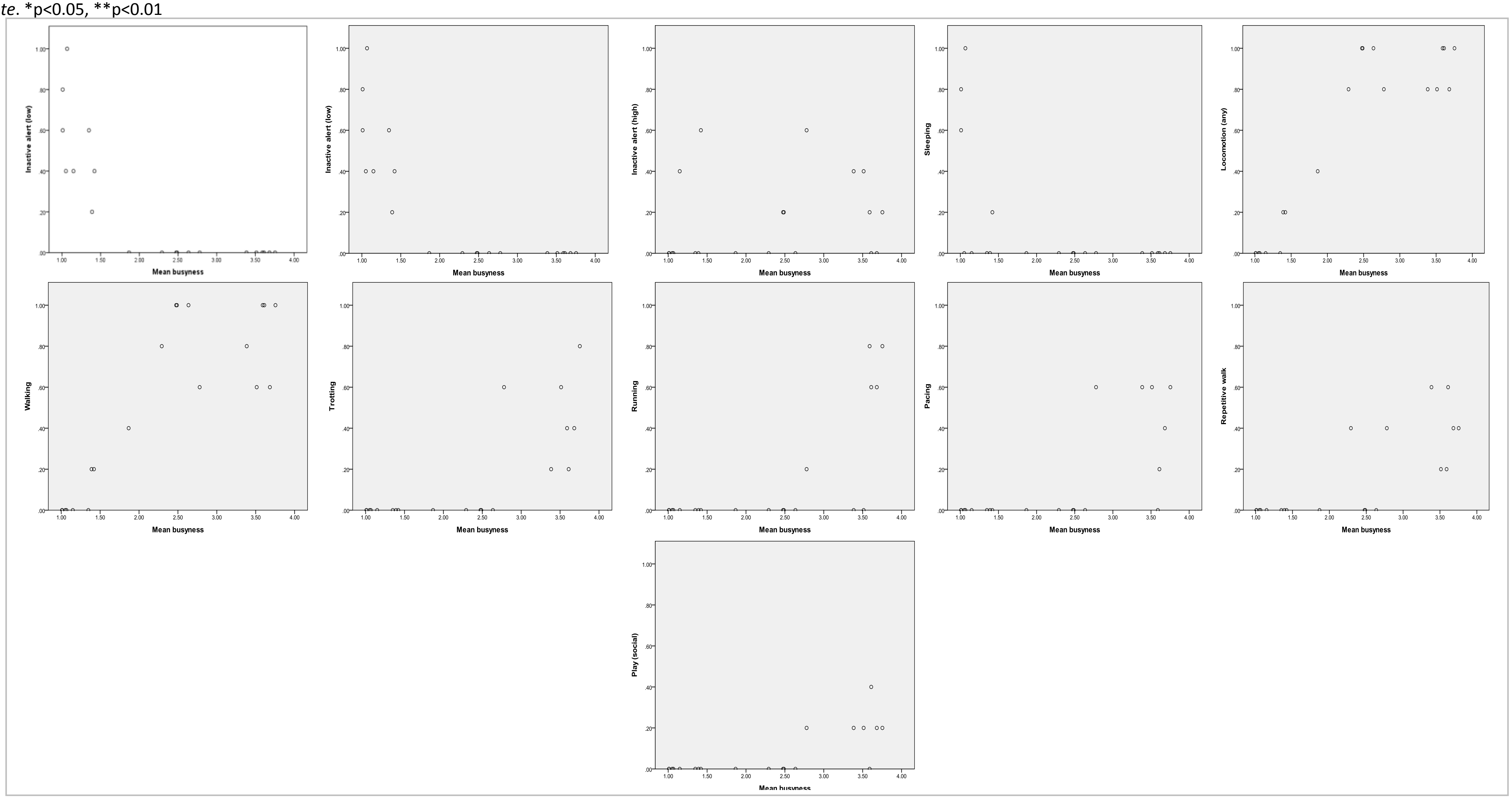


*Figure 4.9: The proportions of the clips in the 5 minute video groups, in which behaviours were observed by one-zero sampling for the female tiger, against mean busyness scores for the video groups. Graphs of stalking, wading, investigating, marking, drinking & object play behaviours are not shown due to the low number of points above zero that were plotted.*

*Table 4.9: Summary of significant positive and negative correlations as extracted from Tables 4.5-4.8, listing the behaviours for which proportions of observations in 5 minute sections of video were significantly correlated with the mean or mode of busyness for that section of video.*

| **Tiger (and behavioural measure)** | **Correlation with mean or modal busyness** | **Positive significance at 0.01 level** | **Positive significance at 0.05 level** | **Negative significance at 0.01 level** | **Negative significance at 0.05 level** |
|---|---|---|---|---|---|
| Male (scan sampling) | Mean | Locomotion (any)<br>Walking | Trotting | Inactive alert (low)<br>Sleeping | |
| | Mode | Locomotion (any)<br>Walking | Trotting | Inactive alert (low)<br>Sleeping | |
| Female (scan sampling) | Mean | Locomotion (any)<br>Pacing | Walking | Inactive alert (any)<br>Inactive alert (low)<br>Sleeping | Inactive alert (medium) |
| | Mode | Locomotion (any)<br>Pacing | Walking<br>Repetitive walk | Inactive alert (any)<br>Inactive alert (low) | |
| Male (one-zero sampling) | Mean | Inactive alert (high)<br>Locomotion (any)<br>Walking<br>Trotting<br>Running<br>Repetitive walking<br>Play (social) | Drinking | Inactive alert (low)<br>Sleeping | Inactive alert (medium) |
| | Mode | Inactive alert (high)<br>Locomotion (any)<br>Walking<br>Trotting<br>Running<br>Repetitive walking<br>Play (social) | Drinking | Inactive alert (low)<br>Sleeping | Inactive alert (medium) |
| Female (one-zero sampling) | Mean | Locomotion (any)<br>Walking<br>Trotting<br>Running<br>Pacing<br>Repetitive walking<br>Play (social) | | Inactive alert (low)<br>Sleeping | Inactive alert (medium) |
| | Mode | Locomotion (any)<br>Walking<br>Trotting<br>Running<br>Pacing<br>Repetitive walking<br>Play (social) | | Inactive alert (low)<br>Inactive alert (medium) | Sleeping |

### 4.3.3. Discussion

Whilst the focus of this study was the validity of busyness rather than reliability, results showed good reliability, thus supporting the data presented in Study 1 (Section 4.2) and providing further evidence of reliability of busyness. Mean and modal scores of busyness showed very little variation between the 3 video orders shown to the participants, and it was rare for scores to differ by more than one busyness level. Intraclass correlation scores for inter-observer reliability also showed good agreement (Portney & Watkins, 2000). It has been noted by several authors that it is important to establish reliability of a method before proceeding to establish its validity (Jones & Gosling, 2005; Meagher, 2009; Wemelsfelder, et al., 2000), and that appears to have been achieved for busyness in Studies 1 and 2.

The current results show that the different levels of busyness are associated with different behaviours. As the scale of busyness increased from 1 to 5, the main behaviours observed in association with busyness changed from inactive behaviours to more active behaviours. As busyness scores increased, the tigers were more alert, as shown by the increase in inactive alert (high) and the decrease in inactive alert (low and medium) behaviours. The tigers also moved more, and with greater speed, as shown by the increase in behaviours such as trotting and running as busyness increased. Significant negative correlations between busyness score and inactive alert (low and medium) and sleeping, and significant positive correlations with busyness and behaviours walking, trotting, running and inactive alert (high) support these associations of the busyness scale with particular behaviours.

Certain behaviours (inactive alert [high], repetitive walk, pacing and social play), whilst correlated with busyness, were only shown in the intermediate levels of busyness (i.e. there were no observations of these behaviours at the extremes of the busyness scale). This gives information about the type of behaviour reflected in these ‘middle’ busyness scores. For example, the behaviour inactive alert (high), whilst a stationary behaviour, involved postural elements and movements that indicated a greater degree of animation and ‘alertness’ from the tigers; hence it was increasingly observed with increasing busyness, but was not observed at low busyness scores (associated with e.g., sleeping) or at high busyness scores (associated with very active behaviours).

A greater diversity of behaviours were observed during clips noted to be of intermediate levels of busyness. For example, behaviours such as marking, playing with objects, stalking and investigating were only observed in clips of modal busyness levels 2-4. These behaviours were not significantly correlated with busyness. As similar behaviours were observed during video clips of busyness levels 2, 3 and 4, presence of the behaviour alone is not sufficient to discriminate between busyness levels. However, the more quantitative results suggest that these levels are intermediary states of alertness and activity. For example, in terms of locomotion, busyness levels 2, 3 and 4 represent a mid-range of speeds. Overall locomotion was increasingly observed in video clips of increasing busyness, but as busyness increased, the specific locomotion behaviours associated with faster movement (e.g., trotting and running) were shown more frequently. Visual analysis of the data was useful in this respect as it showed the distribution of behavioural observations across the busyness scale, with faster locomotion occurring at the higher end of this scale (e.g., Figures 4.6-4.9).

Not all behaviours were found to be correlated with busyness; including marking, stalking, playing with objects and investigating, and also social contact, self maintenance and drinking (female tiger only). It is possible that some of these behaviours such as self maintenance and social contact, are intermediate behaviours, occurring across the mid-range of busyness levels only, and hence would not be expected to correlate with busyness level. It is also possible that some of these behaviours were rare behaviours, observed at a low frequency. Significance may not have been achieved due to the high proportions of zero, and therefore a restricted range. Further work may be necessary if greater detail on the relationship of rare behaviours to busyness is required, using a greater number of video clips to increase the frequency of observations. Nevertheless, if these were rare behaviours, then the video clips were representative of the frequencies of types of tiger behaviour, and so reflected conditions that would be experienced had the participants been observing the animals live.

The present results have therefore shown a relationship between the levels of busyness and different behaviours. Some behaviours have been shown to correlate significantly, whilst others have not. However, taken as a whole these results all give important information on how the different behaviours relate to the levels of busyness. Overall, the data suggest that busyness is related to both the level of activity, and the level of alertness of the animals in an enclosure.

It should be noted that, as used in this instance, busyness was a measure of all of the animals in the enclosure. The current study used video of a pair of tigers, and yet the busyness results were compared to behavioural data for individuals. For this reason it is difficult to directly relate busyness to the behaviour of individual animals. Nevertheless, the present analysis did give an indication of the behaviours and trends that were related to the measure. An aim of the present study was to explore the construct validity of busyness, as there is no exact standard with which to compare busyness, and in this respect it has been successful. Indeed as with the scale developed by Wielebnowski (1999), for ratings of cheetah behavioural traits, busyness was not designed to replicate behavioural measures that are already easily quantifiable, but to provide a measure which includes a range of attributes that are not recorded by traditional measures. Therefore analysis of the behaviours of each tiger was the closest comparison to busyness. As busyness has been shown to be both reliable (Study 1) and now valid (Study 2) it could be considered suitable for use with relatively untrained observers who will not necessarily be able to identify individual animals.

Busyness was correlated against behaviours obtained using both instantaneous, and one zero sampling. Whilst some authors have argued that one-zero sampling is not the best choice for behavioural data collection (Martin & Bateson, 1993). It has received considerable support from others (as discussed in Section 2.4) and in the current study provided a greater number of data points for analysis due to the ability to record more than one behaviour per video clip. The instantaneous scan data resulted in a low number of data points per behaviour for correlation with busyness due to the restriction of only 100 potential scans. Results from scans were also limited due to the need for correction of the data if a tiger was not visible on the sample point. The restricted field of view of the video camera meant that animals were sometimes out of sight (although never for the entire minute), thus reducing the number of data points available for analysis. This affected the scores for proportions of behaviour and meant that, on occasion, modal scores could not be calculated. One-zero results therefore provided a greater number of observations for each behaviour, hence the greater number of correlations for these results.

The present study was designed such that the video clips that were presented to the students showed an even distribution of busyness levels. However, the participants only recorded a modal busyness score of 5 for three out of the 100 video clips. This suggests that the participants were reluctant to use the top end of the busyness scale. Level bias such as this can be a problem when using scales (Dawis, 1987). It is also possible that the participants have an unrealistic expectation of the behaviours that could be seen at the top end of the busyness scale. Further research into the information and instructions that participants are given on busyness is therefore necessary, with particular attention to the examples given for the anchor points of busyness levels 1 and 5. Different levels of instructions on the use of busyness were therefore investigated in Study 3.

## 4.4. Study 3: The effect of level of instruction detail on participants' ratings of busyness

In the following study, different levels of detail in the instructions explaining how to use the measure of busyness were used in order to investigate the effect of instruction detail on participants' ratings of busyness.

### 4.4.1. Methods

*Participants*

The participants were 53 undergraduate psychology students from the University of Plymouth, who received course credit for participation. Participants had not previously taken part in Studies 1 or 2.

### *Materials*

Twenty five minute-long clips of video were selected from the video clips of tigers used in Study 2; the method of filming and video editing was as described in Section 4.3.1. The 25 clips were selected to include 5 clips of each busyness level (1-5), as previously rated by the experimenter. These clips were then edited into a single continuous video, with the order of the clips determined using a random number generator in Microsoft Excel. As the previous studies had demonstrated little effect of the order in which the video clips were shown, and as instruction level was the only independent variable of this study, only one video was produced and the clips were shown in the same order to all participants.

Participants were given one of three different instruction sheets, the detail of which was categorised as low, medium or high, depending on the experimental group (full copies of the instruction sheets are given in Appendix 4.3). The instructions for all categories explained the format of the video and gave basic instructions asking the participants to rate how ‘busy’ the tigers were for each minute clip on a scale of 1 to 5, where 1 was the lowest level of busyness that the animals could show, and where 5 was the highest level of busyness that the animals could show. Busyness was explained as “your subjective opinion of how much you think is going on in the enclosure. For example, you might like to think about what the tigers are doing and how many of them are doing it”. Further detail in the instructions then varied according to the instruction category as follows:

1) Low detail instructions (*N* =18 participants): Basic instructions about the busyness scale (as described above) only.
2) Medium detail instructions (*N* =18 participants): As (1) plus behavioural examples as suggested anchor points for levels 1 and 5 of the scale. These examples were described as: “all or most tigers very quiet e.g., sleeping or inactive” for busyness level 1, and: “all or most tigers very busy e.g., running or active” for busyness level 5.
3) High detail instructions (*N* = 17 participants): As (2), plus an example minute video clip for busyness levels 1 and 5 (as previously rated by the experimenter), which participants watched before starting the study.

### *Procedure*

Participants were briefed (and debriefed) as in Study 1 and received a set of instructions from one of the three categories of detail (low, medium or high). Participants then watched the video clips and rated the busyness of the tigers in each clip. They then had opportunity to write any comments on how easy or difficult they felt the scale was to use.

### *Data analysis*

Mean and modal busyness scores were calculated from all of the participants' scores for each of the 25 video clips in each instruction condition, for descriptive analysis. These mean values were also used in a Friedman's test to analyse for significant differences. *Post hoc* tests were carried out using Wilcoxon's tests, using a Bonferroni correction to account for the multiple comparisons, and effect sizes (*r*) were calculated from the resulting *z*-scores.

Counts and percentages of ratings at each busyness level were taken for all of the participants' responses for all video clips, in each instruction condition.

The inter-observer reliability was tested for the three instruction conditions using intraclass correlations, using the ICC (2,1) model for consistency and absolute agreement.

A simple, qualitative analysis was carried out on the comments left by the participants of their experiences of using the scale. Five categories were created, based on commonly occurring comments and the number of participants leaving a comment in each category was then calculated for each instruction condition. Chi-squared analysis was then used to determine any significant differences in comments between the conditions.

### 4.4.2. Results

Overall, there were few differences between the modal scores for each clip across the different instruction detail conditions (Figure 4.10); only five clips had different modal scores. Clips 2, 14 and 22 had higher busyness modes for the medium and high instructions than for the low instructions. Clip 4 showed the highest modal busyness score in the medium detail condition, and clip 25 showed the highest modal busyness score in the low detail condition.

Mean scores revealed more variation between instruction conditions than the mode (Figure 4.11). Mean busyness ratings for each video clip in the low detail instructions condition were generally lower than those in the medium or high detail conditions. Out of 25 video clips, instruction conditions high and medium resulted in the highest mean busyness score for 12 and 11 of the video clips (respectively), with the low detail instruction condition resulting in the highest mean busyness score for only one video clip. Despite this, differences between the mean busyness scores for each clip across the different instruction conditions were relatively small, with a mean difference between the highest and lowest mean scores for each clip of 0.26 (range 0 – 0.86). Mean busyness scores (overall mean for all participants' ratings, for all clips) also increased with increasing instruction detail (range 2.25-2.46), as did mean standard deviations (mean of standard deviations for all clips) (range 0.46-0.54) (Table 4.10).

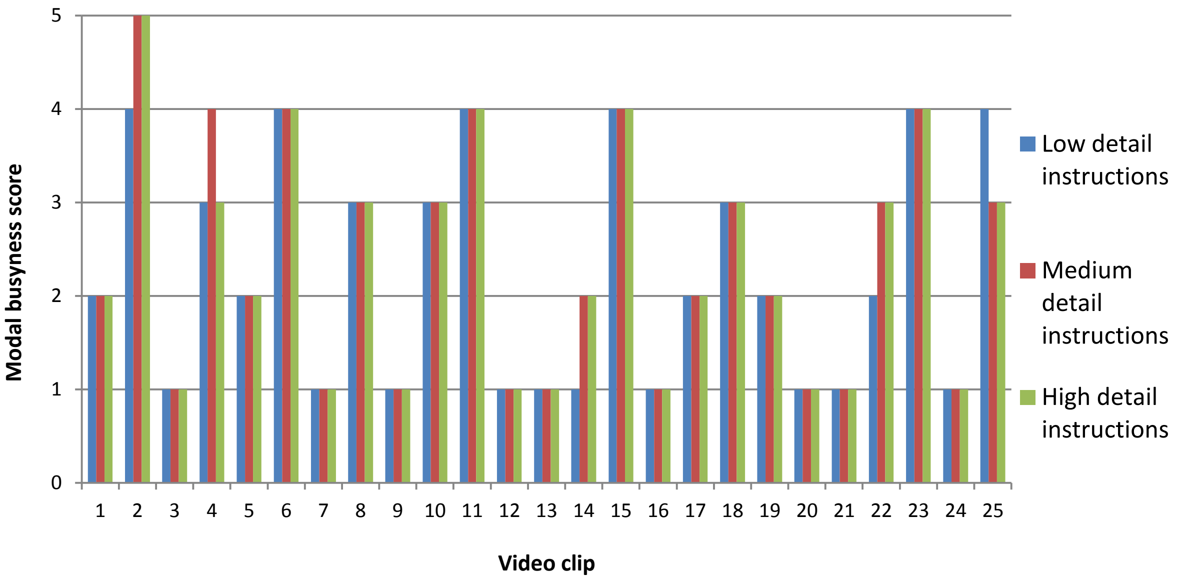


*Figure 4.10: Mode of participants' busyness ratings for each video clip, for each instruction condition.*

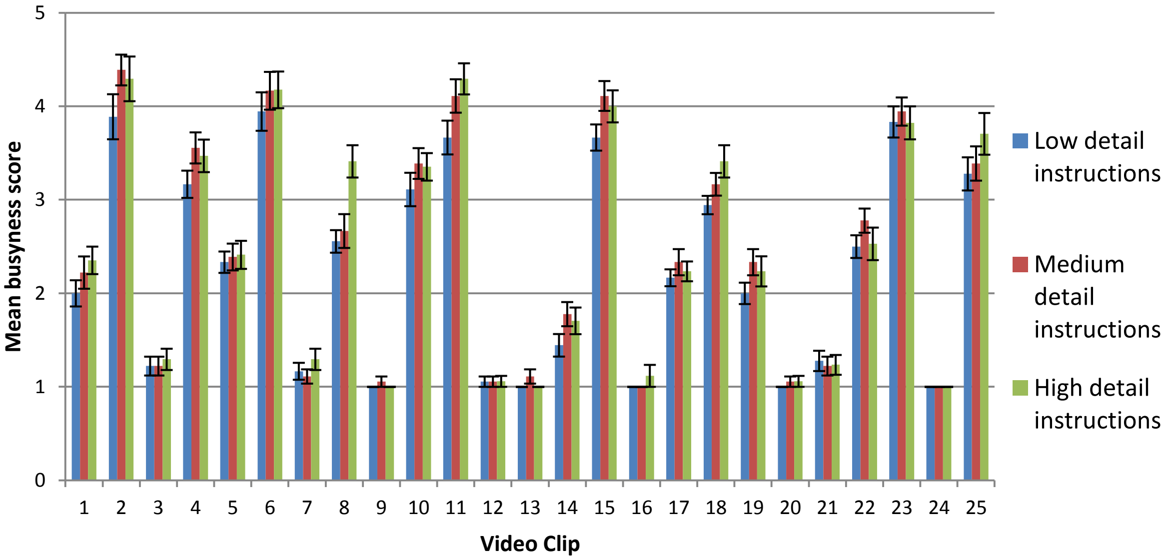


*Figure 4.11: Mean of participants' busyness ratings for each video clip, for each instruction condition. Error bars indicate standard error.*

Intraclass correlation scores (Table 4.10) showed high reliability scores for each instruction condition with no condition giving a reliability score below .80. Intraclass correlation scores for consistency increased with increasing detail of instructions and ICC scores for absolute agreement were highest for the medium detail instruction condition.

*Table 4.10. Descriptive statistics and results of intraclass correlation scores for each instruction condition*

| Instruction level | N | Mean busyness score (over all clips) | Mean standard deviation (over all clips) | ICC model | ICC value | Confidence intervals | | F-test (with true value 0) | P | d.f |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper | | | |
| Low | 18 | 2.25 | 0.46 | (2,1) consistency | .84 | .76 | .91 | 94.67 | <.01 | 24 |
| | | | | (2,1) absolute | .80 | .70 | .89 | 94.67 | <.01 | 24 |
| Medium | 18 | 2.42 | 0.52 | (2,1) consistency | .85 | .77 | .92 | 101.32 | <.01 | 24 |
| | | | | (2,1) absolute | .82 | .73 | .90 | 101.32 | <.01 | 24 |
| High | 17 | 2.46 | 0.54 | (2,1) consistency | .85 | .78 | .92 | 100.25 | <.01 | 24 |
| | | | | (2,1) absolute | .80 | .70 | .89 | 100.25 | <.01 | 24 |

Linked with the increase in mean busyness score, there was also an increase in the number of ratings at the higher end of the busyness scale given by participants in the medium and high detail instruction conditions. As shown in Figure 4.12, in the medium and high detail conditions, the percentage of ratings at busyness level 5 (out of all of the participant responses for all clips), were 7.11% and 8.24% respectively; more than double that recorded in the low detail condition (3.33%). The increased busyness level resulted from an extra 17 and 20 (medium and high conditions respectively) participant responses scoring busyness at level 5, compared to the low detail condition. Percentages of ratings at busyness level 4, were also higher in the medium and high detail conditions, whereas the percentages of responses giving a rating of busyness level 2 or 1, were higher in the low detail condition.  Figure 4.13 also illustrates that participants given the high and medium detail conditions scored fewer video clips at the lower end of the busyness scale (busyness scores 1,2 and 3), and more video clips  at the higher end of the busyness scale (busyness scores 4 and 5). Furthermore, Figure 4.13 also demonstrates how similar the participants' scores were in the medium and high detail conditions, with participants in each condition giving a similar number of ratings at each busyness level.

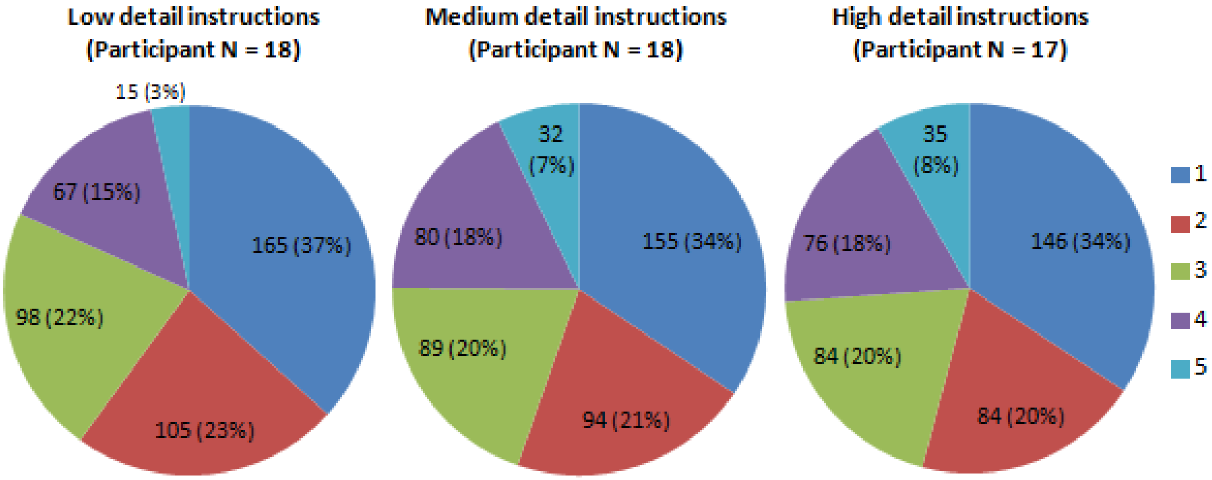


*Figure 4.12: Numbers, and percentage of participants' ratings at each busyness level for all video clips, for each instruction condition (Number of responses for all participants and all clips: 450 [low and medium detail conditions] and 425 [high detail condition]).*

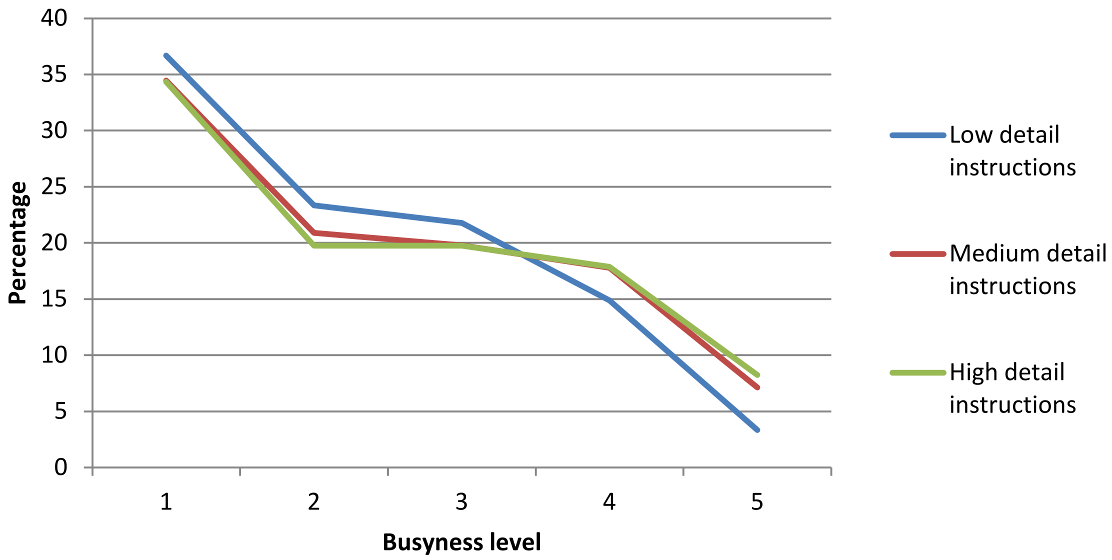


*Figure 4.13: Percentage of the total number of participant ratings over all clips, at each busyness score, for each level of instructions.*

There was a significant difference in the mean ratings given by the participants for each clip in each instruction condition ($\chi^2(2) = 20.33$, $p<.01$). *Post hoc* Wilcoxon tests, using a Bonferroni correction to give an alpha level of .017, revealed significant differences in the mean ratings for the video clips between the low and medium detail instruction conditions ($T= 6.00$, $r = -0.54$, $p<.017$), and between the low and high detail conditions ($T = 6.00$, $r = -0.55$, $p<.017$), but not between the medium and high detail conditions ($T = 135.5$, $r = -0.06$, $p>.017$).

Analysis of the comments left by the participants on their experience of using the measure of busyness (Table 4.11) showed that a high percentage of participants in all instruction conditions noted that the method was easy to use, with no clear difference between conditions. In the low detail instructions, higher percentages of participants commented on: difficulties with interpreting the ratings, problems with using the top of the busyness scale, and increased ease of use as they progressed through the study, and percentages of these comments decreased as the instruction detail increased to medium and high. In particular, this effect of instruction condition was statistically significant for comments regarding difficulties using the top end of the busyness scale. Some participants also reported difficulty assigning a rating if the two tigers were behaving differently, however there was no clear effect of instruction condition on such comments.

*Table 4.11. Percentage of participants leaving comments on their experience of using the measure of busyness, whose comments related to particular common categories.*

| Category of comments | Low Detail Instructions (*n*=18) | Medium Detail Instructions (*n*=16) | High Detail Instructions (*n*=16) |
|---|---|---|---|
| Positive comment on overall ease of use | 72.22% | 50.00% | 62.50% |
| Difficulty interpreting/differentiating ratings | 50.00% | 25.00% | 25.00% |
| Particular mention of/difficulty with the top end of the busyness scale* | 33.33% | 25.00% | 0.00% |
| Method became easier to use as progressed through the study | 22.22% | 18.75% | 6.25% |
| Difficulty assigning rating if tigers were behaving differently to each other | 16.67% | 25.00% | 12.50% |

(* Significant effect of instruction conditions: $\chi^2$=3.21, df=2, Fisher's exact sig = < .05)

### 4.4.3. Discussion

Overall, the results from descriptive and statistical analysis suggest that there was little difference between the ratings given by the participants in the medium and high detail instruction conditions. However, the data from these two conditions differed to those given by participants in the low detail instruction condition. Mean busyness scores for the video clips were generally higher in the medium and high detail conditions than in the low detail condition, and participants gave a higher percentage of responses at busyness levels of 4 and 5 in the medium and high detail conditions.

Therefore, it seems that busyness scores can be affected by the type of instructions given to the participants. It seems that with more detailed instructions, participants may be more inclined to rate video clips with a higher busyness score, and hence make more use of the full range of the busyness scale. However, this difference was only significant between the low detail instructions and the medium and high detail instructions, and not between the medium and high instructions. Therefore it could be concluded that providing participants with details including behavioural examples of anchor points is required in the instructions, as this distinguished low and medium instruction conditions. With additional detail, participants were encouraged to make use of the full range of the busyness scale, but further detail on the anchor points does not significantly affect the ratings scores given by the participants.

Previous research has suggested that survey respondents show a bias for the left hand side of a rating scale (Friedman & Amoo, 1999; Friedman et al., 1994), so participants are more likely to choose a response from the first few options in a rating scale. In the current case this corresponds to the lower numbers of the busyness scale. Therefore, if such left-side bias does exist for busyness, then it seems that providing more detail in the instructions, as given to the raters in medium and high conditions, might mitigate some of the effects of this bias, resulting in greater use of the full range of the busyness scale.

The significant difference found between the mean busyness scores for clips in the three conditions is perhaps surprising when compared to the results of Gilbert, et al. (1984) who found no significant differences between the reliability scores of rating scales in which all of the points of the scale were defined, compared to rating scales in which only the polar points of the scale were defined. However, Gilbert et al. compared different scales, each with a certain level of scale definition, rather than comparing the results within the same rating scale, under different scale definitions, as was the case in the current study. Gilbert, et al. (1984) also compared reliability ratings rather than mean rating scores. In the current study, all three conditions resulted in reliability values indicating almost perfect agreement between observers, and there was little variation between the ICC values in the 3 conditions (range .839-.854 for consistency and .801-.819 for absolute agreement). This is the most comparable result to that of Gilbert, et al. (1984) as there was little difference in the reliability of the results obtained using the different instructions conditions. It is therefore important to note, that whilst the type of instructions used may have little effect on the reliability of the results, it may have an effect on the mean scores obtained and hence may affect the value of the results recorded.

In a study of human behaviour (Shear et al., 2001), two levels of detail were compared on a scale designed to measure somatic and psychic anxiety symptoms using an interview technique; the Hamilton Anxiety Rating Scale. The authors compared the results from the traditional scale which lacks instructions on administration of the interviews and on anchor points, with those of a more structured scale in which guidelines were developed for interview administration along with structured questions and rating criteria. It was found that both techniques produced reliable results. However, the resulting ratings for the more structured version were consistently higher. Whilst this method differed greatly to busyness in terms of its application, method of administration and the fact that all points on the rating scale were defined, not just the polar points, Shear et al.'s study does illustrate that changes in the detail of instructions provided to raters can influence the value of the results, even if reliability is consistently high. These findings and those of the current study reflect the need for careful consideration of, and consistency in, the instructions provided to participants.

Despite the fact that significant differences were found between the mean scores for the video clips in the different instruction detail conditions, when considering the actual values of the mean busyness scores, there is only a small difference in the values for each of the different conditions. The mean difference between the highest and lowest mean busyness score for each clip in each condition was 0.26, with no video clip differing by more than 0.86 between instruction conditions. Mean scores for clips in each condition were always close to the same categorical value of busyness, and if modal scores are considered, the mode of the participants' scores only differed (by no more than one busyness level) for 5 out of 25 video clips. Therefore, the level of instructions made little practical difference to the exact busyness results obtained. Whilst the differences between the instruction conditions are reasonably low, recommendations could be drawn from this study with preference for medium or high detailed conditions. In terms of consistency, both of these instructions conditions would result in similar scores from the participants, and a greater range of the busyness scale would be utilised, making for a more sensitive dataset. Whilst there was little difference between the reliability scores for the 3 conditions, and all scores were high, ICC values were higher for the medium and high detail conditions thus adding further support for their use in future studies. When considering which type of instructions to use, feedback from the participants should also be taken into consideration. Whilst participant feedback was only analysed in simple terms, where participants were free to leave any comments rather than answering specific questions, the results revealed an effect of instruction detail on the participants' ease of interpreting the measure. A high percentage of participants in all instruction conditions found the method easy to use, but as the detail of the instructions increased, fewer participants reported difficulty in interpreting the ratings, or with assigning scores at the top of the scale, or reported that the method became easier as they progressed through the study. This adds further support to use of medium or high detailed instructions, which appeared to aid the participants in using the measure.

Whilst this study used three levels of instruction detail, it was felt that only the anchor points (the polar points on the busyness scale) should be manipulated, and given greater detail. Whilst providing more definition would make this measure more objective, it would then require more training to use and would lose aspects of its descriptive and integrative nature (as discussed in Section 3.4.4.). It is therefore recommended that some broad definition of the anchor points be used (as in the medium instructions) in order to increase the range of the data, without decreasing the inter-observer reliability. High detail (i.e. additional video of the anchor points) could be used if desired but description of the anchor points is sufficient (and less time-consuming to set-up).

This study could be considered to be limited, in that only three conditions were used, and those three conditions were only categories, rather than a clear continuum of instruction detail which could, for example, be correlated with mean busyness score in order to be certain of the relationship between instruction detail and survey result. Further research of this type would be useful, although in practice, the types of instruction used in the present study may be adequate.

Having explored the reliability and validity of busyness and the effects of different instructions in Studies 1, 2 and 3, it was considered desirable, in Study 4, to examine whether busyness could be practically applied to the study of patterns of behaviour.

## 4.5. Study 4: Do busyness ratings reflect patterns of behaviour?

In Studies 1 to 3, sequences of behaviour were artificially segmented. Therefore, Study 4 investigated whether the ratings produced from such segments of video would correspond to ratings produced from intact sequences of behaviour. In other words, investigating whether ratings of clips presented out of sequence (thus removing the risk of observer expectations) would correspond to the original, intact sequence when put back in order. This would help to build confidence in the value of busyness as a field technique, where controlling for order, or expectancy effects may not be possible. In addition, as a further test of the reliability of the busyness, comparisons of ratings between the author and an additional, experienced animal behaviour researcher were made. This was considered important as a test of whether the scale would produce consistent patterns of results with experienced as well as inexperienced observers.

### 4.5.1. Methods

*Participants*

Participants were 43 undergraduate psychology students from the University of Plymouth, who received course credit for participation. Participants had not previously taken part in Studies 1, 2 or 3. A second observer with substantial experience of animal behaviour data collection also watched the video, in addition to the experimenter.

*Animals*

The animals filmed for this study were the same as for Studies 2 and 3 of the current chapter. The female tiger was the same as observed in Chapter 3. However, unlike Chapter 3, the male tiger was her 2 year old cub. The enclosure and husbandry routine of the tigers were the same as described in Chapter 3, where the tigers were let back into their indoor area everyday at approximately 16:40 where they were fed. On the day of filming for this study, the tigers were let inside shortly after filming finished at 16:45.

*Materials*

Video was recorded for one minute, every eight minutes throughout the day from 11:00 to 16:44 on a single day to give a total of 44, one-minute clips of video. These video clips were then taken out of chronological order and edited into a video file in a random order that was determined using a random number generator in Microsoft Excel. Three, additional one-minute clips were added at the beginning of the video file, which served as example clips (as per Study 2, Section 4.3). These three clips were not shown again in the main video. All participants viewed the clips in the same, random order, as Studies 1 and 2 had not indicated any significant effect of the video order on participants' responses.

*Procedure*

Participants were briefed and debriefed as described in Section 4.2.1, and received the same instructions as in Study 2 (Appendix 4.2). Participants then viewed the video and recorded a busyness score for each minute of the study video. Participants were requested to pause the video and to take a break in concentration for approximately 3 minutes, after they had watched 30 minutes of video.

The video was also scored for busyness by the experimenter (first observer) and by another experienced observer (second observer) who had previous experience of animal observations but not specifically of tigers, and no experience of using the busyness scale. The behaviour of the tigers was also recorded by these observers using one-zero sampling every minute according to the ethogram presented in Appendix 4.7. As it was not practical for the second observer to learn to identify the tigers individually, the tigers were identified by their position on the screen (left or right) at the start of each minute and the results were later assigned to the male or female tiger by the first observer, who was able to identify the tigers. Results from the second observer provided a measure of quality control on the behaviours that were recorded for each video clip.

The first observer recorded the busyness of each clip twice, by observing the video twice, with 2 months between observations; and mean values were calculated for each clip. Cohen's Kappa (Cohen, 1960) was used to check for intra-observer reliability.

*Data analysis*

Mean and modal busyness scores were calculated from the participants' results, for each video clip.

Behavioural proportions (from one-zero data) were calculated as the proportion of the two observations made for each video clip, in which each behaviour was observed; if both observers recorded a behaviour such as sleeping for a particular video clip, the proportion of sleeping for that clip would be 1; if only one observer recorded sleeping for that clip, the proportion would be 0.5 and so on.

For data analysis, the results for all video clips were re-ordered and presented in chronological order. Data were presented graphically and analysed qualitatively in order to identify patterns of busyness throughout the day, and any associated temporal patterns in the occurrence of the different behaviours. Behavioural data were presented for behaviours that were observed in a minimum of 3 clips, for at least one of the tigers.

### 4.5.2. Results

Figures 4.14 and 4.15 show the mean and mode of all of the participants' scores for each clip, with the clips arranged back into chronological order. Both graphs show that busyness scores fluctuated around busyness level 1 and 2 during the morning until 13:24, then busyness was consistantly low at around 1 in the middle of the day until 14:28. Busyness then showed a peak of 3 (mode) or 2.63 (mean) at 14:52, then returned to around level 1 after 15:08. There was then an increase in busyness score towards the end of the day from 15:24, to the highest peak of 4 (mode) or 3.93 (mean) at 16:44.

A similar pattern is shown in the busyness results recorded by the first observer and by the second observer (Figure 4.16). The first observer also reported fluctuating busyness scores in the morning, although these scores initially fluctuated around 2 and 3 until 12:12, after which point they then fluctuated around 1 and 2 until 13:16 (with the exception of one score of 3 at 13:16). The second observer recorded a greater variation in busyness scores, with fluctuations around scores of 1, 2 and 3 (with one score of 4) until 11:56, after which point the scores fluctuated around 1 and 2, until 13:24. The scores from both observers were then similar to those of the participants, remaining constantly low in the middle of the day until 14:20 (first observer) and 14:36 (second observer) and showing a peak at 14:44 to 14:52 returning to a low level at 15:16. Both observers then report an increase in busyness towards the end of the day, from 15:32, to a peak of 5 at 16:44.

Figure 4.17 gives the results of the behaviours observed in each minute clip, in chronological order. Inactive alert (low) behaviours were observed in clips throughout most of the day. However, fewer observations of inactive alert (low) were made in clips in the afternoon after 15:32. This corresponded to the afternoon period being a time of increasing busyness. A similar pattern was shown for inactive alert (medium) behaviours, with few observations of this behaviour in clips after 15:32 (female tiger) and 15:40 (male tiger). In contrast, observations of inactive alert (high) behaviours, which were mostly recorded for the female tiger, were recorded more frequently in clips during the afternoon, for example between 14:44 and 15:08, and between 15:40 and 16:28, which corresponded to the reported afternoon peak of busyness, and the increase in busyness towards the end of the day. Sleeping was mostly observed for the male tiger particularly between the times of 12:28 and 14:20, at the times of low busyness (with the exception of one observation at 16:20). Walking was observed in all clips between 14:44 and 15:08 and after 15:48, corresponding to the afternoon peak, and increase in busyness. Walking was not observed in clips between 12:44 and 14:44 (male tiger) or 13:24 and 14:36 (female tiger), corresponding to the period of low busyness in the middle of the observation day. Trotting was only observed in clips after 16:20, at the times of highest busyness. Agonistic behaviour was also only observed in clips at 15:40, and then after

16:28, during the times of high busyness. Self maintenance was only observed in a relatively low number of clips, but occurred in clips between 12:04 and 13:00, and between 14:20 and 14:44, in clips that were rated at busyness levels of 1, 2 or 3. Investigation behaviour was also only observed in a relatively low number of clips and showed no obvious pattern across the day, or with busyness score.

Behavioural patterns for the male and female tiger were similar across the day (Figure 4.17). However, the male tiger was observed to be sleeping in a greater number of clips (13) than the female (4), and the female was observed to show inactive alert (high), agonistic and self maintenance behaviours in a greater number of clips than the male.

The two observers generally agreed on all of the ‘active’ behaviours observed in each clip, and they agreed on when the tigers were inactive, although some differences arose in recording the different levels of alertness when inactive (inactive alert [low], [medium] and sleeping). The results of the two sets of observations made by the first observer differed (by 1 busyness rating) for only 8 out of the 44 clips. All of the other clips received the same busyness score on both observations. Indeed, substantial (Landis & Koch, 1977) intra-observer reliability was reported with Kappa results of .75, $p < .01$, $n = 44$.

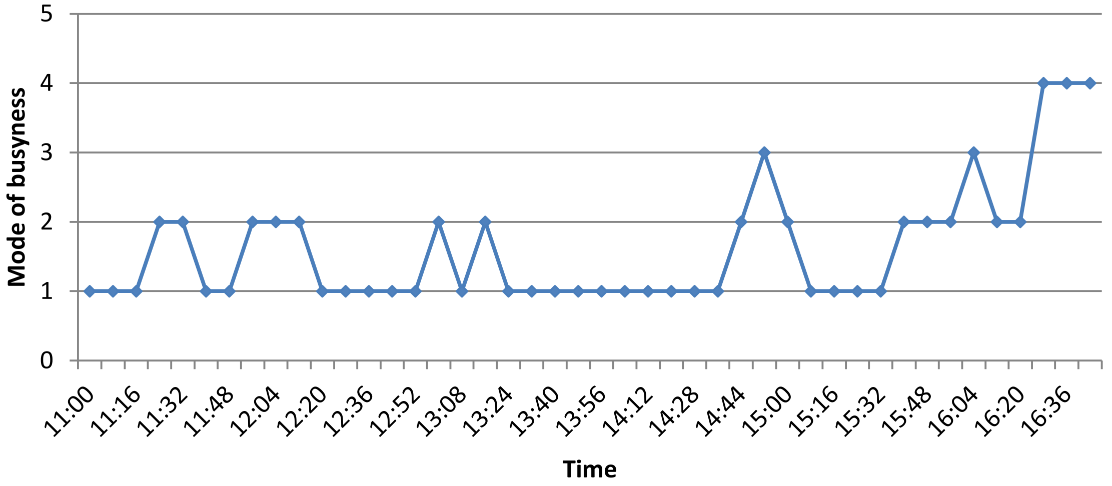


*Figure 4.14: Mode of participants' busyness ratings for each clip, with clips in chronological order at 8 minute intervals.*

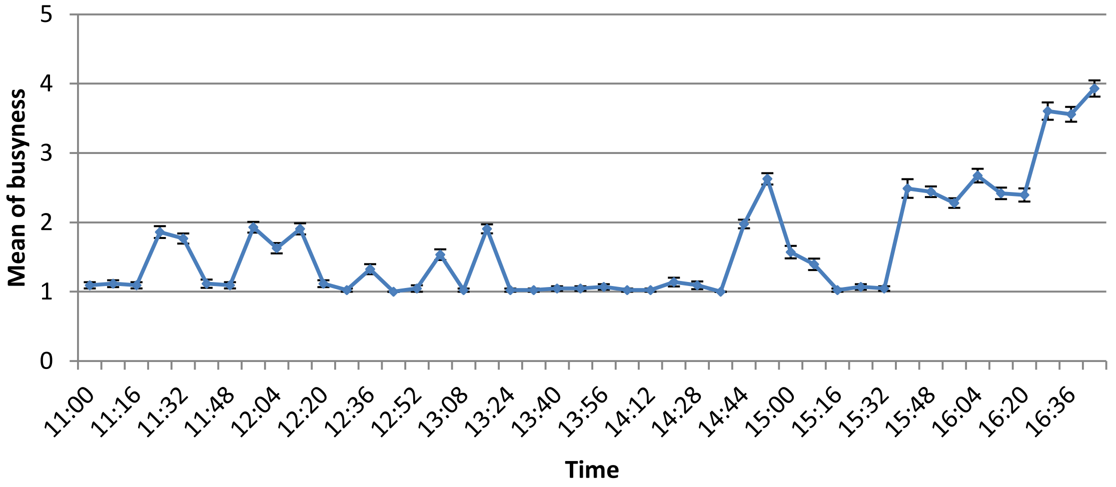


*Figure 4.15: Mean of participants' busyness ratings for each clip, with clips in chronological order at 8 minute intervals. Error bars show standard error*

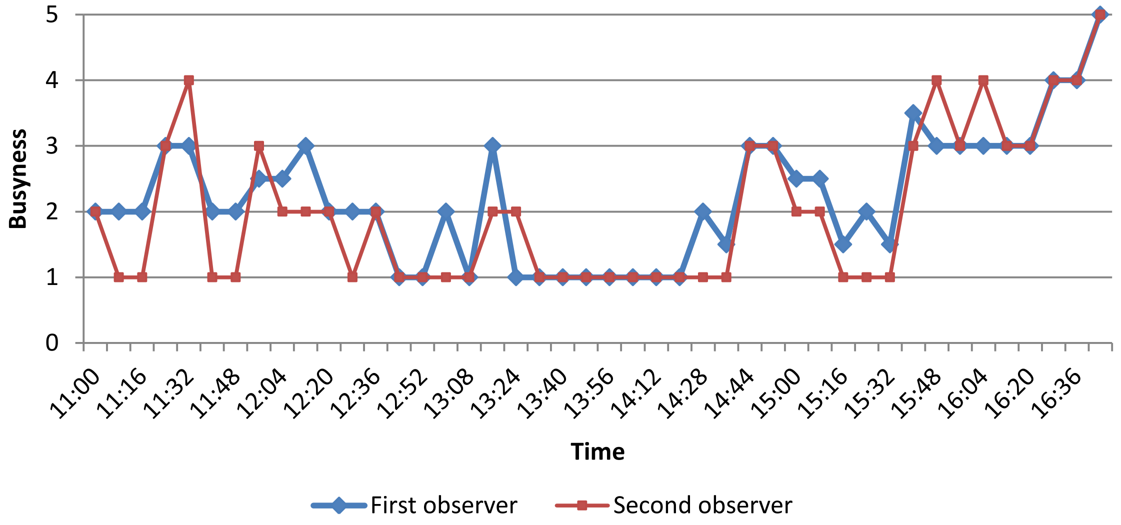


*Figure 4.16: First and second observers' ratings of busyness for each clip, with clips in chronological order at 8 minute intervals (first observer's ratings are the mean of two observations of the video clips).*

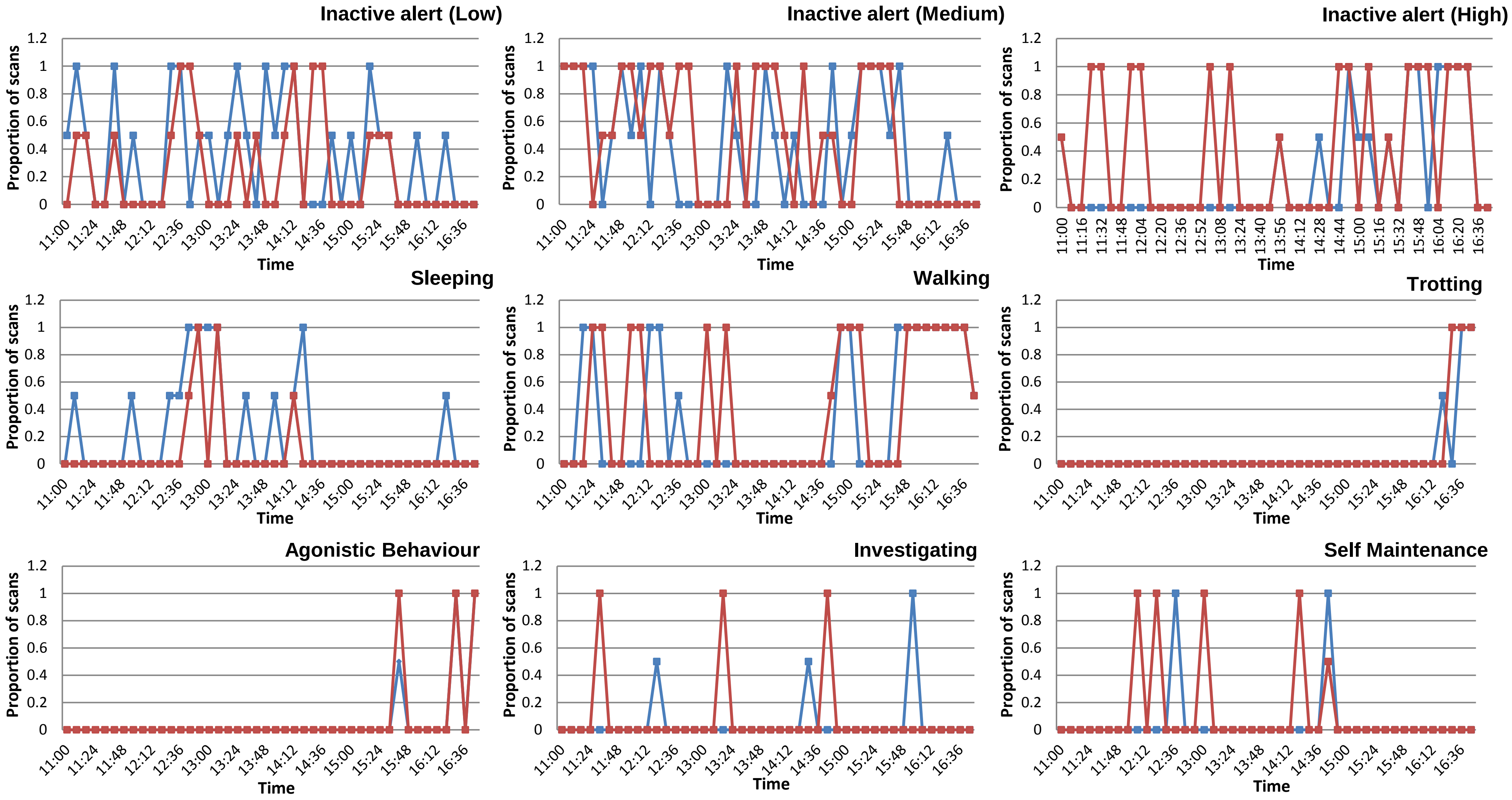


*Figure 4.17: Proportion of observations, as made by two observers, in which behaviours were observed in each minute video clip, with clips in chronological order at 8 minute intervals. Proportions of: 1 indicates both observers recorded the behaviour for that clip, 0.5 indicates one observer recorded the behaviour for that clip, 0 indicates that the behaviour was not observed for that clip.* ■ *Male tiger* ■ *Female Tiger*

### 4.5.3. Discussion

The busyness data generated from the participants formed an obvious pattern of behaviour over the course of the observation day, including an increase in busyness towards the end of the day. This demonstrates that, despite being blind to the chronological order of the video clips, the participant's ratings formed a temporal pattern in tiger busyness. As such, the measure of busyness was successfully utilised to record a clear pattern of behaviour.

This pattern of busyness was similar to that observed in Chapter 3 (Section 3.3.2), with busyness increasing towards the end of the day. Indeed, the husbandry conditions for the current study were the same as for Chapter 3, whereby the tigers were brought inside, and sometimes fed, at around 16:40 each day. It was therefore expected that the tigers would show a pattern of behaviour, and increasing busyness, that anticipated the predictable feeding event, and this was demonstrated. One possible criticism of the results reported in Chapter 3 was the potential for observer bias, as the observers may have been expecting an increase in busyness prior to the feeding time. In the current study there was no opportunity for observer bias, as the participants were blind to the chronological order of the clips, and the order of viewing clips was randomised. That a temporal pattern of busyness was still determined supports the findings of Chapter 3; that tiger busyness increases prior to a predictable feed. It is important to note, that this study is only based on video clips recorded over a single day, therefore there is a possibility that the video clips did not represent a 'normal' day. However, the fact that a similar busyness pattern was recorded to that found in Chapter 3, where observations were carried out during 34 days, under the same husbandry routine and with one of the same tigers as the current study, suggests that confidence in the pattern of busyness reported is warranted.

Patterns of busyness reported over the course of the observation day were very similar between the participants and both observers. Results from the two observers included some higher busyness scores than those reported in the mean and modal scores from the participants. However the temporal patterns were very similar, with peaks and troughs reported around the same time of day, and with all busyness results showing an increase towards the end of the day, prior to the set feed. This agreement between independent observers provides a form of validation for the measure, and indicates that patterns of busyness did reflect actual tiger behaviour patterns.

Further validation of the busyness measure is demonstrated by the traditional behavioural results. The occurrence of particular behaviours in clips during the course of the day coincided with particular patterns of busyness. For example, active, or animated/alert behaviours such as walking, trotting, inactive alert (high) and agonistic behaviours were observed in clips at corresponding times to peaks in busyness. This was particularly the case for the very animated active behaviours such as trotting and agonistic behaviours which were only observed towards the end of the day, as busyness was increasing before the feeding time. In contrast, observations of inactive behaviours or sleeping did not frequently occur in clips corresponding to these high busyness times of day. Indeed sleeping was generally only observed during the times of day corresponding to low busyness scores.

These behavioural results support the results of Study 2 (Section 4.3.2.) in which busyness was positively correlated with active, animated or alert behaviours including walking, trotting and inactive alert (high), and negatively correlated with inactive and sleeping behaviours. In the current study similar behaviours were also observed in video clips corresponding with increases or decreases in busyness respectively. Running was also positively correlated with busyness in Study 2. However observations of running behaviour did not occur at a high enough frequency to be reported in the current study. It can be concluded that busyness does reflect observable aspects of behaviour, and is a measure of a combination of behaviours, with increasing activity and alertness as the busyness scale increases. Busyness has therefore been successfully used to monitor patterns of behaviour over the course of a day, and in the time preceding a temporally predictable feeding event.

## 4.6. Discussion of Chapter 4

The studies presented in this chapter were designed to investigate the utility of busyness, through tests of reliability, validity and suitability for use. The results have shown good supporting evidence for the use of busyness as a measure of animal behaviour, and have been in agreement with predictions made.

In Study 1, busyness showed good inter-observer reliability between the participants' busyness rating scores. Participants generally showed good agreement on their choices of busyness ratings, and Spearman's correlation and intraclass correlation scores indicated good inter-observer reliability. High levels of reliability were also demonstrated between participants' busyness scores in Studies 2 and 3, and also between the experimenters in Study 4, thus demonstrating repeatedly high reliability.

It is essential to establish that a subjective measure is reliable before that measure can be used in scientific study, as the measure is of no use if it is not reliable (Meagher, 2009). Previous tests of some qualitative measures of animal behaviour have found them to be suitably reliable. For example, as mentioned in Section 4.1.1, studies of behavioural trait ratings (Carlstead, Mellen et al., 1999 and Wielebnowski, 1999) have demonstrated good levels of inter-observer reliability. Also, the FCP method has been tested in over 60 trials, with significant agreement demonstrated between observers (Wemelsfelder, 2007), and reliability testing of ratings of e.g., elephant social behaviour and horse personality (Freeman, et al., 2010; Lloyd, et al., 2007) has ensured that further analysis uses only reliable data.

The current chapter has also demonstrated good inter-observer reliability of busyness. Intraclass correlation values were high, and were at the high end of the range of ICC values reported in the literature. As discussed in Section 4.2.3, various ICC values have been reported in tests of qualitative measures. For example King and Figuerdo (1997) reported ICC values of .33 to .88 and McCrae and Costa (1987) reported a range of ICC values for human personality traits of .30 to .65, which they stated as significant. When evaluating reliability ratings, it is important, not only to consider the significance level, but also the value of the reliability score itself. In fact, the reliability score may be more important than the *p*-value, which gives little information on the degree of reliability (Martin & Bateson, 1993). As discussed in Sections 4.1.1 and 4.2.3, requirements for good reliability have been set at over .70 or .75 (Chang, n.d.; Portney & Watkins, 2000), therefore the reliability scores achieved in the current study are good by those criteria. Hence busyness has shown repeatedly high reliability and should thus be considered to be a suitable measure for use, particularly in comparison to some of the measures reported in the literature.

The good inter-observer reliability is particularly notable when it is considered that the student participants in this study had no previous training in animal behaviour, and received only the instruction sheets shown in Appendices 4.1-4.2. As noted in Section 2.7, other discussions of qualitative measures for animal behaviour studies have suggested that observers require prior experience of animal observation e.g., Wemelsfelder (2007). Although Wemelsfelder et al. (2000) earlier reported success in using untrained observers to record behaviour. That the current study demonstrated reliability, despite a lack of experienced participants, demonstrates that the measure of busyness may be suitable for reliable use with relatively untrained observers.

As the measure of busyness is composed of only a single scale, the reliability value is a clear indication that the measure is reliable between observers. This is in contrast to some other qualitative measures in which scoring takes place of multiple traits, some of which may be unreliable, but the focus is on the mean reliability value. For example, Weiss et al. (2006) reported ICC values for items of orang-utan personality ranging from .20 to .79 with a mean reliability of .42. It was concluded that observers were able to reliably assess orang-utan personality; however it is not clear whether all behavioural items were included in the further Principal Components Analysis used to determine the components of orang-utan personality.

Some studies have excluded unreliable traits from the analysis and have thus demonstrated that through reliability testing, the final measure is robust. For example, Wielebnowski (1999) excluded the traits ‘friendly to people’, ‘friendly to conspecifics’ and ‘solitary’ from further analysis of cheetah behavioural variation, as reliability values for these traits were <.48, with *p*-values >.20. In order that the reliability of qualitative measures can be assured, it is preferable for measures to be transparent, with all aspects (e.g., traits) achieving suitable levels of reliability. As a single measure, this was not an issue with busyness. However, it was important (and achieved) that this, single measure could stand alone, showing high reliability.

In addition to showing good reliability, the results in this chapter have demonstrated busyness to be valid and meaningful in terms of animal behaviour. It was predicted that the different levels of busyness would relate to particular behaviours, and that there would be a correlation between busyness and the occurrence of certain behaviours. This was indeed the case, with particular behaviours being observed at each busyness level. More active and animated behaviours occurred at the higher end of the busyness scale, which was also reflected in the correlations. Higher busyness levels were also found to encompass an element of alertness, with more alert behaviours occurring at higher busyness ratings.

As described in Section 4.1.2, relationships between qualitative measures and certain behavioural observations have also been shown in previous validation studies of qualitative measures. For example, Rousing and Wemelsfelder (2006), Carlstead, Mellen et al. (1999) and Wielebnowski (1999) found significant correlations between ratings of behavioural traits and behavioural observations. Also, Lloyd et al. (2007) validated ratings in the Horse Personality Survey (HPS) against behavioural observations, and Capitanio (1999) also found that measures of personality correlated with certain behaviours in the behavioural repertoires of rhesus macaques (*Macaca mulatta*), for example the measure of 'confidence' correlated with aggressive behaviours. In accordance with some of the previous literature on qualitative methods, the current study has gone some way to demonstrating good construct validity of busyness.

Establishing validity and reliability means that the results obtained using a qualitative measure will be useful and meaningful when the measure is applied to a scientific question. In the current chapter, busyness, once validated, was successfully used (in Study 4) to demonstrate a pattern of tiger behaviour over the course of the day, and in the lead up to the feeding time. This further supported the findings of Chapter 3 that tiger busyness increases in the lead up to a predictable feeding event, potentially in anticipation of that event. Study 4 also provided further validation of busyness as demonstrated by the correspondence between peaks in busyness and the occurrence of certain behaviours at particular times of day. As predicted, certain behaviours (such as walking, trotting and inactive alert high) were observed more frequently in video clips later in the day, when busyness scores were higher. These were the same behaviours found to correlate with busyness in Study 2. Hence, this chapter has produced multiple validations of busyness against traditional behavioural results.

Study 3 revealed that greater detail of instructions (on the busyness scale) resulted in higher means of busyness scores, and higher values for inter-observer reliability. Whilst the difference between the means (low to medium, and low to high detail instructions) was statistically significant, the magnitude of the differences between instruction conditions was not large, and there was little difference between modal scores for each video clip for each instruction condition. However, that there was a difference between instruction conditions highlights the importance of taking into account the types of instructions that are to be provided alongside qualitative measures. The types of instructions are also important to consider in terms of providing a method that is easy for participants to use, and as Study 3 reported fewer difficulties in interpretation with more detailed instructions, sufficient detail should be provided to aid participants in using the measure.

As increased detail of the instructions and hence increased ‘training’ resulted in higher busyness scores, it may be useful in future work, to determine whether observers who have undergone more training, and who are more experienced at animal observation, produce different results to untrained participants. Indeed the busyness scores of the two, more experienced observers in Study 4 were often higher than those of the student participants. It may therefore be useful to further test busyness by comparing results given by experienced versus inexperienced observers.

Another area for future research would be to determine the reliability of busyness when used on enclosures containing different numbers of animals. This chapter has demonstrated reliable use of the busyness technique on a group size of two, and also a group size of four, but further work, on different group sizes would further develop the measure. With the exception of Study 1, this chapter focuses on an enclosure containing two tigers. If the two individual animals are performing very different behaviours then there is potential for different interpretations of the different levels of busyness. For example, a rating of 3 could mean that one animal was very active, and the other was not, or that both animals were moderately active. There may be potential for disagreement between observers if the two individual animals are performing different behaviours, as one observer may focus on a particular animal, and another may give an average rating. However, as busyness is defined as a measure of the entire enclosure then this should not be problem, as it is not intended to distinguish particular behaviours and indeed, inter-observer reliability was found to be good, despite using two tigers. This does however highlight the need for instructions to be clear and account for this. Indeed, the participants in Study 3 left feedback regarding difficulty in rating busyness when the two tigers were behaving differently. Guidance should therefore be given for how to interpret this situation. However, it is important that the instructions do not become too prescriptive in order to retain the benefits of a subjective measure that has already proved to be effective and reliably used with multiple observers.

Busyness has so far used a 1 to 5 scale, however observers in the current research questioned whether a score of 0 should be recorded for occasions when no animals were visible. As a measure of the overall busyness of the ‘enclosure’, this might seem sensible, but it is instead recommended that busyness is not given a value for intervals when no animals are visible, as scores of 0 may lead to misleading averages when interpreting behaviour.

Busyness has been demonstrated to be both reliable and valid, and therefore has potential for use in the study of predictability and anticipation. The measure of busyness is particularly useful for such study because it has been demonstrated to encompass a number of different behaviours. As discussed in Chapter 2, a range of behaviours have been reported to change in anticipation of an event, with no, single behaviour constituting an anticipatory response. The measure of busyness therefore can cover a number of behaviours which may comprise a change in the overall behaviour of the animal prior to a predictable event, which may not have been so easily recorded by a method in which behaviours are analysed individually. Such 'overall' observation has been cited as an advantage of qualitative methods. In their basis of human interpretation, they become integrative measures of aspects of behaviour, and so provide a level of information that is not available from objective measures (Wemelsfelder, 1997).

Another advantage of busyness is that it can be used as a 'real time' sampling measure. Busyness provides data for each minute of observation, and as such can provide information on temporal patterns of behaviour (as demonstrated in Chapter 3, and Study 4 of the current chapter). Some other, previously validated qualitative measures require observation over a period of time, followed by an overall rating value. For example ratings of behavioural traits and personality provide a single rating value for each trait, per animal, for which the observer is often required to have substantial experience of the animal (e.g., King & Landau [2003]). Wemelsfelder et al. (2009) were able to use a qualitative measure (the FCP technique) to record the behaviour of pigs after just 3 minutes of observation. However, this measure, whilst quick to use, still only achieved a single measure per individual. An advantage of busyness is that it can provide an ever-changing measure of behaviour, in accordance with the animal's behavioural changes over time.

In summary, this chapter has demonstrated on multiple occasions, that busyness shows substantial reliability between observers and hence it is suitable for use with multiple observers. Validations of busyness against traditional measures of animal behaviour data collection have demonstrated busyness to be meaningful in terms of behaviour, and to be related to active, animated and alert behaviours. Busyness ratings have also been shown to correspond to observable patterns of animal behaviour derived using more traditional methods, and hence shows potential for use in studies of predictability and anticipation. The studies reported in this chapter have also demonstrated that busyness can be used by relatively untrained observers, and that increasing the detail provided to the observers on the anchor points of the scale, increases (although only slightly) the inter-observer reliability, and increases use of the full range of the busyness scale. Thus, the results of this chapter demonstrate that people have the capacity to record animal behaviour in a consistent fashion using subjective measures. The busyness ratings chosen by observers were not based on individual beliefs or biases, but rather a shared view of what constitutes busyness. Therefore, by testing the reliability and validity of busyness, it has been demonstrated that busyness can be used, by multiple, relatively untrained observers to record observable aspects of behaviour.

This process of testing for reliability and validity is therefore important in the development of useful qualitative measures. A qualitative measure of animal well-being using keeper scores is currently being tested by the Chicago Zoological Society at Brookfield Zoo (Whitham & Wielebnowski, 2009), which is being compared with instantaneous and all occurrences sampling of behaviours and faecal glucocorticoid levels. If it is found to be reliable and valid, this measure will then be circulated to other zoos to be used to monitor and manage welfare issues. Similarly, it is hoped that the measure of busyness can now be used in further work, to provide useful data on animal behaviour, particularly in relation to anticipation and predictability. This was achieved in Chapter 5 of the current thesis, where busyness was used in a ‘field’ situation, to record the behaviour of meerkats, and as a further test of the application of busyness.

# Chapter 5

## *The effect of predictable feeding times on meerkat (Suricata suricatta) behaviour*

### 5.1. Introduction

Chapter 3 revealed changes in tiger behaviour in the time leading up to a predictably timed feeding event, such that the behavioural changes anticipated the feeding time. However, in order to confirm anticipation and establish that the feeding time was controlling behaviour, there was a need for an experimental study in which feeding times are manipulated. As mentioned in Chapter 3, manipulation of the tiger feeding regime was not possible for management reasons and also due to the birth of tiger cubs. The current chapter therefore describes research that was carried out on captive meerkats (*Suricata suricatta*); a carnivore species whose feeding time could be manipulated. In the current chapter feeding times were manipulated to give two experimental conditions: temporally predictable and unpredictable feeding regimes. Two experiments are described; an initial comparison of meerkat behaviour on predictable and unpredictable routines, and a repeat study carried out at a time in which there were fewer visitors in the zoo (as described further in Section 5.5).

The current chapter used a number of measures to investigate the effect of a predictable feeding time on meerkat behaviour, including busyness and as such, provided a further test of the utility of busyness on a different species.

As described in Chapter 3, predictable feeding times can lead to changes in felid behaviour that anticipate the feeding time. However behavioural changes have also been demonstrated in other animal taxa, as described in Section 1.4.2. For example, coyotes, François' langurs, sea bream and hamadryas baboons (Gilbert-Norton, et al., 2009; Krishnamurthy, 1994; Sánchez, et al., 2009; Wasserman & Cruikshank, 1983 respectively). Unpredictability of feeds, in terms of early or late deviations from a set feeding time has also been shown to affect behaviour, for example, Waitt and Buchanan Smith (2001) demonstrated that stump-tailed macaques showed increased self-directed behaviour, inactivity and abnormal behaviours when waiting to be fed.

As described in Chapter 1, there is a lack of agreement in the literature over the welfare implications of predictability in a captive environment, and hence whether predictable or unpredictable routines are better for animal welfare. For example, Ulyan et al. (2006) tested brown capuchins on predictable and unpredictable feeding schedules and suggested that predictable schedules are better for animal wellbeing. This was because the capuchins spent more time in proximity, activity, and social behaviour and had lower cortisol concentrations in the predictable condition. In contrast to this, Bloomsmith and Lambeth (1995) found that groups of chimpanzees fed on an unpredictable schedule showed lower levels of inactivity and abnormal behaviours in the period prior to feeding, than chimpanzees fed on a predictable schedule.

Whilst anticipatory behaviour prior to set feeds has been documented for several species, it has not, to the researcher's knowledge, been specifically studied in meerkats and hence the effect of predictable feeding times on captive meerkat behaviour is unknown. Anecdotally, meerkats appear to be popular zoo exhibits (e.g., Murden, 2010), and many UK zoos are now offering 'Meet the Meerkat' experiences in which visitors are able to feed the meerkats. Such zoos include Banham Zoo (2009), Paignton Zoo (2011), Paradise Wildlife Park (nd) and The Wildlife and Dinosaur Adventure Park, Combe Martin (nd) to name a few (it is important to note that the meerkat experience at Paignton Zoo was not running at the time of this study). In addition to feeding for husbandry, such definite feeding events can become fixed at set times. These set times may then have consequences for meerkat behaviour, as in other species, which furthers the interest in studying the effects of predictable feeding on meerkat behaviour.

### 5.1.1. Introduction to the meerkat

The slender-tailed meerkat (*Suricata suricatta*) is a small carnivore from the family Herpestidae (mongoose). Meerkats are widespread across western parts of southern Africa, including areas of Namibia, Botswana, South Africa and Angola, in habitats of shrubland and desert (Macdonald & Hoffmann, 2008). There are no major threats to the conservation of this species and hence the conservation status, given by the International Union for Conservation of Nature (IUCN) is “Least concern” (Macdonald & Hoffmann, 2008).

Meerkats are diurnal, although they have been observed to avoid activity outside of their burrows during the middle of the day, when temperatures in southern Africa are high (Doolan & Macdonald, 1997). Foraging activity occupies most of the active period, and diet in the wild consists of mostly insects plus some reptiles, small mammals and birds (Doolan & Macdonald, 1997; Kingdon, 1997). Meerkats forage individually but in close proximity to other meerkats (Kingdon, 1997) and foraging is a highly active process, involving sniffing the ground, scratching and raking at the soil surface and digging holes, whilst making contact vocalisations with other members of the group (Doolan & Macdonald, 1997; Ewer, 1963).

Meerkats are co-operatively breeding animals living in groups of 2-50 individuals (Thornton, 2008). A dominant male and female meerkat are the parents of around 75% of the young meerkats in a group (Clutton-Brock et al., 1999) and the other adults in the group, both male and female, assist in the rearing and provisioning of food to these offspring (Brotherton et al., 2001; Clutton-Brock, et al., 1999). Group members also assist in predator detection duties. Meerkats raise themselves into a high posture, standing on their hind feet with their whole body extended vertically. This is used as a look-out posture (Ewer, 1963). Group members alternate in keeping guard for predators from this, raised position (Clutton-Brock, et al., 1999) and they alert the other meerkats with alarm calls if a predator approaches.

Meerkats in captivity are often held in smaller groups than found in the wild (Wemmer & Fleming, 1975). A husbandry guide produced by Bristol Zoo states that feeding should take place once or twice per day and that food types given in zoos are varied, and can include: live invertebrates, such as mealworms and crickets; meat items, such as day-old chicks; fish, eggs, fruit and concentrated commercial feeds (Brown & Partridge, n.d.).

Research on captive meerkats has shown that they display a similar repertoire of behaviours as those observed in the wild. For example, captive meerkats will spend a large proportion of time foraging, particularly if provided with feeding enrichment (Brown & Partridge, n.d.). Vigilance behaviours are also shown by captive meerkats. Moran (1984) observed that at least one member of a captive group of meerkats would be situated in a prominent, elevated position at almost all times, engaged in vigilance behaviours. In association with vigilance, captive meerkats have been reported to give all alarm calls that have been documented in the wild (Hollén & Manser, 2007). Vigilance behaviours have been connected to keeper activities as well as predators, and in an undergraduate research dissertation Hatersley (2005) noted a high proportion of vigilance behaviours prior to a predictable feed.

### 5.1.2. Rationale, aims and predictions

The current study was designed to allow comparison of behaviour on predictable and unpredictable feeding routines in an experimental study. This study was carried out on meerkats, a species for which the effect of predictable feeding has not yet been determined, and for which it might be expected that predictable feeding might be used in zoos for husbandry reasons and for visitor feeding experiences.

An aim of this study was therefore to investigate the effect of a predictable feeding time on the behaviour of captive meerkats, by investigating behavioural changes throughout the day on a predictable feeding routine, and by comparing meerkat behaviour prior to predictable and unpredictable feeding times. Another aim was to investigate the types of behaviour that are affected by predictable routines, in order to identify which behaviours are indicative of an anticipatory response.

Due to the lack of previous research on meerkat anticipatory behaviour, and the lack of consensus in the literature over the effects of predictable routines, the current research was largely inductive in its exploration of the effect of predictability on meerkat behaviour. As predictability has been shown to affect behaviour in other species, it was predicted that patterns of meerkat behaviour would show differences between the feeding conditions, with certain behaviours increasing or decreasing in the time leading up to the predictable feeding time. However, due to the lack of previous research, the direction of change of specific behaviours could not be predicted (see later in the current section). As a consequence of these behavioural changes (and as a comparison to previous research which has often only compared sample observation sessions) it was hypothesised that meerkat behaviour in the half hour directly preceding a predictable feed would differ significantly from their behaviour in the equivalent observation sessions prior to feeds on an unpredictable schedule.

Given that meerkats adopt look-out postures (referred to as vigilance in the current study), it was possible that the meerkats would show this behaviour when looking out for their keepers. Hence it could be expected that the meerkats would show a pattern of increased vigilance behaviours, including increased proportions of time spent in vigilance, and increased acts or rates of rising up into the standing posture (termed standing in the current study), in the period leading up to the predictable feed, as the meerkats anticipated, and looked out for, the arrival of their keepers. These vigilance behaviours could therefore be expected to be greater in the observation sessions prior to feeding on a predictable schedule as compared to an unpredictable schedule. On the other hand, it could also be argued that vigilance behaviours might decrease prior to feeding; Clutton-Brock et al. (1999) observed that the provision of food can increase the contribution of meerkats to vigilance behaviours. Hence it could be assumed that the inverse might happen when nearing a feeding time; when the longest time has passed since the previous feed, the meerkats might decrease their contribution to vigilance. Therefore, it was predicted that predictable feeding would cause a change in meerkat vigilance, but the direction of that change could not be predicted.

The methods employed in this study, again consisted of extended periods of observation in order to determine more detailed patterns of behaviour than in previous literature. This study also aimed to investigate the effect of a predictable feeding time on meerkat busyness and to further develop the measure of busyness by testing it on a different species, and in conditions where the feeding schedule had been manipulated in a manner that might be expected to alter behaviour. An important question was whether busyness ratings would be capable of reflecting patterns of behavioural change prior to feeding in a field setting. The current study therefore aimed to further validate busyness ratings against traditional measures of behaviour.

## 5.2. Methods for Study 1

### 5.2.1. Subjects, housing and husbandry

Subjects were initially two male and three female, adult (aged 3-6 years) slender-tailed meerkats (*Suricata suricatta*), marked with hair dye to enable individual identification. These meerkats were housed at Paignton Zoo Environmental Park, Devon, UK, in a walled enclosure, composed of 2 sections (sections 1 and 2) of approximately 11m x 7m each, joined by 2 pipes running through a dividing wall. Enclosure furnishings consisted of mud and bark chip areas, in which the meerkats could tunnel and forage for insects; bushes and small trees; rocks and logs; raised areas underneath heat lamps, and a house. One section of the enclosure was shared with a pair of porcupines who also had a house area, which the meerkats could enter. Visitors could view the meerkats along two sides of the enclosure by looking over the wall. Appendix 5.1 shows photographs of these enclosures.

The meerkats were kept in section 1 of the enclosure overnight and were given access to both sections in the morning, when the enclosure was also cleaned. On the 13th day of the unpredictable feeding condition (see Section 5.2.2 for the feeding timetable), it became necessary to separate the meerkats due to serious aggression in the social group. After this separation, it was decided to continue to study only one male and one female meerkat, now in a pair, since they experienced the least disturbance likely to affect behaviour as a result of this separation. This pair of meerkats was kept in section 1 of the enclosure for the remainder of the study, with access to section 2 on alternate days. The remaining meerkats also had alternate access to section 2 on alternate days but were housed in a new enclosure section and were mixed with a new female meerkat; hence observation of these meerkats was discontinued. The focus of the current studies reported in this chapter was therefore on the pair of meerkats.

### 5.2.2. Feeding routines

Prior to the study, the meerkats were fed in the morning and afternoon but with no clearly defined feeding times. A preliminary pilot study of the meerkats on their existing husbandry routines was used in order to develop an ethogram and ensure that there were no other, pre-existing daily patterns of behaviour that occurred around the time that was to become the predictable feeding time. The meerkats were then studied in two different feeding conditions; unpredictable and predictable, as described in Table 5.1. The manipulated feeding time of interest for this study was the second feed (afternoon) of the day.

*Table 5.1. Details of the two feeding routines used in this study.*

| Feeding routine | Duration | Time of first (morning) feed | Time of second (afternoon) feed |
|---|---|---|---|
| Unpredictable | 30 days | 10:00 | Random time at any, 15min interval between 12:30 and 17:45 (pre-determined using a random number generator in Microsoft Excel) |
| Predictable | 92 days | 10:00 | 15:30 |

The predictable condition followed directly after the unpredictable condition in a within subjects design. The keepers fed the meerkats at the pre-determined times as closely as their other duties permitted. A keeper log was kept, recording the actual time at which the keepers arrived at the enclosure to feed the meerkats. The results were reviewed after 30 days of the predictable condition and it was found that none of the predicted trends of anticipation were apparent. Johnston and Pennypacker (2009) suggest that “phases should last until satisfactory stability has been obtained” (p217), and this was not achieved after 30 days. As this study was somewhat inductive in design, and no previous research exists on the speed of learning in meerkats, it was decided to extend the length of the predictable condition, to further investigate meerkat behaviour over this time, in case the meerkats had not yet had enough time to learn the routine or had been affected by a long period of bad weather, which occurred during observations.

### 5.2.3. Behavioural data collection

*Behavioural trends throughout the day*

Meerkat behaviour was recorded using instantaneous scan sampling of state behaviours at 1 minute intervals, in 30 minute observation periods throughout the day between 08:30 and 18:00, organised so that over the course of the study, data were collected to cover each 30 minute session in the day with either 15 or 16 repeats in the predictable condition and 5 or 6 repeats in the unpredictable condition. This different number of repeats was due to the differing lengths of the conditions as detailed in Table 5.1. Data were collected throughout the day in order to both investigate any patterns in behaviour, and to habituate the meerkats to the presence of the observer and prevent them from associating the arrival of the observer with the imminent arrival of food and hence using the observer as a feeding cue. The meerkats were fed by a keeper and not by the observer to further avoid this association. Instantaneous scan sampling was used as the most appropriate method to obtain data on all five meerkats (as observed at the start of the study) at the same time, as it allowed each individual to be scanned on the sampling interval.  This method also enabled the calculation of proportions of behaviours in 30 minute periods throughout the day.

Recorded behavioural states included 3 vigilance states; standing in a sentry position, ‘high vigilance’ and ‘low vigilance’, as well as locomotion, foraging/investigating, feeding, resting, enrichment-directed, social, agonistic and self maintenance behaviours (see Table 5.2 for full descriptions of behaviours).

The event behaviour ‘standing’ (the act of rising up into a full standing, sentry position) was recorded by all occurrences recording for each minute of the 30minute observation period, for any meerkat (see Table 5.2).  The presence or absence of keeping staff was also recorded each minute, as was the number of meerkats not visible for the entire minute.

### *Busyness*

Busyness (as previously described in Section 2.9 and Chapters 3 and 4) was recorded for every minute of the 30 minute observation periods on a scale of 1 to 5 (very quiet – very busy) based on the behaviour of all meerkats in the enclosure.

### *Effect of feeding condition on pre-feed behaviour.*

The data collection described above included observation sessions during the 30 minute period immediately prior to the feed time.  In the unpredictable feeding condition this involved the 30 minutes prior to a randomly chosen (unpredictable) feeding time (see Table 5.1), and in the predictable condition this involved the 30 minute session at 1500 to 1530, immediately prior to the set feed.  Over the course of the study, data were collected in 16 pre-feed sessions in the unpredictable condition (7 before the meerkat group was split up, and 9 after the split) and 38 pre-feed sessions in the predictable condition.  Data collection always started early, and if necessary finished late so that, in addition to observing behaviour prior to the timetabled feeding time, data were obtained for the 30mins immediately prior to the exact feeding time (for days when feeding was not exactly on time), in order to record any effects of slight deviations from the routine.

*Table 5.2. Descriptions of meerkat behaviour categories*

| **Behavioural Category** | **Description of Behaviour** |
|---|---|
| ***State behaviours*** | |
| Not Visible | Animal is obstructed from view. |
| Vigilance | Sentry: Meerkat is standing on hind feet in the tallest bipedal stance, looking at or around environment.<br>High sit vigilance: Meerkat is sitting upright with back straight and forelimbs off the ground, looking at or around environment.<br>Low sit vigilance: Meerkat is sitting on hind legs with all 4 paws on the substrate or is sitting with its lower quarters supported by the substrate, looking at or around environment. |
| Resting | Meerkat is stationary, sitting or lying in the enclosure. Eyes may be closed. |
| Locomotion | Activity involving movement of the whole body from one spot to another that is greater than one body length of the animal. This may include running, trotting, walking, jumping and climbing. |
| Self-Maintenance | Meerkat uses mouth or paws to groom and lick fur. |
| Foraging / investigating | Meerkat sniffs, scratches or digs at the ground or enclosure objects. Meerkat may walk whilst foraging with its head and nose to the ground or enclosure object. |
| Feeding | Consuming food. |
| Enrichment-Directed Behaviour | Any behaviour that is directed towards an environmental enrichment object (separate to normal enclosure furniture) in the enclosure. Behaviours may include moving the object with paws, body or head, carrying, holding, licking, chewing, ripping, etc. |
| Social Activity | Behaviour involving interaction between two or more meerkats excluding agonistic behaviour. |
| Agonistic Behaviour | Behaviour between meerkats related to fighting or defence. |
| ***Event behaviours*** | |
| Standing | Meerkat raises forelimbs off the ground and stands up on hind paws to rise up into in the tallest bipedal stance; the act of standing up |

### 5.2.4. Data analysis

*Behavioural trends throughout the day*

In each feeding condition, the data were collated in the following way: the proportion of scans of each state behaviour was calculated for each 30 minute observation session. Mean proportions were then calculated for all of those 30 minute sessions at each time of day. These means were then plotted and used to determine patterns of behaviour over the course of the day, and prior to the predictable feeding time.

A rate of standing for a single meerkat was calculated for each minute of observation in the predictable condition by dividing the number of standing events by the number of meerkats visible for that minute. Mean rates were then calculated for each minute of observation over all of the days of observation in the predictable condition. Minutes in which no meerkats were visible were excluded from the analysis. Standing behaviour was analysed twice, firstly using the full set of data and again, omitting data in which zoo staff were present at the enclosure. This was to remove any effect on meerkat behaviour associated with keeper presence. The mean rate of standing events was also calculated in each of the 30 minutes leading up to the exact (rather than timetabled) feed time, over all days of observation (with the data collected when the keepers were present removed), and descriptive analysis was used to determine any patterns in standing behaviour leading up to the exact feed time.

Mean busyness scores were calculated for each minute of the day, over all observations in the predictable condition. These scores were analysed descriptively to determine any patterns of meerkat busyness over the course of the day, in particular, prior to the feeding time. As in Chapter 3, descriptive analysis of the data was suitable to determine detailed patterns of behaviour. As discussed in Chapter 2, the study of predictability would benefit from consideration of behaviour over an extended period of time, hence descriptive analysis enabled the investigation of the extent of patterns of behaviour prior to feeding, in context with patterns throughout the day.

### *Effect of feeding condition on behaviour*

To determine any patterns of change in pre-feeding behaviour across the duration of the study, the proportion of scans spent in each state behaviour were plotted for the pre-feed 30 minutes on each day of observation, in both feeding conditions. For the unpredictable condition this was the pre-feed 30 minutes at a random time, and for the predictable condition this was the 30 minutes starting at 15:00, prior to the timetabled feed at 15:30. In order to statistically determine any effect of the feeding condition on pre-feeding behaviour (as demonstrated in previous literature such as Bloomsmith & Lambeth [1995]), randomisation analyses (Design 3) (Todman & Dugard, 2001) (chosen for their suitability for use with small-n designs) were performed to compare the mean proportions of each state behaviour in the pre-feed observation sessions, in the predictable and unpredictable feeding conditions for both meerkats (using 2000 re-randomised pseudosamples). Randomisations were run three times, and average scores were calculated.

Analysis of early and late feeds (when feeding was not exactly on time in the predictable condition) was carried out by plotting the proportion of scans spent in all vigilance behaviour for the exact 30mins of observation before the feeds that were: a) early by 2-10mins, b) on time plus or minus one minute, c) late by 2-10 mins or d) late by over 10 mins. Randomisation analyses (Design 6) (Todman & Dugard, 2001) were used to determine any significant differences between these on time, early and late conditions for both meerkats (using 2000 re-randomised pseudosamples). Randomisations were run three times, and average scores were calculated.

In the following section (5.3), results from the predictable feeding condition are considered first, followed by comparison to the unpredictable condition, since predictable feeding was the main condition for the investigation of patterns of anticipatory behaviour.

## 5.3. Results of Study 1

Sections 5.3.1 and 5.3.2 (below) report data from the patterns of mean proportions of state behaviours throughout the day in the predictable and unpredictable feeding conditions (Figures 5.1-5.8). Section 5.3.1 focuses on patterns of behaviour in observation sessions up to 1.5 hours prior to the 15:30 feed time, whereas Section 5.3.2 reports daily patterns of behaviour, in order to place the findings of Section 5.3.1 into context.

### 5.3.1. Behavioural trends prior to 15:30

Of all state behaviours recorded for both meerkats, there were few clear patterns of increase or decrease in proportions in the observation sessions leading up to the feeding time in the predictable condition, in comparison to other variation in proportions of the behaviours throughout the day (Figures 5.1,5.3, 5.5 and 5.7). There were no clear patterns of increase or decrease prior to predictable feeding for the male meerkat, although a slight pattern of increase in proportions of foraging behaviour was shown leading up to the feeding time. However, this was not clearly discernible from patterns throughout the rest of the day. The female meerkat showed patterns of increase in ‘vigilance high’, ‘vigilance low’ and ‘all vigilance’ behaviour in the observation sessions leading up to the 15:30 feeding time (Figures 5.1 and 5.3). However it should be noted that there was variation in the data and other, greater peaks in proportions of these behaviours were shown at other times of the day (as described in Section 5.3.2).

Whilst the female meerkat showed some patterns of increased proportions of vigilance behaviours in the observation sessions leading up to the predictable feeding time (15:30), comparisons with a comparable time in the unpredictable feeding condition revealed only limited differences between the two conditions. Proportions of ‘vigilance high’ behaviours were higher immediately prior to 15:30 in the predictable condition, than the unpredictable condition, however there was variation in the data in the unpredictable condition (Figure 5.2). Proportions of ‘vigilance low’ behaviour prior to 15:30 for the female meerkat were marginally higher in the predictable than the unpredictable feeding condition, however the same pattern of increase in sessions leading up to 1530 was demonstrated in both conditions.  Proportions of ‘all vigilance’ behaviour for the female meerkat appeared to be higher prior to 15:30 in the predictable feeding condition.  There were no other clear differences in proportions of behaviour prior to 15:30 in the two feeding conditions.

### 5.3.2. Behavioural patterns throughout the day in the predictable and unpredictable feeding conditions

Both meerkats showed some regular patterns in the proportions of different state behaviours throughout the day, which require description in order to place the pre-feed behaviours into context.  Overall, similar patterns were shown throughout the day in both feeding conditions; however those differences that did exist will be described here.

The male meerkat showed similar patterns of ‘vigilance high’ in both feeding conditions, with low proportions (<.01) in the morning, gradually increasing throughout the day, but reaching no higher than .09 in either condition (Figures 5.1-5.2).  Proportions of ‘vigilance high’ were higher, but more variable for the female meerkat, with peaks shown during the predictable condition at 10:30, 13:00, 15:00 and 16:30 and low levels at 10:00, 12:30, 14:00 and 15:30 (Figure 5.1).  Similar variation was also shown in the unpredictable condition; however the peak proportion of .38 at 16:30 was not shown in the unpredictable condition.

In contrast to the ‘vigilance high’ results, the male meerkat showed higher levels of ‘vigilance low’ behaviour over the day than the female meerkat in both conditions (Figures 5.1-5.2).  In the predictable condition, peaks were shown in the morning (maximum of .43 in the 11:00 session), with the exception of low levels in the 10:00 session (.13) (Figure 5.1).  Proportions were then fairly constant throughout the rest of the day, with slightly lower levels in the 15:00, 15:30, 16:00 and 17:30 sessions (.19, .17, .18 and .16 respectively).  Similar patterns were shown in the morning in the unpredictable condition (Figure 5.2) however proportions were lower in the middle of the day (11:30-15:00), and a peak of .39 was shown at 16:00 in the unpredictable condition compared to a peak of 0.30 at 17:00 in the predictable condition.   The female meerkat showed low levels of ‘vigilance low’ behaviour throughout the day in the predictable condition (Figure 5.1), with a minimum proportion of 0.03 in the 12:30 and 17:30 sessions, with .04 in the 10:00 session.  There was a slight peak of .11 in the 15:00 session.  More constant proportions of ‘vigilance low’ were shown throughout the day for the female meerkat in the predictable condition compared to the unpredictable condition, where levels dropped to .03 at 14:00 and then rose to .13 at 15:30 (Figure 5.2).

## Predictable feeding condition

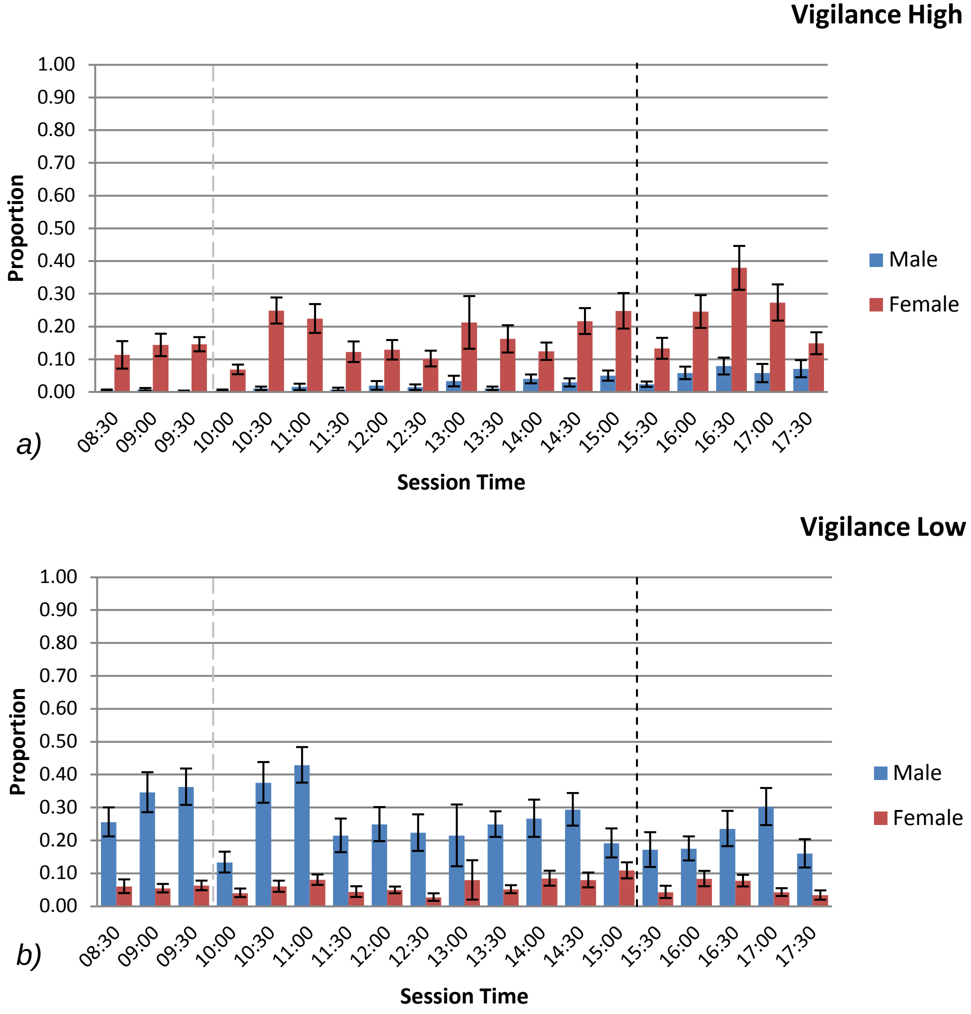


*Figure 5.1: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Vigilance High' and b) 'Vigilance Low' behaviour in the predictable feeding condition. Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time. n = either 15 or 16 for each data point.*

## Unpredictable feeding condition

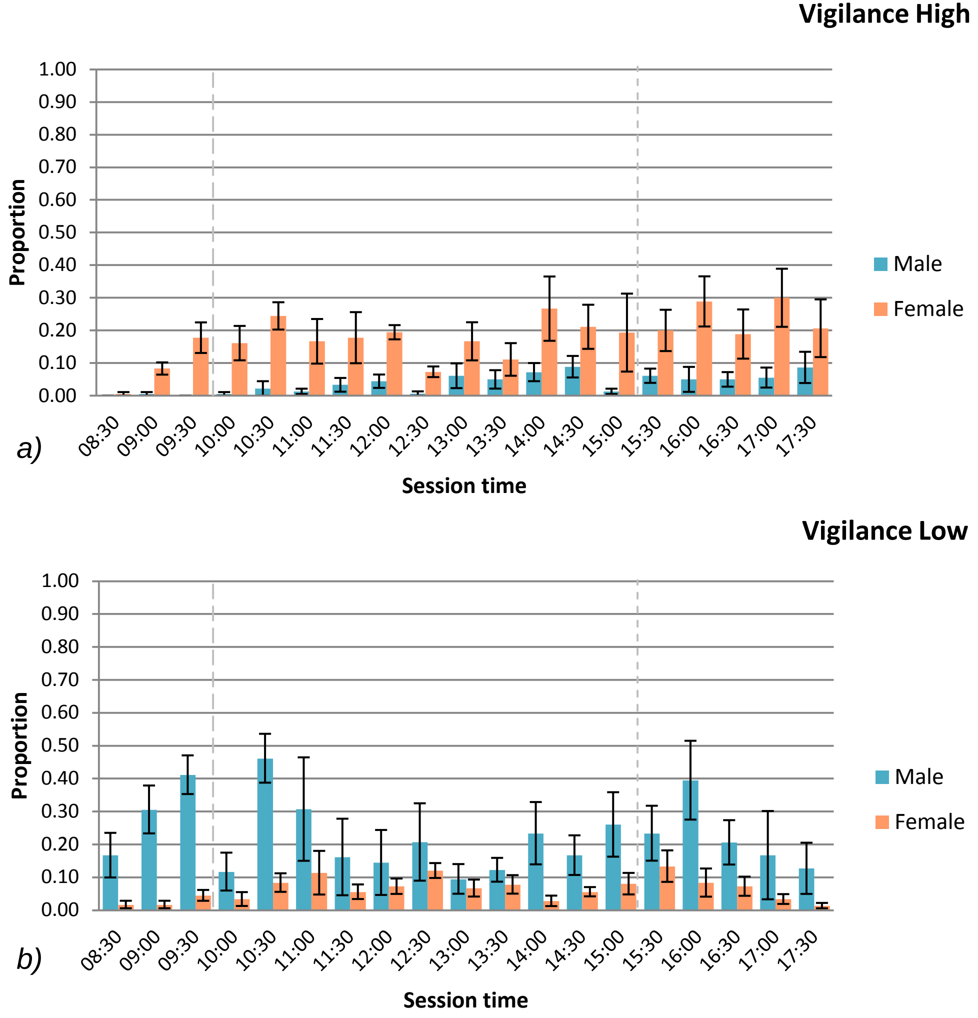


*Figure 5.2: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Vigilance High' and b) 'Vigilance Low' behaviour in the unpredictable feeding condition. Dotted line shows the time that would become the set afternoon feed time, dashed line shows the morning feed time. n = either 5 or 6 for each data point.*

The male meerkat showed lower proportions of sentry behaviour throughout the day than the female meerkat in both conditions, particularly in the morning up to 10:30 (Figures 5.3-5.4). In the predictable condition, a peak of .18 was shown in the 10:30 session, and relatively constant and lower proportions of sentry behaviour were shown for the rest of the afternoon. Again, a lower proportion of this vigilance behaviour was shown at 10:00 for both meerkats (male = .06, female = .24). The female meerkat showed a similar pattern of sentry behaviour throughout the day, to the male meerkat, but at higher levels (Figure 5.3). A peak of .49 was shown at 09:30 with minimum values of .08 in the 12:30 and 17:30 sessions. Sentry behaviour showed similar patterns throughout the day in both conditions for both meerkats, with the exception that afternoon peak proportions of .12 (male) and .21 (female) were shown in the unpredictable condition at 16:30 and 16:00 respectively, but not in the predictable condition (Figure 5.4).

Despite differences in the proportions of the different vigilance behaviours shown by the male and female meerkats, both meerkats showed a similar level of ‘all vigilance’ behaviours, both to each other, and across conditions (Figures 5.3-5.4). Both conditions showed high levels of ‘all vigilance’ in the morning (maximum .70), although slightly higher levels were then shown between 12:30 and 13:30 in the predictable condition compared to the unpredictable condition. Again, lower proportions of vigilance behaviours were shown by both meerkats in the 10:00 session, in both conditions. Both conditions resulted in a peak of ‘all vigilance’ in the afternoon, however this was shown slightly later in the predictable condition (16:30) than in the unpredictable condition (16:00).

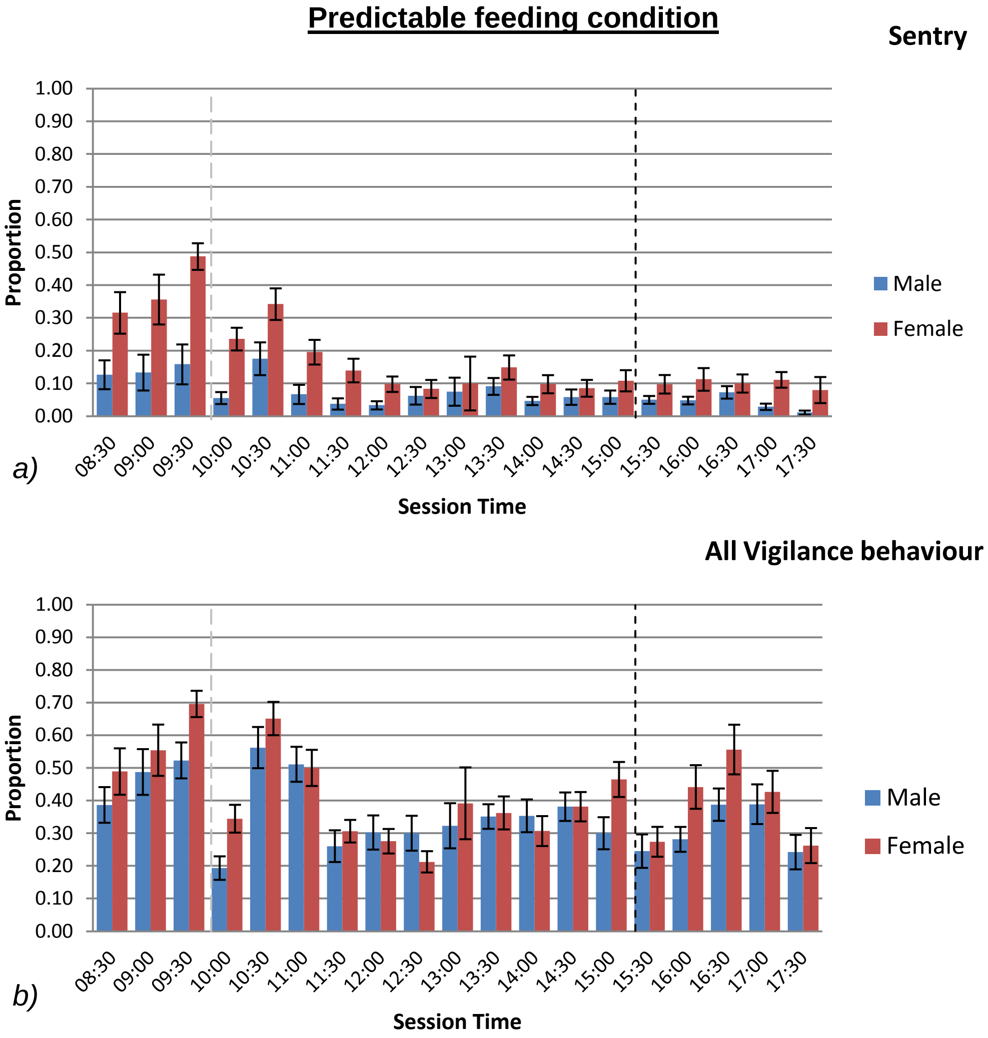


*Figure 5.3: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Sentry' and b) 'All vigilance' behaviours (vigilance high + vigilance low + sentry), in the predictable feeding condition. Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time. n = either 15 or 16 for each data point.*

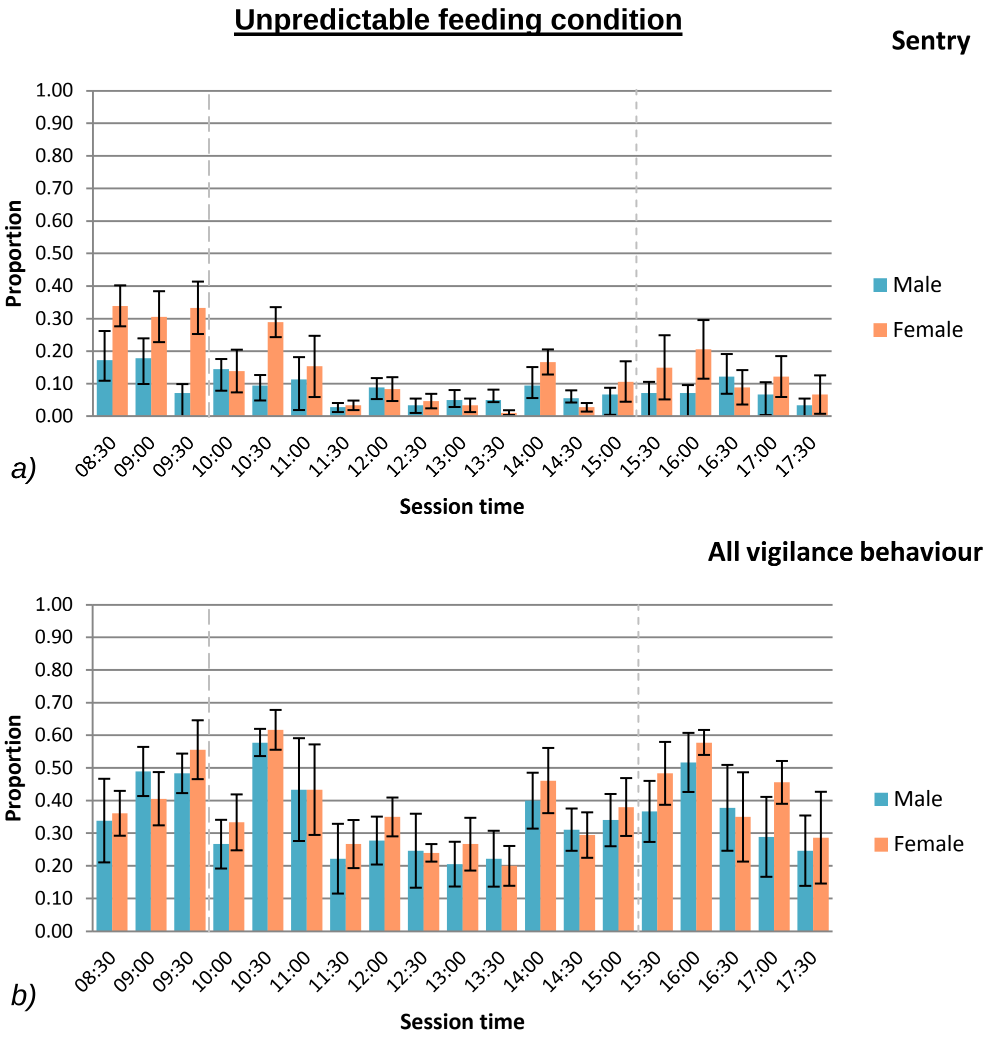


*Figure 5.4: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Sentry' and b) 'All vigilance' behaviours (vigilance high + vigilance low + sentry) in the unpredictable feeding condition. Dotted line shows the time that would become the set afternoon feed time, dashed line shows the morning feed time. n = either 5 or 6 for each data point.*

Both meerkats showed a similar pattern of foraging/investigating behaviour throughout the day, with the male meerkat showing slightly higher levels of the behaviour (Figures 5.5-5.6). Lower levels of foraging were shown in the morning by both meerkats in both conditions (<.21), with the exception of the 10:00 session where foraging increased to 0.32 and 0.28 (male and female respectively) in the predictable condition, and 0.30 (male only) in the unpredictable condition. Foraging levels remained more constant throughout the afternoon in the predictable condition, with more varied proportions in the unpredictable condition. A peak in foraging was shown at 11:30 in both conditions, however this peak was lower in the predictable condition (0.37, male and 0.32, female) compared to the unpredictable condition (.49, male and .39, female). A peak of foraging was shown at 15:30 in the predictable condition (.39, male and .37, female), which was only demonstrated for the male in the unpredictable condition (.36).

Feeding behaviour was observed at very low proportions over most of the day for both meerkats, in both feeding conditions. However both animals showed peaks in feeding in the predictable condition in the 10:00 and 15:30, post feed sessions (Figure 5.5). In the unpredictable condition, a peak at 10:00 was shown for the male meerkat only (Figure 5.6).

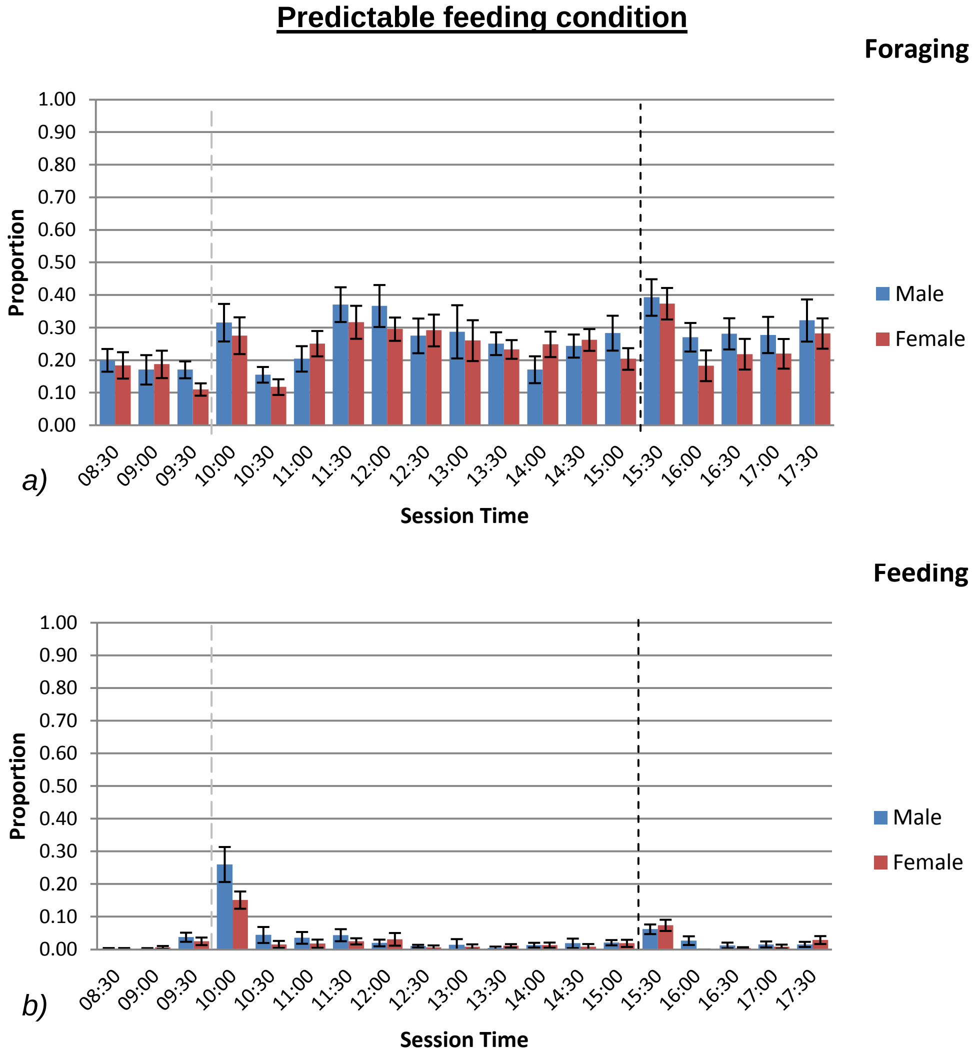


*Figure 5.5: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Foraging and b) Feeding behaviour in the predictable feeding condition. Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time. n = either 15 or 16 for each data point.*

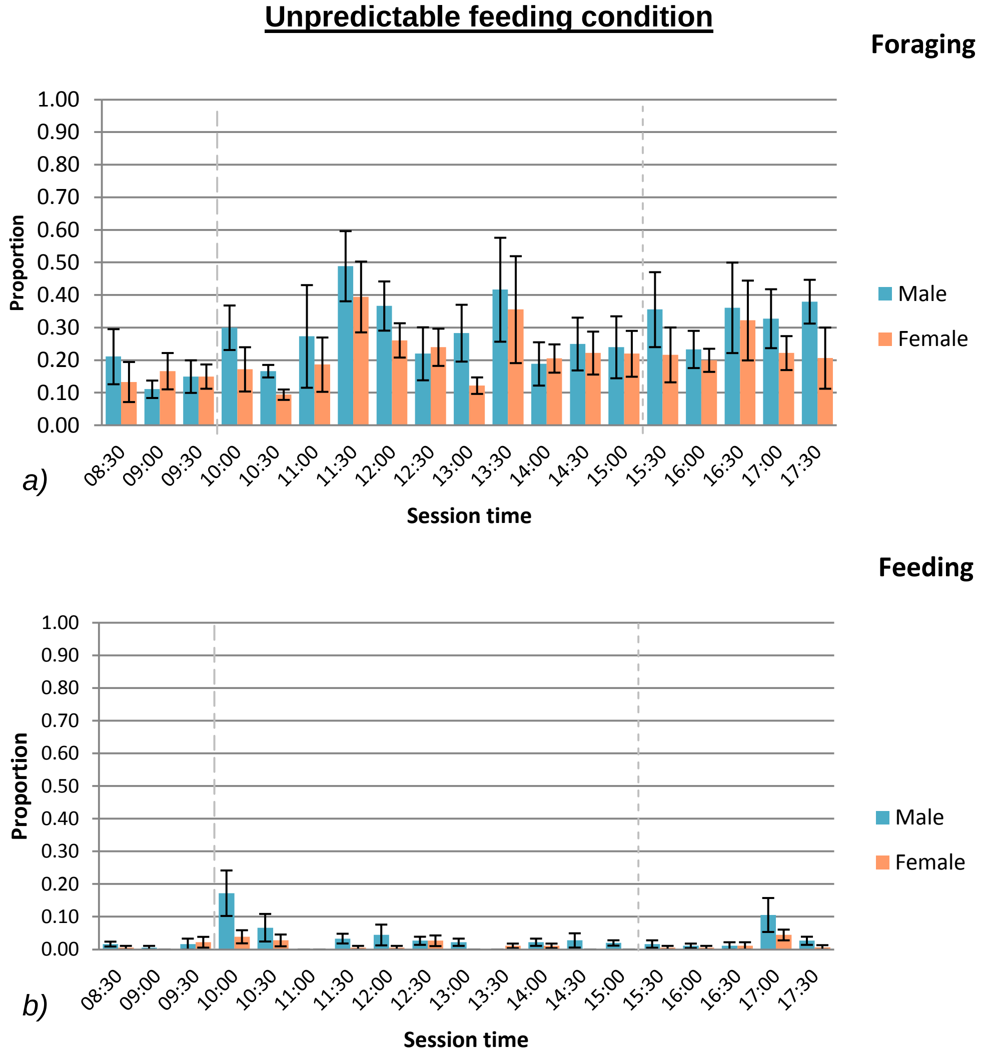


*Figure 5.6: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Foraging and b) Feeding behaviour in the unpredictable feeding condition. Dotted line shows the time that would become the set afternoon feed time, dashed line shows the morning feed time. n = either 5 or 6 for each data point.*

Locomotion was observed at fairly low proportions in the predictable condition, but both meerkats showed an increase over time from the morning to the middle of the day, followed by a decrease to the end of the day.  Peaks in locomotion were shown at 14:00 (0.11) and 12:30 (0.16) for the male and female respectively (Figure 5.7).  Similar patterns and proportions of locomotion behaviour were shown throughout the day in both conditions (Figures 5.7-5.8), with slightly lower proportions of locomotion at the end of the day (16:00-17:30) in the predictable condition.

Similar proportions and patterns of ‘not visible’ were shown throughout the day for both meerkats and for both feeding conditions.  Proportions of ‘not visible’ were lowest during the morning and late afternoon, with peaks in the middle and end of the day.  Higher proportions of ‘not visible’ were shown in the predictable condition from 14:00 onwards than in the unpredictable condition (Figures 5.7-5.8).

Resting, self maintenance, social, agonistic and enrichment-directed behaviours were shown at very low proportions with no obvious trends throughout the day and hence are not presented here.

## Predictable feeding condition

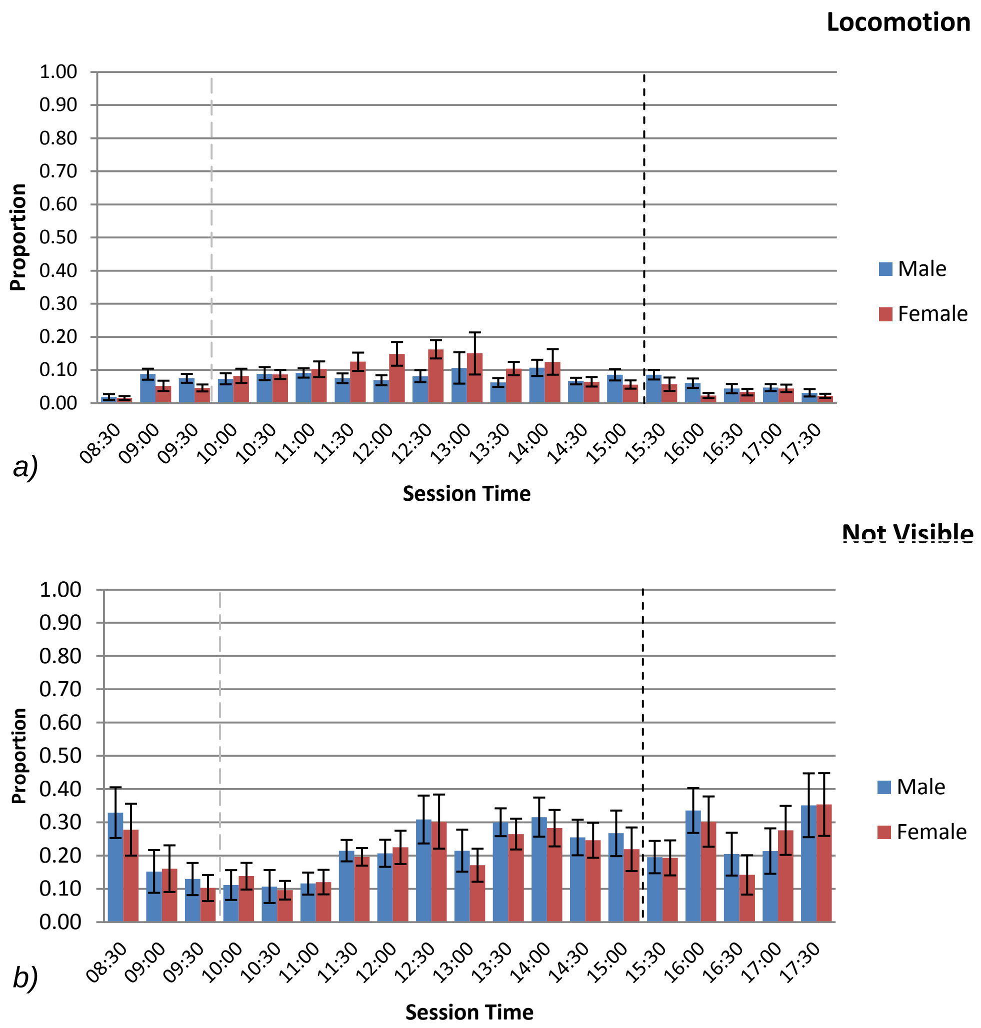


*Figure 5.7: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Locomotion behaviour or were b) Not visible in the predictable feeding condition. Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time. n = either 15 or 16 for each data point.*

## Unpredictable feeding condition

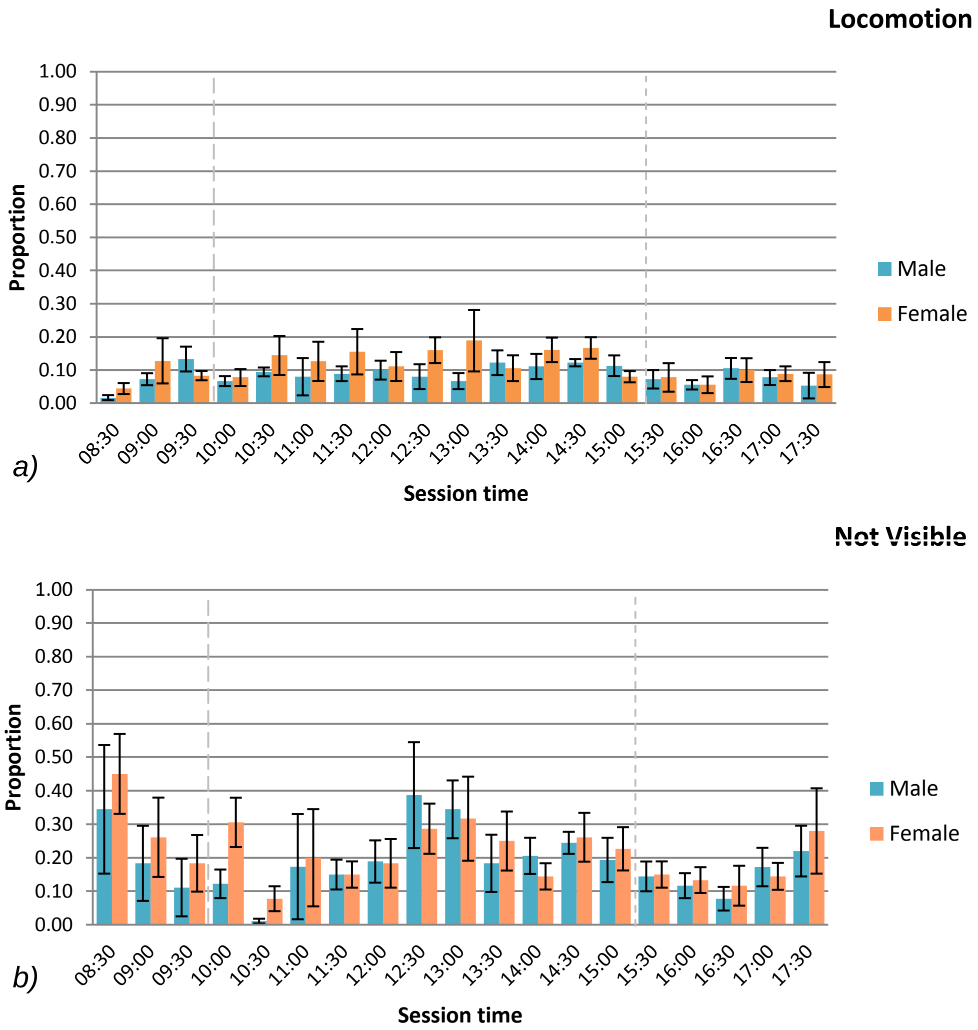


*Figure 5.8: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Locomotion or were b) Not visible in the unpredictable feeding condition. Dotted line shows the time that would become the set afternoon feed time, dashed line shows the morning feed time. n = either 5 or 6 for each data point.*

### 5.3.3. Patterns of event behaviour (standing) and busyness throughout the day in the predictable condition

There was an increase in the rate of standing during the early morning from 08:30 (0.07 stands/min) to a peak at 10:08 (1.1 stands/min) during the predictable condition (Figure 5.9). This comprised the period leading up to the morning feed. The rate of standing then dropped to 0.07 at 10:14, then rose again to a peak of 1.2 stands/min at 10:57. A third, but less well defined increase in standing events was shown from approximately 15:12 to 15:36, where the rate of standing rose from 0.11 to 0.69 stands per minute, with another peak of 0.73 stands per minute at 15:55. However, as shown in Figure 5.10, when data are removed that were collected when staff were present, this trend of increase around 15:30 is no longer shown and the trends in the morning are much reduced. This is also demonstrated in Figure 5.11 which plots the mean number of standing events in each of the 30 minutes leading up to feeding in the afternoon, with keeper present data removed; there is no trend to suggest an increase in the number of standing events leading up to the feeding time.

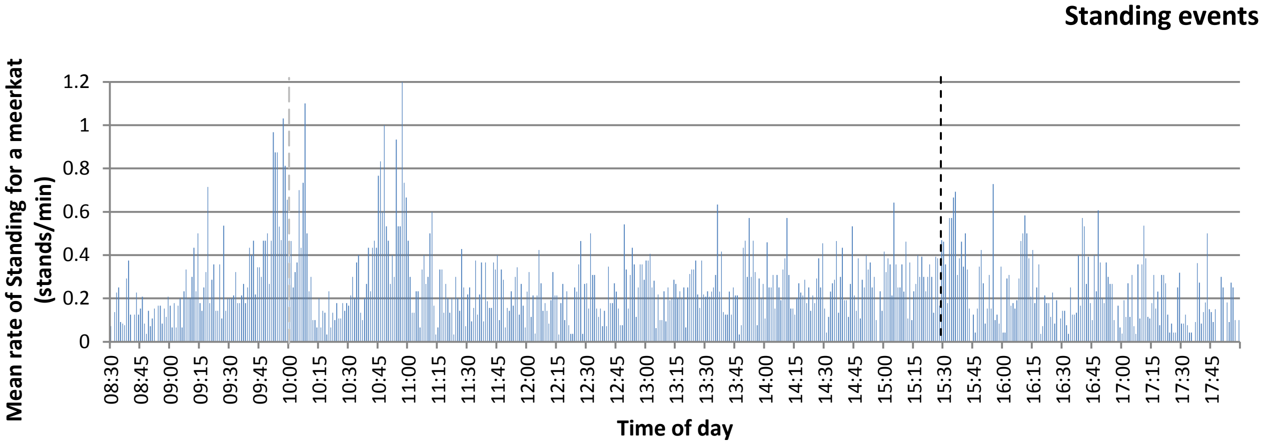


*Figure 5.9: Mean rate of standing for a single meerkat (stands/min) for each minute of the day (mean of all days of observation on predictable schedule). Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time.*

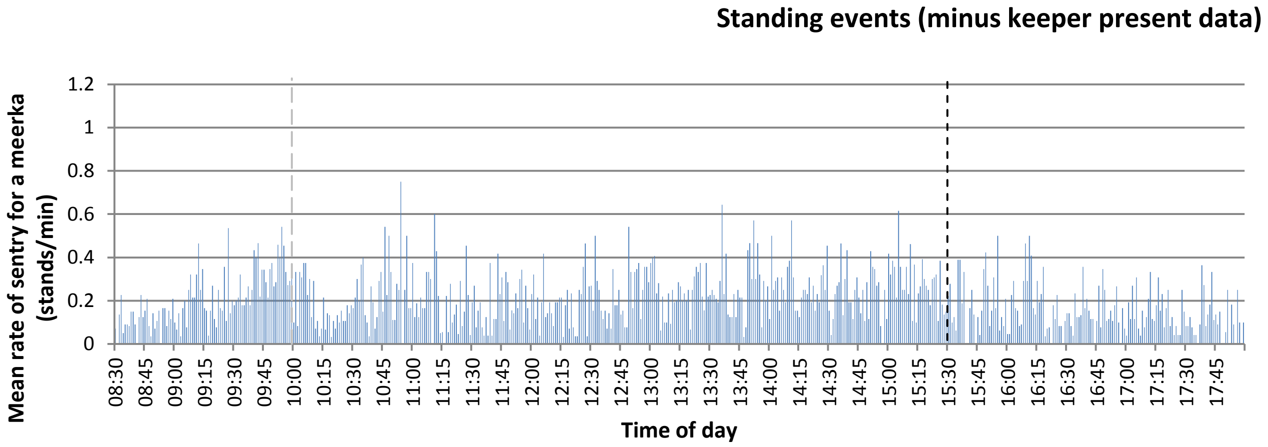


*Figure 5.10: Mean rate of standing for a single meerkat (stands/min) for each minute of the day (mean of all days of observation on predictable schedule) (minus keeper present data). Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time.*

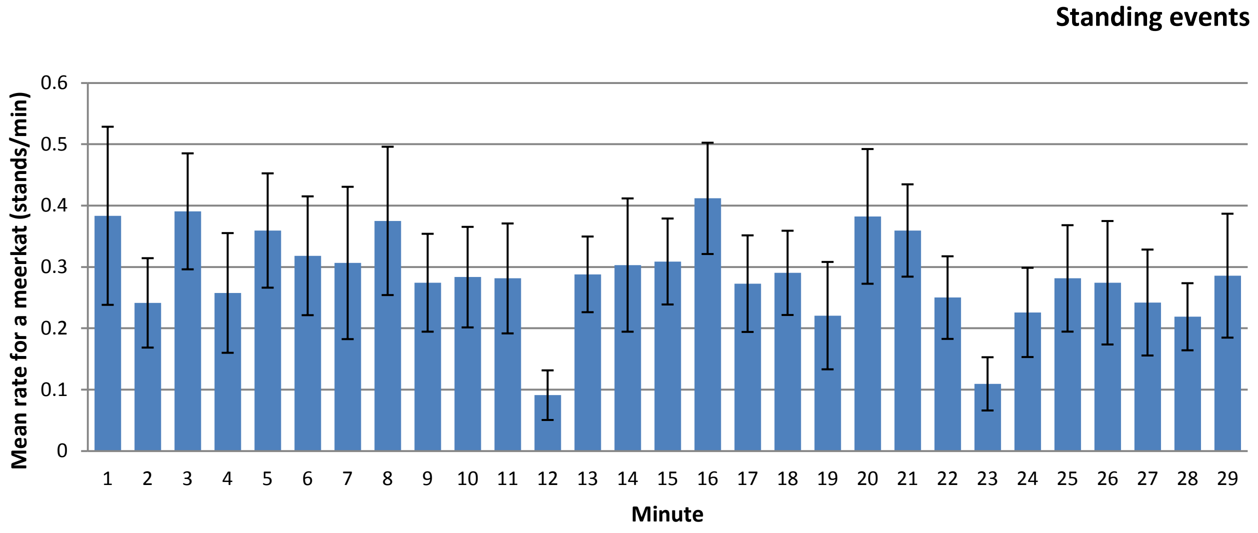


*Figure 5.11: Mean (+/- SE) rate of standing for a single meerkat (stands/min) for each minute of the 30 minutes leading up to feeding (mean of all days of observation on predictable schedule) (minus keeper present data).*

Mean busyness ratings showed some patterns throughout the day in the predictable feeding condition (Figure 5.12).  For example, busyness increased during the early morning to a peak of 3.93 at 10:07, followed by a decrease to 2.53 at 10:31.  For the rest of the day, busyness showed a gradual, but fluctuating decrease to 1.93 at 17:59.  Fluctuations of busyness during this time were fairly regular and low, with a maximum difference between adjacent minutes of 0.98.  A second peak of mean busyness was shown at 15:29 (3.69), with a preceeding increase in busyness from 1.94 at 15:08.  However, it was only at 15:28 that busyness rose above the general fluctuating level of busyness as shown over the rest of the day.  Figure 5.13 demonstrates that when the busyness scores are plotted for only the observation minutes in which keepers were not present at the enclosure, whilst the peak of mean busyness in the morning remains, the afternoon peak in busyness is now shown at 15:45 (3.46) and there is no clear increase in mean busyness in the period prior to the set feeding time, discernible from the general fluctuation of busyness.  Busyness increased after the feeding time to the peak of 3.46 at 15:45, followed by a decrease in mean busyness to 1.81 at 16:25.

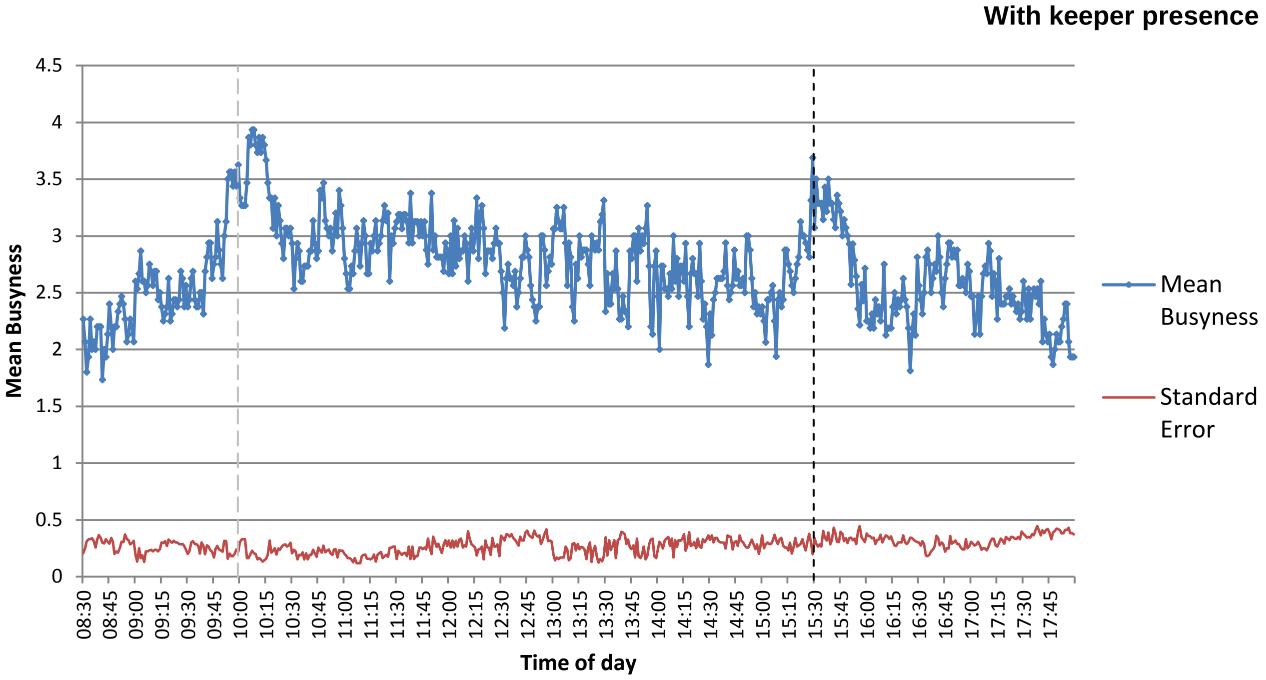


*Figure 5.12: Mean busyness for each minute of day (over all days of observation in the predictable feeding condition). Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time.*

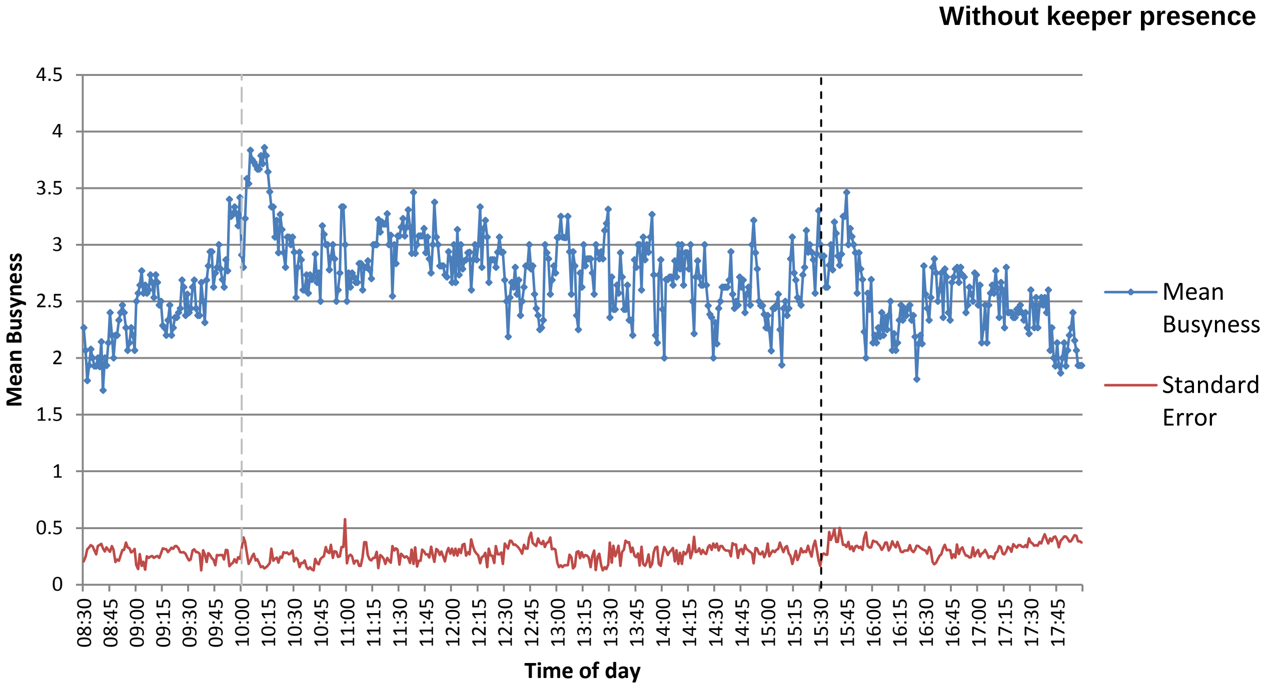


*Figure 5.13: Mean busyness for each minute of day (over all days of observation in the predictable feeding condition) (minus keeper present data). Black dotted line shows the set afternoon feed time, grey dashed line shows the morning feed time.*

### 5.3.4. Comparison of pre-feed 30-minute observation sessions across all days of observation

Proportions of scans of state behaviours in the 30 mins prior to the feeding time showed great variation over each day of observation, with few behaviours showing consistent levels over several days (Figure 5.14). There was no clear trend across all days of observation for ‘vigilance high’ (male), ‘vigilance low’ (male and female), and locomotion (male and female), with days of high proportions of these behaviours interspersed with days of low proportions.

Other behaviours generally showed no trend across the days of observation but with a few days of high proportions. For example, the female meerkat showed higher proportions of ‘vigilance high’ behaviour between days 29 and 33 of the predictable condition, compared to the previous observation days. The male meerkat showed higher proportions of sentry behaviour after day 26 of the predictable condition and also in the unpredictable condition (pre-split) but observation days varied in the proportion of sentry behaviours from .00 on some days to a maximum of .37 on day 33 of the predictable condition. The female meerkat showed less variation in sentry behaviour in the last 6 days of the study, with proportions mostly above .13, with a maximum of .37. Higher percentages of ‘all vigilance’ behaviour were shown in the 4 days from day 30 of the predictable condition for the male meerkat, and between days 29-33 of the predictable feeding condition for the female meerkat. No obvious trends in foraging behaviour were shown for either meerkat, with the exception of lower percentages in the unpredictable condition, prior to the split of the group, and between days 29 (28 for the male) and 33 of the predictable condition (Figure 5.14).

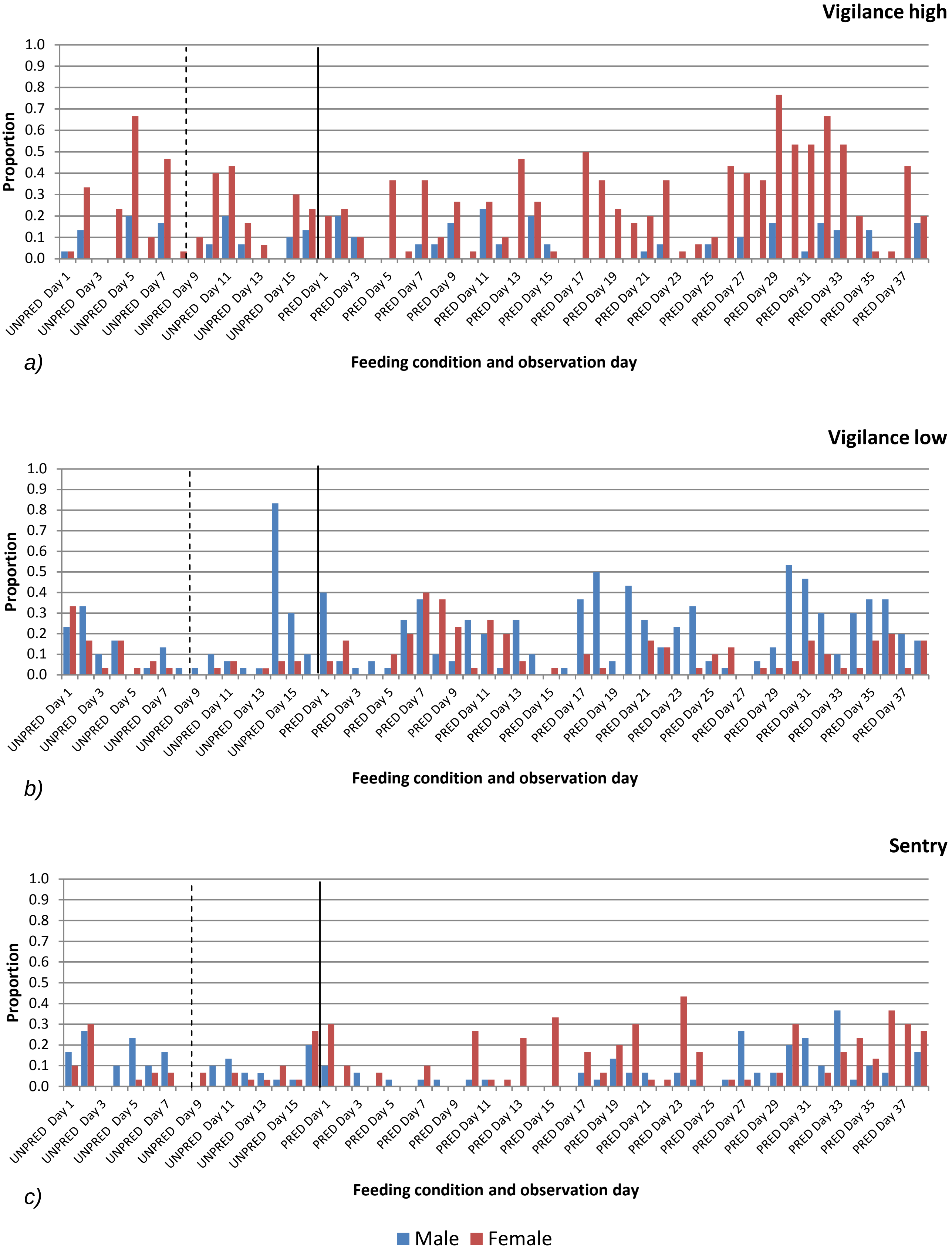
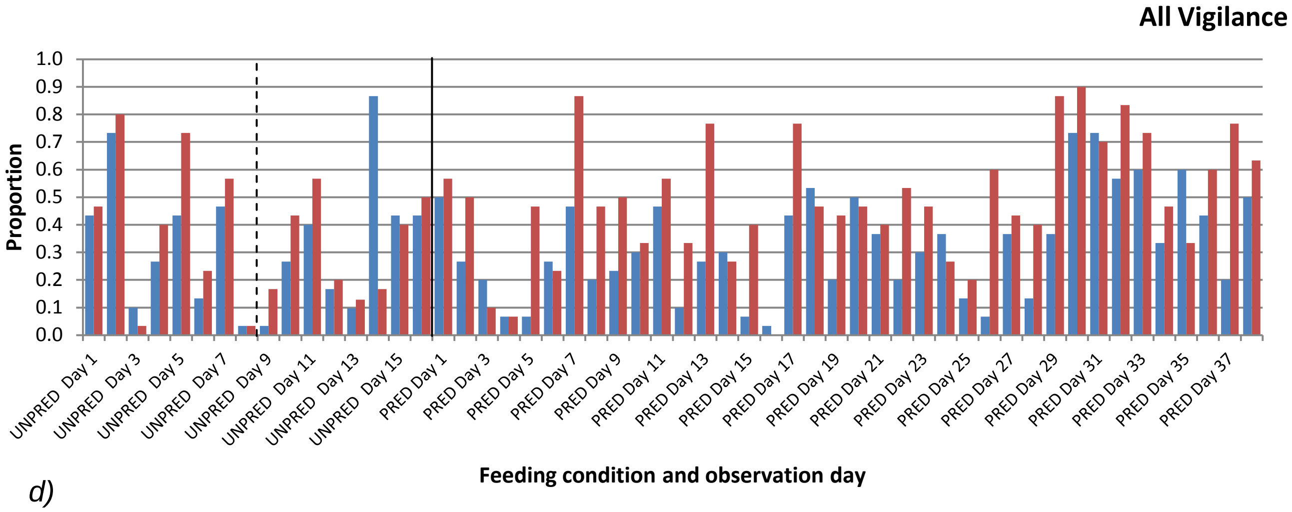
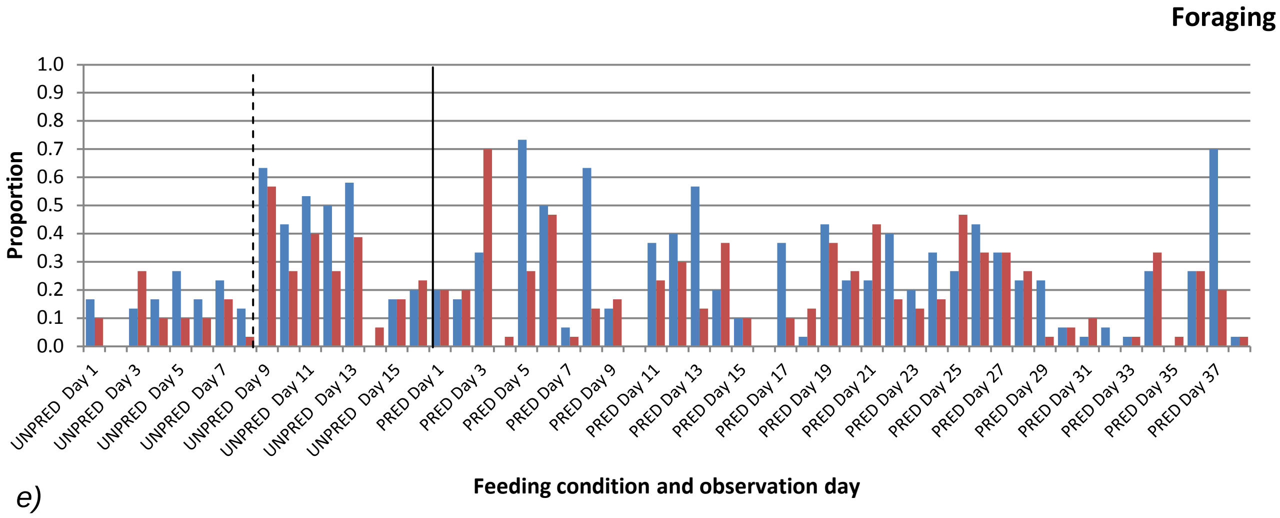


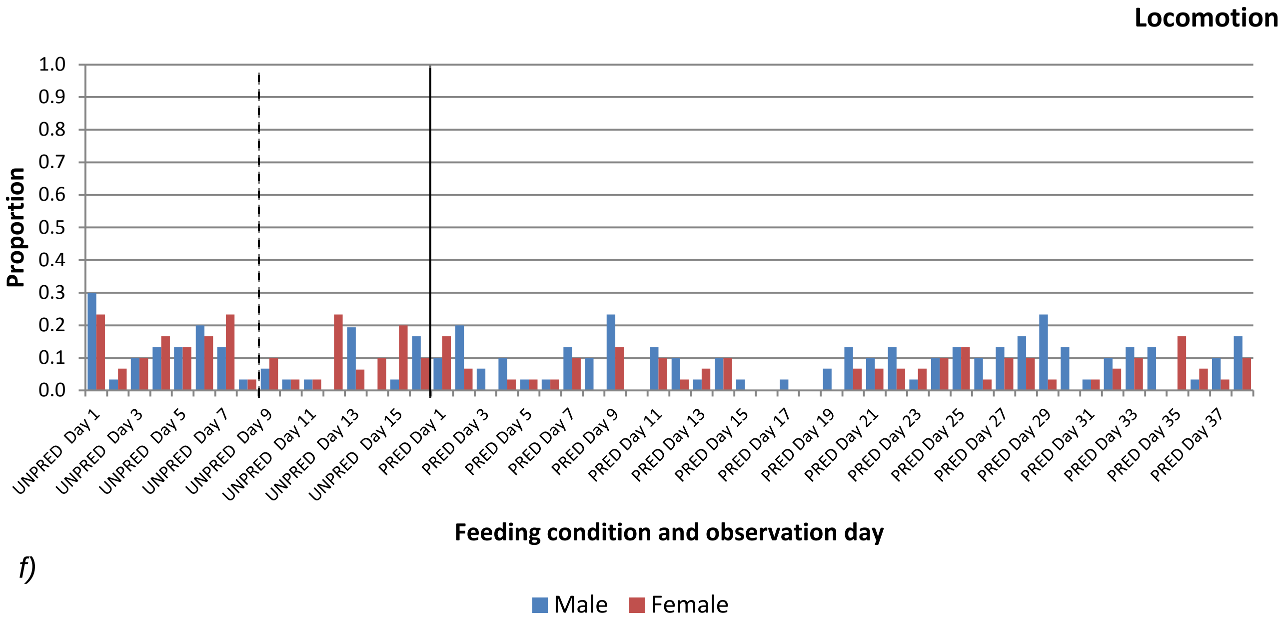
*Figure 5.14: Proportion of a) 'Vigilance high', b) 'Vigilance Low', c) 'Sentry, d)'All vigilance', e) Foraging and f) Locomotion behaviour shown in pre-feed/1500 observation sessions on each day of observation in all feeding conditions for both meerkats. The dotted line shows when the group of meerkats was separated to leave a pair of meerkats and the continuous line shows the start of the predictable feeding condition.*


All Vigilance
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
UNPRED Day 1
UNPRED Day 3
UNPRED Day 5
UNPRED Day 7
UNPRED Day 9
UNPRED Day 11
UNPRED Day 13
UNPRED Day 15
PRED Day 1
PRED Day 3
PRED Day 5
PRED Day 7
PRED Day 9
PRED Day 11
PRED Day 13
PRED Day 15
PRED Day 17
PRED Day 19
PRED Day 21
PRED Day 23
PRED Day 25
PRED Day 27
PRED Day 29
PRED Day 31
PRED Day 33
PRED Day 35
PRED Day 37
Feeding condition and observation day

*d)*


Foraging
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
UNPRED Day 1
UNPRED Day 3
UNPRED Day 5
UNPRED Day 7
UNPRED Day 9
UNPRED Day 11
UNPRED Day 13
UNPRED Day 15
PRED Day 1
PRED Day 3
PRED Day 5
PRED Day 7
PRED Day 9
PRED Day 11
PRED Day 13
PRED Day 15
PRED Day 17
PRED Day 19
PRED Day 21
PRED Day 23
PRED Day 25
PRED Day 27
PRED Day 29
PRED Day 31
PRED Day 33
PRED Day 35
PRED Day 37
Feeding condition and observation day

*e)*


Locomotion
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
UNPRED Day 1
UNPRED Day 3
UNPRED Day 5
UNPRED Day 7
UNPRED Day 9
UNPRED Day 11
UNPRED Day 13
UNPRED Day 15
PRED Day 1
PRED Day 3
PRED Day 5
PRED Day 7
PRED Day 9
PRED Day 11
PRED Day 13
PRED Day 15
PRED Day 17
PRED Day 19
PRED Day 21
PRED Day 23
PRED Day 25
PRED Day 27
PRED Day 29
PRED Day 31
PRED Day 33
PRED Day 35
PRED Day 37
Feeding condition and observation day
Male
Female

*f)*

Results of the randomisation analysis revealed no significant difference in any state behaviour in the 30 minute period prior to feeding in the predictable condition compared with the unpredictable condition for the two meerkats (*p* >.05) (see Table 5.3).

*Table 5.3. Descriptive data giving the mean proportion and standard error of each state behaviour for both meerkats in the pre-feeding 30 minutes in the predictable and unpredictable feeding conditions, plus results of the randomisation analysis (Design 3) (Todman & Dugard, 2001)*

| | **Predictable** | | **Unpredictable** | | | | |
|---|---|---|---|---|---|---|---|
| | **Mean proportion** | **SE** | **Mean proportion** | **SE** | **Test statistic** | **Count of arrangement statistics as least as large in abs value as abs (test)** | ***P*** |
| Vigilance High | 0.16 | 0.02 | 0.15 | 0.03 | 0.03 | 1621 | >.05 |
| Vigilance Low | 0.15 | 0.02 | 0.11 | 0.03 | 0.08 | 726 | >.05 |
| Sentry | 0.10 | 0.01 | 0.09 | 0.02 | 0.02 | 1757 | >.05 |
| All Vigilance | 0.41 | 0.03 | 0.35 | 0.04 | 0.13 | 1734 | >.05 |
| Foraging | 0.23 | 0.02 | 0.24 | 0.03 | 0.02 | 1832 | >.05 |
| Feeding | 0.02 | 0.00 | 0.01 | 0.00 | 0.02 | 1025 | >.05 |
| Locomotion | 0.08 | 0.01 | 0.11 | 0.01 | -0.07 | 369 | >.05 |
| Not Visible | 0.22 | 0.03 | 0.25 | 0.04 | -0.06 | 1712 | >.05 |

### 5.3.5. Variation of feeding around the set time

There was no significant difference in ‘all vigilance’ behaviour for both meerkats in the 30 mins prior to feeding when scheduled, predictable feeding was early (minus 2-10mins), on time (plus or minus 1 minute) or late (plus 2-10 or over 10 minutes) (p >.05) (Figures 5.15-5.16).

*Table 5.4. Descriptive data giving the mean proportions of ‘all vigilance’ behaviour in the pre-feed 30 mins in the predictable condition for both meerkats, as grouped into categories of time deviation from the set feed time.*

| **Time deviation from set feed time** | **MALE** | | **FEMALE** | |
|---|---|---|---|---|
| | **Mean Proportion of All Vigilance** | **Standard Error** | **Mean Proportion of All Vigilance** | **Standard Error** |
| Minus 2-10 mins | 0.46 | 0.04 | 0.49 | 0.09 |
| On time +/- 1 min | 0.33 | 0.04 | 0.49 | 0.06 |
| Plus 2-10 mins | 0.32 | 0.06 | 0.49 | 0.07 |
| Plus over 10 mins | 0.41 | 0.11 | 0.42 | 0.08 |

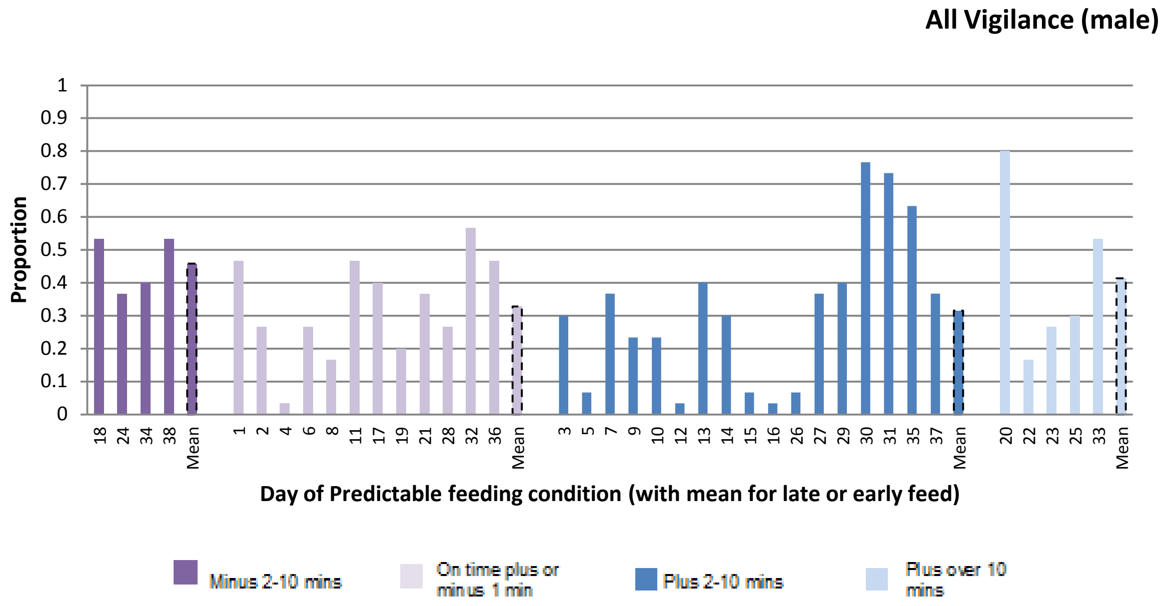


*Figure 5.15: Proportion of 'all vigilance' behaviour in the pre-feed 30 mins in the predictable condition for the male meerkat. Bars are grouped into categories of time deviation from the set feed time with a mean for each category shown with a dashed border.*

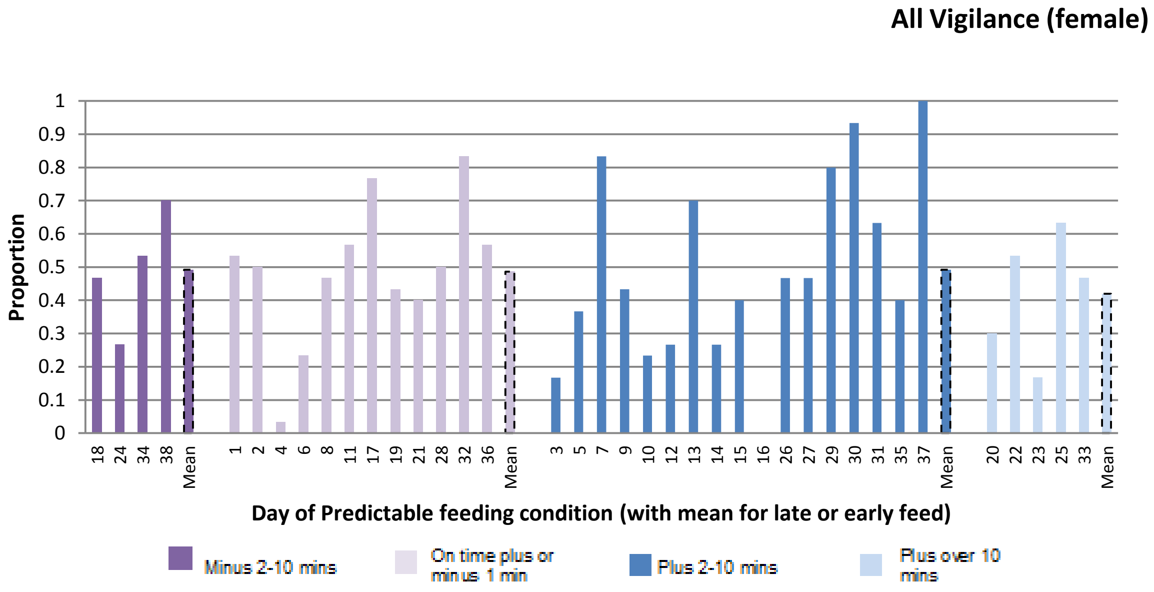


*Figure 5.16: Proportion of 'all vigilance' behaviour in the pre-feed 30 mins in the predictable condition for the female meerkat. Bars are grouped into categories of time deviation from the set feed time with a mean for each category shown with a dashed border.*

## 5.4. Discussion of Study 1

### 5.4.1. Behaviour throughout the day and prior to feeding

This study revealed patterns in meerkat behaviour throughout the day in both feeding conditions, however, it is important to note that, as discussed in Section 3.4.2., interpretation of the observed patterns of behaviour (in both Study 1 and Study 2 of the current chapter) should be made with caution, since much of the data was analysed using descriptive analysis, based on the judgement of the experimenter alone, rather than with inferential statistics. However, as for the study described in Chapter 3, a substantial number of repeat observations were made and, in the current chapter, the mean results were described whilst taking the standard error of the data into consideration. Hence, it is reasonable to interpret the patterns of behaviour described. Inferential statistics were used for some parts of the analysis which add support to the descriptive analysis. These results are discussed in Section 5.4.2.

Before discussing the main findings, there are specific data points that should be noted, as they may be explained as a direct effect of husbandry events and activities. For example, both meerkats showed low proportions of scans of vigilance behaviours in the observation sessions at 10:00 and 15:30 (predictable condition only). This is likely to be because these are the sessions during which the meerkats were fed and hence spent more time in food-related behaviours than vigilance. This suggestion is supported by the higher levels of foraging and feeding at these times. Proportions of scans of feeding are higher after the morning feed than after the afternoon feed, and foraging results show the opposite trend. This is likely to be because the afternoon feed was generally a feed of mealworms or other invertebrates which were scatter fed. The actual act of consuming these food items was quick, but more time was spent foraging for the invertebrates. In contrast, the meerkats were generally fed vegetables or fruit such as celery or tomatoes at the 10:00 feed. This required the meerkats to stay in one location and chew pieces from the food item.

The morning (before 10:00) peaks in various vigilance behaviours shown by both meerkats could be due in part to anticipation of the morning feed at 10:00.

However, proportions of vigilance behaviours after the feed (up to 11:00) were just as high which suggests that other events, rather than feeding time may have affected this pattern of vigilance behaviour. For example, there was a lot of activity outside of the enclosures during the morning such as keepers commencing their morning routines and animals in other enclosures being let out or fed, with the corresponding noises. The meerkats' keepers spent more time near the meerkat enclosure during the morning, to clean out their enclosure, and to give a public talk at the nearby baboon enclosure at 10:45, which could be heard at the meerkat enclosure and may have resulted in increased vigilance. Finally, the meerkats spent a lot of time underneath their heat lamps in the cooler mornings. Whilst under the heat lamps they showed vigilance behaviours. In this case it was not possible to separate warming from vigilance behaviours. For these reasons, and the many factors potentially affecting meerkat behaviour in the morning, the focus of the following discussion of predictability will be on the afternoon feeding time.

With the exception of these morning behaviours, the male meerkat showed no apparent trends in any vigilance behaviour over the course of the rest of the day, with no peaks in vigilance prior to the 15:30 feed time. This is in contrast to the suggestion that vigilance behaviours would show a pattern of change leading up to, and in anticipation of the afternoon feed. This suggests that either the behaviour of the male meerkat did not anticipate the feed, or that vigilance is not an anticipatory behaviour. However the results do not show a particular peak of any other observed behaviour prior to the feed time suggesting that the male meerkat did not show observable behaviours that anticipated the 15:30 feed time. The female meerkat however, showed some increases in 'vigilance high', 'vigilance low' and 'all vigilance' behaviours in the pre-feed observation period. When taken in isolation, this suggests that the female meerkat may have learnt to predict the time of the afternoon feed and hence showed increased vigilance behaviour prior to the set feed. However, these vigilance behaviours also showed peaks of similar, or higher proportions at other times of the day in which anticipation would not necessarily be expected, which reduces the certainty with which these behaviours can be considered to be food anticipatory behaviours. Also, some similarities in patterns and proportions of behaviours are shown between comparable times in the predictable and unpredictable feeding conditions, with some variation in the unpredictable condition, making it difficult to confirm differences in behaviour, caused by predictable feeding.

Neither meerkat showed a pattern of change in sentry behaviour in the pre-feed observation sessions in the predictable condition, and there was little difference in patterns of behaviour between the predictable and unpredictable conditions. Again, this is contrary to the suggestion that, as a look-out posture (Ewer, 1963), the meerkats would spend more time in this behaviour, looking out for their keepers, in anticipation of the feed. Bloomsmith and Lambeth (1995) found that chimpanzees were more inactive prior to a predictable feeding time than an unpredictable time, and suggested that the chimpanzees were “waiting” for the feed. If vigilance behaviour performed a similar function in looking out for keepers (in addition to its predator detection function, as discussed in Section 5.1.1) then it seems logical to suggest that vigilance behaviour would show a similar trend to that of inactivity, as found by Bloomsmith and Lambeth. However, this was not clearly demonstrated. It was also suggested in Section 5.1.2, that vigilance behaviour could decrease prior to feeding as meerkats increased their contribution to foraging; meerkats that have been provisioned with food have been shown to increase vigilance (Clutton-Brock, et al., 1999), therefore a decrease in vigilance was a possibility towards the end of the inter-feed interval (prior to predictable feeding). However, no clear trends of decreased vigilance, or increased foraging were demonstrated, discernable from the variation in behaviours over the day, and foraging levels prior to the feed were similar to those after the feed (with the exception of the 15:30 session in which the meerkats were foraging for the mealworm feed that they had just received). In addition, vigilance behaviours did not show patterns of decrease prior to the morning feed. This therefore suggests that meerkat vigilance does not decrease in favour of foraging prior to set feeds as might be expected from the results of Clutton-Brock et al. (1999).

Rates of ‘standing’ did not show a pattern of increase as the feeding time approached. This was contrary to predictions that, as a vigilance behaviour, rates would increase prior to feeding. Previous laboratory studies in which an event behaviour such as lever pressing is used to measure anticipation, have shown the behaviour to increase as the feeding time approaches. For example, Lejeune and Wearden (1991) described a study in which wood mice pressed levers to receive food rewards according to fixed interval schedules. The results showed an increase in lever pressing at an accelerated rate as the interval elapsed. However, it should be considered that in this laboratory example, receiving the reinforcement was contingent on the mice pressing the lever, whereas in the current study, the provision of the feed was not contingent on the behaviour of the meerkats; as is often the case in zoos, and might explain the difference in the results. Anticipatory responding has, however been demonstrated when reinforcement was independent of the animal's response. For example, Zeiler (1968) tested pigeons on both response-dependent (key pecking), fixed interval reinforcement schedules and response-independent, fixed time reinforcement schedules. Zeiler found that when the reinforcement was not dependent on the behaviour of the pigeon, the pigeon still showed a increase in pecking rate as the time of reinforcement approached, albeit at lower levels overall, than when reinforcement was dependent on the pigeons' response. However it should be noted that Zeiler's study used pigeons that had prior experience of key pecking being associated with reinforcement, and it was suggested that this response, originally made to food, became anticipatory.

All occurrence sampling of standing events was used in the current study, to record a pattern of occurrences that could be plotted in a similar fashion to data obtained using operant responses such as key pecks. As no pattern of increase in occurrences prior to feeding pattern was observed, this suggests that the rate of standing was not indicative of anticipation in the current study. Anecdotally it appeared that the meerkats responded with an increased number of standing events when the keepers could be heard on their approach to the enclosure and the data showed increased rates of standing when the keepers could be seen. It may therefore be the case that standing behaviour is more of a reactive than anticipatory behaviour, which occurs in response to the signal of keeper presence. Bassett and Buchanan-Smith (2007) describe signalled predictability as the reliability of a signal preceding the event, and it may be that in this study, the meerkats were responding in anticipation of a feed occurring with signalled predictability, with the signal being the arrival of the keepers. This is supported by the fact that the graphs of standing events over the course of the day showed peaks around the feeding times, which are no longer shown when the data is viewed without the data collected when the keepers were present. However signalled predictability was not specifically tested in this study so this is difficult to conclude with a level of reliability.

Groups of wild meerkats have been observed to alternate vigilance duties between group members (Clutton-Brock, et al., 1999), and a report of captive meerkats observed at least one member of the group to be engaged in vigilance behaviour at almost all times (Moran, 1984). It was therefore possible in this study that the meerkats could have taken turns in expressing vigilance behaviours to keep a sentry posted throughout the day, and if so, an increase in vigilance behaviours prior to feeding would not be expected. However, whilst these meerkats were observed to alternate vigilance duties, they were also observed to be gregarious in their behaviour, and the data show many observations where both meerkats were vigilant or when both were foraging at the same time. Indeed the current data showed that combined scores of all vigilance proportions for the male and female in each observation session, did not result in a meerkat constantly on vigilance duty throughout the day. For some of the morning observations if the proportions of male and female all vigilance behaviours were combined, then proportions would effectively reach over 1.00, showing that both meerkats were often vigilant at the same time. Therefore the need for taking turns to keep one meerkat on vigilance duty throughout the day is unlikely to be a reason for the lack of a clear change in vigilance behaviour prior to feeding, and it could have been possible for either, or both meerkats to show changes in vigilance behaviour in anticipation of the feeding time.

There were no obvious trends in feeding, locomotion, resting or self maintenance behaviours in the observation sessions leading up to the set feed. Therefore these behavioural measures did not suggest anticipation.

Busyness scores showed a peak in the morning which was likely associated with the morning activity (as described above). There was also a pattern of increase in busyness prior to the predictable, afternoon feeding time. However, there were other increases and decreases in busyness over the rest of the day that were not associated with feeding time. Also, the peak of busyness did not occur until after the feed time (when the meerkats were feeding), which may have exaggerated the magnitude of the pre-feed increase when analysed descriptively. Once data in which the keepers were present at the enclosure were removed, this pre-feed increase was no longer distinguishable from the general daily fluctuations in busyness, and the main increase in busyness was then shown after the feed. It therefore cannot be concluded that meerkat busyness increased in anticipation of feeding. This removal of peak behaviours in the absence of keepers was similar to the results shown for the standing behaviour. It is therefore possible that, as for standing behaviour, meerkat busyness increases in response to signalled predictability of an imminent feeding time.

### 5.4.2. Comparisons of feeding conditions

Comparisons of meerkat behaviours throughout the day in both feeding conditions did not reveal major differences, particularly in the middle of the day and prior to feeding, although vigilance and foraging behaviours were less variable at this time in the predictable condition. This once again gives little evidence to support a difference in behaviour in the two feeding conditions as a result of anticipation of the predictable feeding times. A more pronounced peak and then decrease in vigilance behaviours was shown in the afternoon in the unpredictable condition than the predictable condition. It is possible that the set feeding time may have disrupted general afternoon patterns of behaviour (after 15:30) as the meerkats were always feeding or foraging at this time in the predictable condition.

Meerkat behaviours in the pre-feed observation sessions on each day of observation showed a great deal of variety in all behaviours over the duration of the study, and there was no significant difference in any state behaviour between the pre-feeding observation sessions in the predictable and unpredictable conditions. However, the female meerkat did show higher levels of vigilance high and all vigilance behaviours in days 29-33 of the predictable feeding condition, and less variation in sentry behaviour in the last 6 days of the predictable condition. These data suggest that towards the end of the study, the female meerkat may have learnt, and started to anticipate the time of the predictable feed. However, this conclusion is based on very few days at the end of the study in which there may have been other factors affecting the behaviour of the meerkats, and the noise in the data makes it difficult to hold this as a firm conclusion.

Whilst there were no clear patterns of behavioural change prior to feeding demonstrated in the current study, some increases in vigilance behaviours have been discussed above. If the meerkats had been anticipating the feed (for example with increased ‘all vigilance’ behaviour), then it would be logical to suggest that if the feeding time was delayed for any reason, then such behaviour would further increase whilst the meerkats were waiting to be fed, and that prior to early feeds the occurrence of such behaviours would be lower. Indeed, Waitt and Buchanan Smith (2001) demonstrated increases in behaviours such as self-directed behaviours, inactivity and abnormal behaviours in stump-tailed macaques that were waiting for delayed feeds. However, the current results comparing early, on-time and late feeds showed no significant difference in ‘all vigilance’ behaviours in the different deviations of feeding time. This reinforces that a conclusion of anticipatory vigilance behaviour cannot be supported from this study. Whilst there are some occurrences of high vigilance behaviour in the late feed observations (plus 2-10mins) for both meerkats, it should be noted that these occur on those days of observation already reported to show high vigilance behaviours that are inconsistent with the rest of the study period.

### 5.4.3. Effect of environmental factors

The current study does not show the clear evidence of anticipation prior to set feeds that was expected. An alternative reason for this may be *ad lib*. feeding of the meerkats by the visiting public. An unexpected result of this study was the observation that visitors to this exhibit feed the meerkats more often than was supposed, despite the signs around the zoo asking visitors not to feed the animals. This potential for feeding meant that the meerkats often responded to the visitors with increased vigilance and increased standing; the behaviours that were also associated with keeper presence and predicted to be associated with anticipation of the keepers. It was not generally possible to separate out vigilance for visitor feeds and for keeper feeds in the data set. As there was potential for food from the visitors at any time of the day, the meerkats may have been vigilant for feeds throughout the day. This may explain why the levels of vigilance, particularly for the male meerkat were more consistent across the day than was expected, without the large peaks prior to the 15:30 feed. It may have been the case that whilst, experimentally, the meerkats were on a predictable feeding routine, the *ad lib*. nature of the visitor feeds meant that the meerkats were actually on an unpredictable feeding routine. This would have resulted in irregular lengths of time between feeds and would have prevented the expression of anticipatory behaviours.

Environmental stimuli such as visitor feeding may have influenced the relative importance of the manipulated feed to the meerkats and in this study the predictable feed may have been less important to the meerkats than predicted. There was also the possibility of the meerkats foraging and finding an insect that had entered the enclosure which may have also reduced the relative importance of anticipating the predictable feed from the keepers, and hence caused the lack of a clear anticipatory response noted in this study. The ability to ‘beg’ for food from the visitors or to find food through foraging may have afforded the meerkats a level of control over their environment. Control and predictability are linked, as defined by Bassett and Buchanan-Smith (2007) (and in Section 1.4.3). Waitt and Buchanan-Smith (2001) suggest that predictability is more important to animals that do not have control, hence if the meerkats had some control over their environment, their need for predictability and tendency to express anticipatory behaviours may have been reduced.

### 5.4.4. Conclusions

An aim of this thesis was to clarify the debate on the effect of predictable and unpredictable routines on animal behaviour. No clear evidence was shown to suggest that the male meerkat changed his behaviour in anticipation of a predictable feeding time. It may have been the case that this meerkat was unable to predict the feed time, or the behaviour may have been influenced by environmental factors and potential extraneous variables such as the visitors feeding the meerkats. There is some evidence to suggest that the vigilance behaviours of the female meerkat increased prior to feeding in the predictable condition, potentially suggesting prediction of the feeding time. However, there are several caveats to this, such as the observation of other, larger peaks in vigilance behaviours at other times of the day, the large variation in vigilance behaviours across the duration of the study, with increased vigilance shown only in a few days at the end of the study. Also the lack of a significant difference in behaviour in the pre-feeding observation session in the two feeding conditions.

There was also no clearly defined change in meerkat busyness prior to the predictable feed, suggesting that meerkat busyness did not change in anticipation of a set feed. However, meerkat busyness and standing behaviour did increase around the predictable feeding time in response to keeper presence, suggesting that meerkats may be affected by signalled predictability of the arrival of food.

This brings to light some important factors for consideration when studying the effects of predictability. For example: the level of environmental variability and external stimuli affecting behaviour, potential cues to feeding and reactive responses, opportunities for foraging or food outside of the keeper feeds, the level of control that the animal can exert over its feeds, and the importance of the feed event to the animal.

This study was designed as a further validation test of the measure of busyness. Despite the lack of a change in busyness prior to the predictable feeds, the fact that this busyness result is in agreement with the lack of behavioural observations of anticipatory behaviour, further supports the utility of busyness as a potential measure of daily patterns of behaviour.

In order to address some of the concerns regarding visitor influences on behaviour, it was decided to repeat this study (Study 2) at a time of year where visitor numbers were lower than for Study 1.

## 5.5. Introduction to Study 2

The results of Study 1 suggested that environmental variables, namely the presence of visitors, may have had an effect on the behaviour of the meerkats, such that it may have reduced any possible expression of anticipation behaviours prior to the predictable feed. A second study was therefore carried out using a similar method to Study 1 but during winter months, when fewer visitors were present at the zoo (mean weekly visitor number to the whole zoo = 14830 and 4158 for studies 1 and 2 respectively). Aims and predictions were the same as for Study 1 and it was hoped that in the presence of fewer visitors, any patterns of behavioural change prior to predictable feeding would be more clearly apparent, as there should be less meerkat vigilance for food from the visitors.

## 5.6. Methods for Study 2

### 5.6.1. Subjects, housing and husbandry

Subjects were the same male and female meerkat as used for Study 1, housed in the same conditions (Section 5.2.1). However, on the 25th day of the predictable feeding condition, two additional meerkats (male and female) joined the study pair, and all meerkats were given access to an extra enclosure section. This extra section was also approximately 11m x 7m, walled and joined to section 2 via pipes. This section (section 3) contained sand as the main flooring substrate and contained logs and rocks as enclosure furnishings (similar to sections 1 and 2). The two extra meerkats were not observed in the current study.

*Observers*

Data were collected by six student observers from the final year of a psychology degree course at the University of Plymouth. Student observers received a minimum of three training sessions plus independent practice time, to learn the behavioural data collection methods and meerkat identification (although the busyness method required less training time), and to ensure that individual observers were consistently observing the correct behaviours. An additional eight volunteers from the first and second year of the same university course collected additional data with a focus on busyness, and hence were trained, and collected data on all aspects except state behaviours.

### 5.6.2. Feeding conditions

As in Study 1, the meerkats were observed in two different feeding conditions; unpredictable and predictable (as described in Table 5.5) and the predictable condition followed directly after the unpredictable condition in a within subjects design. Data were reviewed after 30 days of the predictable condition and, as for Study 1, no clear trends related to the predictable feed had developed, therefore it was decided to extend the length of the predictable condition for a further 15 days; the maximum time available for this study.

*Table 5.5. Details of the two feeding routines used in this study.*

| Feeding routine | Duration | Time of first (morning) feed | Time of second (afternoon) feed |
|---|---|---|---|
| Unpredictable | 14 days | 10:00 | Random time at any, 15min interval between 12:30 and 17:45 (pre-determined using a random number generator in Microsoft Excel) |
| Predictable | 45 days | 10:00 | 15:30 |

### 5.6.3. Behavioural data collection

*Behavioural trends throughout the day*

State and event behaviours, and busyness were recorded using the method described for Study 1, in 30 minute observation sessions between the hours of 11:30 and 16:00. This was a truncated portion of the day compared to Study 1.

Each minute of the day was observed with a minimum of 1 and maximum of 3 repeats in the unpredictable condition, and a minimum of 5 and maximum of 15 repeats in the predictable condition. A minimum of 4 and maximum of 21 repeats of each minute were recorded for busyness in the predictable condition.

#### *Effect of feeding condition on pre-feed behaviour*

As in Study 1, data were collected during the 30 minute period prior to the feed time (prior to the random feed time and from 15:00, prior to the 15:30 feed). Data were collected in 3 pre-feed sessions in the unpredictable condition, and 15 pre-feed sessions in the predictable condition.

### 5.6.4. Data analysis

The mean proportions of scans of state behaviours for both feeding conditions, throughout the day, and prior to feeds, were analysed as described in Section 5.2.4, as were the busyness scores. Randomisation analysis (Design 3) (Todman & Dugard, 2001) was used to compare the mean proportions of state behaviours for both meerkats in the pre-feed observation sessions, in both feeding conditions (2000 re-randomised pseudosamples). Randomisations were run three times, and average scores were calculated.

## 5.7. Results of Study 2

### 5.7.1. Behavioural trends prior to 15:30

With the exception of ‘vigilance low’ and ‘all vigilance’ behaviours, no state behaviour showed a clear increase in the proportion of scans prior to the 15:30 feeding time in the predictable condition (see Figures 5.17, 5.19, 5.21 and 5.23). ‘Vigilance low’ behaviour for the male meerkat showed a gradual increase across the day to a peak of .23 prior to 15:30, after which time a decrease was shown to .03. A pattern of increase was shown for the female but at lower proportions, with an increase from .02 to .11 between 14:00 and 15:00, followed by a drop to .00 at 15:30 (Figure 5.17). 'All vigilance' behaviour showed an increase up to .44 in the 15:00 observation session for the female meerkat, with a similar, albeit more variable pattern for the male, followed by a drop to .20 (female) and .09 (male) in the 15:30 session (Figure 5.19). High variability was shown in the 'not visible' data for both meerkats, however a pattern of decrease was shown leading up to 15:30 (Figure 5.23).

There were few clear differences in behavioural patterns prior to 15:30 between the two feeding conditions (Figures 5.17-5.24). Of those behaviours (described above) showing increases prior to predictable feeding, 'vigilance low' behaviour in the unpredictable condition showed similar, but more variable patterns prior to 15:30 compared to the predictable condition (Figure 5.18), although proportions of scans were higher for the male meerkat in the unpredictable condition (reaching .30 in the 15:00 observation session). Patterns of 'all vigilance' behaviour for the male meerkat in the unpredictable condition were also similar to those in the predictable condition, and again were higher in the unpredictable condition (.53 in the 15:00 observation session) (Figure 5.20). There was more variability in female 'all vigilance' behaviour in the unpredictable condition, compared with the predictable condition, with no clear increase prior to 15:30. Proportions of scans of 'not visible' were lower prior to 15:30 in the unpredictable condition compared to the predictable condition, and no clear pattern was shown (Figure 5.24).

### 5.7.2. Behavioural patterns throughout the day in the predictable and unpredictable feeding conditions

With the exception of those patterns described in Section 5.7.1 above, there were few clear patterns of any behaviour shown throughout the observation day in the predictable feeding condition (Figures 5.17, 5.19, 5.21 and 5.23), and there was variability in the proportions of behaviours across the day (as demonstrated by the large error bars on Figures 5.17, 5.19, 5.21 and 5.23). The only other notable patterns were that each vigilance behaviour showed lower proportions in the two observation sessions after 15:00, and locomotion and enrichment-directed behaviour showed peaks at these times.

Overall there was more variability in the data, across the day in the unpredictable feeding condition (Figures 5.18, 5.20, 5.22 and 5.24) compared to the predictable condition, however some similarities and differences could be determined. Similar patterns were shown throughout the day in both feeding conditions for 'vigilance high' (male) and 'vigilance low' (female). The main differences in vigilance behaviours were shown for 'vigilance low' (male), 'sentry' (female) and 'all vigilance' (both meerkats) where a decrease in proportions after 15:00 was not observed in the unpredictable condition as it was in the predictable condition. In addition, the female showed higher proportions of 'vigilance high' and 'all vigilance' behaviour, and the male showed lower proportions of 'all vigilance' behaviour, prior to 14:00 in the unpredictable feeding condition than the predictable condition.

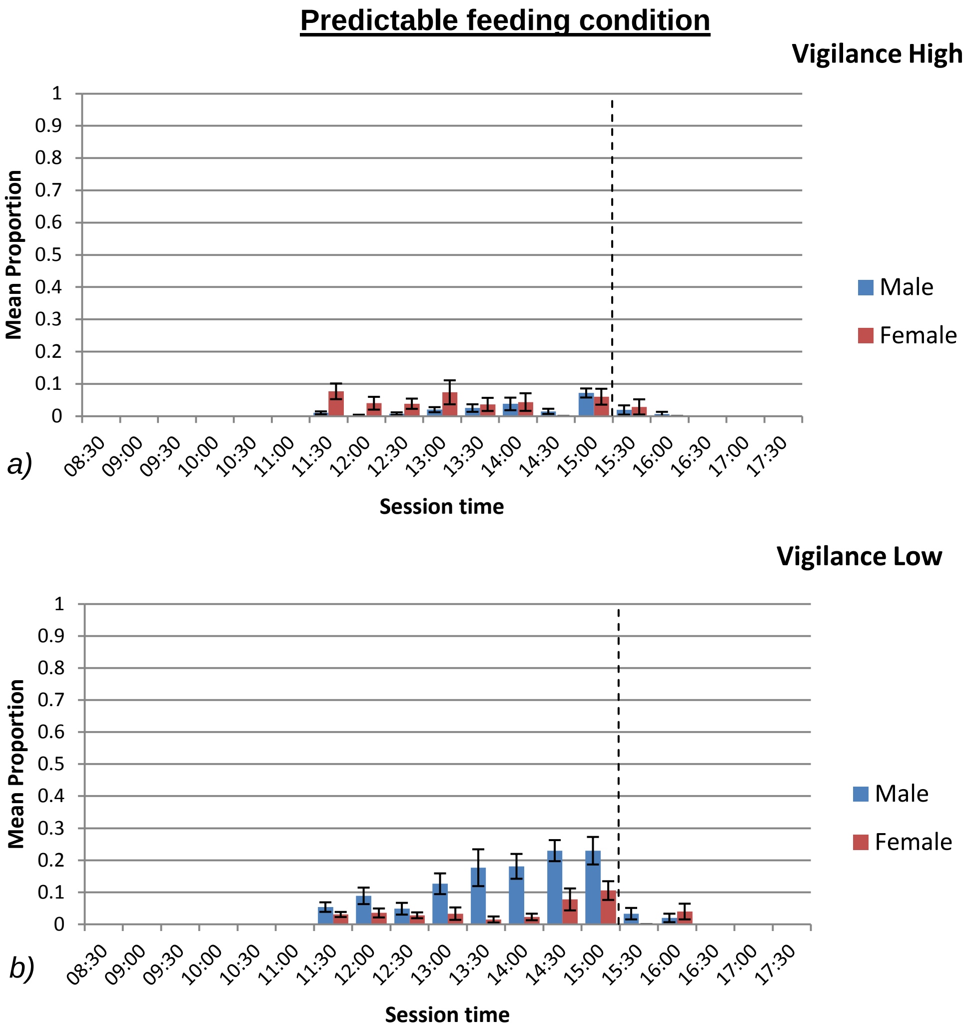


*Figure 5.17: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Vigilance High' and b) 'Vigilance Low' behaviour in the predictable feeding condition. Dotted line shows the set feed time. n min = 5, n max = 15. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison.*

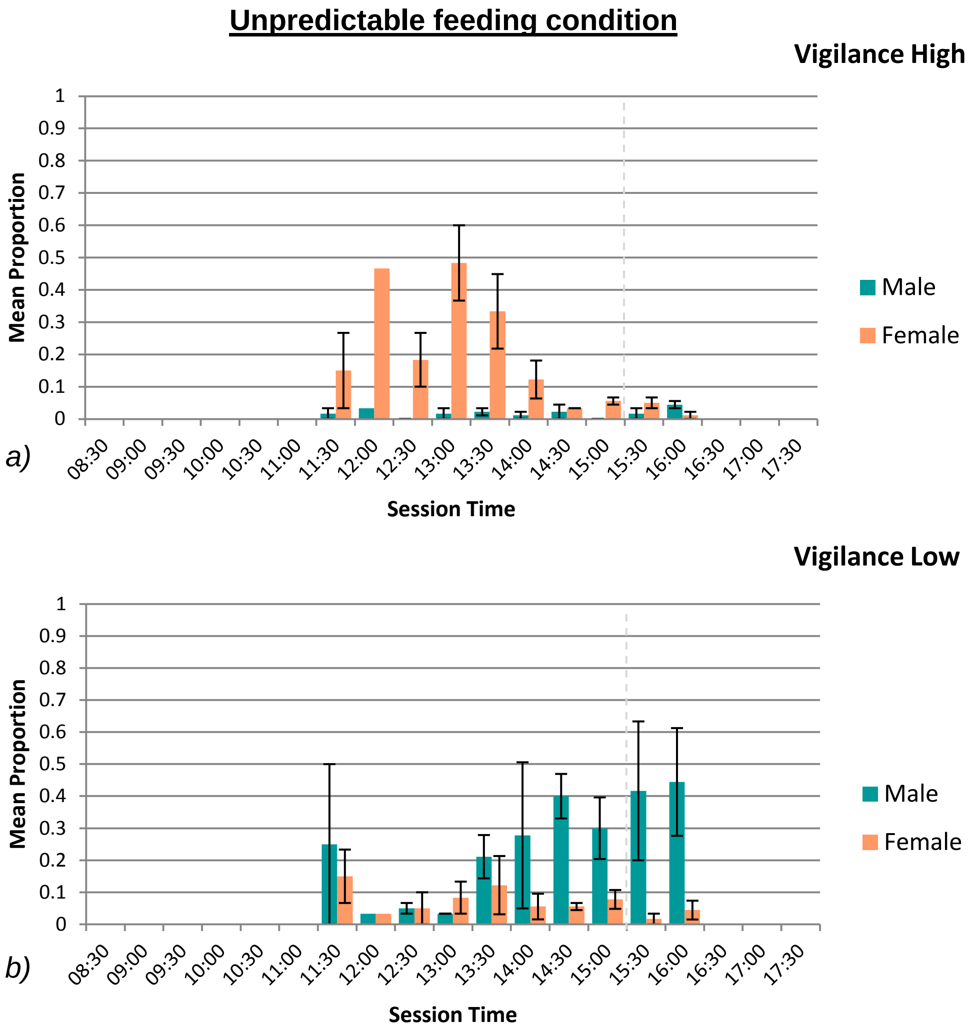


*Figure 5.18: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Vigilance High' and b) 'Vigilance Low' behaviour in the unpredictable feeding condition. Dotted line shows the time that would become the feeding time in the predictable feeding condition. n min = 1 (at 11:30 and 12:00), n max = 3. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison.*

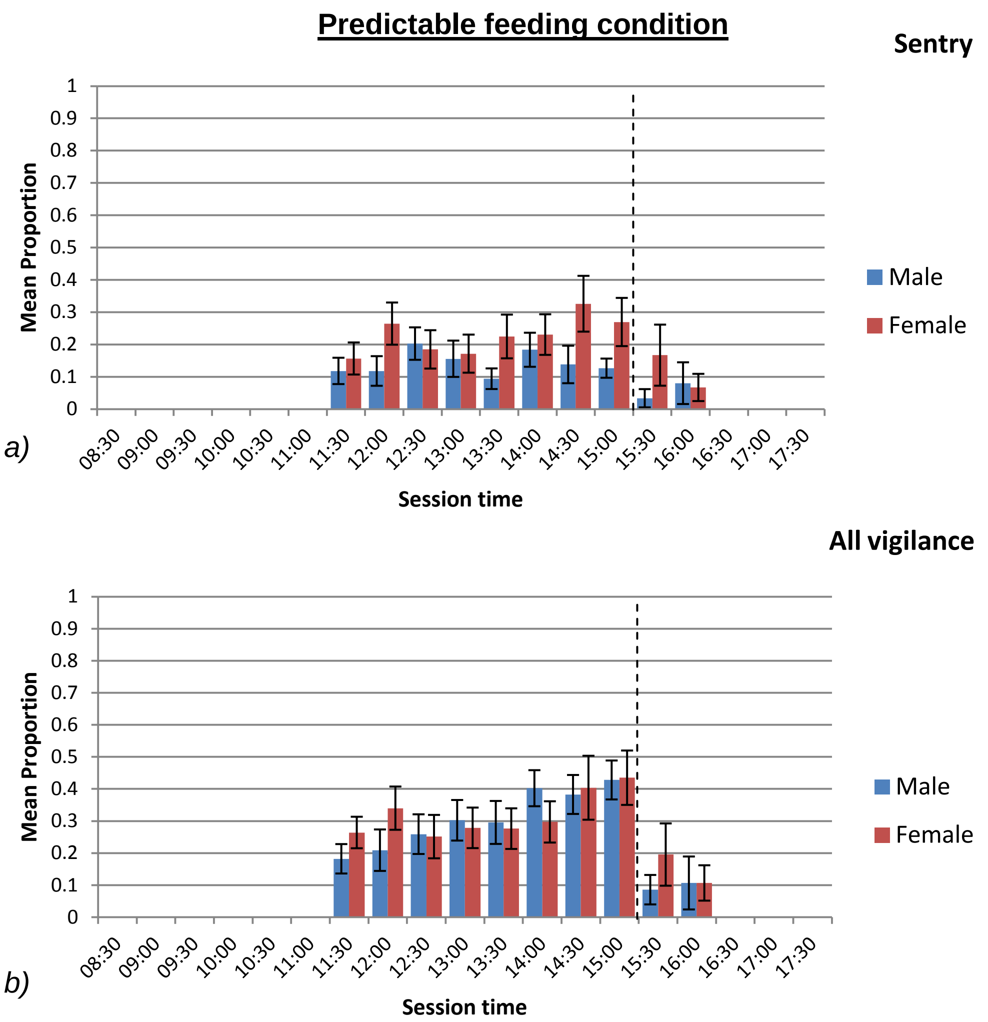


*Figure 5.19: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Sentry' and b) 'All vigilance' behaviour in the predictable feeding condition. Dotted line shows the set feed time. n min = 5, n max = 15. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison*

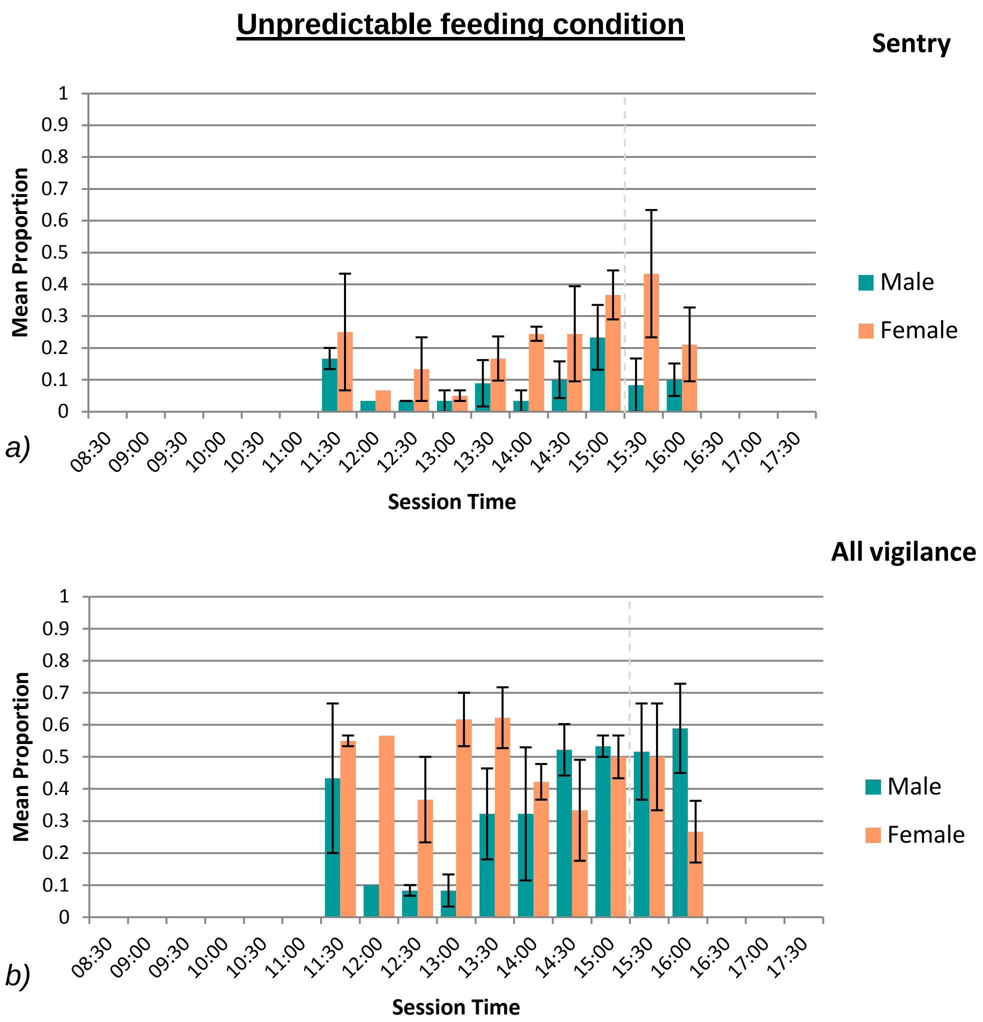


*Figure 5.20: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) 'Sentry' and b) 'All vigilance' behaviour in the unpredictable feeding condition. Dotted line shows the time that would become the feeding time in the predictable feeding condition. n min = 1 (at 11:30 and 12:00), n max = 3. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison.*

Due to variability in the data, no clear differences in daily patterns of foraging, feeding or locomotion could be determined between the feeding conditions (Figures 5.21-5.24). However, higher proportions of scans of foraging were shown for the male meerkat up to 13:00 in the unpredictable condition compared to the predictable condition (up to a maximum of .80). Also, afternoon proportions of feeding were higher for both meerkats in the unpredictable condition, and the peak of locomotion as shown in the 15:30 observation session in the predictable condition, was not shown in the unpredictable condition. Proportions of 'not visible' were lower throughout the day in the unpredictable feeding condition (Figures 5.23-5.24).

All other behaviours (resting, self maintenance, social and agonistic behaviours) were observed at low proportions, and hence are not reported here. However, it is worth noting that enrichment directed behaviour was not observed in the unpredictable feeding condition, in contrast to the predictable condition, where it occurred in the observation sessions at 15:30 and 16:00.

## Predictable feeding condition

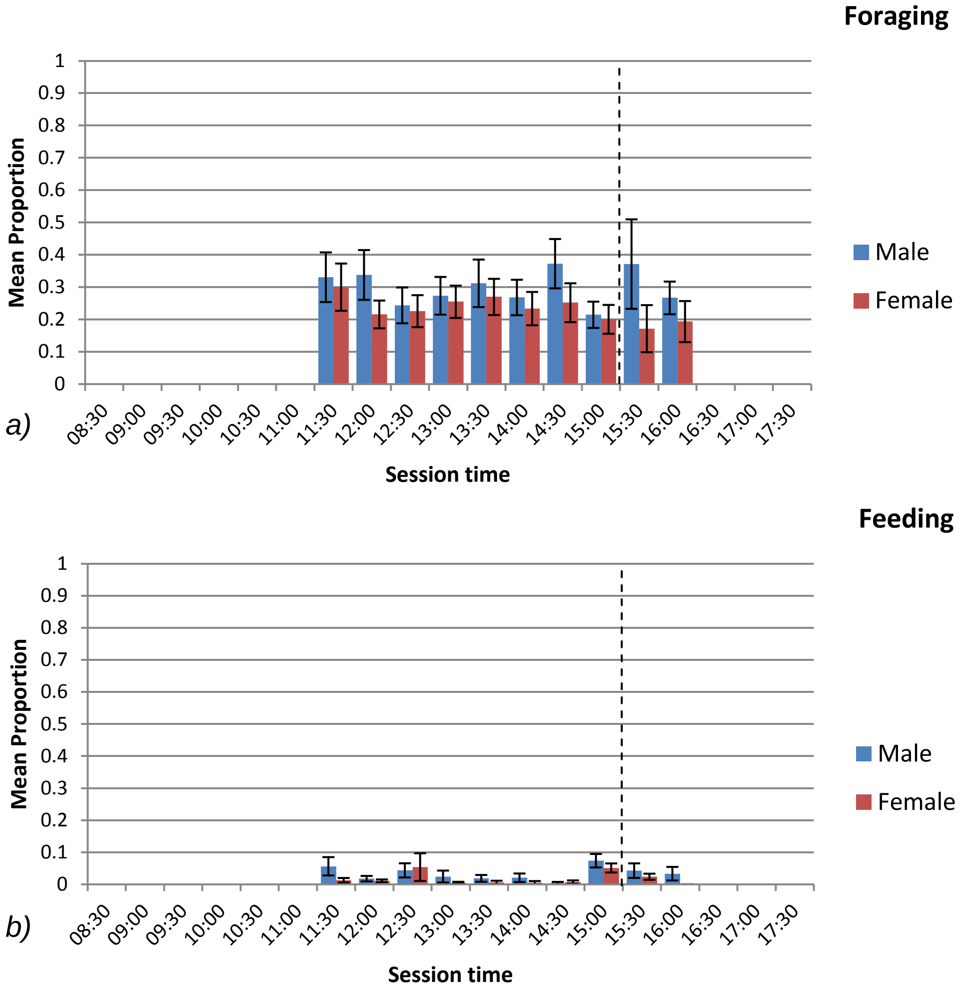


*Figure 5.21: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Foraging and b) Feeding behaviour in the predictable feeding condition. Dotted line shows the set feed time. n min = 5, n max = 15. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison.*

## Unpredictable feeding condition

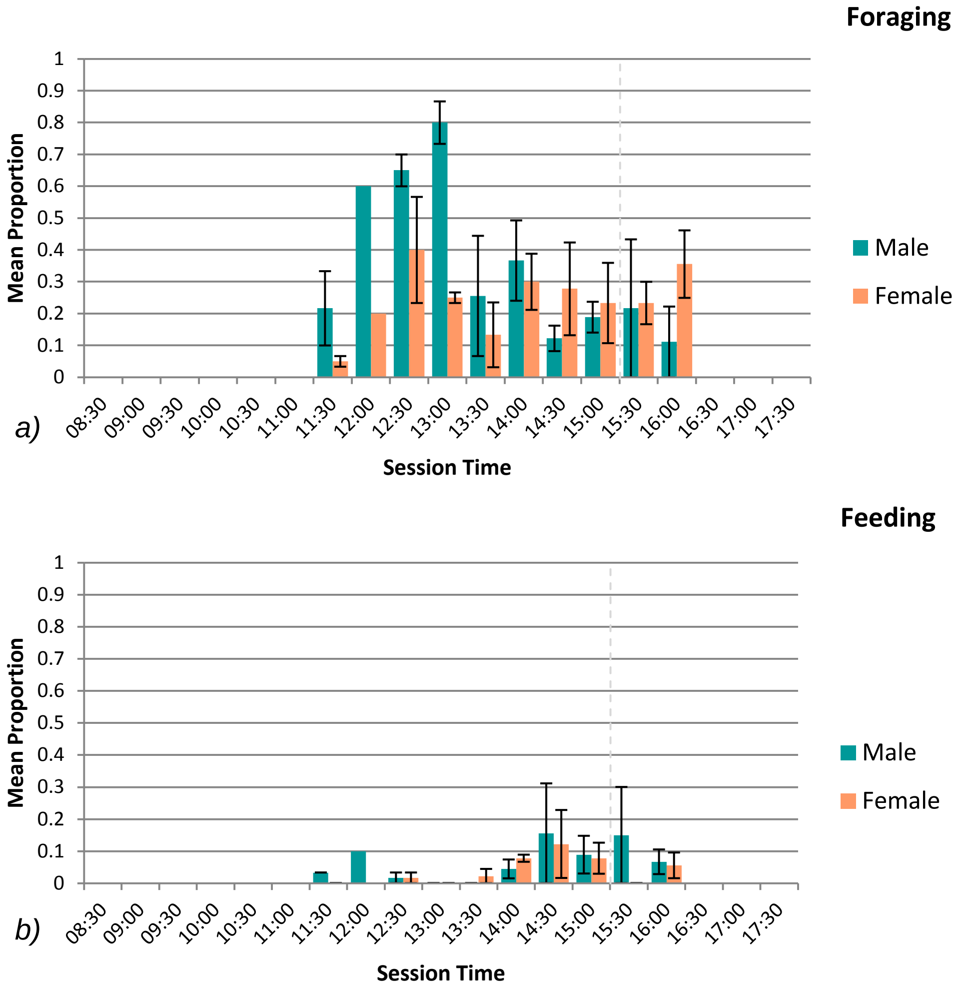


*Figure 5.22: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Foraging and b) Feeding behaviour in the unpredictable feeding condition. Dotted line shows the time that would become the feeding time in the predictable feeding condition. n min = 1 (at 11:30 and 12:00), n max = 3. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison.*

## Predictable feeding condition

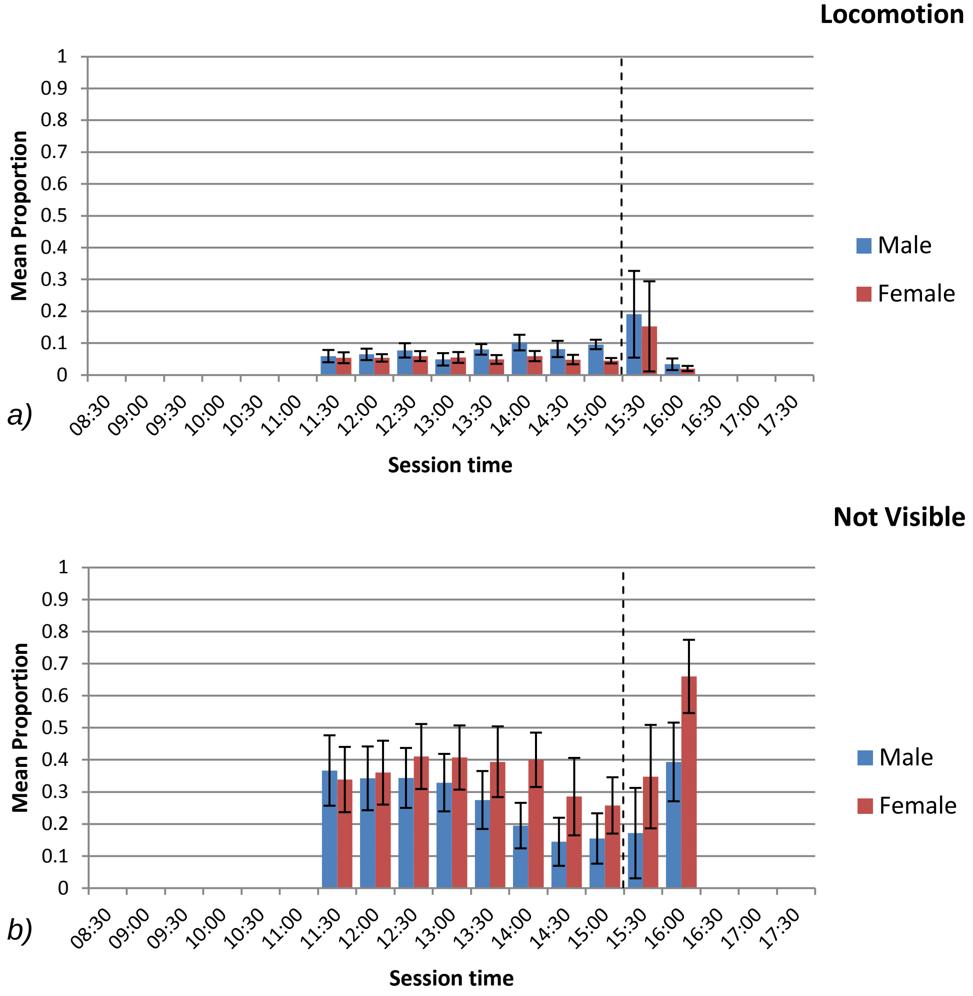


*Figure 5.23: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Locomotion and b) 'Not visible' behaviour in the predictable feeding condition. Dotted line shows the set feed time. n min = 5, n max = 15. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison.*

## Unpredictable feeding condition

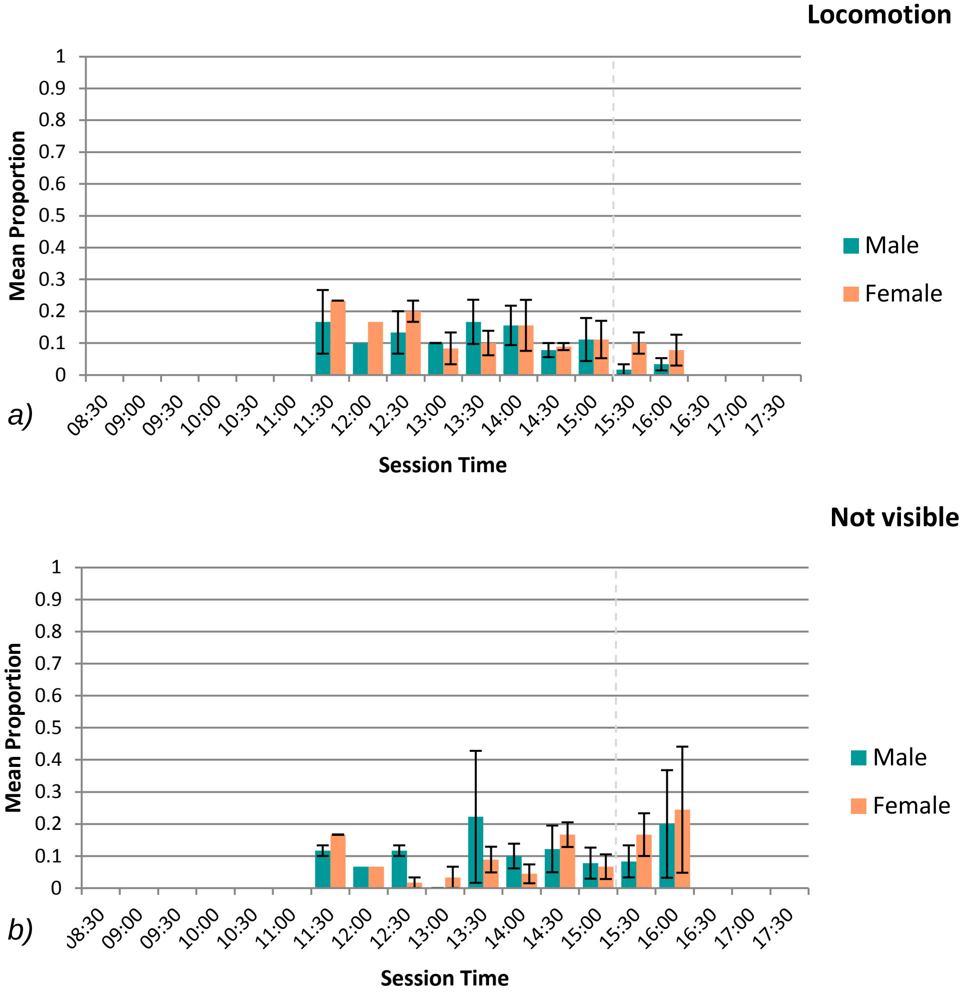


*Figure 5.24: Mean (+/- SE) proportion of scans in 30min sessions throughout the day, where meerkats were observed in a) Locomotion and b) 'Not visible' in the unpredictable feeding condition. Dotted line shows the time that would become the feeding time in the predictable feeding condition. n min = 1 (at 11:30 and 12:00), n max = 3. There were no observations prior to 11:30 or after 16:30, however the same axes are given as for Study 1, for comparison.*

### 5.7.3. Patterns of busyness throughout the day in the predictable condition

Busyness increased throughout the day in the predictable condition, with peaks at 14:38 (1.83) and at 15:31 (3.67) (Figure 5.25). Busyness then decreased, and became more variable over the rest of the afternoon, with a low at 16:25 (0.60). The data show an increase in busyness in the 45 minutes preceding the feeding time. However, similarly to Study 1, when the data are presented with those observations in which keepers were present excluded, mean busyness does not rise above the general fluctuating level of busyness (throughout the rest of the day) until around 15:30. In addition, busyness scores are at their highest level after the feeding time, with no clear pre-feeding increase discernible from the general fluctuating level of busyness (Figure 5.26).

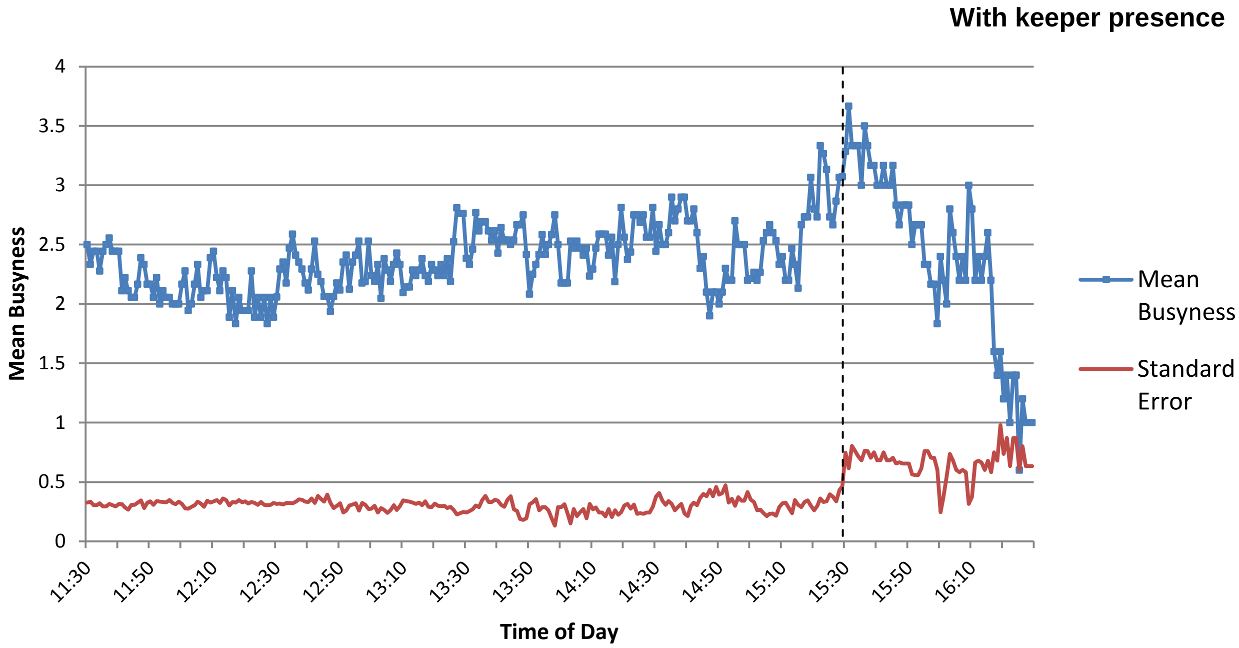


*Figure 5.25: Mean busyness for each minute of day (over all days of observation in the predictable feeding condition). Vertical dashed line indicates the predictable feeding time.*

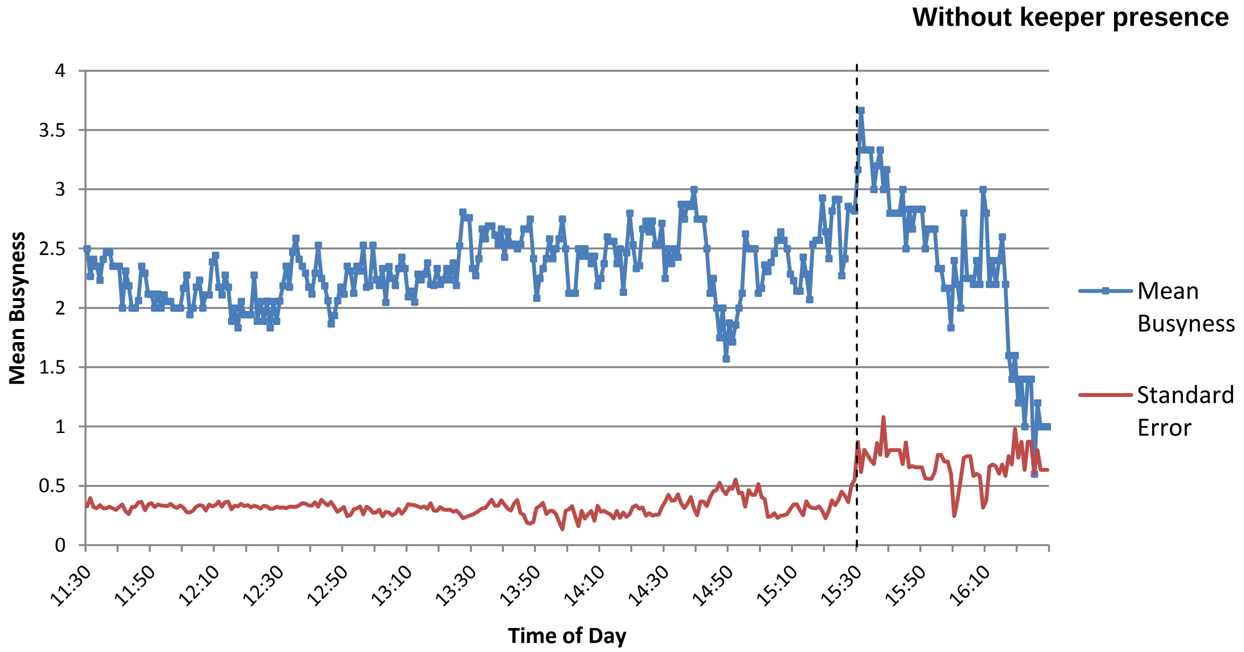


*Figure 5.26: Mean busyness for each minute of day (over all days of observation in the predictable feeding condition) (minus keeper present data). Vertical dashed line indicates the predictable feeding time.*

### 5.7.4. Comparison of pre-feed observation sessions across all days of observation

The data collected in the 30 mins prior to the feeding time showed variability over the days of study, with no obvious increase or decrease in any state behaviour as the days of the predictable condition progressed (Figure 5.27). A period of comparatively higher 'vigilance high' (male meerkat) and 'vigilance low' (both meerkats), than in the unpredictable condition, occurred around predictable observation days 8-13 and 9-11 respectively. A period of comparatively lower sentry behaviour compared to the unpredictable condition occurred around observation days 11-14 of the predictable condition. However, consequent decreases/increases in proportions of these behaviours in the following days prevent the development of a pattern of continual increase/decrease or 'levelling off' of the behaviours. Foraging, feeding, locomotion and not visible showed no clear change over the course of the predictable condition.

Results of the randomisation analysis revealed no significant difference in any state behaviour in the 30 minute prior to feeding in the predictable feeding condition compared to the unpredictable feeding condition for the two meerkats (*p* >.05) (Table 5.6).

*Table 5.6. Descriptive data giving the mean proportion and standard error of each state behaviour for both meerkats in the pre-feeding 30 minutes in the predictable and unpredictable feeding conditions, plus results of the randomisation analysis (Design 3) (Todman & Dugard, 2001).*

| | **Unpredictable** | | **Predictable** | | | | |
|---|---|---|---|---|---|---|---|
| | **Mean proportion** | **SE** | **Mean proportion** | **SE** | **Test statistic** | **Count of arrangement statistics as least as large in abs value as abs (test)** | ***P*** |
| Vigilance High | 0.11 | 0.06 | 0.06 | 0.01 | -0.09 | 353 | >.05 |
| Vigilance Low | 0.07 | 0.03 | 0.17 | 0.03 | 0.20 | 221 | >.05 |
| Sentry | 0.21 | 0.05 | 0.19 | 0.04 | -0.05 | 1606 | >.05 |
| All Vigilance | 0.39 | 0.09 | 0.42 | 0.05 | 0.06 | 1454 | >.05 |
| Foraging | 0.38 | 0.11 | 0.21 | 0.03 | -0.35 | 392 | >.05 |
| Feeding | 0.06 | 0.02 | 0.06 | 0.01 | 0.01 | 1685 | >.05 |
| Locomotion | 0.10 | 0.03 | 0.07 | 0.01 | -0.06 | 227 | >.05 |
| Not Visible | 0.06 | 0.03 | 0.21 | 0.06 | 0.31 | 322 | >.05 |

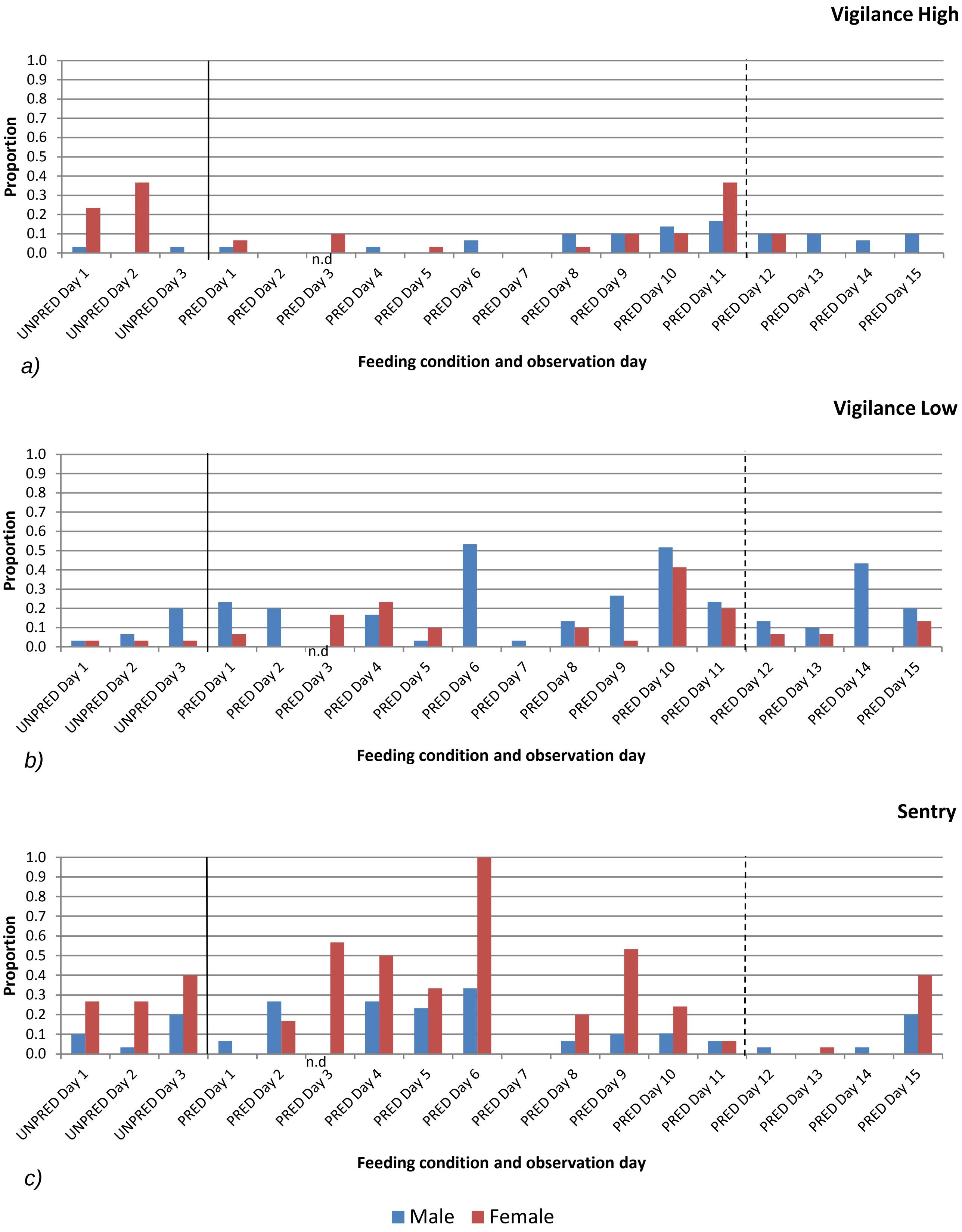
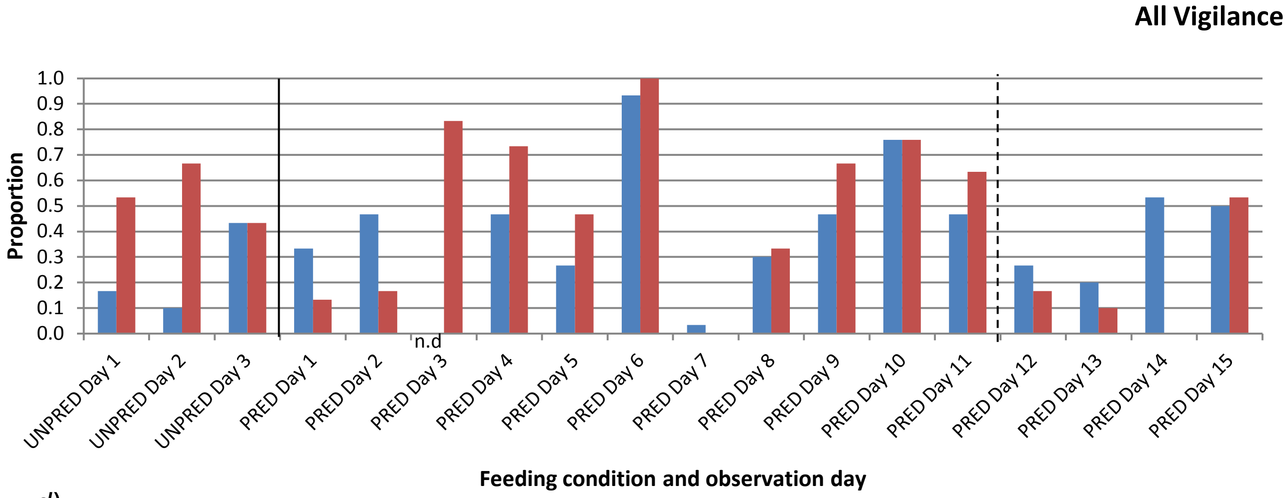
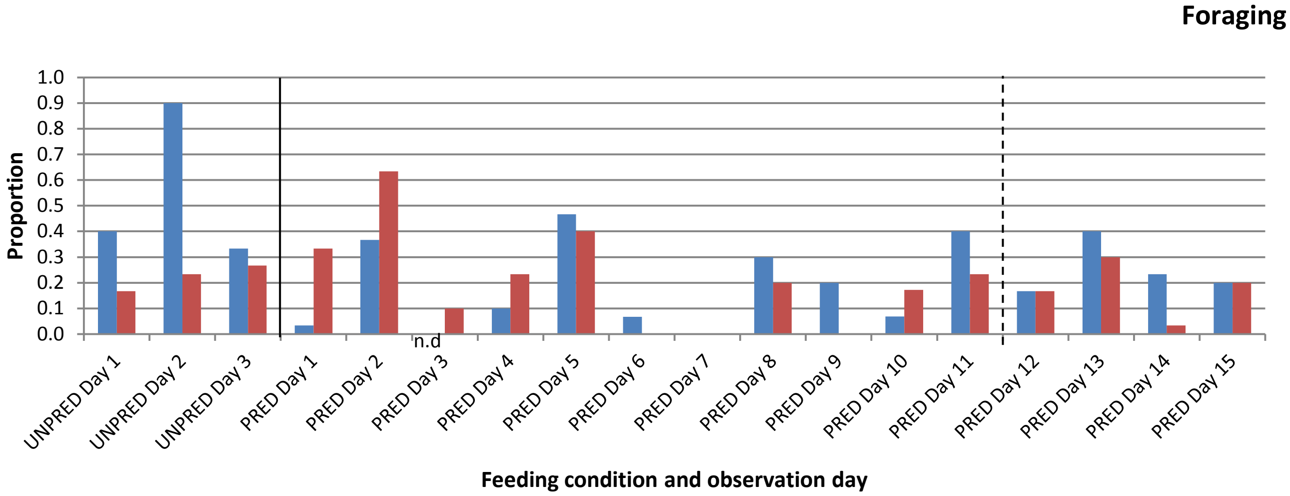


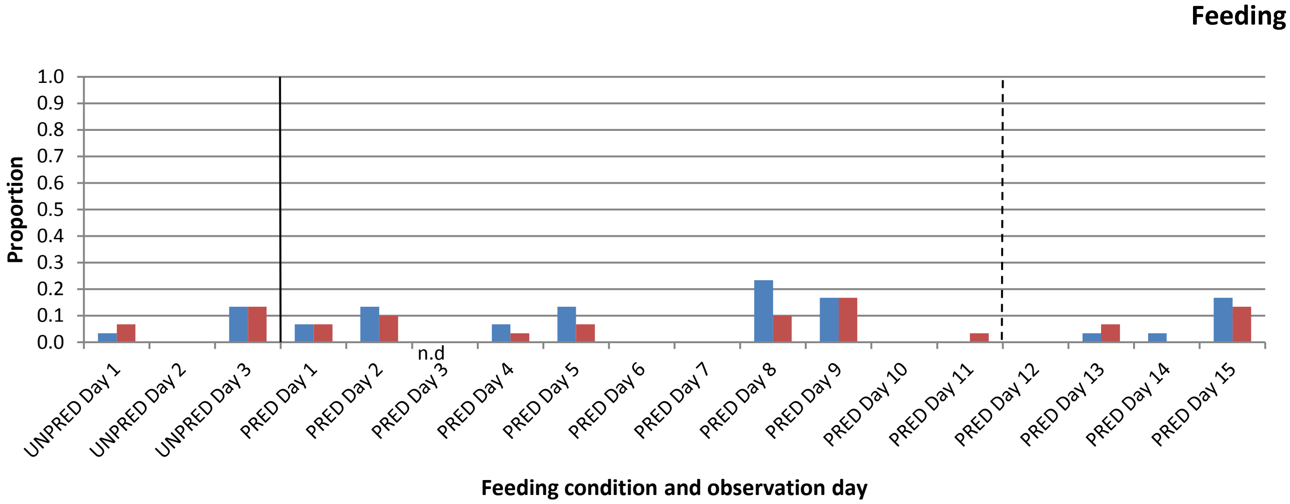
*Figure 5.27: Proportion of a) 'Vigilance high', b) 'Vigilance Low', c) 'Sentry, d)'All vigilance', e) Foraging, f) Feeding, g) Locomotion, and h) Not visible behaviours shown in pre-feed/1500 observation sessions on each day of observation in all feeding conditions for both meerkats. The continuous line shows the start of the predictable feeding condition and the dotted line shows when the additional meerkats joined the study pair (n.d indicates that no data was recorded on this day).*


All Vigilance
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
n.d
UNPRED Day 1
UNPRED Day 2
UNPRED Day 3
PRED Day 1
PRED Day 2
PRED Day 3
PRED Day 4
PRED Day 5
PRED Day 6
PRED Day 7
PRED Day 8
PRED Day 9
PRED Day 10
PRED Day 11
PRED Day 12
PRED Day 13
PRED Day 14
PRED Day 15
Feeding condition and observation day

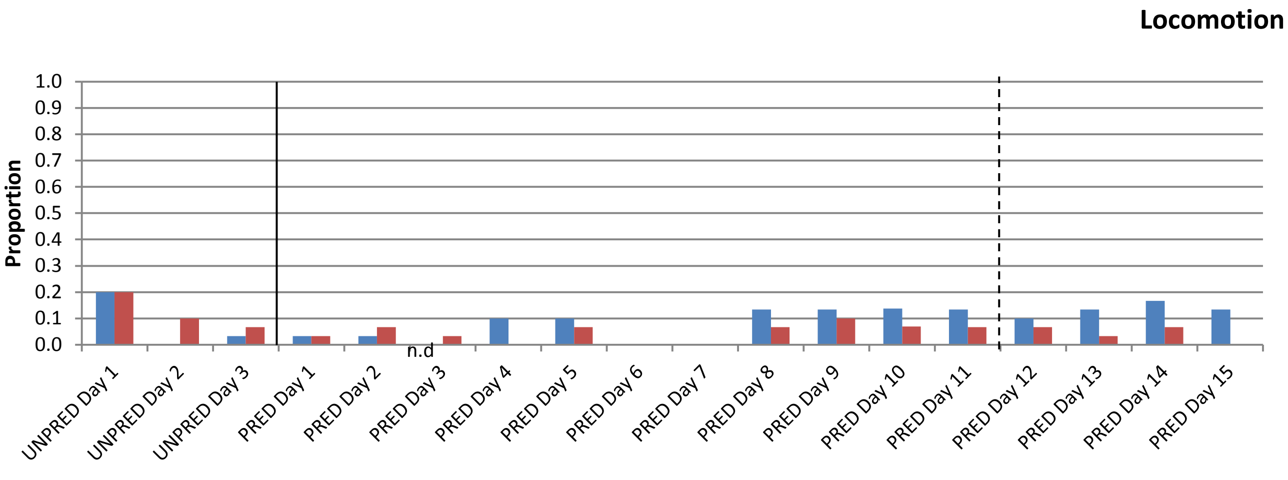
*d)*


Foraging
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
n.d
UNPRED Day 1
UNPRED Day 2
UNPRED Day 3
PRED Day 1
PRED Day 2
PRED Day 3
PRED Day 4
PRED Day 5
PRED Day 6
PRED Day 7
PRED Day 8
PRED Day 9
PRED Day 10
PRED Day 11
PRED Day 12
PRED Day 13
PRED Day 14
PRED Day 15
Feeding condition and observation day

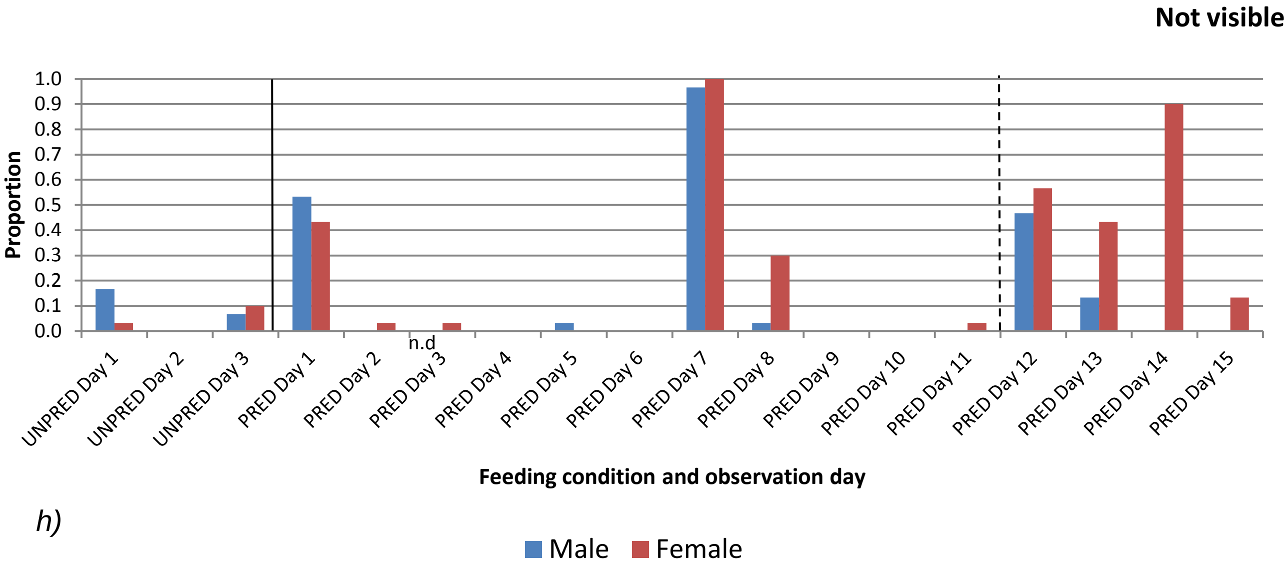
*e)*


Feeding
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
n.d
UNPRED Day 1
UNPRED Day 2
UNPRED Day 3
PRED Day 1
PRED Day 2
PRED Day 3
PRED Day 4
PRED Day 5
PRED Day 6
PRED Day 7
PRED Day 8
PRED Day 9
PRED Day 10
PRED Day 11
PRED Day 12
PRED Day 13
PRED Day 14
PRED Day 15
Feeding condition and observation day
Male
Female

*f)*


Locomotion
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
n.d
UNPRED Day 1
UNPRED Day 2
UNPRED Day 3
PRED Day 1
PRED Day 2
PRED Day 3
PRED Day 4
PRED Day 5
PRED Day 6
PRED Day 7
PRED Day 8
PRED Day 9
PRED Day 10
PRED Day 11
PRED Day 12
PRED Day 13
PRED Day 14
PRED Day 15
Feeding condition and observation day

*g)*


Not visible
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
n.d
UNPRED Day 1
UNPRED Day 2
UNPRED Day 3
PRED Day 1
PRED Day 2
PRED Day 3
PRED Day 4
PRED Day 5
PRED Day 6
PRED Day 7
PRED Day 8
PRED Day 9
PRED Day 10
PRED Day 11
PRED Day 12
PRED Day 13
PRED Day 14
PRED Day 15
Feeding condition and observation day
Male
Female

*h)*

### 5.7.5. Comparison of daily behavioural trends (predictable condition) in Studies 1 and 2; a summary of main patterns

For the comparable period of the day in studies 1 and 2 (11:30-16:00), the proportion of scans of state behaviours was compared. ‘Vigilance high’ behaviour for the male meerkat showed very low proportions in both studies, with no clear change in the sessions leading up to the feeding time. ‘Vigilance high’ behaviour for the female meerkat showed higher proportions in Study 1, plus slight evidence of an increase in ‘vigilance high’ in the sessions leading up to the feeding time, not shown in Study 2. In both studies, ‘vigilance low’ behaviour was observed at higher proportions in the male meerkat than the female. Low proportions of ‘vigilance low’ behaviour for the male meerkat in the 11:30 to 13:00 sessions in Study 2 resulted in a pattern of increase in ‘vigilance low’ behaviour in the male meerkat up to the 15:00 session, which was not shown in Study 1. However the actual values of pre-feed proportions of ‘vigilance low’ were similar in both studies. Decreased proportions of ‘vigilance low’ were shown at 15:30 in both studies. The female meerkat showed similar patterns and low proportions of ‘vigilance low’ in both studies, with a slight increase in the sessions leading up to feeding time.

Proportions of scans of sentry behaviour showed no pattern across the time period of comparison in both studies, with no increase or decrease prior to feeding. However, proportions of sentry were generally lower in Study 1 compared to Study 2. Proportions of ‘all vigilance’ behaviour were similar between meerkats in both studies. The female meerkat showed more variation in ‘all vigilance’ proportions between observation sessions in Study 1; however a similar general increase is shown leading up to the feeding time in both studies. Similar proportions of all vigilance for the male meerkat were shown in both studies.

Foraging behaviour showed no clear pattern in both studies, evidenced by overlapping error bars between successive sessions. Feeding behaviour was shown at very low proportions, with no clear pattern across the observation day in either study, with the exception that higher proportions were shown at 1500-15:30 in both studies. Locomotion for the male meerkat showed similar proportions and a similar lack of pattern across the comparable time period in both studies, however a peak in locomotion (0.19 and 0.15 for the male and female respectively) was shown at 15:30 in Study 2, but not in Study 1. Proportions of locomotion for the female meerkat were generally higher in Study 1 (but still below 0.2) and showed a pattern of decrease from 0.16 at 12:30 to 0.02 at 16:00, a pattern which was not clearly demonstrated in Study 2. Patterns of not visible behaviour were similar in both studies; however proportions were generally higher across the day in Study 2.

## 5.8. Discussion of Study 2 and General Discussion

### 5.8.1. Discussion of anticipatory behaviour

The predicted patterns of behaviour were not supported by the data, and Studies 1 and 2 of the current chapter demonstrated few, clear patterns of behaviour throughout the day, in the lead up to the predictable feeding time. Thus, these results suggest that either the meerkats' behaviour did not anticipate the feed, or that the observed behaviours were not indicative of anticipation in these meerkats. Study 1 suggested a possible increase in female 'vigilance high' behaviour in the lead up to the feeding time; however this was not demonstrated in Study 2, where proportions of 'vigilance high' were much lower (see Section 5.7.5). Furthermore, it would have been expected that any behavioural change in response to the predictable event would be greater in Study 2, when visitor numbers were lower, but this was not demonstrated.

The only pre-feeding patterns suggested in Study 2 were patterns of increase in 'vigilance low' and 'all vigilance' behaviours leading up to the feeding time. These data are similar to Study 1 for the female meerkat, but the increase for the male meerkat, was not shown in Study 1. Despite these increases, it cannot be concluded that these vigilance behaviours increased prior to, and in anticipation of the predictable feeding time, for a number of reasons. Firstly there was no progressive pattern of increased proportions of vigilance behaviours in each of the pre-feed observation sessions across the course of the study in the predictable condition (for both Studies 1 and 2). Likewise, there was no trend for either a decrease, or increase in any other behaviour across the course of study. Secondly, vigilance and other behaviours showed similar patterns across the day in the predictable feeding condition, as in the unpredictable condition. Finally, there was no statistically significant difference between proportions of any behaviour in the pre-feed 30 minutes in the unpredictable feeding condition and the predictable condition. Therefore there was no clear evidence, in either study to suggest that behaviour anticipated the feeding time.

The fact that the patterns of ‘vigilance low’ and ‘all vigilance’ in the predictable condition were not concluded to be anticipatory patterns when the data from both conditions were compared, demonstrates the importance of comparing unpredictable and predictable feeding conditions when inferring causality from behavioural patterns.

It should be noted that some differences were shown between conditions in Study 2. For example, proportions of ‘vigilance high’ for the female meerkat and foraging for the male meerkat were higher in the earlier observation sessions in the unpredictable condition, and proportions of ‘all vigilance’ for the male, were lower between the 12:00 and 13:00 observation sessions in the unpredictable condition than the predictable condition. However, results for the unpredictable condition in Study 2 are based on a low number of repeats, and differences in the earlier part of the day may reflect factors specific to those observation days. Proportions of scans of feeding behaviour were higher, and more variable in the afternoon of the unpredictable condition compared to the morning, probably due to the varying time of the feed, hence feeding behaviour was reported at a greater range of times compared to the predictable condition. For the unpredictable feeding days observed, all random feeds occurred at a time after 13:20. Not visible behaviour was observed at higher proportions in the predictable condition, and correspondingly some other behaviours (such as locomotion and ‘all vigilance’) were observed at higher proportions in the unpredictable feeding condition. These proportions of not visible may have been due to colder or wetter weather during the predictable condition, which took place later into the winter months of the year. However the important comparisons for this chapter concern the patterns in the data rather than the absolute proportions.

Data from the predictable condition of Studies 1 and 2 showed similar patterns for most of the behaviours. One difference of note was that in Study 2, whilst proportions of vigilance behaviours dropped in the 15:30 and 16:00 observation sessions, there was no large, corresponding increase in feeding or foraging at this time, as seen in Study 1, and as would be expected after the provision of food. This is due to the type of feed that the meerkats received on some of the predictable feeding days; that of an enrichment feed, whereby food was hidden in an enrichment object. Enrichment-directed behaviours and locomotion were therefore observed to increase after the feed in Study 2, in place of feeding and foraging behaviours.

The overall conclusions of Study 1 were that there was no evidence of anticipatory behaviour for the male meerkat, and only limited evidence of changes prior to feeding for the female meerkat, although these were not supported when compared to other aspects of behaviour during the day and in the unpredictable condition. That Study 2 showed similar patterns and proportions of behaviours to Study 1 strengthens these conclusions, and suggests that neither meerkat showed behavioural changes prior to the predictable feeding time, such that it anticipated the feed.

### 5.8.2. Revisiting objectives of Study 2

Study 2 was designed to test the original hypotheses of Study 1, during winter months (when fewer visitors were present at the zoo), in order to prevent the variability in the data as obtained in Study 1. If the meerkats were vigilant for unpredictably timed visitor feeds throughout the day, it would be expected that in Study 2, meerkat vigilance would be lower and less variable throughout the day, with a clear increase leading up to the feeding time. This was demonstrated to some extent; proportions of ‘vigilance high’ for the female, and ‘vigilance low’ for the male were generally lower in Study 2 compared to Study 1. However, proportions of sentry behaviour were higher in Study 2, perhaps due to the colder winter weather, and the need to stand under the heat lamps, where the meerkats were also vigilant (as discussed in Section 5.4.1).

Vigilance behaviour showed less variability in Study 2 than Study 1. It is therefore possible that observation in the low-visitor season (winter) resulted in less variable data across the day. However, standard error bars do still demonstrate variation in the data at each observation time, and no clear evidence of an increase in vigilance in anticipation of the feed was found. Therefore it is possible, that the meerkats were still vigilant for feeds from those visitors that were present, and occasional (even if less frequent) feeding from visitors may have reinforced this behaviour. However, this study was not designed to determine the exact relationship between zoo visitors and meerkat behaviour, and hence causality cannot be confirmed in this case, only considered as a potential influencing factor. Indeed other factors (such as temperature) also varied between the seasons. Study 2 was necessary to clarify the patterns of meerkat behaviour under potentially more predicable (rather than *ad lib*) feeding conditions, however anticipation of the predictable feed could not be concluded.

### 5.8.3. Post-feeding behaviour

One clear behavioural pattern in the predictable condition of both studies, particularly apparent in Study 2, was a change in behaviour in the 15:30 and 16:00 observation sessions. For example, vigilance behaviours decreased, and foraging and feeding (or, alternatively, enrichment-directed in Study 2) behaviours increased. This change in behaviour was a result of the meerkats being fed at 15:30. This change in proportions was not shown in the unpredictable conditions. Under visual analysis, this drop in vigilance at 15:30 exaggerates the increase in pre-feed vigilance in Study 2; hence what visually appears to be an increase in vigilance before feeding may actually be a result of a drop after feeding. When compared to Study 1, where observations were continued until later in the day, it was demonstrated that after the initial, post feed observation sessions (15:30 and 16:00), proportions of behaviours seemed to resume their pre-feed patterns, after the deviation from this pattern caused by the feed. For example, in Study 1, female ‘all vigilance’ and ‘vigilance high’ behaviour continued to increase until 16:30. Similar patterns are shown in the unpredictable feeding condition (without the deviation at 15:30). It therefore seems likely that, as suggested in Section 5.4.1, the meerkats used a reactive, rather than anticipatory strategy in relation to keeper feeds, and the main behavioural change was in response to feeding time. This was also demonstrated in the busyness results whereby busyness increased around the feeding time, but only when keepers were present, and then after the meerkats were fed. This reactive strategy could explain the lack of a clear difference in behaviour patterns between the unpredictable and predictable conditions prior to the feeding time, and could explain the greater variability in the data during the unpredictable condition, as the deviation in ‘normal’ behaviour, caused by the feed, occurred at different times on different days. Study 1 suggested an increase in standing from the meerkats upon arrival of the keepers, and both studies suggest an increase in busyness when keepers were present, followed by decreases in vigilance and increased foraging and feeding, upon being fed. Therefore these meerkats may have been responding to the signalled predictability of the arrival of the keepers, followed by feeding, as discussed in Section 5.4.1 and Section 1.4.3. In this case the exogenous cue of keeper arrival may have been a stronger influence on meerkat behaviour than any endogenous cue.

### 5.8.4. Discussion of methodology

Patterns of pre-feed state behaviours and busyness were similar in both studies, and neither showed a clear change prior to feeds. This similarity is further validation of the busyness measure. Busyness data also demonstrated an effect of keeper presence and hence the potential response of the meerkats to signalled predictability, which was not identified in the scan sampling data. The measure of busyness and its continuous rating for each minute of the day was therefore of benefit in this study as it helped to identify this relationship.

Study 2 was carried out by a group of student observers. Whilst a case for the benefits of using multiple observers has been made earlier in this thesis (Chapters 2 and 4), the current study has highlighted an important difficulty that can arise with such methods; that of obtaining the raw data from the observers. In this study, despite careful planning, only limited data were retrieved from the student observers covering observations in the unpredictable condition (minimum *n* = 1, maximum *n* = 3), hence the results presented here are based on a low number of repeats. This study, as with other projects requiring multiple observers such as the Chimpanzoo project (ChimpanZoo, 2003) relied on observers emailing their data to the experimenters. In future work requiring multiple observers, it is recommended that focus should be placed on methods of immediately obtaining raw data from all observers, for example with the use of handheld mobile internet devices which can be synced to instantly update and monitor records.

It should be noted that Study 2 covered a truncated portion of the day than Study 1, thus when analysed visually, patterns of behaviours appeared more prominent, than when compared with the complete day of data from Study 1. The greater range of data points as provided in Study 1 enabled a more substantial picture of the daily patterns of behaviour, and enabled identification of the continuation of daily patterns after the pre-feed session. This provides support for the need to study temporal patterns of behaviour over as long a period as possible, as discussed in Chapter 2. This can be achieved with multiple observers. Whilst the current study used only an opportunity sample of observers, future work could specifically recruit observers in order to cover a greater time period of observation.

### 5.8.5. Conclusions

In conclusion, Studies 1 and 2 have demonstrated that, in contrast to predictions, there was no clear demonstration of anticipatory behaviour in the study group of meerkats. Whilst patterns of vigilance low and all vigilance behaviour suggest some increases in proportions in the lead up to the feeding time, comparisons of behaviours across the predictable condition, and with the unpredictable feeding condition reveal few clear differences between feeding conditions prior to the feeding time, and no significant differences in pre-feeding behaviour between the different feeding conditions, suggesting a lack of anticipatory behaviour. It could be concluded that either the behaviour of the meerkats did not anticipate the predictable feeding time, or that the behaviours observed were not indicative of anticipation. It seems likely that instead, the meerkats responded to the signalled predictability of the arrival of the keepers and then deviated from their afternoon behaviour patterns, in response to the feed. This study would benefit from further work in which the time of day of the predictable feed is changed, in order to determine whether anticipation would develop at a different time of day, or whether the daily patterns of behaviour would remain, but with a post feed deviation in behaviour. The latter would support the conclusion of a reactive, rather than anticipatory strategy in response to keeper feeds. It would also be beneficial to repeat the study on a group of meerkats where visitor feeding could be eliminated as an influencing factor, to thus confirm whether visitor feeds influence patterns of behaviour.

Of the available literature on predictability, there are no specific reports of a lack of feeding anticipation, to the authors' knowledge; where predictability is documented it is to report observation of anticipatory behaviour. However, in the debate over the positive or negative effects of predictable feeding, it is also important to acknowledge the possibility of a neutral effect (at least as defined by behavioural indicators), and management decisions may be aided with further knowledge of which species or environmental conditions can result in this neutral effect. As discussed in Section 5.4.3, control and predictability are linked, and in the current example, the ability of the meerkats to forage in their enclosure for any interloper insects (and potentially gain food from visitors) may have enabled the meerkats to exert control over their environment, and hence the need to anticipate feeding time may have been reduced. Future work observing meerkats in different enclosure types, with varying levels of environmental complexity and opportunities for foraging control or visitor influence would enable determination of this effect of environmental control.

The current chapter has further demonstrated the utility of busyness and has demonstrated the importance of observing behavioural patterns over as much of the day as possible. It has also demonstrated the need for an organised, and ideally automated system for obtaining raw data, when using multiple observers.

# Chapter 6

## *The effect of predictable feeding times on the behaviour of the gold dust day gecko (Phelsuma laticauda)*

## 6.1. Introduction

One conclusion of Chapter 5 was that environmental variability, and factors such as visitor feeds, may have affected the response of the meerkats to the predictable feeding times. In order to test the effects of predictable feeding times in a more controlled situation where potential extraneous variables could be minimised, animals housed in an indoor and less publicly accessible enclosure were selected. These animals were gold dust day geckos (*Phelsuma laticauda*) which were housed in a glass terrarium, behind a viewing window, which prevented visitor contact, reduced visitor noise, and allowed control over climatic variables such as light, temperature and importantly, husbandry regime feeding. The feeding times of *P. laticauda* were manipulated to give two experimental conditions presented in an ABA design: temporally unpredictable feeding, then predictable feeding, followed by a return to unpredictable feeding.

### 6.1.1. Reptile circadian rhythms

There is a paucity of literature available on reptile timing. That which does exist is mainly focused on circadian rhythms, but such reports are scarce compared to availability of information for other vertebrates such as mammals, birds and fish (Oishi et al., 2004). Examples of reptile circadian rhythms observed in the wild include regular night time emergence from burrows in the Western shovel-nosed snake (*Chionactis occipitalis*) (Norris & Kavanau, 1966), and diving behaviour which is linked to sunrise and sunset in loggerhead turtles (*Caretta caretta*) (Sakamoto et al., 1990). Examples of reptile circadian rhythms demonstrated in laboratory and other captive conditions include: moving out of, and into water at sunrise and sunset in American alligators (*Alligator mississippiensis*), a pattern which was experimentally phase shifted by artificially altering the length of light cycles (Lang, 1976); daily cycles of locomotion in the Plains garter snake (*Thamnophis radix hayendi*) in relation to light onset, the timing of which was dependent on the temperature, with diurnal activity occurring when temperatures were set at 15°C and 20°C, and nocturnal activity at 31°C (Heckrotte, 1975); and robust daily rhythms of diurnal locomotor activity in sleepy lizards (*Tiliqua rugosa*), red-eared turtles (*Trachemys scripta elegans*) and Japanese grass-lizards (*Takydromus tachydromoides* and *Takydromus smaragdinus*), in 12-hour light:dark cycles (Ellis et al., 2007; Gopar-Canales et al., 2010; Oishi, et al., 2004 respectively).

A specific example of timing ability in geckos has been reported in three *Ptyodactylus* species (*P. hasselquistii puiseuxi, P. h. cf. hasselquistii* and *P. h. guttatus*) (Frankenberg, 1979). These geckos showed cycles of activity under conditions of: light:dark (LD) (set according to the actual sunrise and sunset times of the geographic area) with a daily temperature gradient; constant light (LL) and constant dark (DD) with a daily temperature gradient; and DD with a constant temperature; but not in conditions of LL with a constant temperature.

Whilst the above examples demonstrate circadian patterns, it is important to note that reptiles are a diverse class of vertebrates in terms of physiology and ecology and variation exists within the Gekkonidae family alone. Care should therefore be taken when comparing the behaviour of different species. Indeed, even between the above described *Ptyodactylus* species, Frankenberg (1979) reported differences in the time of peak activity.

Reptiles are therefore able to show endogenously controlled patterns of behaviour associated with time of day. Circadian timing in reptiles, as in mammals is thought to be controlled by the suprachiasmic nucleus in the brain, but additionally (in reptiles) by the pineal complex, and can be entrained by light and/or temperature (Oishi, et al., 2004; Tosini et al., 2001; Underwood, 1992). Light and temperature can therefore affect patterns of reptile behaviour related to time. As ectotherms, temperature also affects reptile behaviour patterns in relation to thermoregulation (e.g., Willmer et al., 2004). Underwood (1992) advises caution when inferring that a behaviour such as activity is under the control of an individual's biological clock, as temperature and light can stimulate or suppress behaviour. Temperature and light were therefore controlled in the current study.

### 6.1.2. Effects of feeding times on reptile behaviour

Whilst the effect of light and temperature on the timing of reptile behaviour has been studied, very few reports exist on the effects of feeding regime. Those that do, vary in the emphasis placed on feeding as an experimental variable. For example, Terezow et al. (2008) observed captive juvenile tuatara (*Sphenodon* spp.) to make frequent movements during the daytime, whilst in contrast, wild tuatara are predominantly nocturnal, and are active and feed around sunset. It was suggested that the observed daytime movements were stimulated by the diurnal provision of feeds. However the authors noted that the tuatara did show peak emergence from burrows at night, and so suggest that the feeding regime did not govern all aspects of the behavioural rhythms. In a study of whiptail lizards (*Aspidoscelis* spp.) (Winne & Keck, 2004) the prediction that, once satiated, the lizards would cease activity (defined as use of the surface of the enclosure) and retreat underground, was not supported by observations; there was no difference found between the retreat time of lizards fed early and those fed late. Therefore the timing of feeding was not considered to have an effect on patterns of activity in these lizards, which were thought to be entrained by the photothermoperiod. However this study focused on behaviour after feeding, rather than behaviour in anticipation of feeding, and feeding times were varied.

To our knowledge, there are only two examples specifically investigating reptile food anticipatory behaviour. Firstly, wild marine iguanas (*Amblyrhynchus* *cristatus*) feed on algae present in intertidal areas at low tide, and have been demonstrated to time their movements from distant resting sites to the tidal areas, such that they anticipate the low tide (Wikelski & Hau, 1995). The authors suggest that the iguanas possessed a circatidal oscillator which controlled this behaviour. When a sample of iguanas were separated into a nearby enclosure, with no access to the feeding grounds, a bitidal rhythm of activity was still demonstrated, but after some days of separation, behavioural patterns became out of phase with the free-living conspecifics, suggesting that food intake or related stimuli had an entraining role on the circatidal oscillator.

Secondly, freshwater turtles (*Pseudemys scripta elegans*) have been shown to push cylinders with their heads to receive pieces of meat on fixed interval schedules of either 30, 60 or 90 seconds (Laurent, 1983, as cited in Lejeune & Wearden, 1991). Head pushing increased as the time of reinforcement approached, indicating a timing ability by the turtles. However, when compared with data collected on mammals and birds, the rate of response was relatively high throughout the interval (rather than showing the temporal differentiation shown by other species), and coefficients of variation (see Section 1.2) were high, indicating poorer temporal control. High individual variation in turtle timing ability was also noted.

Anticipatory activity has been reported in red-eared turtles (Gopar-Canales, et al., 2010), but to light onset rather than feeding time. Turtles kept under cycles of light and dark showed increases in activity prior to light onset. However it should be noted that these data were highly variable between individuals, with occurrences of both high and low levels of activity prior to light onset, of which the low levels matched points in the dark period. The above examples indicate that certain reptile species can anticipate timed events, and that patterns of behaviour can be influenced by feeding. However, little is known about reptile anticipation, and the presence or absence of such behavioural patterns seem to be variable between species and individuals.

### 6.1.3. Introduction to *Phelsuma* spp. with a focus on *Phelsuma laticauda*

The genus *Phelsuma* (day geckos) is the most diverse of the gecko genera on Madagascar, consisting of over 20 species (Ikeuchi et al., 2005). One such species is the gold dust day gecko (*Phelsuma laticauda*). *P. laticauda* is a medium-sized day gecko up to 130mm in length (Bartlett & Bartlett, 2001; Van Heygen, 2004). Colouration is bright green to yellow-green with gold-coloured speckles on the nape and shoulder area. The head shows two red lines, with blue eye rings, and red tear drop-shaped marks are present on the lower back (Bartlett & Bartlett, 2001; Ota & Ineich, 2006; Van Heygen, 2004). *P. laticauda* is native to northern Madagascar, but is also found within the Comoros archipelago, the southern Seychelles and Réunion, Hawaii, French Polynesia and Mauritius, where the species has been introduced (Gerlach et al., 2011; Van Heygen, 2004). Preferred habitats are palms, banana trees and some smaller deciduous trees, although a range of habitats are utilised, with the exception of dense forest. Urban gardens and houses also provide suitable habitats (Gerlach, et al., 2011; Van Heygen, 2004). *P. laticauda* has been given the conservation status of Least Concern by the IUCN (Gerlach, et al., 2011).

As the name suggests, day geckos, are generally considered to be diurnal (Bartlett & Bartlett, 2001; Ikeuchi, et al., 2005), however *P. laticauda* have also been observed to be active and forage at night, under conditions of man-made illumination (Seifan et al., 2010). Such deviation from expected diurnal behaviour patterns is also seen in other gecko species, for example *P. guentheri* (Carpenter et al., 2003).

Geckos can therefore demonstrate flexibility in their behavioural response to environmental variables. Similar variation has been shown in gecko foraging tactics. *Phelsuma* spp. feed on insects, pollen, nectar, fruits, juice and sap (Bartlett & Bartlett, 2001; D'Cruze et al., 2009; Howard, 1980); however different hunting tactics have been reported for the insect component of gecko diets. Many lizard species, including geckos, either adopt a sit-and-wait foraging strategy (SW) whereby they are stationary and then ambush their prey, or a widely-foraging (WF) strategy whereby they actively seek out their prey (Seifan, et al., 2010; Werner et al., 2004). Reported SW species include *Phelsuma* spp.

such as *P. laticauda* (Seifan, et al., 2010) and *P. madagascariensis kochi* (Ikeuchi, et al., 2005) and WF species include *Cnemaspis kendallii* (Werner & Chou, 2002) and *Goniurosaurus kuroiwar orientalis* (Werner, et al., 2004). Some species have been observed to vary their foraging tactics, or demonstrate intermediate tactics (Seifan, et al., 2010; Werner et al., 1997). Indeed wild *P. laticauda* have been shown to deviate from expected foraging tactics at night, in the vicinity of artificial light. Extreme SW tactics were expected based on geckos waiting for phototactic insects, but this was not observed (Seifan, et al., 2010). This serves again to demonstrate that geckos can adapt their behaviour to suit environmental variables such as changes in the availability of food.

### 6.1.4. Geckos in zoos

In zoos, *Phelsuma* can be housed in ventilated glass vivariums, with substrates of bark, shale, soil, sand or paper towels, with cork bark hides and branches as furnishings (Howard, 1980; Seipp, 1990). In terms of environmental conditions: humidity can be maintained by misting, recommended temperatures range from 24-32°C, and UV lamps should be provided (Howard, 1980; Seipp, 1990). Captive diets can include small invertebrates such as fruit flies, second instar crickets and mealworms, dusted with a calcium, vitamin and mineral powder; and a fruit component such as fruit puree or fruit juice. Food can be provided either daily or on alternate days (Howard, 1980; Seipp, 1990). *Phelsuma* individuals can be highly territorial and aggressive, therefore are usually housed either individually or in pairs (Bartlett & Bartlett, 2001; Howard, 1980).

Few reports exist of gecko behaviour in zoo environments. Behaviour and enclosure use of *P. madagascarensis grandis* has been documented in a large (11,000m$^2$), semi-natural rainforest exhibit (Furrer et al., 2006; Wanger et al., 2009) but use of more comparable (to the current study) vivarium enclosures has been documented in *P. guentheri* (Wheler & Fa, 1995). Wheler and Fa (1995) described the behaviour of *P. guentheri* housed in individual glass vivaria of either 0.03m$^3$ or 0.22m$^3$. General findings reported enclosure use preferences for cage furniture or hiding places and avoidance of glass walls (in comparison to available glass area), potentially to avoid contact with neighbours, or due to a dislike for the substrate (however it should be noted that despite the authors' conclusion of glass avoidance, geckos spent over 25% of their time on the glass, a figure which is consistent with the use of other substrate types). Data revealed high levels of concealment during daylight hours, with a high percentage (96%) of time exposed during night time hours. Nocturnal activity cycles were demonstrated, with peak activity between 1800 and 2100, however activity was also observed during daylight hours, and there was no positive relationship between temperature and activity.

### 6.1.5. Rationale and aims

The current study was designed as a further experimental study (comparing unpredictable and predictable feeding) in conditions in which environmental variables could be controlled and visitor influence was prevented. This experimental study was therefore carried out on *P. laticauda*, and as the effect of predictable feeding has not yet been determined in reptiles, this work would be a valuable addition to the literature on reptile behaviour.

This study aimed to investigate the effect of predictable feeding times on the behaviour of *P. laticauda* by comparing behaviour and testing for food anticipatory behaviour, on temporally unpredictable and predictable routines in an ABA design.

In order to determine whether the subjects were affected by predictable feeding, behaviour in the 30 mins prior to the predictable feed was compared with: a) Behaviour in the 30 mins prior to feeding in both unpredictable conditions, b) Behaviour in 30 mins at a random time of day in the predictable feeding condition. In addition, behaviour in the 30 mins prior to unpredictable feeding was compared with a set time of day (equivalent to the pre-feed 30 mins in the predictable condition), in both unpredictable feeding conditions.

The measure of Busyness was not recorded in the current study as video footage was used to record gecko behaviour. Therefore it was thought that multiple observers would not be needed to record live behaviour, and a simple method such as busyness was not required.

## 6.2. Methods

### 6.2.1. Subjects, housing and equipment

Four male *P. laticauda* geckos were housed individually in 30 x 30 x 45 cm Exo Terra® glass terrariums in a reptile nursery building at Paignton Zoo Environmental Park, UK. Enclosure furnishings were the same in each terrarium and comprised of a bark chip flooring substrate, branches, and a cork bark hide on the floor. Terrariums were positioned in a row with visual barrier screens between them to prevent aggressive behaviour between neighbouring males, and two ultra violet (UV) (plus visible spectrum) lamps were hung above, one between the two left-most terrariums, and one between the two terrariums on the right of the row (photographs shown in Appendix 6.1). The UV lamps were automatically set to turn on at 08:25 (left lamp) and 08:01 (right lamp), and turn off at 18:04 (left lamp) and 18:10 (right lamp) (changed to 08:37 and 08:05, and 18:17 and 18:15 after a power cut on 06/10/10). The main room lighting was also set to come on automatically at 07:00 and off at 19:00. However there was a dim light on at all times over the 24 hour period. There were no windows to outside light sources but tanks were placed behind a viewing window, through which zoo visitors (standing in a separate, indoor viewing area) could see the geckos (and the rest of the reptile nursery). Daylight was briefly visible when visitors opened the door to access the viewing area, or when keepers entered the reptile nursery.

The terrariums were housed in room with a thermostat temperature of 26°C and humidity was maintained by spraying water by hand at intervals throughout the day. Temperature and UV readings were monitored throughout the study period to ensure that heating and UV systems were functioning consistently. In addition, the temperature at the top and bottom of each tank was recorded at half hour intervals between 09:00 and 17:00 on four days over the course of the study in order to obtain a reference daily temperature gradient, which showed average increases of up to 2.6°C across the day (Appendix 6.2)

The geckos were fed daily at times that depended on the experimental condition (as described below). A segment of orange was provided in a set location in each terrarium, and *Drosophila* that had been dusted with Nutrobal® calcium balancer and vitamin supplement were placed on to the orange.

The geckos were monitored using four video cameras, one set up directly above each terrarium. The cameras were connected, via FTS VTD100 timestamp devices, to a PC with an Osprey Capture Card (with four output slots), which was programmed to record the live video from each camera on to a hard drive.

### 6.2.2. Data collection

Two feeding conditions were provided: unpredictable and predictable feeding, presented in an ABA design (37, 42 and 30 days, respectively), as detailed in Table 6.1. Unpredictable feeding times were selected using a random number generator in Microsoft Excel, with the proviso that feeds on adjacent days were not in the same hour. If this occurred then the randomisation was re-run. Geckos were fed by the observer and keeping staff, starting from the left-most tank at the planned time. Feeding times in the predictable condition were relatively constant; the earliest feeding time was 11:00 and the latest feeding time (on a single occasion) was 11:30 (Mean = 11:02, Standard deviation = 6.08).

Behavioural observations were made during 30minute periods termed: ‘pre-feed’, ‘pre-set time’ and ‘pre-random time’, as detailed in Table 6.1. Data were collected in pre-feed sessions in order to compare behaviour prior to predictable and unpredictable feeding. The pre-random time observations were used to enable comparisons within a day in the predictable condition, to investigate whether gecko behaviour changed within a day (i.e. prior to predictable feeding, compared to another time of day) and also to control for any more general changes in gecko behaviour between conditions. In addition, the pre-set time sessions in the unpredictable feeding conditions were matched with the pre-feeding time in the predictable condition, to provide a comparison where the daily temperature gradient was comparable, as temperature changes might affect behaviour at other times of the day.

*Table 6.1. Details of feeding conditions used in this study*

| Feeding condition | Duration of geckos' experience of condition | Explanation | Video footage observed | Number of consecutive days of observation at end of feeding condition |
|---|---|---|---|---|
| 1st Unpredictable | 37 days | Geckos fed at a unpredictable time of day between 08:45 and 17:15 | - 30 mins prior to unpredictable feed time (pre-feed)<br>- 30 mins at a set-time (10:30-11:00) (pre-set time) | 5 (4 for Gecko 2 due to a problem with the video) |
| Predictable | 42 days | Geckos fed at 11:00 each day | - 30mins prior to predictable-feed time (10:30-11:00) (pre-feed)<br>- 30 mins at random-time during the day (pre-random time) | 5 |
| 2nd Unpredictable | 30 days | Geckos fed at a unpredictable time of day between 08:45 and 17:15 | - 30 mins prior to unpredictable feed time (pre-feed)<br>- 30 mins at a set-time (10:30-11:00) (pre-set time) | 5 |

Instantaneous sampling was used every minute during each 30 minute observation session to record the state behaviour of each gecko (see Table 6.2). One-zero sampling was used each minute to record event-type behaviours, including locomotion (Table 6.2). The movements of the geckos were often short-lived and infrequent, therefore one-zero sampling was used to ensure occurrences were noted, as they might have been missed using instantaneous sampling alone. All occurrence sampling was used to record jumping and tail flick behaviour (Table 6.2).

*Table 6.2. Descriptions of state and event-type behaviours recorded.*

| *Behaviour* | *Description* |
|---|---|
| Locomotion | Gecko moves from one point to another, covering a distance of at least one body length (head to end of body length). |
| Stationary | Gecko is not moving. |
| Feeding on orange | Gecko uses tongue to lick juice from orange segment. |
| Not visible | Gecko is obstructed from view. |
| Movement on the spot | Gecko moves body or limbs in any direction but movement in any one direction does not cover a distance of greater than one body length. |
| Stationary but moves head | Gecko moves head but body and limbs do not move. |
| Feeding on invertebrates | Gecko consumes invertebrate food |
| Tongue flick | Gecko protrudes tongue out of mouth and then draws it back in again |
| Jumping | Gecko leaps from one point to another with all 4 feet off the ground simultaneously at some point during the jump |
| Tail flick | Gecko flicks tail sideways or up and down. Body is stationary. |

### 6.2.3. Data analysis

Due to the time required to analyse the individual video recordings, it was not practically possible to record data on all four geckos throughout the day in detail as it was for Chapters 3 and 5, hence the above-described sampling was used (and the controlled conditions of this study facilitated a clear ABA design). As this study involved different experimental conditions, comparison of the sampling periods in the different conditions was possible using inferential statistics (as also used in Chapter 5). Whilst statistical analysis was possible with this study design, with only four subjects, the power of conventional statistical analysis would be low. Therefore, as in Chapter 5, randomisation analysis was used as a statistical method that is suited to small-n designs (see below). Proportions of behaviours recorded using instantaneous and one-zero sampling were calculated for each 30 minute session; mean (+/- standard error) proportions were calculated for each observation time (pre-feed, pre-set time and pre-random time) from the five (or four) days of data per feeding condition. Randomisation analysis (Design 6) (Todman & Dugard, 2001) was used on the one-zero data, to compare mean proportions for each observation time, in each feeding condition, using data from all four geckos (2000 re-randomised pseudosamples). Randomisations were run three times, and mean scores were calculated.

Proportions of one-zero behaviours observed in each minute during the pre-feed observation sessions, over the five days of observation, were calculated and plotted in order to determine any pattern of behaviour within the 30 mins prior to feeding.

## 6.3. Results

There were no, clear differences in any behaviour, as recorded by instantaneous sampling, between the pre-feed sessions in predictable and unpredictable feeding conditions. In addition, substantial variation between individuals across these phases of the study was apparent (Figure 6.1). The subjects were not visible in a high proportion of the scans, and few occurrences of other state behaviours were observed on the sampling intervals. Hence this method was not considered to be appropriate and was not used in further analysis.

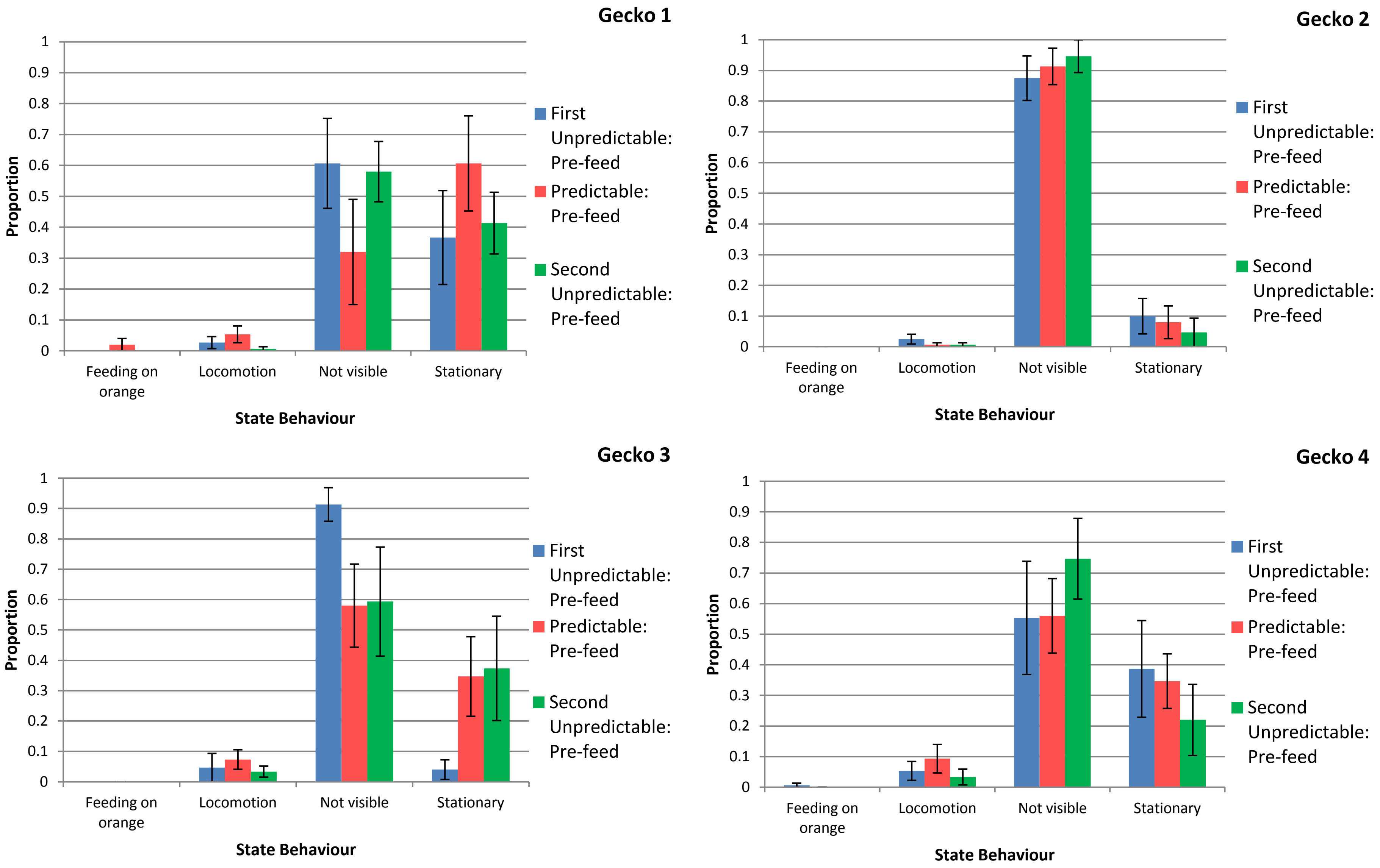


*Figure 6.1: Mean (+/- SE) pre-feed proportions of behaviour for Geckos 1, 2, 3 and 4 in each condition, achieved by instantaneous sampling.*

*Table 6.3. Descriptive data (one-zero data) giving the mean proportion (plus standard error across the mean proportions for each gecko) of each behaviour for all 4 geckos in each condition, plus mean data from three runs of randomisation analysis (Design 6) (Todman & Dugard, 2001).*

| | First Unpredictable: Pre-feed | First Unpredictable: Pre-set time | Predictable: Pre-feed | Predictable: Pre-random time | Second Unpredictable: Pre-feed | Second Unpredictable: Pre-set time | RSS | Count of RSS as least as small | P |
|---|---|---|---|---|---|---|---|---|---|
| Locomotion | 0.196 | 0.168 | 0.300 | 0.117 | 0.182 | 0.225 | 0.059 | 30.67 | <.05 |
| Standard Error | 0.034 | 0.024 | 0.076 | 0.024 | 0.039 | 0.060 | | | |
| Movement on spot | 0.088 | 0.101 | 0.177 | 0.095 | 0.120 | 0.152 | 0.040 | 284.3 | >.05 |
| Standard Error | 0.032 | 0.025 | 0.050 | 0.037 | 0.032 | 0.044 | | | |
| Stationary but moves head | 0.055 | 0.048 | 0.168 | 0.103 | 0.085 | 0.117 | 0.047 | 82.33 | <.05 |
| Standard Error | 0.022 | 0.013 | 0.058 | 0.040 | 0.028 | 0.038 | | | |
| Not visible | 0.821 | 0.761 | 0.708 | 0.822 | 0.790 | 0.768 | 0.149 | 1201 | >.05 |
| Standard Error | 0.080 | 0.073 | 0.103 | 0.054 | 0.068 | 0.062 | | | |
| Feeding on orange | 0.003 | 0.007 | 0.008 | 0.025 | 0.002 | 0.015 | 0.002 | 278.7 | >.50 |
| Standard Error | 0.003 | 0.005 | 0.008 | 0.011 | 0.002 | 0.011 | | | |
| Tongue flick | 0.003 | 0.003 | 0.010 | 0.035 | 0.000 | 0.005 | 0.004 | 5.333 | <.01 |
| Standard Error | 0.003 | 0.002 | 0.008 | 0.018 | 0.000 | 0.002 | | | |
| Jumping | 0.400 | 0.500 | 1.050 | 0.200 | 0.808 | 0.400 | 2.445 | 75.33 | <.05 |
| Standard Error | 0.337 | 0.238 | 0.608 | 0.141 | 0.491 | 0.337 | | | |
| Tail flick | 1.863 | 1.163 | 0.850 | 0.550 | 0.000 | 0.866 | 38.463 | 1590 | >.05 |
| Standard Error | 1.646 | 0.654 | 0.544 | 0.377 | 0.000 | 0.866 | | | |

Randomisation analysis, carried out on the mean proportions of behaviours from all four geckos, in each feeding condition revealed significant differences between the different conditions for the one-zero data for the behaviours: locomotion (RSS = 0.059, *p* < .05), stationary but moves head (RSS = 0.047, *p* < .05) and tongue flick (RSS = 0.004, *p* < .01), and for the all occurrences data for jumping behaviour (RSS = 2.445, *p* < .05) (see also Table 6.3). *Post hoc*, pair-wise randomisations did not reveal any significant differences, however descriptive analysis of resulting graphs reveals patterns between conditions, which are suggestive of where these differences lie. The following section describes the differences between conditions for each gecko (as illustrated in figures 6.2 to 6.13).

Within the first unpredictable feeding condition, there were no clear differences in any behaviour observed in the pre-feed observation sessions, compared to observations in the pre-set time sessions for any gecko (Figures 6.2a, 6.5a, 6.8a and 6.11a), although Gecko 4 showed higher proportions of locomotion in the pre-feed session than in the pre-set time session. However, there was variation in the mean proportion of locomotion for Gecko 4, and the standard error overlapped. Similarly, for the second unpredictable condition, there was no clear difference between proportions of any behaviour in the pre-feed observation sessions, and observations in the pre-set time sessions for any gecko (Figures 6.2c, 6.5c, 6.8c, and 6.11c), although Gecko 3 showed slightly higher proportions of locomotion, ‘movement on the spot ‘and ‘stationary but moves head’ behaviours in the pre-set time observations.

Due to the lack of differences shown between behaviours in the pre-feed and pre-set time observations, particularly in the first unpredictable feeding condition, it was decided to continue analysis of the effect of the predictable feeding routine, using only the pre-feed and pre-random time observation sessions from the predictable condition, and the pre-feed sessions across conditions. Gecko 2 showed no clear differences in any behaviour across the observation sessions and feeding conditions, and hence will not be mentioned further in this section.

Locomotion, ‘movement on the spot’ and ‘stationary but moves head’ behaviours showed higher proportions in the pre-feed than the pre-random time sessions in the predictable feeding condition (Figures 6.2b, 6.5b, 6.11b), and higher proportions in the pre-feed (predictable) sessions compared to both pre-feed (unpredictable) sessions for Geckos 1, 3 and 4 (Figures 6.3, 6.6, 6.12), (although Gecko 4 did not show this difference for ‘stationary but moves head’). It is likely that these differences are the source of the significant difference across conditions for locomotion and ‘stationary but moves head’ behaviour. Proportions of pre-feed behaviours in the two unpredictable conditions showed similar proportions to each other for Geckos 1 and 4, however for Gecko 3, proportions of these behaviours do not return to the levels of the first unpredictable condition, in the second unpredictable condition (Figure 6.6). This result for Gecko 3 in the second unpredictable condition is consistent with the higher proportions of behaviours observed in the pre-set time observations than the pre-feed observations in the second unpredictable condition (as mentioned above).

Proportions of ‘not visible’ were lower in the pre-feed than the pre-random time sessions in the predictable feeding condition (Figures 6.2b, 6.5b, 6.11b), and in the pre-feed (predictable) sessions compared to the first pre-feed (unpredictable) session for geckos 1,3 and 4 (Figures 6.3, 6.6, 6.12). Proportions of ‘not visible’ were also lower in the pre-feed (predictable) session than in the second pre-feed (unpredictable) session for Gecko 1 only; for geckos 3 and 4 proportions in the second pre-feed (unpredictable) session remained at a similar level (Gecko 3), or higher (Gecko 4) than the pre-feed (predictable) level. ‘Feeding on orange’ behaviour was observed at low levels, with no clear pattern across conditions for any gecko. ‘Tongue flick’ behaviour was also observed at low levels, with variation in proportions across observation sessions and feeding conditions for the different geckos, however there was a significant difference in ‘tongue flick’ behaviour between the observation sessions in the different conditions which may have been a result of high ‘tongue flick’ behaviour in the pre-random time (predictable) sessions, compared to all other sessions across conditions.

Results of all occurrences sampling (Figures 6.4, 6,7, 6.10 and 6.13) showed higher proportions of jumping behaviour in the pre-feed (predictable) sessions compared to both pre-feed (unpredictable) sessions for geckos 1,3 and 4, and it is likely that this is the source of the significant difference in jumping behaviour across sessions in each condition.  'Tail flick' behaviour showed no clear pattern across conditions for any gecko (comparisons of pre-feed and pre-set time are given, for reference in Appendix 6.3).

# Gecko 1

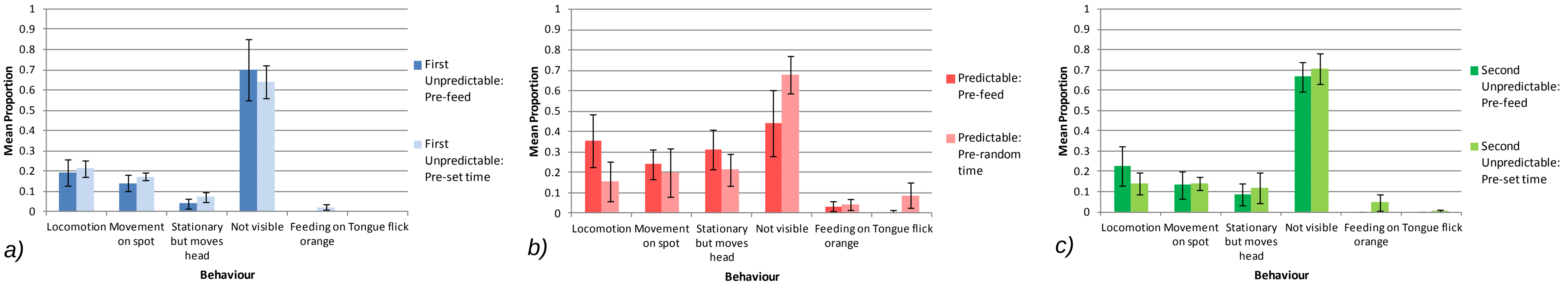


*Figure 6.2: Mean (+/- SE) proportions of behaviours recorded using one-zero sampling for Gecko 1 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*

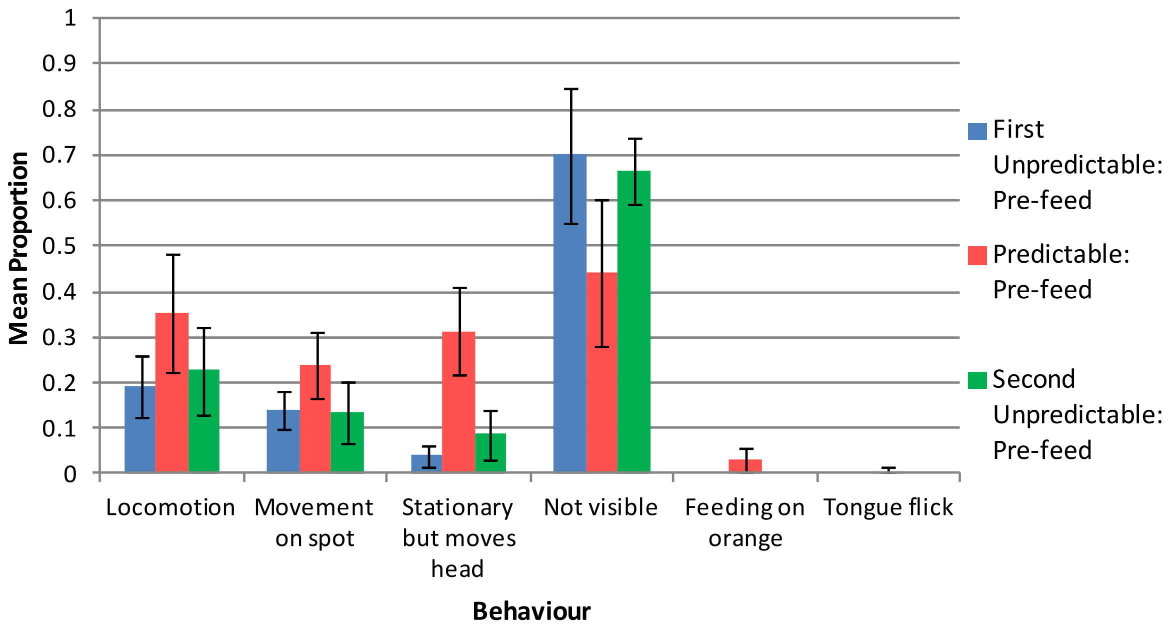


*Figure 6.3: Mean (+/- SE) pre-feed proportions of one-zero behaviours for Gecko 1 in all feeding conditions.*

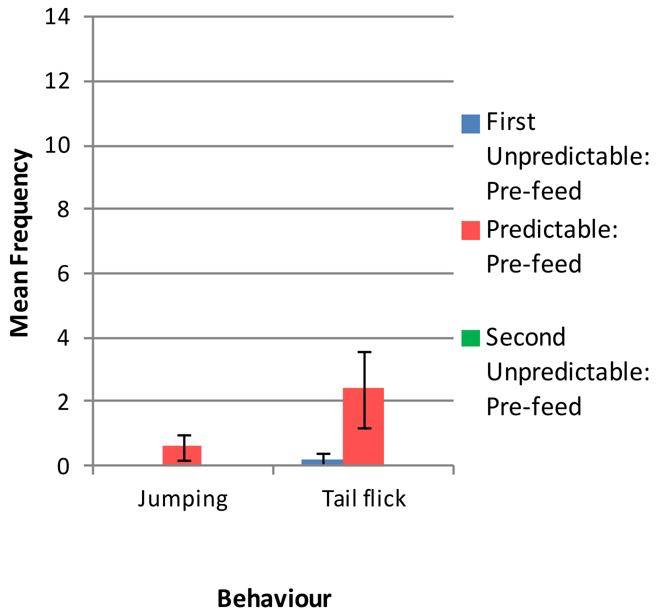


*Figure 6.4: Mean (+/- SE) pre-feed frequency of jumping and tail flicking for Gecko 1 in all feeding conditions.*

# Gecko 2

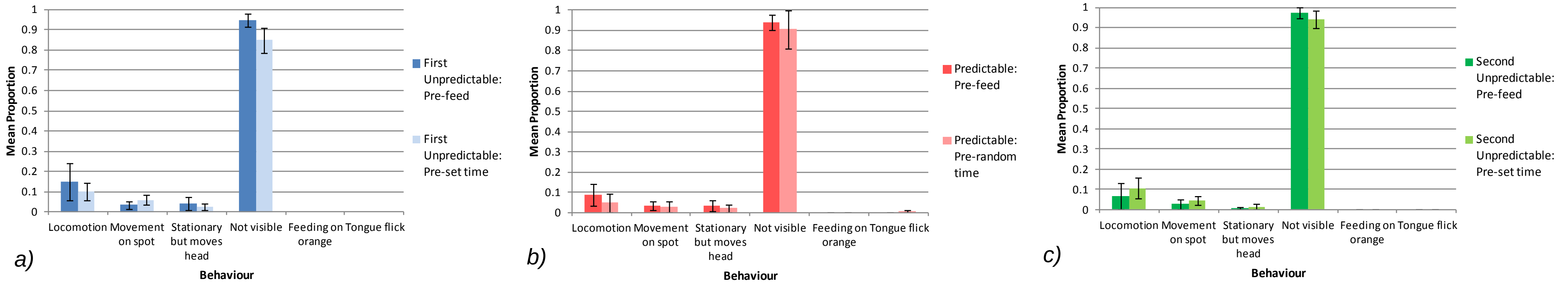


*Figure 6.5: Mean (+/- SE) proportions of one-zero behaviours for Gecko 2 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*

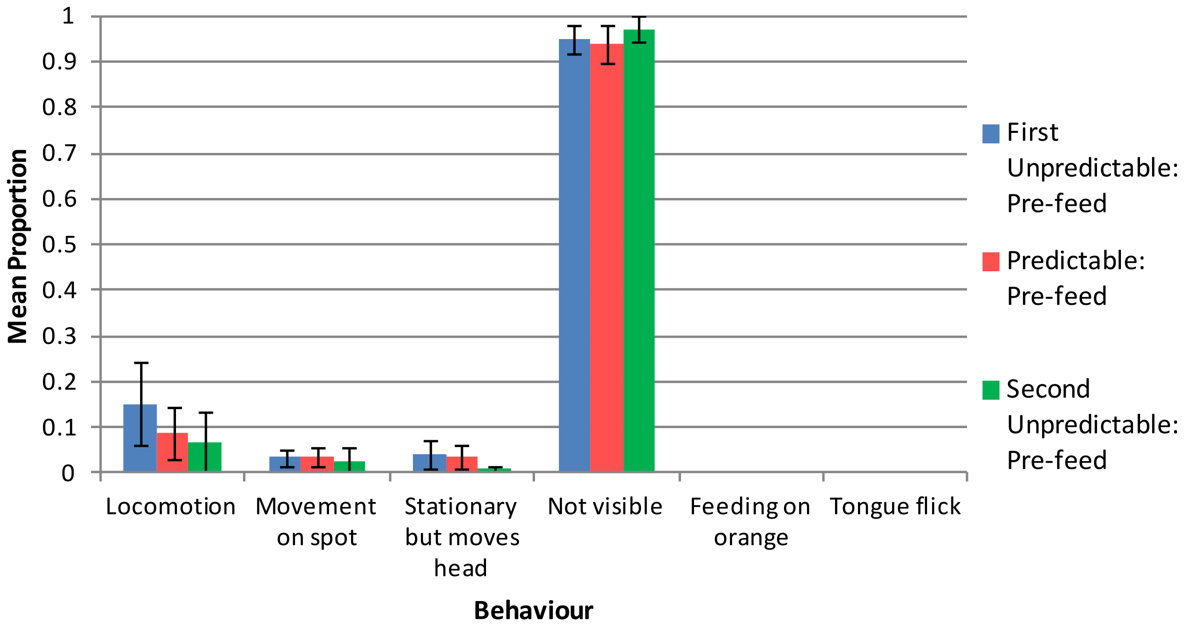


*Figure 6.6: Mean (+/- SE) pre-feed proportions of one-zero behaviours for Gecko 2 in all feeding conditions.*

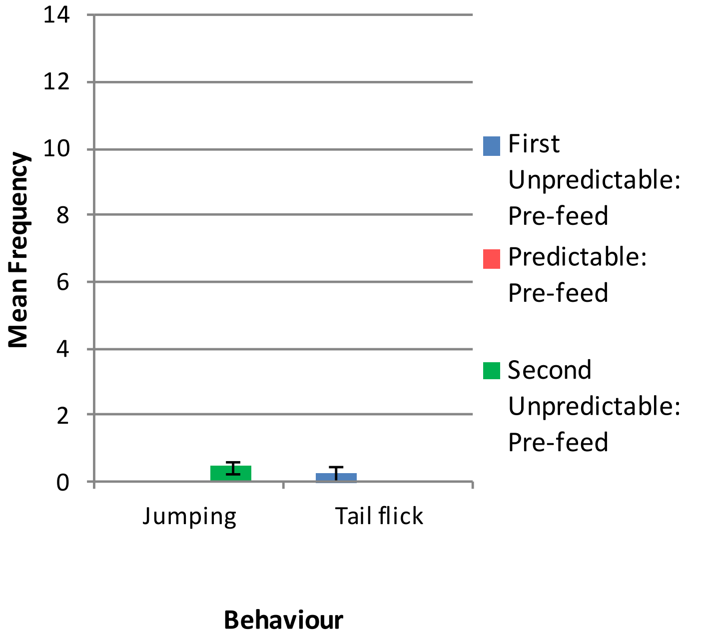


*Figure 6.7: Mean (+/- SE) pre-feed frequency of jumping and tail flicking for Gecko 2 in all feeding conditions.*

## Gecko 3

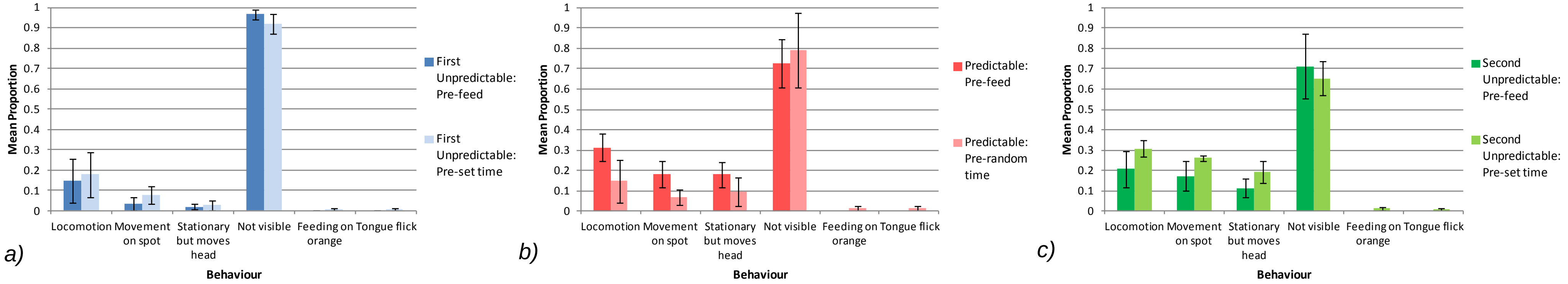


*Figure 6.8: Mean (+/- SE) proportions of one-zero behaviours for Gecko 3 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*

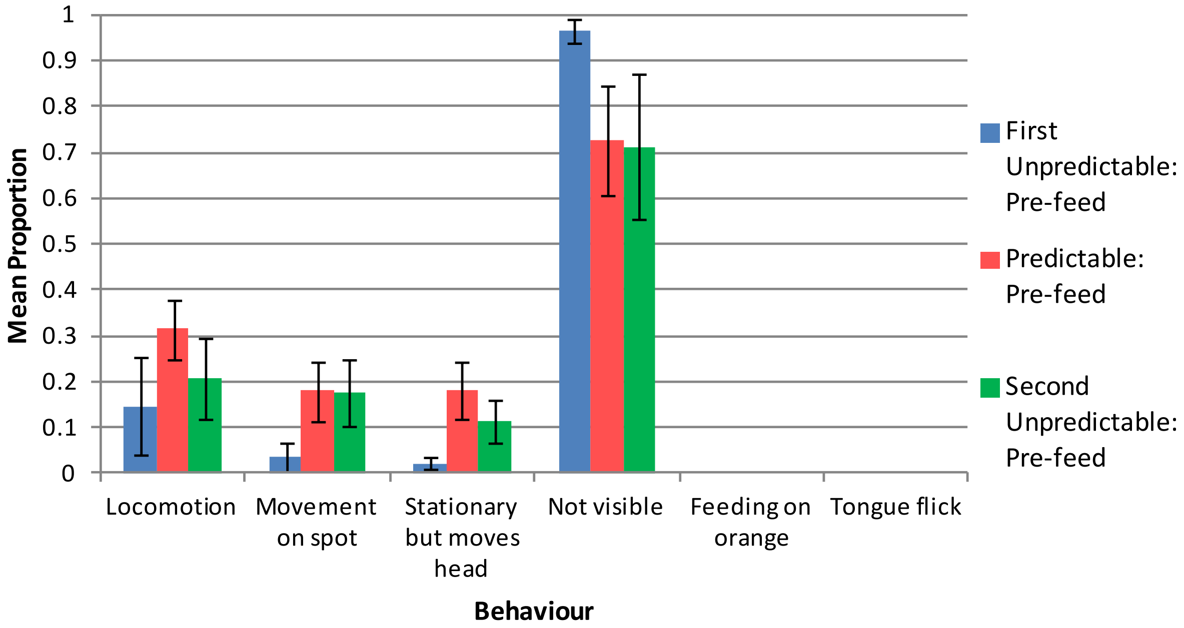


*Figure 6.9: Mean (+/- SE) pre-feed proportions of one-zero behaviours for Gecko 3 in all feeding conditions.*

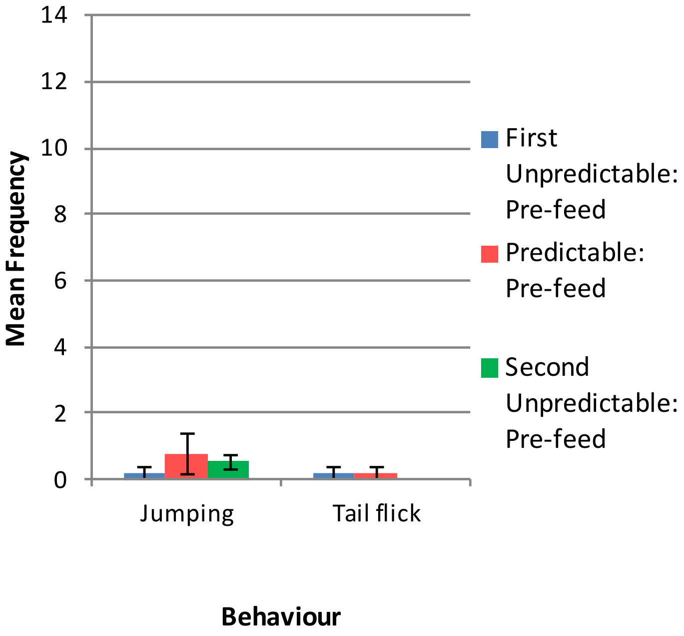


*Figure 6.10: Mean (+/- SE) pre-feed frequency of jumping and tail flicking for Gecko 3 in all feeding conditions.*

# Gecko 4

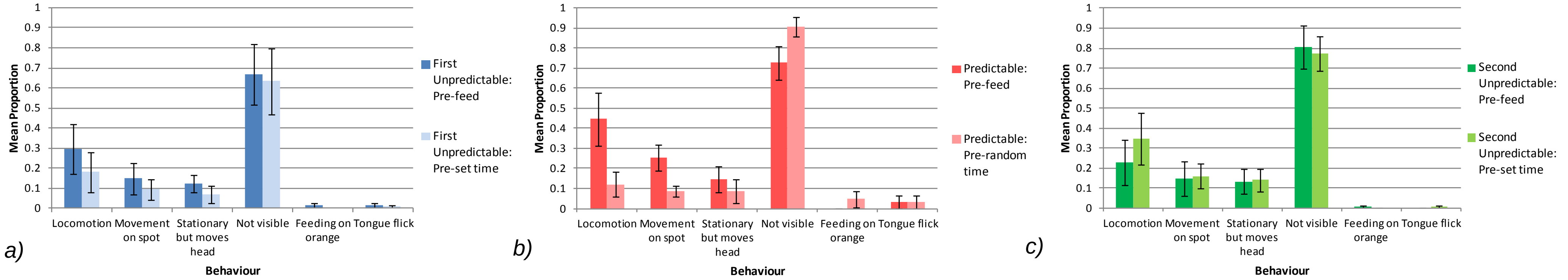


*Figure 6.11: Mean (+/- SE) proportions of one-zero behaviours for Gecko 4 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*

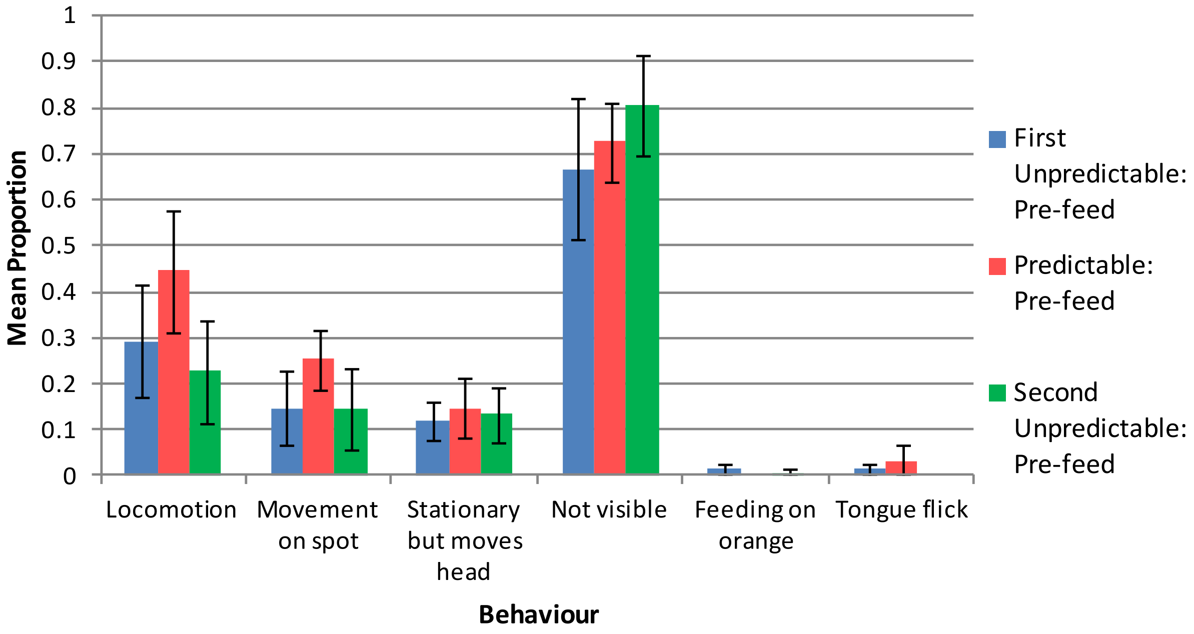


*Figure 6.12: Mean (+/- SE) pre-feed proportions of one-zero behaviours for Gecko 4 in all feeding conditions.*

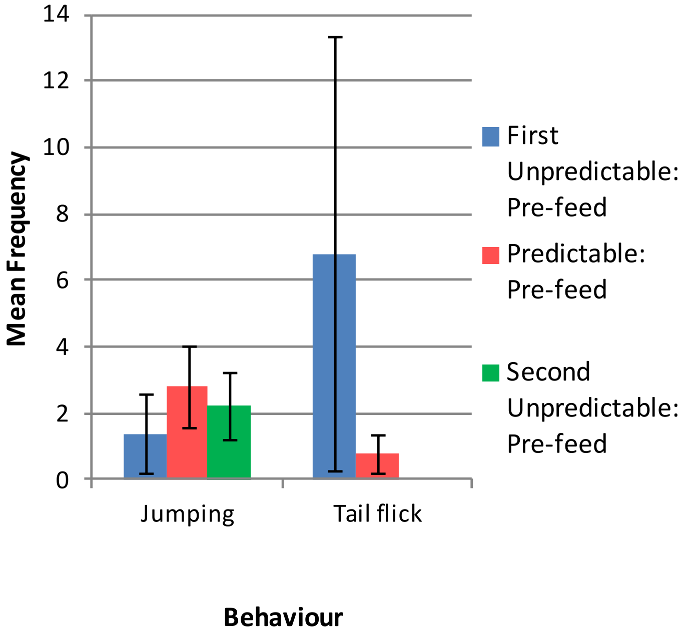
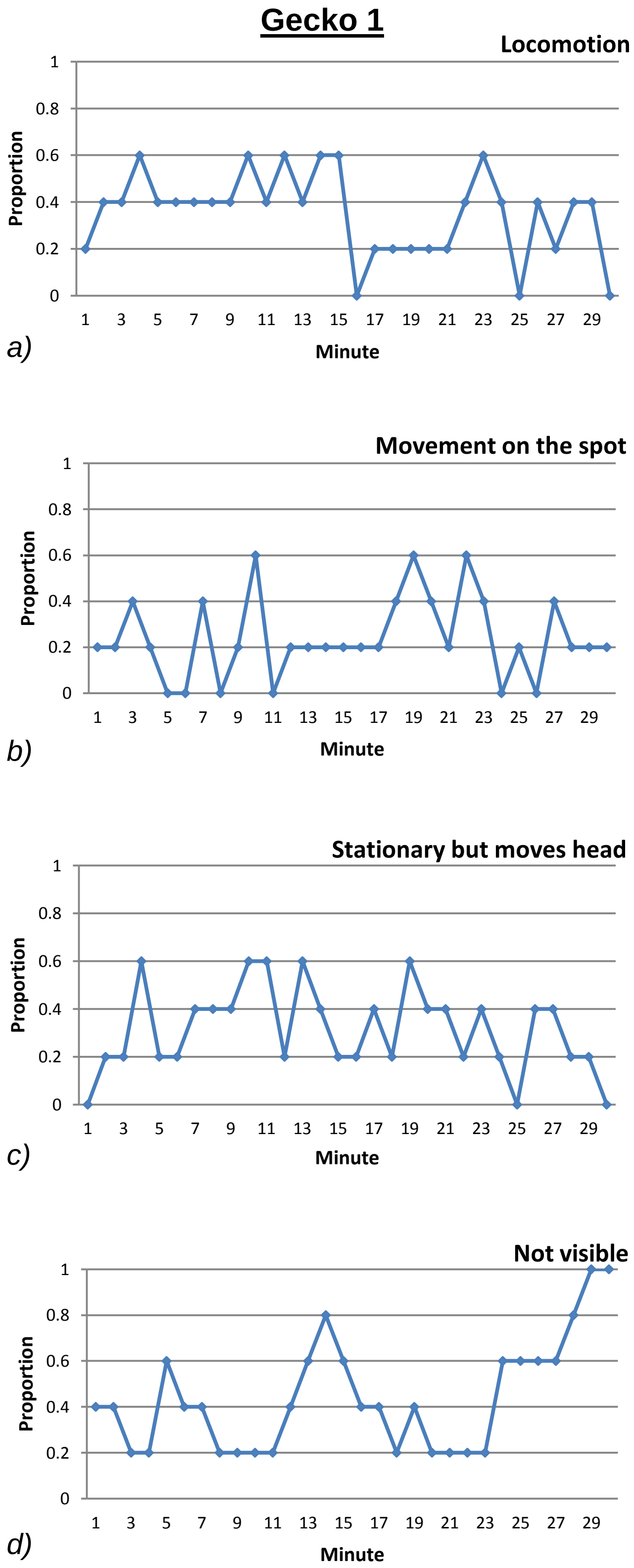


*Figure 6.13: Mean (+/- SE) pre-feed frequency of jumping and tail flicking for Gecko 4 in all feeding conditions.*

Figures 6.14-6.17 illustrate the patterns of one-zero behaviours across the pre-feeding observation session in the predictable feeding condition. These figures demonstrate the proportions of occurrence of each behaviour in each minute prior to the feeding time. No clear pattern is demonstrated in any behaviour for any gecko, and there were individual differences, although some slight patterns could be noted. For example: Gecko 1 showed an increase in the proportions of not visible in the last 7 minutes prior to feeding; Gecko 3 showed an increase in the proportions of locomotion, ‘movement on the spot’ and ‘stationary but moves head’ within the last 9 minutes prior to feeding, and a decrease in not visible at this time; and Gecko 4 showed a general decrease in ‘movement on the spot’ across the 30 minute observation period. No patterns of behaviour prior to feeding were shown for Gecko 2, which was mostly not visible at this time.



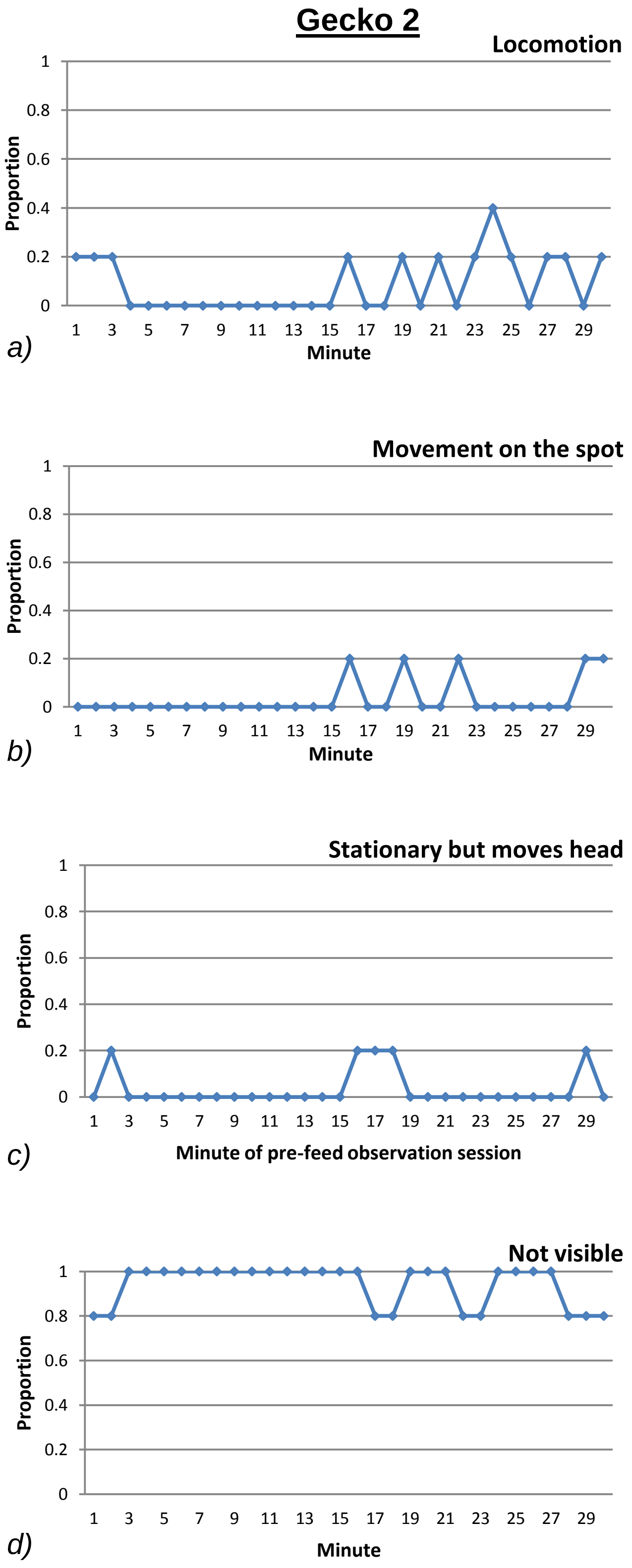


*Figure 6.14: Proportion of observed pre-feed minutes in which a) locomotion, b) 'movement on the spot, c) 'stationary but moves head and d) 'not visible' was observed for Gecko 1. x-axis indicates successive minutes of the observation period. Feeding time occurred after minute 30.*

*Figure 6.15: Proportion of observed pre-feed minutes in which a) locomotion, b) 'movement on the spot, c) 'stationary but moves head and d) 'not visible' was observed for Gecko 2. x-axis indicates successive minutes of the observation period. Feeding time occurred after minute 30.*

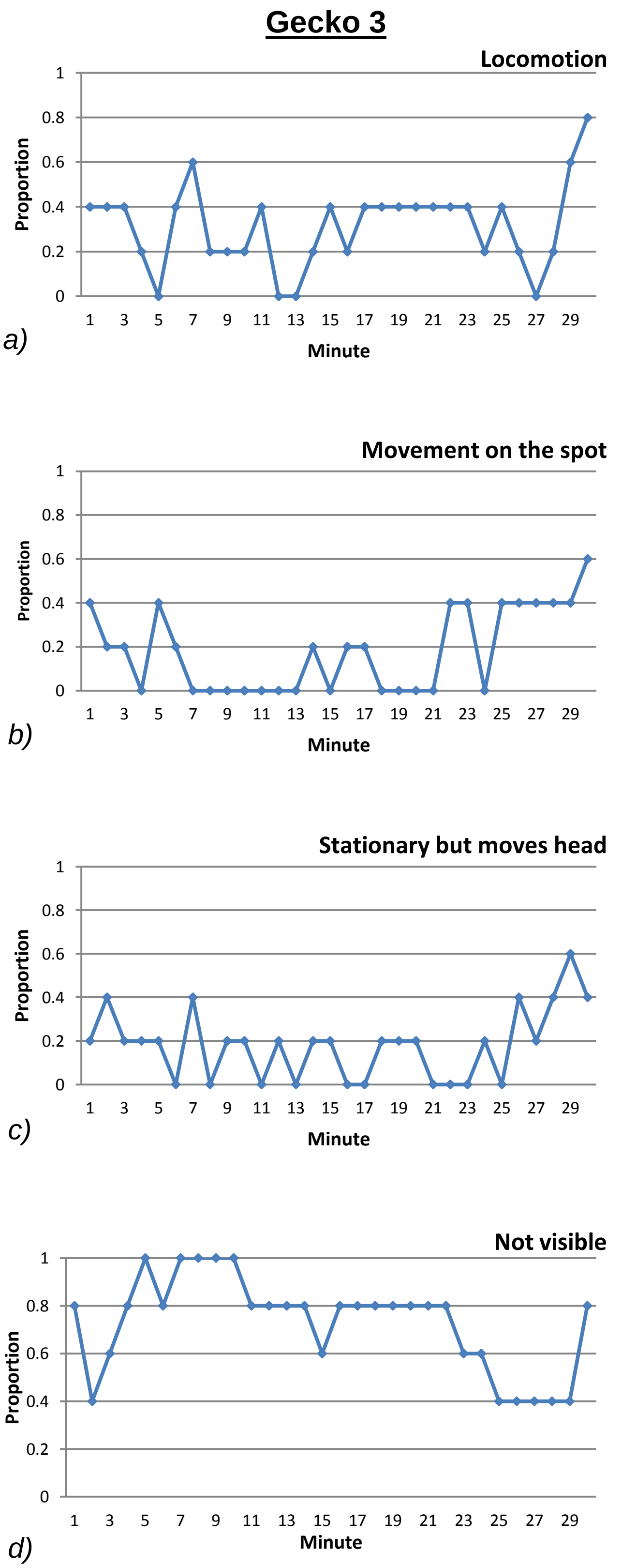


Figure 6.16: Proportion of observed pre-feed minutes in which a) locomotion, b) 'movement on the spot, c) 'stationary but moves head and d) 'not visible' was observed for Gecko 3. x-axis indicates successive minutes of the observation period. Feeding time occurred after minute 30.

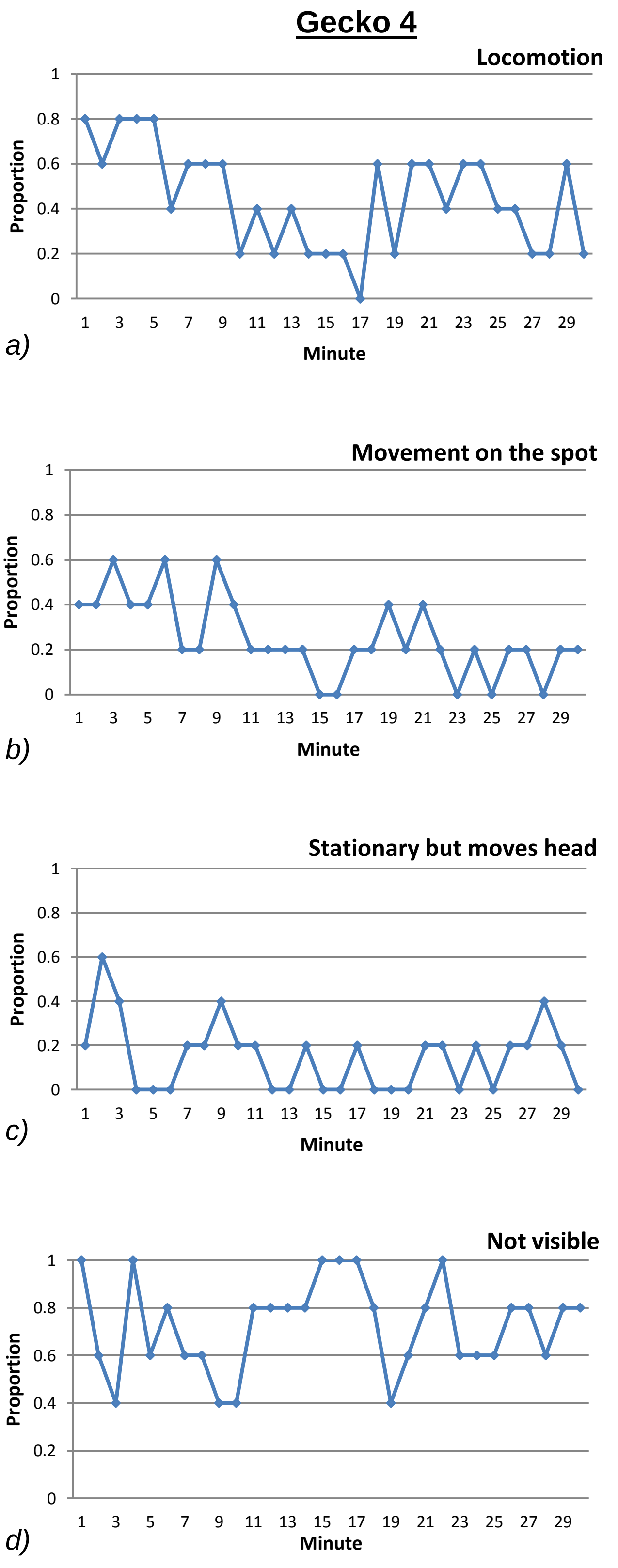


Figure 6.17: Proportion of observed pre-feed minutes in which a) locomotion, b) 'movement on the spot, c) 'stationary but moves head and d) 'not visible' was observed for Gecko 4. x-axis indicates successive minutes of the observation period. Feeding time occurred after minute 30.

## 6.4. Discussion

### 6.4.1. Discussion of behaviour

Proportions of ‘active’ behaviours, including locomotion, ‘stationary but moving the head’ and jumping were shown to be significantly different between observation sessions in the different feeding conditions.  These behaviours were higher in pre-feed observation sessions in the predictable, compared to the unpredictable feeding conditions.  Gecko 2 was the exception, and no difference was noted, and Gecko 4 did not show a difference in ‘stationary but moving head’ behaviour.  ‘Movement on the spot’ was also higher in the pre-feed (predictable condition) although this was not statistically significant.  The above behaviours were also observed at a higher level prior to predictable feeding, compared to random times of day (within the same predictable condition); again with the exception of Gecko 2.  The above results suggest that the geckos showed changes in behaviour that anticipated the predictable feeding time.

As expected, there was very little difference in behaviour between the pre-feed and pre-set time sessions in the unpredictable feeding conditions; with the exception of Gecko 3 in the second unpredictable feeding condition.  Reptile behaviour patterns have been reported to be entrained by temperature as well as light (e.g., Oishi, et al., 2004; Tosini, et al., 2001; Underwood, 1992) and, as ectothermic animals which rely on ambient temperature to obtain heat (e.g., Willmer, et al., 2004), it would be expected that activity levels may vary at different temperatures.  A temperature gradient was demonstrated across the day (Appendix 6.2), however the maximum difference between the average maximum and minimum of the recorded temperatures, across the observation day was only 2.60°C.  That no clear differences in behaviours were observed between pre-feed and set-time observations in the first unpredictable feeding condition (in comparison to the predictable pre-feed observations) suggests that temperature was not an explanation for the differences observed, and also that there were no other circadian events affecting behaviour at the set-time of day.  Hence any difference between unpredictable and predictable pre-feeding behaviour was likely due to the predictable feeding time.

In should be noted that *post* hoc analysis on the data revealed no significant differences in any behaviours between conditions. It is likely that this is due to the low sample size for paired comparisons. Therefore, exactly which conditions accounted for the statistically significant differences cannot be confirmed. However, descriptive analysis helped to reveal patterns in the data. Had a larger sample size been available for *post hoc* analysis, any resulting significant differences would have been useful, particularly for interpretation of ‘tongue flick’ behaviour. In the current data, it is unclear which conditions accounted for the significant difference in ‘tongue flick’ behaviour, although this difference may have been the result of higher proportions of ‘tongue flick’ in the pre-random time sessions. Higher proportions of ‘tongue flick’ behaviour in the pre-random time sessions cannot be interpreted in relation to feeding time, however it is possible that ‘tongue flick’ behaviour was affected by other environmental factors, not recorded in the current study. In addition, this behavioural category which involved only a small movement, was often difficult to distinguish on the video recording, therefore the data may contain an underestimate of the occurrence of tongue flicking, and interpretation should be made with caution. Future work would be needed, with clearer video, and a focus on tongue flick observations, in order to clearly determine the effect of predictability on tongue flick behaviour.

The increase in activity prior to predictable feeding time shown in the current study is a similar result to that found by Gopar-Canales et al., (2010) where red-eared turtles demonstrated increased activity in anticipation of light onset. There is no literature of non-operant food anticipatory behaviour in reptiles available for comparison with the current study; therefore these data are new information in this field. The observed increases in locomotion, moving on the spot, and moving the head may relate to the foraging strategy of *Phelsuma* geckos. Whilst sometimes considered to be sit-and-wait foragers (Ikeuchi, et al., 2005; Seifan, et al., 2010), *Phelsuma* have been demonstrated to show flexibility in locomotory activity and hence their foraging strategy. It is possible that the observed change in ‘active’ behaviours reflected more time spent foraging prior to feeding. It is important to note that not only did the feeding event involve the provision of food at a predictable time, it also involved the predictable occurrence of a keeper opening the vivarium and putting a hand inside it, a process which may have caused a flight response in the geckos, and may also have temporarily changed the microclimate in the vivarium. It is therefore possible that the geckos' behaviour prior to feeding was a preparatory response to any or all of these events.

To the author's knowledge, there are no published comparisons of reptile behaviour under predictable and unpredictable feeding regimes. The current research has demonstrated that predictability does appear to affect gecko behaviour, and therefore should be considered in husbandry management practices. More research would be necessary to determine whether predictability has positive or negative ramifications, as the current research is sufficient only to demonstrate a behavioural difference. These results do invite further research on gecko/reptile behavioural welfare, and on the effects of predictability, akin to the research mentioned throughout this thesis on mammals, birds and fish.

Previous research has noted individual variation in reptile timing ability (e.g., Gopar-Canales et al. 2010; Laurent, 1983, as cited in Lejeune & Wearden, 1991). Individual variation was also reflected in the current study, as the behaviour of Gecko 3 differed in the second unpredictable condition compared to Geckos 1 and 4, and Gecko 2 showed a lack of behavioural differences between conditions, unlike the other individuals. Previous research has also demonstrated that reptiles may have poorer temporal control than mammals and birds (Lejeune & Wearden, 1991) and this may be a reason that the behaviours recorded for Gecko 3 did not return to the same levels in the second unpredictable condition as in the first unpredictable condition.

The results from the all occurrence sampling of tail flicking, showed no clear pattern of performance between experimental conditions, despite the detailed level of recording. Tail flicking was variable across feeding conditions and was therefore concluded to be unrelated to anticipation. Tail flicking behaviour has been associated with aggressive interactions in *P. laticauda* (Howard, 1980) and with anti-predator and social behaviour in the yellow-headed gecko (*Gonatodes alogularis*) (Bohórquez Alonso et al., 2010). In the current study, geckos were housed individually with visual barriers between tanks preventing aggression and social behaviour, and no predators were present, therefore the cause of tail flicking in the current study is unclear. Bohórques Alonso et al. (2010) observed tail waving displays directed at the human observers in their study therefore an increase in tail flicking prior to the keepers attending the predictable feeds may have been a logical conclusion in the current study. However, no such association was observed, and indeed tail flicking was variable across conditions. Further research would be necessary to determine the causes of tail flicking in these individuals.

Proportions of behaviour observed across the 30 minute pre-feed periods showed no defined pattern of increase or decrease in the lead up to the feeding time (e.g., as was seen for the tigers in Chapter 3). However, overall levels of ‘active’ behaviours were generally elevated during this time. One conclusion may be that this is consistent with the findings of Lejeune & Wearden (1991) who reported that when anticipating a food reward on a fixed interval (Laurent, 1983, as cited in Lejeune & Wearden, 1991), turtles showed higher levels of responding throughout the interval, and poorer temporal control than in equivalent studies of mammals and birds. Hence gecko timing ability may not be precise enough to develop a distinct behavioural pattern over time. However, another conclusion is that patterns of gecko behaviour may have already reached an asymptote during the short sample period of 30 minutes prior to feeding. Had observations taken place over a greater length of time during the day, patterns of behavioural change may have been clearer. Future work would therefore benefit from the use of multiple observers, using simple methods such as busyness (as developed throughout this thesis, e.g., Sections 2.9 and Chapter 4), in order to increase the volume of data that could be collected throughout the day. This point is further developed in Section 6.4.2.

Future work may benefit from using an automatic feeder for dispensing food. It is possible that more precision in the time at which food is dispensed may create more optimal conditions for the development of clear anticipation. The use of such a feeder would also remove the need for keepers to open the tank in order to provide food, which in itself may have introduced effects on the behaviour of the geckos that interacted with any effects of regular feeding *per se*. Future work could also investigate the provision of different quantities of food. In the current study, the geckos had no competition for food, and were fed every day with a live food source which did not rapidly degrade; therefore there may have been low incentive for anticipation. In a study of mice, reduction of the caloric density of restricted feeds led to greater activity in anticipation of the feeding time (Holmes & Mistlberger, 2000). Smaller meal size has also been shown to result in a greater likelihood of food anticipatory behaviour at restricted feeds of different lengths of availability in greenback flounder (*Rhombosolea tapirina*) (Purser & Chen, 2001). Greater motivation for food may therefore increase anticipatory behaviour in the geckos. Alternatively, feeding could be restricted to alternate days, which is also a suitable feeding timeframe for captive geckos (Seipp, 1990). Further research could then determine whether such a change increases the anticipatory response, and whether geckos are capable of adapting their behavioural patterns to a 48 hour inter-feed interval.

### 6.4.2. Discussion of methodology

Of the sampling methods used in the current study, one-zero data sampling resulted in a greater number of data points per behaviour, which revealed clearer patterns of change in each behaviour between conditions, compared to data recorded using instantaneous sampling; hence it was a preferable data collection method for the current research. The erratic nature of gecko behaviour with frequent pauses between movements, meant that active behaviours were often missed using instantaneous sampling whereas one-zero recording captured these behaviours. Whilst it could be argued that shorter sampling intervals might solve this problem, true instantaneous sampling would likely still miss these behaviours, and one-zero behaviour allowed coverage of the entire minute interval. One-zero sampling was also more appropriate than all occurrences sampling for certain behaviours, since the erratic movements of the geckos made events, states or bouts of these behaviours difficult to distinguish, and for the purposes of this research it was sufficient to record whether or not the behaviour occurred each minute. One-zero recording also enabled a record of each behaviour for each minute interval, hence allowing the determination of patterns of each behaviour across the pre-feed observation sessions. One-zero recording was therefore appropriate for use in the current study, in contrast to those criticisms sometimes afforded to this method (see Section 2.4).

The current experimental set-up recorded behaviour at different times of day (i.e. pre-feed and set-time observations) for control purposes, and this enabled greater confidence in the effect of predictable feeding rather than other extraneous factors. However in future research it would be beneficial to analyse daily or 24-hour patterns of gecko behaviour to determine the extent to which predictable feeding causes deviation from cycles of behaviour as demonstrated on an unpredictable routine (see Sections 2.2, 3.4.2 and 5.8.4) . This would be a very time intensive method; hence it was not undertaken in the current study. It would also be beneficial to analyse more observation days and to observe a greater number of geckos, to provide more data for statistical analysis.

It was not initially deemed necessary for the current study to include a team of observers to record data, or to use the measure of busyness, since it was possible to record video of each gecko. Despite this, the current study again required a large volume of data, and a high investment of time spent in observation, which limited the number of observation sessions that were feasible (as mentioned above). These circumstances emphasise the need for a suitable methodology which would allow multiple observers to record behaviour, hence reducing the observation load on individuals. There is scope to use the concept of citizen science (see Section 2.8) to obtain data in studies such as this, for example by asking volunteers to watch video of the geckos, which could be made available over the internet, in a similar fashion to that of the citizen science project ‘Galaxy Zoo’ where volunteers classify galaxies from images available online (Raddick, et al., 2010). Chapter 7 presents a pilot investigation of such citizen science research which tests the feasibility of using volunteers to collect behavioural data, not via the internet, but using a touch screen computer adjacent to an enclosure.

### 6.4.3. Conclusions

In conclusion, this study has demonstrated a pattern of increase in active behaviours such as locomotion, ‘movement on the spot’ and ‘stationary but moving the head’, prior to predictable feeding times that was not demonstrated prior to unpredictable feeding times. This behavioural pattern appears to be anticipation of the predictable feeding routine. Three of the four geckos showed similar patterns in behaviour between feeding conditions, suggesting an effect of feeding routine. More work is required on gecko behaviour, behavioural indicators of welfare, and natural behavioural rhythms in order to further interpret these results and to determine the effect of the change in behaviour, and deviation from natural rhythms that was caused by predictable feeding. One-zero sampling was deemed to be a useful method in this study, however this chapter has once again identified the need for methods where multiple observers can collect data on patterns of behaviour over time. Chapter 7 will therefore investigate an approach of citizen science, to the study of predictability.

# Chapter 7

## *Use of a touch screen computer to collect data on animal behaviour via citizen science*

### 7.1. Introduction

Throughout this thesis, the case has been made that data collected by multiple observers, over prolonged periods, may be useful in the study of patterns of behaviour that are greatly extended in time. In Chapter 4 a simple, subjective measure that required little or no training to use was evaluated and found to be a suitable technique by which multiple observers could reliably obtain valid data. In Chapters 2 and 6 a citizen science approach was suggested as a possible method. The current study was therefore designed to explore the value of a broader 'Citizen Science' approach, in which data were collected by members of the public, in order to investigate the effect of predictable feeding times in an aquarium exhibit. The current research was conducted in an aquarium because: it presented a complex subject which would usually be difficult to record using more traditional approaches, clear predictions could be made (see below) and the environment was suitable for the installation of a touch screen computer (dry and equipped with suitable power outlets).

As described in Section 2.8., citizen science is a term given to research projects in which multiple observers (volunteers and members of the public) collect a small amount of data which contributes to a larger study (Bonney, et al., 2009). Such projects are often aided, or made possible by the use of technology, for example the Valley of the Khans and Galaxy Zoo projects (National Geographic, 2011; Raddick, et al., 2010) (as described in Section 2.8) use the internet to allow multiple observers access to a large library of digital photographs.

Presenting zoo visitors with technology is not a new concept, and a variety of interactive technologies have been used in visitor areas for education purposes (Clay et al., 2011). The use of multiple observers or citizen science in zoo research has also been undertaken previously. For example the Chimpanzoo project required multiple observers to collect behavioural data at different zoos on laptop computers using bespoke software (ChimpanZoo, 2003), and the Zoological Society of London's (ZSL) Instant Wild and iBats projects, required multiple observers to use smart-phone apps to identify animals in camera trap images, and to monitor bats (ZSL, 2012). However, to the experimenter's knowledge there are no examples where zoo visitors at exhibits have been used to systematically record data on animal behaviour.

The current study investigated anticipatory behaviour and time-place learning in a multi-species exhibit of fish. Data were collected by visitors who used a touch screen computer to record fish behaviour. Time-place learning occurs when animals learn to associate a particular location with a particular time of day (Barreto et al., 2006), for example they may learn that food is available in a specific location at a certain time of day. Individuals may therefore demonstrate anticipation of a timed event in a particular location. Time-place learning has been demonstrated in fish, for example Reebs (1996) fed golden shiners in different sides of their tanks at different times of day, and found that when food was withheld, the fish were positioned at the correct side of the tank, in anticipation of the time and location of food release. Similar results were found by Barreto et al. (2006) in angelfish (*Pterophyllum scalare*), although pearl cichlids (*Geophagus brasiliensis*) were not found to demonstrate this behaviour. In another study on golden shiners, Reebs and Gallant (1997) demonstrated that the fish spent more time close to the surface, where food was to be delivered, prior to a predictable feeding time. Also Brännäs et al. (2005) found that Arctic charr increased aggressive behaviour in the vicinity of a feed trigger, prior to a predictable feeding time.

In the current experimental design the fish had historically been fed in the same location and at the same times every day, and anecdotal reports by aquarium staff suggested that the fish congregated in a particular area of the tank for most of the day, but particularly prior to feeding. The aim of the current study was to test whether visitor responses on a touch screen could be used to determine patterns in fish location and activity. Principally, two main research questions were asked of the visitors: which area of the tank contained the most fish (in order to test time-place learning), and how ‘busy’ were the fish (in order to determine patterns of anticipatory behaviour). It was predicted that visitors would report that fish spent most time on the right of the tank (next to the feeding area) prior to, and in anticipation of feeding times, and that ratings of busyness would also increase at this time. This study required the development of appropriate touch screen software as well as suitable and accessible research questions and methods of quality control.

## 7.2. Methods

### 7.2.1 Subjects, housing and equipment

Subjects were mixed species of fish housed in the Atlantic Ocean exhibit at the National Marine Aquarium (NMA) in Plymouth. This exhibit housed 40 species of fish (approximately 1000 individuals) from the Western Atlantic, including smaller species such as ocean surgeon fish (*Acanthurus bahianus*), porkfish (*Anisotremis virginicus*) and banded butterfly fish (*Chaetodon striatus*) and larger species such as tarpon (*Megalops atlanticus*) and sand tiger sharks (*Carcharias taurus)*. The smaller fish species were the focus of this study. The Atlantic Ocean tank was the largest tank in the aquarium (holding 2.5 million litres of water) and as such, could be viewed from different levels. The focus of the current study was the top third of this tank where there was a large visitor viewing window and presentation area (see Appendix 7.1), and suitable space to set up the touch screen. Two feeding pipes were present in the tank, positioned to the left and the right of the visitor viewing window (as viewed when facing the window). Prior to, and for the majority of the current study, feeding only took place from the right-hand feeder.

Food was released from the feeder either automatically or by hand, at 11:00 and 15:00 daily, and the sharks also received a pole feed at approximately 11:00 on Mondays, Wednesdays and Fridays; but the latter were not a part of the study.

The touch screen apparatus initially consisted of a desktop PC with a touch-responsive monitor (with the processor locked in a cabinet to prevent public access); however this was updated to a purpose-built, enclosed, Xen X5 touch screen unit (see Appendix 7.1) later in the study (07/07/2011), when this equipment became available; to be referred to as the touch-screen hereafter. Computers in both apparatus ran the same software, however the enclosed touch screen unit was preferred for this research due to its aesthetics and design which clearly designated it as a piece of professional university research equipment. The touch screen ran the operating system Windows XP, and the program containing the research questions was written in Microsoft VB.Net. The programme opened immediately on start-up. The touch screen was positioned towards the left of the visitor viewing area and was occasionally repositioned slightly according to the space use requirements of the NMA, although it generally remained within approximately 5 metres of its original location.

### *Participants*

Participants were visitors to the aquarium. The touch screen was available for use by all visitors, and as such there was no pre-screening of participants. Participants were free to approach and use the touch screen as they wished, and (aside from the occasional presence of aquarium staff) the unit was not supervised. The information available on the screen was all that was used to advertise the project, although aquarium staff could direct people to the unit, if interested. In addition to, and independently of the touch screen citizen science study, a group of six psychology undergraduate students (plus assistants) from the University of Plymouth also carried out research on the behaviour of fish. This work is described further in Section 7.2.5.

### 7.2.2. Touch screen programme and research questions

The touch screen programme was designed to give as much useful information to the participants as possible, whilst remaining concise. Therefore a combination of descriptions, photographs and strategic placement of the touch buttons on the screen were used to aid the participants. A sequence of eight screens was presented to participants on the touch screen (as shown in Figures 7.1 and 7.2). The first two screens presented to the visitors invited them to take part and gave a brief introduction, and participants were then free to choose whether to take part or not. The following screens presented either questions or instructions, including the two research questions: 1) Which area of the tank (1, 2 or 3) has the most fish in it, or are they evenly distributed across the areas? and 2) On a scale of 1 to 5, how busy do you think the fish have been for the last minute? The final screen thanked the participants for taking part, gave a short debrief and invited the participants to either answer the questions again, or view some of the results so far.

Whilst the fish were fed on the right of the tank for the majority of this study, a feeder was also present on the left (which was used during the student study, as described in Section 7.2.5). Therefore research question 2 asked visitors about the area on the left and the right of the tank. It was decided that it would be too confusing to ask individual visitors to observe the left *and* right sides, therefore each participant was asked to report busyness on one side only, and the computer programme alternated the side of the tank to be observed, for each use of the touch screen (see Figure 7.2).

Prior to being asked research question 2, regarding busyness, participants were shown a screen which explained what was meant by the term. They were then asked to watch either the left or the right third of the tank for 1 minute while a timer counted down on the screen. After the 1 minute countdown had elapsed, the visitors were asked to press a button to move on to the next screen, which showed research question 2. It should be noted that participants could press the button to move on before the minute had elapsed. This was done to prevent a loss of interest, or the feeling that the touch screen had stopped working.

However, it was the intention that visitors watched for one minute (indeed this formed a quality control measure as described in Section 7.2.3).

Each time a participant pressed an answer on the screen, their response was recorded, along with the date and time of their response (see Figure 7.2 for details of data recorded from each screen). If the participant did not complete the questions, the program was set to ‘timeout’ and return to the start screen, and no data were recorded (see Figure 7.2 for timings).

### 7.2.3. Quality control

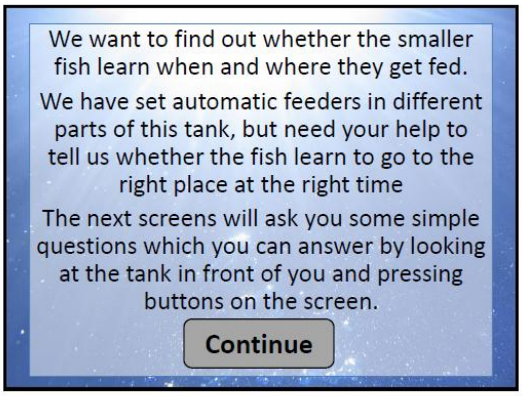
It was expected that a high number of visitors would not use the touch screen for the purpose it was intended, and that their button presses may therefore not match the behaviour of the fish; for example it was expected that some children would play with the touch screen and not follow the directions provided. Therefore two ‘quality control’ measures were put in place to minimise the inclusion of unsuitable data in the final dataset:

1) The first question screen asked visitors “What day of the week is it today?” The participant’s response was recorded along with the actual date, and it was assumed that if the two did not agree, then the participant was not engaged in the research, and any data they contributed was discarded from further analysis.
2) Prior to research question 2, the participants were asked to observe the tank for 1 minute. As mentioned above, the participants could skip forward to the next screen before the countdown timer reached 0. However, the time between starting the timer and answering research question 2 was recorded, and data from any participant who spent ≤ 30 s observing the fish was discarded from further analysis.

**Could you help us learn more about our fish?**

If you can spare a couple of minutes of your time to contribute to a study of the behaviour of the fish in the Atlantic Oceans tank, please touch the 'yes' button below.

Screen 1: Title screen and invitation (welcome screen)

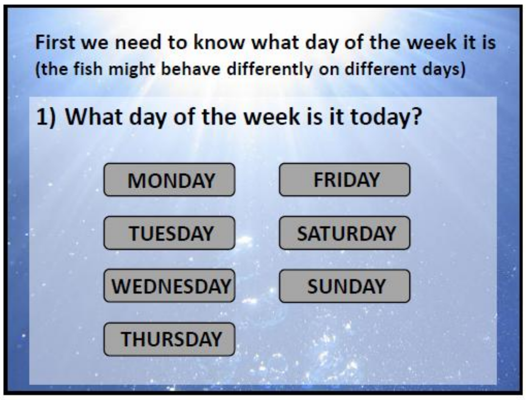


We want to find out whether the smaller fish learn when and where they get fed.

We have set automatic feeders in different parts of this tank, but need your help to tell us whether the fish learn to go to the right place at the right time

The next screens will ask you some simple questions which you can answer by looking at the tank in front of you and pressing buttons on the screen.

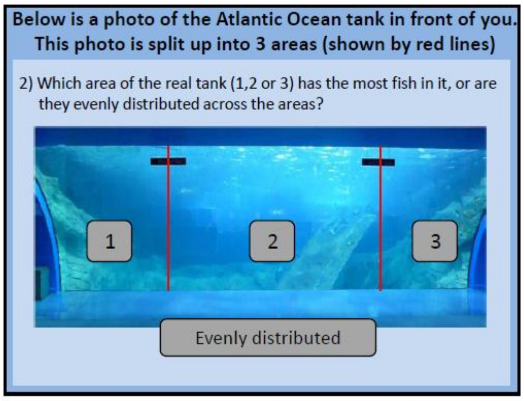
Screen 2: Brief (welcome screen)



First we need to know what day of the week it is (the fish might behave differently on different days)

1) What day of the week is it today?

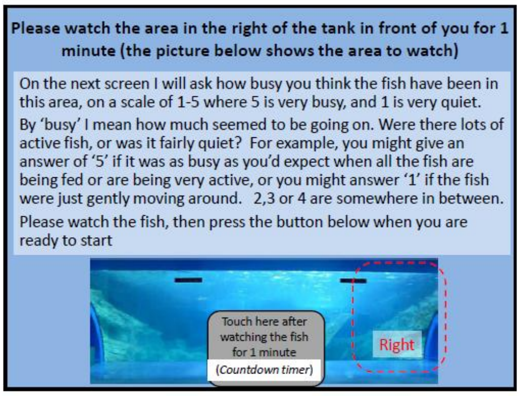
Screen 3: Quality control question



**Below is a photo of the Atlantic Ocean tank in front of you. This photo is split up into 3 areas (shown by red lines)**

2) Which area of the real tank (1,2 or 3) has the most fish in it, or are they evenly distributed across the areas?

Screen 4: Research question 1



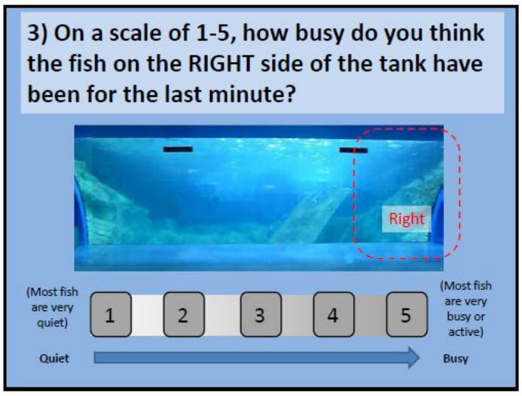
**Please watch the area in the right of the tank in front of you for 1 minute (the picture below shows the area to watch)**

On the next screen I will ask how busy you think the fish have been in this area, on a scale of 1-5 where 5 is very busy, and 1 is very quiet.

By 'busy' I mean how much seemed to be going on. Were there lots of active fish, or was it fairly quiet? For example, you might give an answer of '5' if it was as busy as you'd expect when all the fish are being fed or are being very active, or you might answer '1' if the fish were just gently moving around. 2,3 or 4 are somewhere in between.

Please watch the fish, then press the button below when you are ready to start

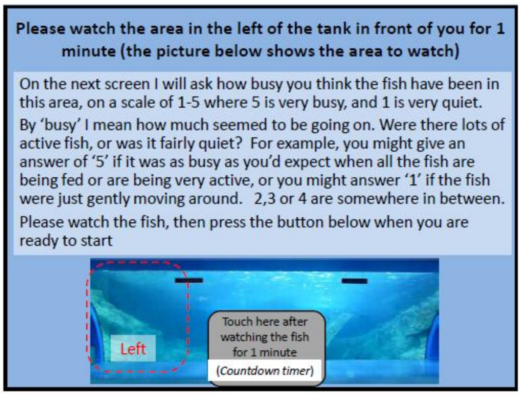
Screen 5: Explanation of busyness



**3) On a scale of 1-5, how busy do you think the fish on the RIGHT side of the tank have been for the last minute?**

Screen 6: Research question 2 for the right of the tank

*Figure 7.1: Screens presented to participants on the touch screen computer*



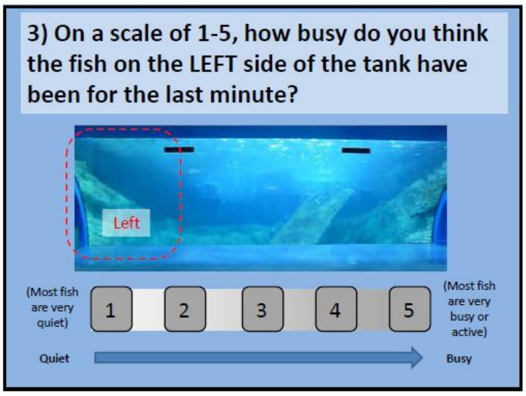
**Please watch the area in the left of the tank in front of you for 1 minute (the picture below shows the area to watch)**

On the next screen I will ask how busy you think the fish have been in this area, on a scale of 1-5 where 5 is very busy, and 1 is very quiet.

By 'busy' I mean how much seemed to be going on. Were there lots of active fish, or was it fairly quiet? For example, you might give an answer of '5' if it was as busy as you'd expect when all the fish are being fed or are being very active, or you might answer '1' if the fish were just gently moving around. 2,3 or 4 are somewhere in between.

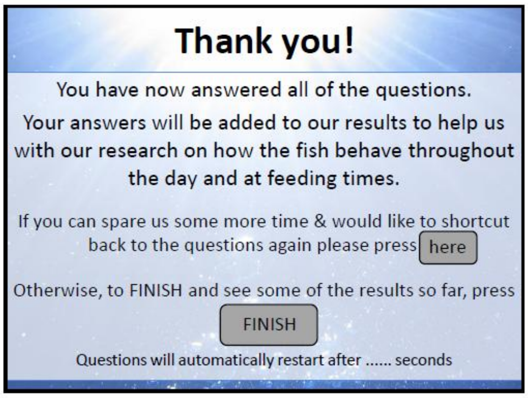
Please watch the fish, then press the button below when you are ready to start

Screen 7: Explanation of busyness



**3) On a scale of 1-5, how busy do you think the fish on the LEFT side of the tank have been for the last minute?**

Screen 8: Research question 2 for the left of the tank



**Thank you!**

You have now answered all of the questions.

Your answers will be added to our results to help us with our research on how the fish behave throughout the day and at feeding times.

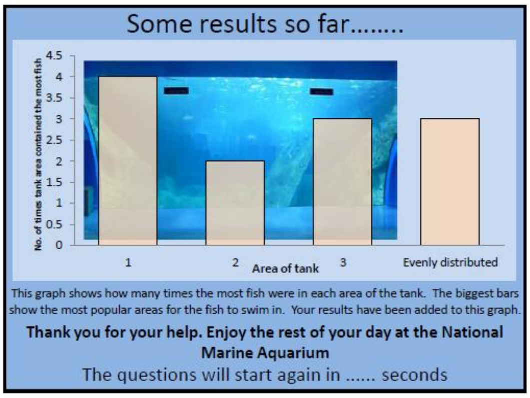
If you can spare us some more time & would like to shortcut back to the questions again please press here

Otherwise, to FINISH and see some of the results so far, press

FINISH

Questions will automatically restart after ...... seconds

Screen 9: Thank you and debrief, and invitation to answer again or view results



Some results so far........

This graph shows how many times the most fish were in each area of the tank. The biggest bars show the most popular areas for the fish to swim in. Your results have been added to this graph.

**Thank you for your help. Enjoy the rest of your day at the National Marine Aquarium**

The questions will start again in ...... seconds

Screen 10: Graph produced from participants' responses to date

*Figure 7.1 continued.*

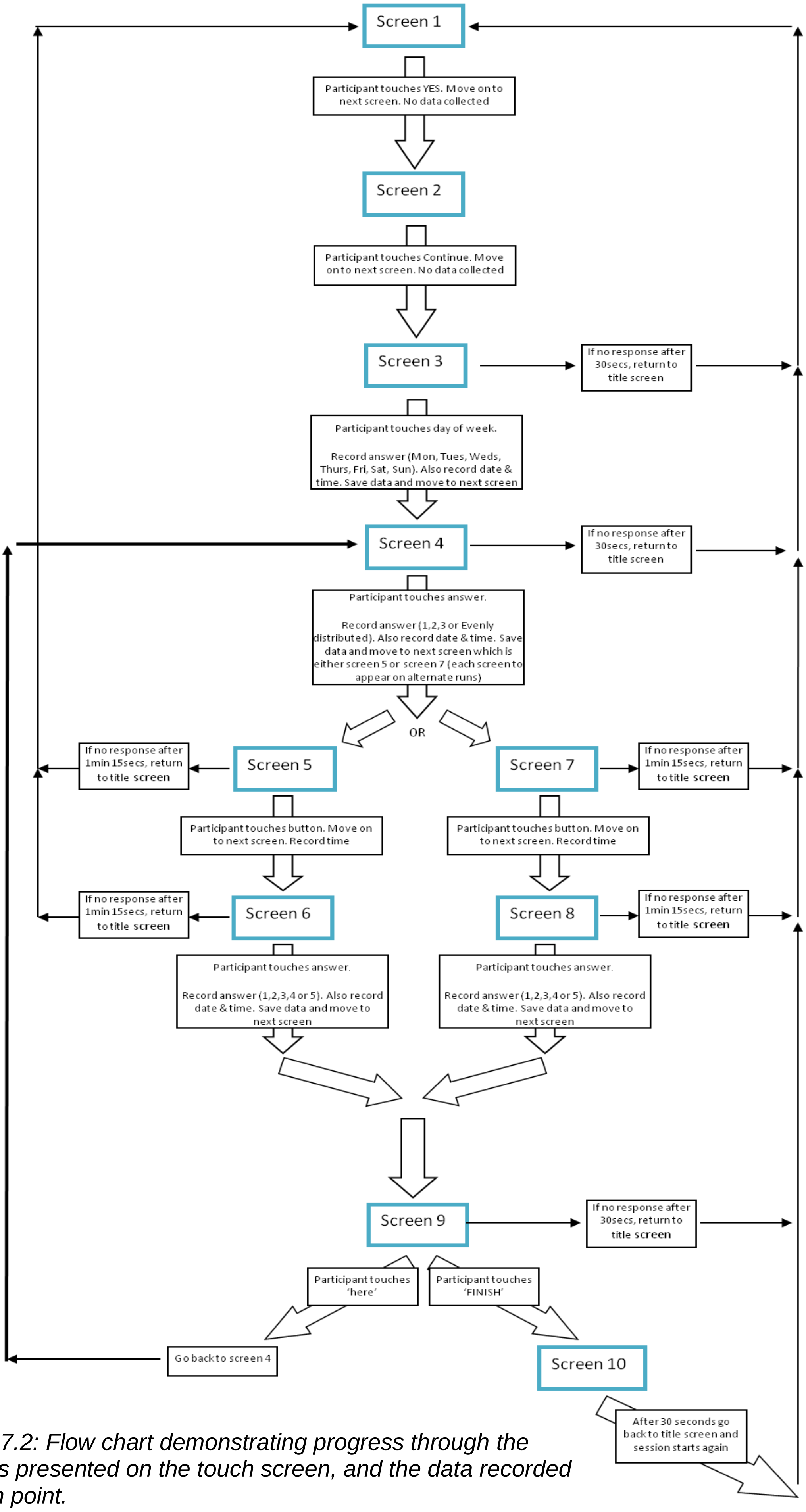


*Figure 7.2: Flow chart demonstrating progress through the screens presented on the touch screen, and the data recorded at each point.*

### 7.2.4. Procedure

The touch screen unit was at the aquarium from 18/01/2011 to 07/03/2012, during which time 16616 complete uses (i.e. participant answered both research questions) of the touch screen were recorded. This number included participants who answered the questions more than once (as was identifiable in the data file), and was before quality control. During this time, the touch screen was switched on most days, however it was turned off on certain days according to the requirements of the NMA. Visitors touched the screen to give their answers which were recorded in a Microsoft Office Excel comma separated values file.

### 7.2.5. Student study

Data were also collected by undergraduate students from the University of Plymouth, which are reported here as a validation measure (see Chapter 4). Students observed the fish during 30 min observation periods in the morning (09:30-11:30) and afternoon (13:30-15:30) during two conditions: 1) a baseline phase where the fish were fed according to their normal feeding routine as described above (22/11/10 – 06/02/11, *n*=17, although only 18/01/11 – 06/02/11, *n*=5 overlapped with the touch screen study); and 2) an intervention phase where the fish were fed from the left side feeder at the 11:00 feeding time as well as continuing to be fed on the right at 15:00 (07/02/11 - 04/03/11, *n*=15). Data collected were comparable to those collected in the touch screen study, as described above; every minute during the 30 minute observation the students a) indicated which third of the tank had most fish, and b) graded how busy they were.

### 7.2.6. Data analysis

Data were imported from the touch screen to an Excel spread sheet which was designed to automatically analyse the data as it was inputted. Some data were discarded according to the quality control measures described in Section 7.2.3). Whilst the fish were fed from the left feeder for a period of time during the current study, this period was not separated from the dataset used in analysis, as the time period of feeding on the left was short in comparison to length of the touch screen study, and feeding from the left resulted in only slight changes in fish behaviour (as described in Section 7.3.4).

Responses were grouped into 10 minute bins throughout the day, totalling 44 bins for the touch screen results, and 24 bins for the student study results. Calculations made of these data were: a) the percentage of participant responses for each area of the tank, where that area was reported to contain the most fish, and b) the mean busyness score. Data regarding the area of the tank containing the most fish were plotted for only those 10 minute bins in which the number of visitors using the touch screen (touch screen responses) equalled 5 or more, as percentages calculated from low numbers of responses would have given disproportionately high or low values. Descriptive analysis of the resulting data was used in order to investigate detailed patterns throughout the day. This enabled interpretation of visitor records of fish behaviour in relation to the temporally predictable feeding routine of the fish. As previously described throughout this thesis, descriptive analysis of data throughout the day was mostly used rather than statistical comparison of a limited number of groups of data at particular times of the day, since such comparisons would not reveal the details of behavioural patterns over the day. Indeed, the nature of the touch screen apparatus meant that visitors could record data at any minute throughout the day; hence data were collected over a long period of the day, enabling the descriptive analysis of detailed patterns of behaviour.

## 7.3. Results

### 7.3.1. Touch screen use and quality control

Throughout the duration of the study, 16616 complete uses of the touch screen were recorded. This included participants who answered the series of questions more than once. Of these data, 5500 responses showed the wrong weekday for the first quality control question, and 11977 spent ≤30 seconds observing the fish. When these data were removed, the remaining data set consisted of 4093 responses (a violation of either or both quality control conditions resulted in the discarding of that participant’s data). As the focus on the left or right side of the tank, alternated between touch screen uses for research question 2, after quality control 2246 responses of busyness remained for answers regarding the left of the tank and 1847 for the right.

### 7.3.2. Research Question 1

Results for research question 1 (enclosure use) (Figure 7.3) revealed a higher percentage of participants reporting the fish to be in either the middle third or the right third of the tank (mean of all 10 min bins = 37.05% and 31.46% respectively). Responses for the left third of the tank or evenly distributed were low (mean of all 10 min bins = 10.36% and 21.13% respectively), and the distribution was similar throughout the day. Responses reporting that most fish were in the right third of the tank demonstrated a bimodal-style pattern across the day with peaks between 10:50 to 11:00 (57.58%) and 14:40 to 15:20 (maximum 57.75%). Responses indicating most fish were in the middle third of the tank showed an opposite pattern, with low percentages at these times (15.15% and minimum 23.97% respectively).

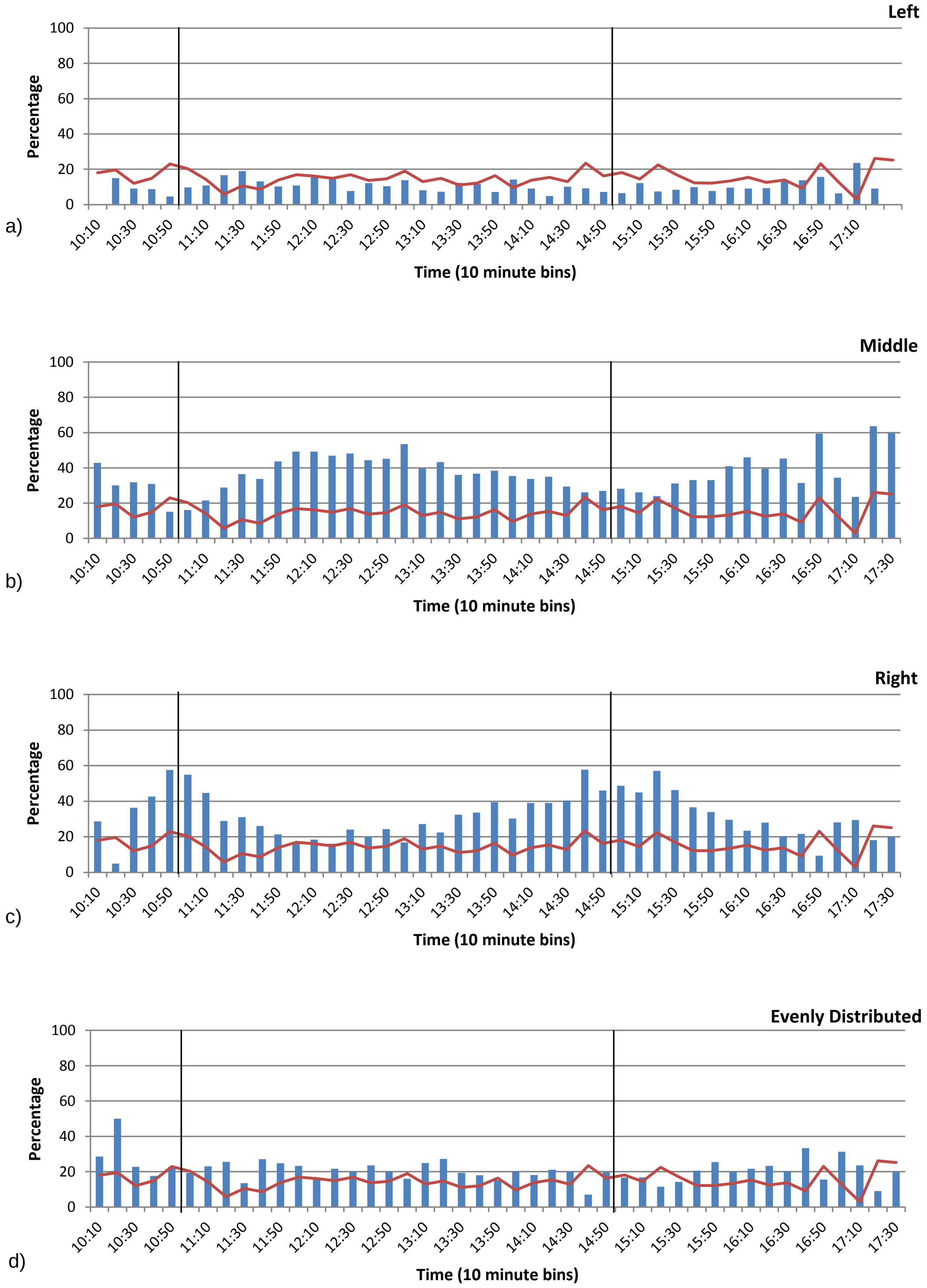


*Figure 7.3: Percentage of participant responses in 10 minute bins throughout the day where participants reported the most fish in the: a) right, b) left, c) middle of the tank, or d) evenly distributed across the tank. Vertical lines indicate feeding times. n max = 155, n min = 3 (n for each data point is shown in Appendix 7.2.1.). The red line shows the standard deviation across responses for the 4 tank areas.*

### 7.3.3. Research Question 2

Responses to research question 2 (Figures 7.4-7.5) revealed lower mean busyness scores on the left compared to the right of the tank; but there was no clear pattern of busyness throughout the day. Busyness scores attributed to fish in the right of the tank showed high levels (around 3.6) prior to 11:00 which then decreased to around 2.9 in the middle of the day before increasing to a peak of 3.81 between 15:30 and 15:40, followed by another decrease to the end of the day.

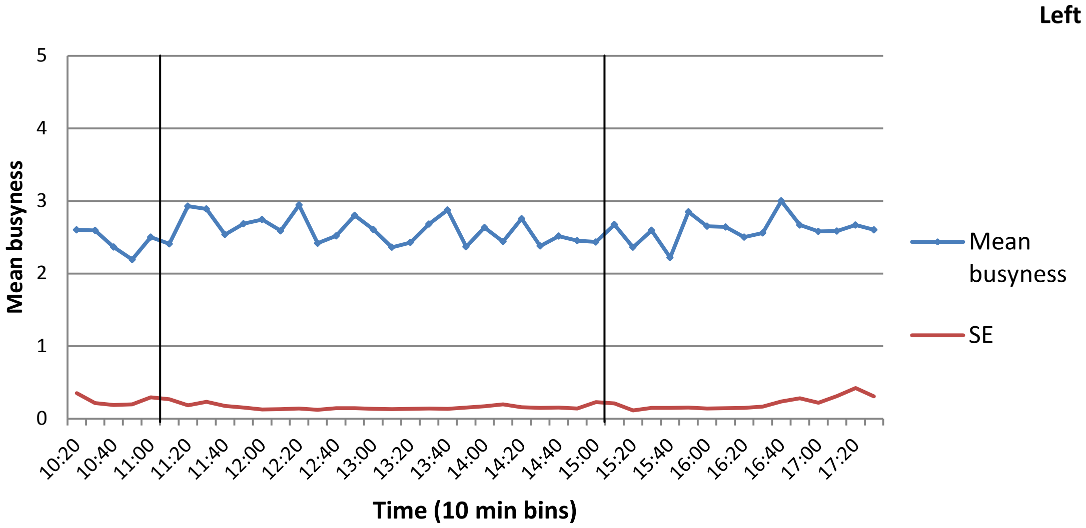


*Figure 7.4: Mean participant score for busyness on the left of the tank in 10 minute bins throughout the day. Vertical lines indicate feeding times. n max=87, n min=2 (n for each data point is detailed in Appendix 7.2.2.).*

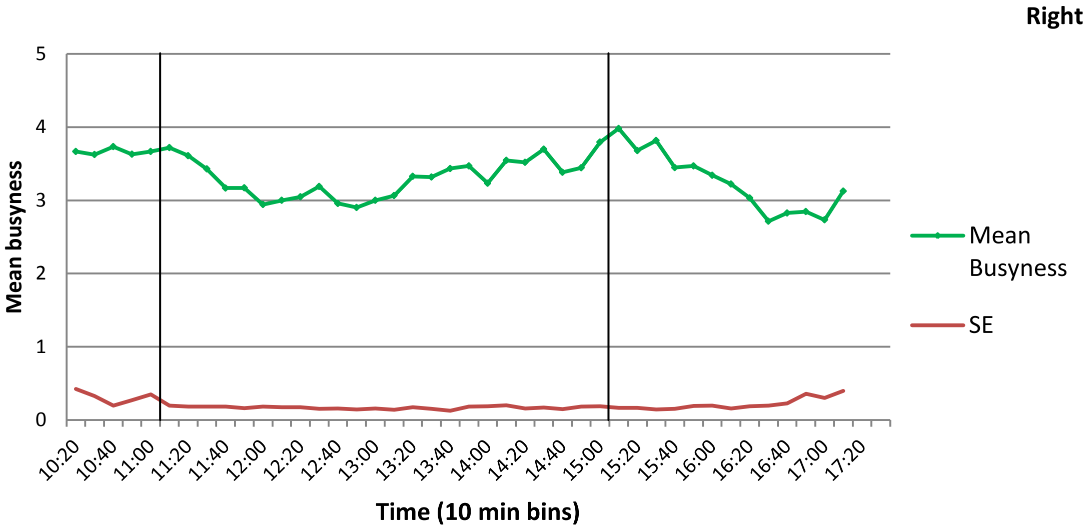


*Figure 7.5: Mean participant score for busyness on the right of the tank in 10 minute bins throughout the day. Vertical lines indicate feeding times. n max = 71, n min = 1 (n for each data point is detailed in Appendix 7.2.3.).*

### 7.3.4. Comparison between touch screen and student data

Observations recorded by students in both study phases indicated that the most fish were seldom reported in the left third of the tank, and were most frequently reported in the right third of the tank (Figure 7.6); similar patterns were reported using the citizen science approach (over the entire touch screen data collection period). In contrast to the touch screen results, the student study reported relatively high percentages of observations when fish were evenly distributed throughout the tank, but low percentages of observations when most fish were in the middle third of the tank.

In agreement with the touch screen results, the students reported lower busyness scores in the left of the tank compared to the right of the tank (Figure 7.8). In addition, data collected by students and the touch screen showed an increase in busyness for fish in the right third of the tank and an increase in percentage of observations of fish on the right, in the time leading up to the feeding times (11:00 and 15:00) on the right.

Data collected by students revealed mostly similar patterns between the baseline (fed on the right for both feeds) and intervention (fed on the left for the 11:00 feed) phase, however some differences were observed. For example, higher percentages of observations reported most fish in the left third of the tank between 10:40 and 11:00 in the intervention phase compared to baseline (max. 25% compared to 2.5%). The percentage of observations where the most fish were said to be on the right, showed a pattern of decrease over the morning in the intervention phase, which was not demonstrated in the baseline phase. Also mean busyness on the left of the tank showed a peak of 3 at 10:40 in the intervention phase, which was not present in the baseline phase.

Figures 7.6-7.9 plot data generated from the touch screen and student study, for matched days. Whilst low numbers of responses on the touch screen during this period resulted in missing data points, thus making comparison difficult (particularly for the morning observations), some similarities can be seen. For example both the touch screen and student results indicated that: a) most fish were in the right of the tank for the highest percentage of observations, with the lowest percentage on the left; b) in the intervention period, the percentage of responses reporting that the most fish were on the left in the morning was greater than in the baseline period; c) mean busyness was higher (and at a similar level to the student data) on the right than on the left (although mean busyness on the left was at a higher level in the touch screen data than the student study). Also, a similar peak of busyness was shown on the left of the tank in the morning in the touch screen results as the student results.

## Student Study

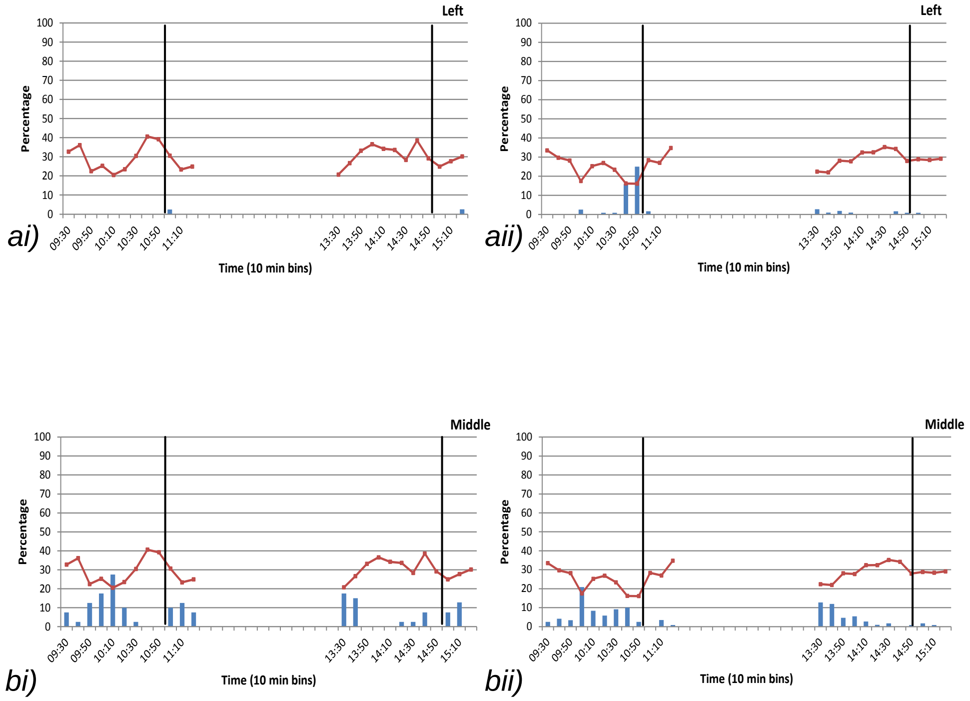


Figure 7.6: Student study. Percentage of responses in 10 minute bins throughout the day where students reported the most fish in the a) left, b) middle or c) right of the tank, or d) evenly distributed across the tank, in the baseline (i) and intervention (ii) phases. Vertical lines indicate feeding times. Red line shows the standard deviation across responses for the 4 tank areas. n = 5 (baseline phase) and n = 15 (intervention phase).

## Touch Screen Study

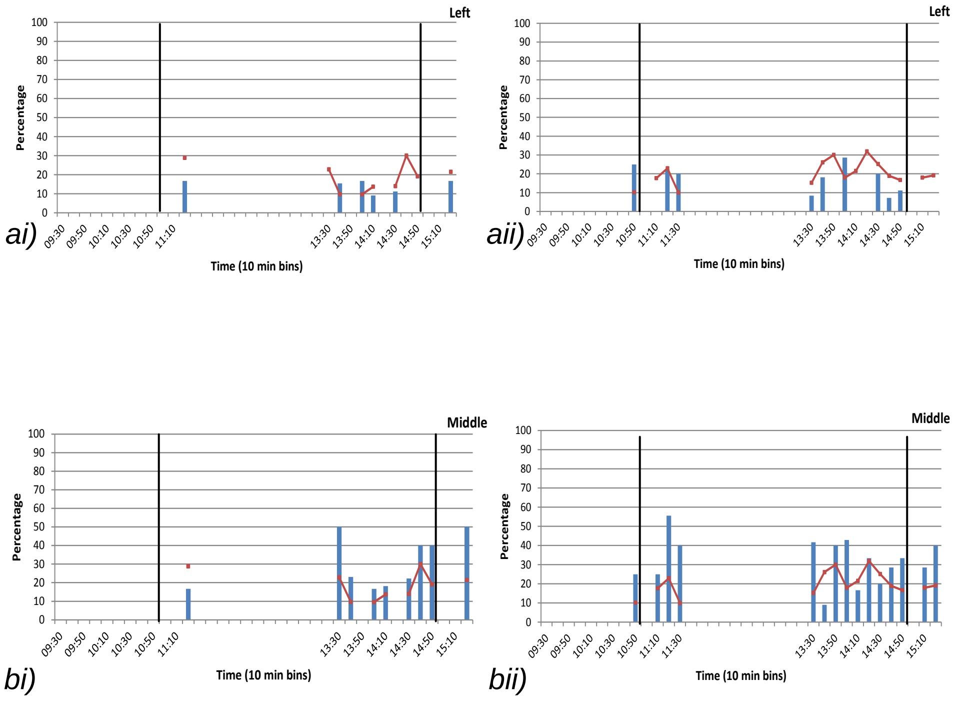


Figure 7.7: Touch screen study. Percentage of responses in 10 minute bins throughout the day where participants reported the most fish in the a) left, b) middle or c) right of the tank, or d) evenly distributed across the tank, in the baseline (i) and intervention (ii) phases. Vertical lines indicate feeding times. Red line shows the standard deviation across responses for the 4 tank areas. Data are plotted for only those bins in which n ≥ 5: Baseline phase: n max = 13, n min = 0 (minimum shown on graph = 5) Intervention phase: n max = 14, n min = 5 (minimum shown on graph = 5) (n for each data point is detailed in Appendix 7.3.1).

## Student Study

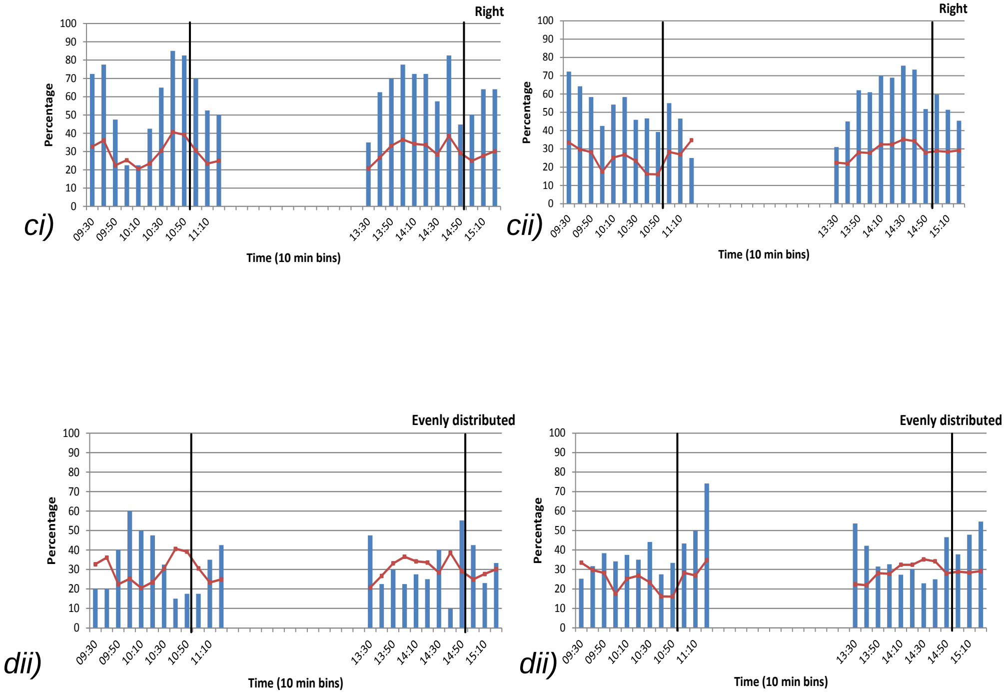


*Figure 7.6: Continued.*

## Touch Screen Study

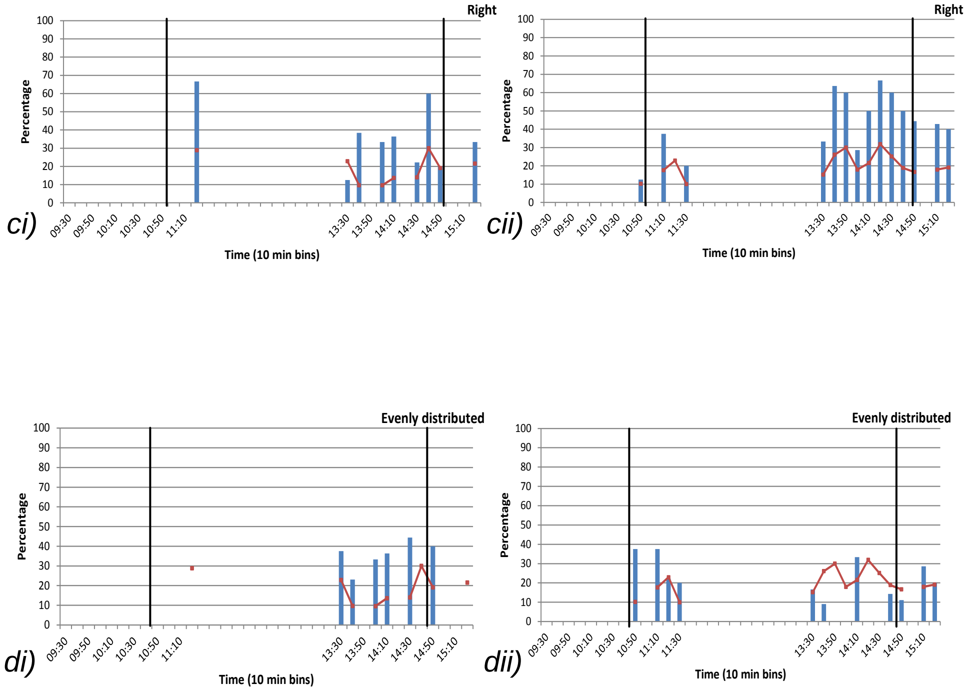


*Figure 7.7: Continued*

## Student Study

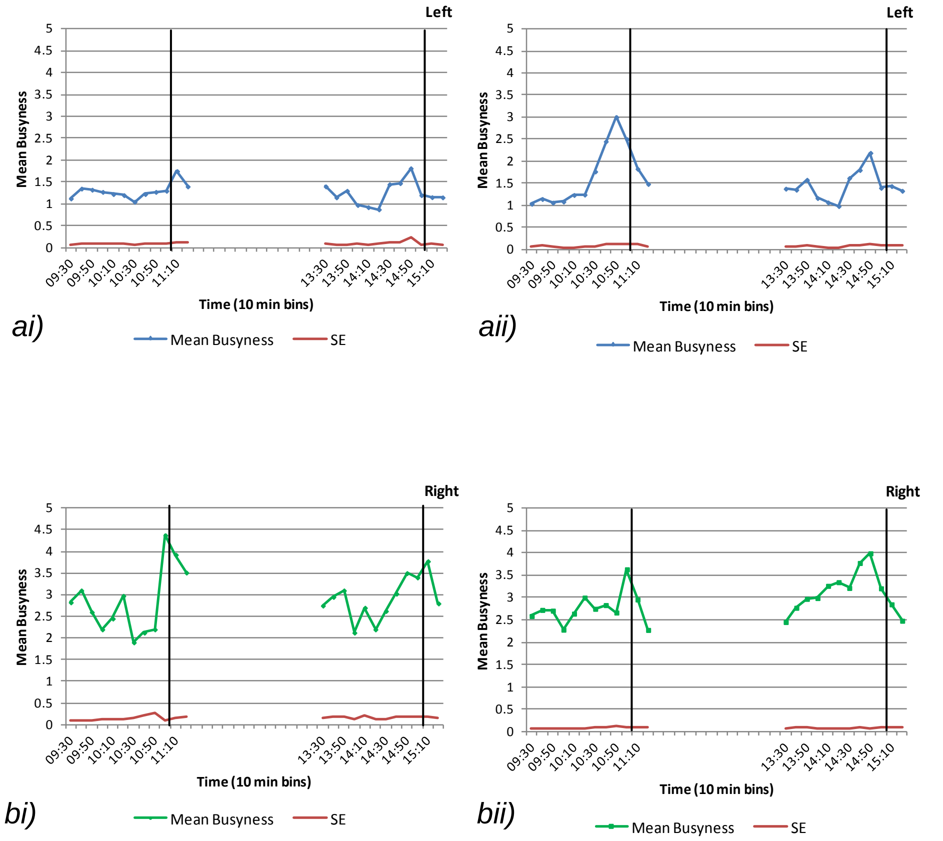


Figure 7.8: Student study. Mean busyness in 10 minute bins throughout the day in the a) left and b) right of the tank in the baseline (i) and intervention (ii) phases. Vertical lines indicate feeding times. n = 5 (baseline phase) and n = 15 (intervention phase).

## Touch Screen Study

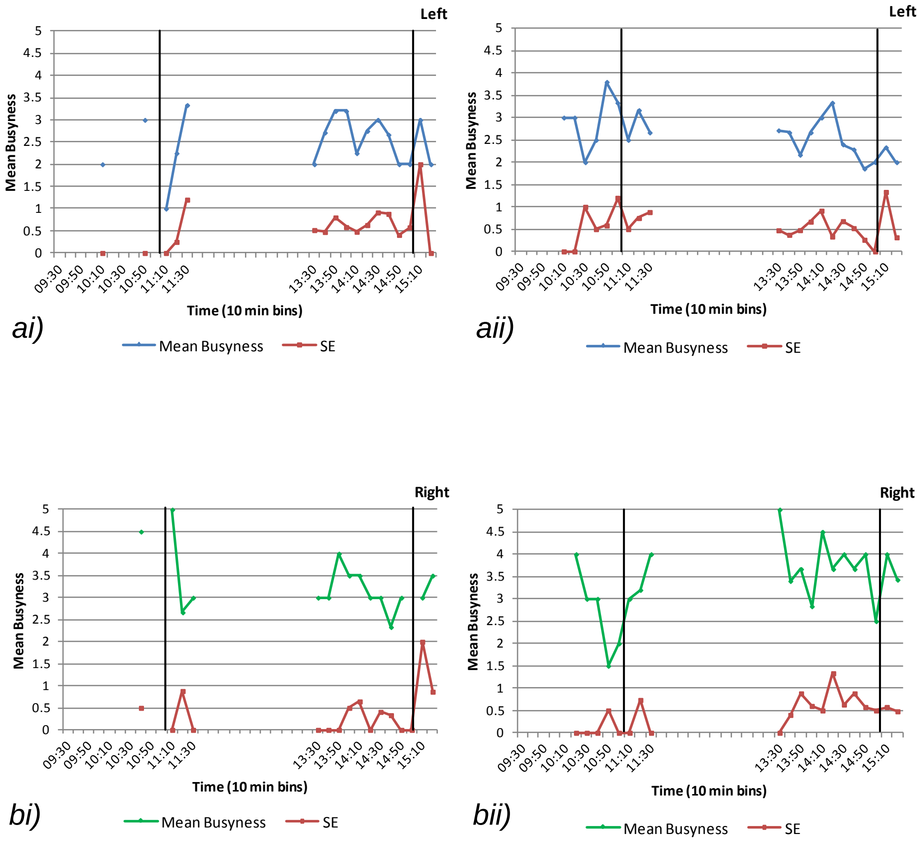


Figure 7.9: Touch screen study. Mean busyness in 10 minute bins throughout the day in the a) left and b) right of the tank in the baseline (i) and intervention (ii) phases. Vertical lines indicate feeding times. Baseline phase: Left n max = 7, n min = 0, Right n max = 4, n min = 0. Intervention phase: Left n max = 9, n min = 0, Right n max = 7, n min = 0 (n for each data point is detailed in Appendix 7.3.2 and 7.3.3).

Whilst similarities existed between the student and touch screen data, the gaps in the data would make interpretation of the touch screen data difficult in isolation from the student study data. Figures 7.10-7.21 show the accumulation of data generated from the touch screen in one month increments between one and six months. This is provided to illustrate the effect of study length on the patterns obtained. Graphs of the percentage of responses reporting the most fish on the right, and the busyness on the right, are plotted for reference. There were substantial gaps in the data when the touch screen had been available for only one month (Figures 7.10-7.11), particularly for the busyness data (Figure 7.11). By 4 months, a much clearer pattern of behaviour was demonstrated, with a minimum of 7 (figure 7.16) and 6 (figure 7.17) visitor responses in each 10 minute bin throughout the day. When the touch screen had been available for longer than 4 months (Figures 7.27-7.20), whilst the number of responses increased each month, there was little change in the patterns of data presented, and standard error remained low.

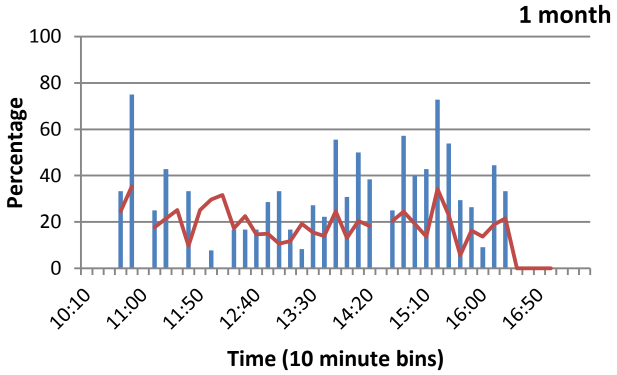


Figure 7.10: Data from 1 month of usage showing the percentage of participant responses reporting the most fish in the right of the tank. n max = 19, n min = 2 (minimum shown on graph = 5). Total n = 365.

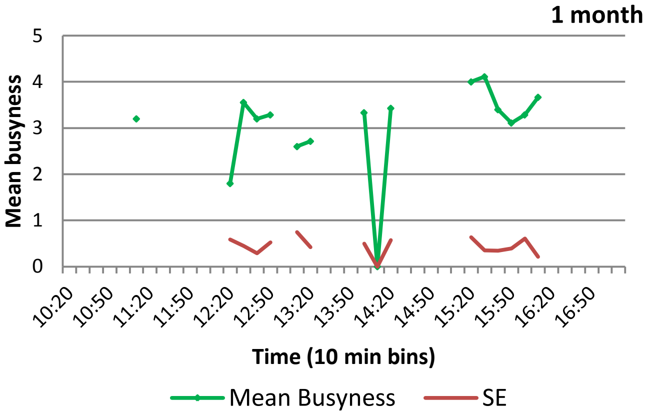


Figure 7.11: Data from 1 month of usage showing the mean busyness in the right of the tank. n max = 10, n min = 1 (minimum shown on graph = 5). Total n = 178.

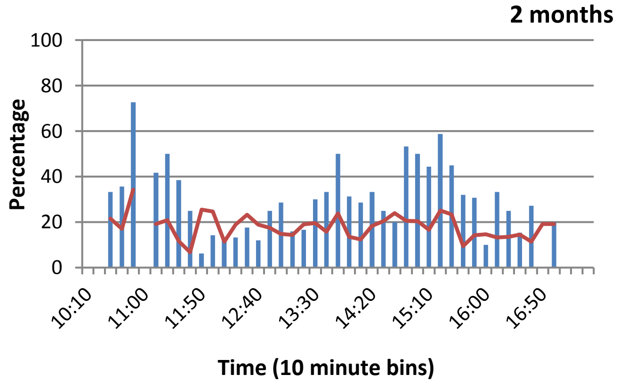


Figure 7.12: Data from 2 months of usage showing the percentage of participant responses reporting the most fish in the right of the tank. n max = 30, n min = 2 (minimum shown on graph = 5). Total n = 631.

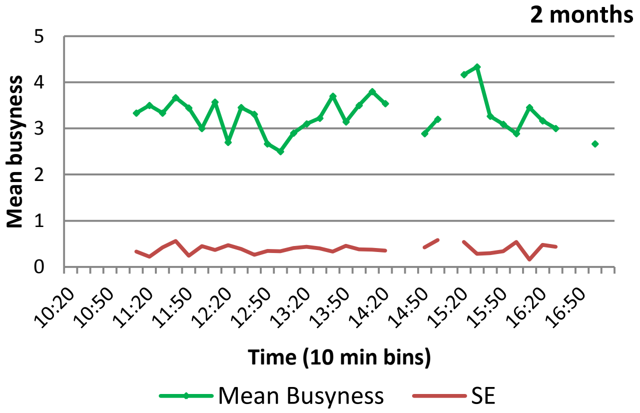


Figure 7.13: Data from 2 months of usage showing the mean busyness in the right of the tank. n max = 15, n min = 1 (minimum shown on graph = 5). Total n = 302.

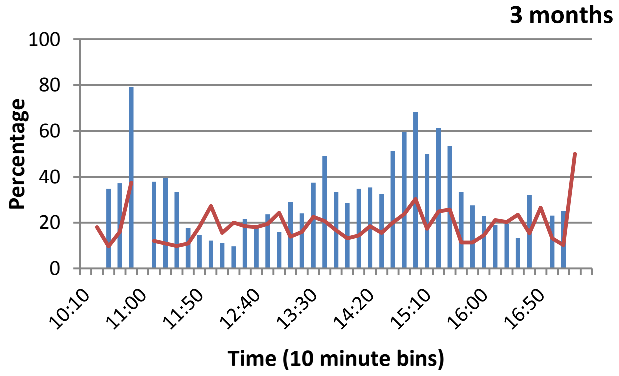


Figure 7.14: Data from 3 months of usage showing the percentage of participant responses reporting the most fish in the right of the tank. n max = 55, n min = 2 (minimum shown on graph = 5). Total n = 1446

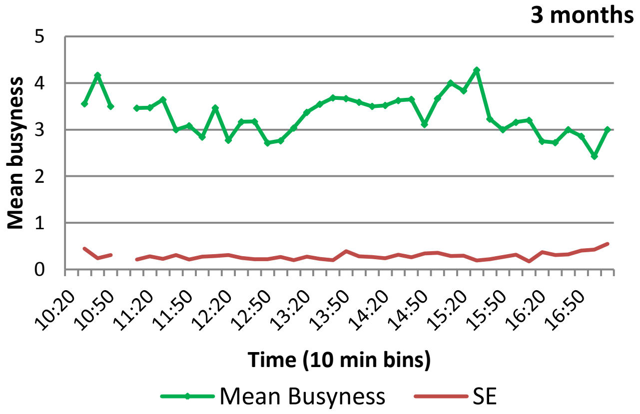


Figure 7.15: Data from 3 months of usage showing the mean busyness in the right of the tank. n max = 29, n min = 1 (minimum shown on graph = 5). Total n = 705.

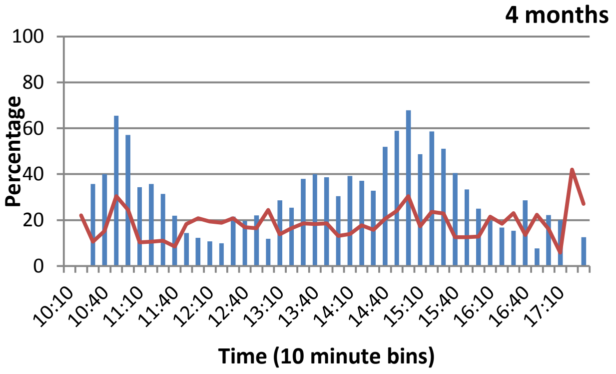


Figure 7.16: Data from 4 months of usage showing the percentage of participant responses reporting the most fish in the right of the tank. n max = 70, n min = 2 (minimum shown on graph = 7). Total n = 1867.

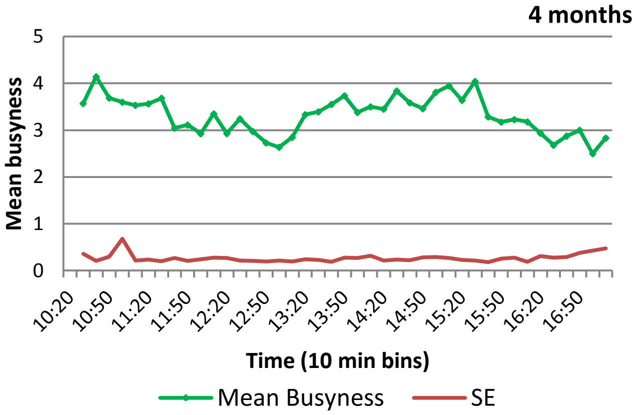


Figure 7.17: Data from 4 months of usage showing the mean busyness in the right of the tank. n max = 37, n min = 1 (minimum shown on graph = 6). Total n = 925.

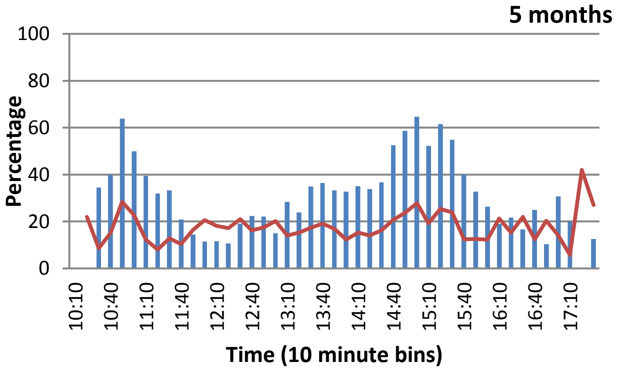


Figure 7.18: Data from 5 months of usage showing the percentage of participant responses reporting the most fish in the right of the tank. n max = 77, n min = 2 (minimum shown on graph = 8). Total n = 2094.

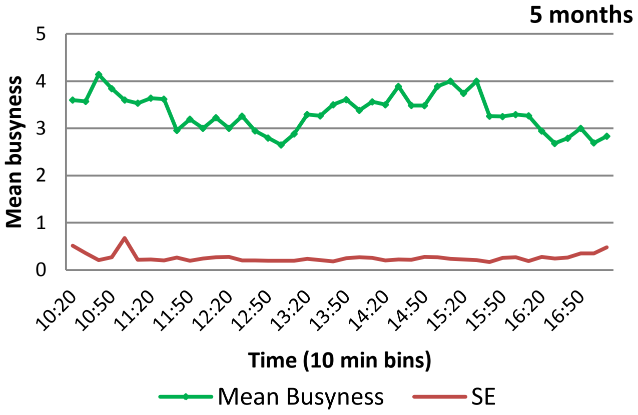


Figure 7.19: Data from 5 months of usage showing the mean busyness in the right of the tank. n max = 39, n min = 1 (minimum shown on graph = 5). Total n = 1016.

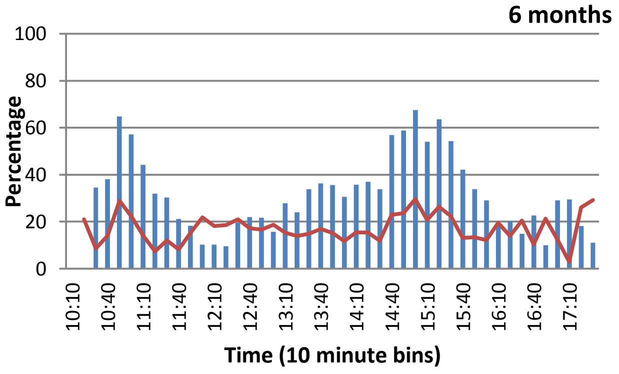


Figure 7.20: Data from 6 months of usage showing the percentage of participant responses reporting the most fish in the right of the tank. n max = 83, n min = 2 (minimum shown on graph = 9). Total n = 2355.

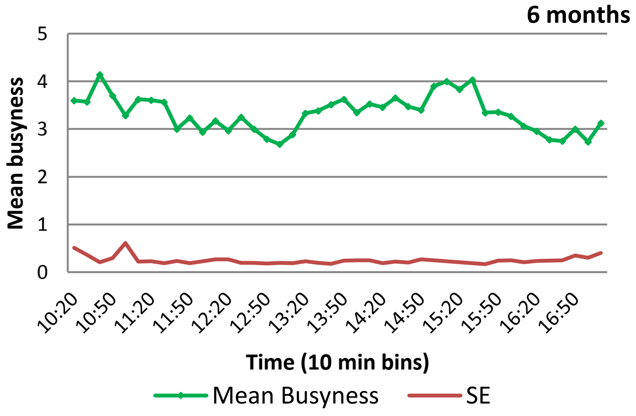


Figure 7.21: Data from 6 months of usage showing the mean busyness in the right of the tank. n max = 39, n min = 1 (minimum shown on graph = 5). Total n = 1107.

## 7.4. Discussion

Data collected using the touch screen allowed determination of patterns of fish behaviour over the day. The results showed increased use of the right of the tank, and increased busyness on the right, particularly in the time leading up to feeding times. The fish were predominantly fed on the right hand side of the tank, therefore these results fit with the predicted pattern of anticipatory behaviour and time-place learning. Whilst time-place learning was not tested using a withdrawal of food in order to test location in the absence of food, for example as tested by Reebs (1996), a pattern of increase prior to, and decrease after feeding time was demonstrated. Results in the current study are consistent with previous literature, where fish have been demonstrated to increasingly use the side of the enclosure where feeding will take place, in anticipation of the feeding time (e.g., Barreto, et al., 2006; Reebs, 1996; Reebs & Gallant, 1997) (see Section 7.1). Increased busyness prior to feeding also agrees with previous literature demonstrating FAA in fish (e.g., Chen & Purser, 2001; Chen & Tabata, 2002; Herrero, et al., 2005; Reebs & Lague, 2000), and other behaviours such as increased aggression (Brännäs, et al., 2005) prior to predictable feeding (see Section 1.2).

Data generated from the touch screen computer were successful in revealing behavioural patterns in a large mixed-species fish exhibit, with clear patterns of enclosure use and busyness demonstrated in anticipation of feeding. Whilst the analysis of this study relied on descriptive analysis and hence (as previously discussed in sections 3.4.2. and 5.4.1.), patterns should be interpreted with caution, these patterns were clearly defined, and standard error for mean busyness was low and consistent; suggesting high agreement between responses at particular times of the day.

Results of the student study provided some evidence for time place learning; there was a slight increase in fish use and busyness of the left side of the tank in the lead up to feeding, when feed location was changed to the left. Agreement between data generated using the touch screen and by students suggests that the touch screen participants were, indeed recording the correct fish behaviour. Whilst matched comparison of these two datasets was hindered by a low number of data points from the touch screen, some similarities were seen, and comparison of the student data with the entire touch screen dataset clearly demonstrated these similarities. However, it is recommended that further work to evaluate the accuracy of the touch screen results be carried out, perhaps by comparing a more extended period of researcher observation of the fish with the touch screen data.

One benefit of the data generated from the touch screen over that collected by the students was that the touch screen ran continuously throughout the day; it was not limited to morning and afternoon periods as it was in the student study due to manpower restrictions, as is often the case for observational studies. This limitation associated with the data collected by the students meant that, whilst increases in right-side enclosure use and busyness were demonstrated around feeding time, the data lacked detailed information on the patterns of commencement and cessation of these increases during the day. By using a large number of untrained observers throughout the day, the touch screen study provided detail on patterns of behaviour that might otherwise not have been observable.

In the matched comparisons of the touch screen and student data, some differences were noted. For example the touch screen data showed most fish to be on the right or in the middle, whereas the students reported most fish to be either on the right of the tank or evenly distributed. Also, busyness scores on the left, whilst consistently lower than on the right, were generally recorded to be higher up the busyness scale from the touch screen results than the student results. A possible reason for these differences may have been due to the location of the touch screen, which was positioned towards the left of the tank. Whilst all observers had a clear view of the tank, visitors standing at the touch screen may have had a slightly different perspective of the tank than the view that could be obtained by a student observer, due to the curved ‘demi-tunnel’ nature of the viewing window. Factors such as touch screen placement are therefore important considerations for future work.

The current study has demonstrated that whilst visitors may have had only a short amount of experience of the behaviour of the fish, compared to a student who had experienced the entire range of fish busyness, they were able to successfully use the measure of busyness. The instructions presented on the touch screen used behavioural descriptions of the anchor points of the busyness scale, as recommended in Section 4.4. Results in this current study suggest these descriptions were successful, however if necessary it would be possible to incorporate video examples of busyness in future studies as a further aid to the visitors (as also suggested in Section 4.4).

The quality control measures employed, led to the creation of a data set which showed clear patterns in fish behaviour over the day; thus they seemed to have successfully removed irrelevant data. However these measures did result in a large reduction in the data set, accounting for 12523 discarded entries. Future research should look to improve the quality of data obtained from each participant in order to reduce the number of discarded data points. This could be achieved by a number of measures, such as clearer instructions on how long the participants should observe the fish, or a clearer count-down timer. Interestingly, it was noted that fewer children played with the touch screen unsupervised when the computer was updated to the purpose-built unit, which was taller that the original PC.

Analysis of the data accumulated during 1-6 month periods revealed that a more complete data set was achieved after 4 months of touch screen use. This gives an approximate guide to the length of use required from the touch screen for a single experimental condition. This can only be an approximate guide as visitor numbers vary between months and institutions (the mean number of usable responses per month in the current study was 395, with a minimum of 228 and a maximum of 811). Advertising could be used to increase the number of people using the touch screen each day, for example with the use of posters, or awareness raised by staff. Low data times of the day were at the beginning and end of the day when there were fewer visitors in the aquarium, and during the public talk given at the time of the shark feed. Again, such factors must be taken into consideration in future work.

Throughout this thesis, much emphasis has been placed on the need for a suitable methodology with which to study the effects of predictability, using multiple observers, in order to determine patterns of behaviour over extended periods of time. The current chapter both demonstrated the potential for visitors to use a simple, subjective measure (busyness), and demonstrated the potential for using touch screen technology in order to record such behavioural patterns. As such, the current chapter has developed the original idea of using a group of volunteer observers to collect data, to using a much larger number of observers. Many citizen science projects aim, not only to collect useful data, but to provide participants with an opportunity to gain knowledge, and understanding of scientific procedures (Bonney, et al., 2009; Brossard et al., 2005). Future research could therefore investigate the effect of citizen science studies (such as the current study) on visitors' knowledge and also, on their attitudes towards topics such as research, conservation and animal management, topics which are particularly relevant to the zoo and aquarium environment.

In conclusion, the current touch screen set up was successful in producing a data set in which clear patterns of fish behaviour were demonstrated. Once programmed, this method of data collection required little effort to run, and produced a large data set. Hence this citizen science approach to collecting data using a touch screen computer showed great potential for future use. Visitors to aquariums, and similar organisations, could therefore be used to provide large volumes of data on animal behaviour by answering simple questions about the animals in an enclosure. This study has also demonstrated that the measure of busyness can be used successfully by untrained members of the public, and may therefore be a useful tool in future research.

# Chapter 8

## Concluding discussion

### 8.1. Summary of work

Animals can show regular daily patterns of behaviour, with the rhythmicity of such behaviour synchronised with the rhythmicity of the external environment. For example, predictable events such as scheduled feeding times can entrain patterns of behaviour, such that they anticipate the occurrence of these predictable events. However, outside of a laboratory situation, we have few methodological tools for investigating such patterns of behaviour.

The present programme of research has supported the suggestion that the temporal predictability of events such as feeding is an important consideration for the management of captive animals due to its potential to influence patterns of behaviour, although the literature reveals a lack of consensus on whether the effect of predictable feeding on animal behaviour has positive or negative welfare implications. This lack of consensus may in part be because there is little research on the effect of predictable and unpredictable feeding routines, particularly in the zoo environment, where predictable feeding is often a management practice. Studies that exist, often report only incidental evidence, are confounded by other variables, or do not focus specifically on anticipatory behaviour. Studies on this subject also face challenges to the methodology, such as insufficient study or observation lengths (as discussed in Section 2.2). The aims of the current programme of research were therefore to extend the body of research investigating the effects of predictable and unpredictable feeding times on patterns of behaviour and anticipation in zoo animals, and to develop suitable methodology to overcome some of the challenges to the study of predictability. These aims were successfully addressed.

### 8.1.1. Effect of predictable feeding routines

The current programme of research identified a number of patterns of behaviour which appeared to be related to predictable feeding times. For example, tigers showed some patterns of increased locomotion and pacing, decreased inactivity and sleeping, and increased use of particular enclosure areas in the time leading up to a predictable feeding time (Chapter 3). These data are consistent with previous studies of felid behaviour, demonstrating increased pacing and decreased inactivity prior to feeds (e.g., Altman, et al., 2005; Carlstead, 1998; Lyons, et al., 1997; Weller & Bennett, 2001). However the study reported in Chapter 3 provided more detail on the commencement and duration of behavioural patterns than most other literature, and whilst the study was not designed to investigate the relative effects of signalled and temporal predictability, results demonstrated the onset of anticipation prior to the arrival of keepers. The effects of temporal predictability were therefore demonstrated, separately to signalled predictability which may, in other literature, be a confound to interpretations of temporal predictability (Section 1.4.3).

In order to fully understand the relationship between feeding time and patterns of behaviour, and to investigate the effects of predictable and unpredictable routines, manipulation of feeding times was required. This was not feasible with the tigers, but could be accommodated within the husbandry routine of a group of meerkats. When fed according to predictable and unpredictable feeding routines, the meerkats demonstrated variability in behaviours over the course of the day, with no clear patterns related to the predictable feeding routine, and no clear differences in behaviour between the two feeding routines (Chapter 5). Predictable feeding therefore cannot be assumed to result in anticipatory behaviour, and there may be other environmental factors which affect an animal's response to predictability. In terms of captive animal management, it is important to try to identify these factors, and evaluate their influence on behaviour. In terms of the study of temporal predictability, research should identify and control for these factors in order to obtain a true representation of the effect of temporal predictability on animal behaviour. Two suggestions arose from the conclusions of Chapter 5: that the meerkats did not respond to the temporal predictability of the feed, but instead reacted to the signalled predictability of the arrival of the keepers; and that environmental variability including the potential of *ad hoc* feeds received from zoo visitors, affected meerkat behaviour such that clear patterns of anticipatory behaviour were not established.

In order to eliminate any effects of extraneous variables such as visitor feeds, it was necessary to investigate the effects of predictable and unpredictable routines under controlled conditions. *P. laticauda* day geckos were housed in an environment where visitor feeds were prevented, and environmental variables and feeding times could be controlled (Chapter 6). Under controlled conditions, *P. laticauda* showed changes in behaviour, such that increased locomotion, ‘movement on the spot’ (not significant), ‘stationary but moving the head’ and jumping behaviour occurred prior to predictable feeding times compared to unpredictable feeding times (Chapter 6). Whilst some individual variation was present (Sections 6.3 and 6.4), these changes in pre-feeding behaviour on a predictable feeding routine suggest an effect of feeding time on ‘active’ gecko behaviour, in anticipation of the feed. This is similar to the increased locomotion reported in red-eared turtles prior to light onset (Gopar-Canales, et al., 2010), however to the author’s knowledge, the current research represents the first report of a difference in reptile behaviour prior to predictable versus unpredictable feeding times.

The current research successfully investigated the effects of predictable feeding time on animal behaviour. However, these results could only be generalised with extreme caution due to the small number of individuals studied. Despite this, the current work has identified that an animal’s response to predictability may be dependent on the specific circumstances (Sections 1.4.3, 1.5 and 8.2), therefore there is value in analysing these small sample sizes.

This programme of research has explored effects of predictability on behaviour in a number of species, in an applied (zoo) setting. As in previous research (as discussed in Sections 1.4.2 and 2.2), changes in a range of behaviours prior to predictable feeding times were identified, with increases in locomotion and pacing in tigers, and patterns of increased locomotion, movement on the spot, moving the head and jumping in geckos. Previous research has not reached a consensus on what constitutes an anticipatory response and indeed it is likely that, as in the current research, several behaviours collectively show anticipatory changes. The study of predictability and anticipation would therefore be facilitated with the use of a compound measure of behaviour, which integrates the changes in several behaviours to provide an indicator of anticipation. In the current thesis, the measure of busyness was successful as one such measure, indeed the current programme of work developed a number of aspects of methodology, to benefit the study of predictable feeding routines, as discussed below.

### 8.1.2. Development of suitable methodology for the study of predictable feeding routines

The current programme of work has addressed some of the drawbacks of previous methodologies such as short study periods, insufficient lengths of observation across the day, and sampling only before and after feeding (as discussed in Section 2.2). In the current research, animals were exposed to the feeding routines for long periods (minimum 3 weeks) to ensure that behaviour changes were an effect of the routine itself rather than an effect of changing the routine, and behaviours were observed over long periods of time. Observation throughout the day, rather than in restricted sampling periods, has been a clear benefit of the current research, as it enabled the determination of detailed daily patterns of behaviour. For example, daily patterns in tiger locomotion and pacing were demonstrated, with increases which commenced over 2 hours prior to the feeding time, and before the arrival of the keepers (Chapter 3). Observation of meerkat behavioural patterns throughout the day enabled the conclusion that there was no clear pattern of any behavioural change prior to predictable feeding, in comparison to patterns in those behaviours at different times of the day that were unrelated to the feeding time (Chapter 5). Whilst some behaviours (such as 'all vigilance' behaviour) demonstrated slight increases in proportions in the time prior to the feeding time, increases were also observed some time after the feeding time (with a disruption in the pattern immediately after feeding) suggesting that any increase in behaviours prior to the feed, may be a part of a more general afternoon increase. Had pre-feeding behaviour been observed in isolation from other times of the day, a different interpretation may have been reached.

In Chapter 5, behavioural changes were also demonstrated immediately after the feeding time, as a consequence of the animals being fed. Had this study compared only pre- and post- feeding observation sessions (e.g., as discussed in Section 2.2) an interpretation of anticipatory behaviour may have been reached in error. Hence, observation of behaviour across the day in Chapter 5, enabled the conclusion that the meerkats showed no clear pattern of behaviour in anticipation of feeding, and raised awareness of other potential influencing factors (as discussed further in Section 8.2). Indeed this study supported an important finding; that it cannot be assumed that all situations of predictable feeding (as determined by human observers) will lead to anticipatory behaviour, despite the lack of literature reporting non-significant results.

Whilst observation throughout the day should be recommended, this may not be feasible in some circumstances, therefore suitable sampling periods should be chosen. In some previous research, data collection only sampled times such as the period immediately before and after predictable feeds, or the period immediately before predictable and unpredictable feeding times (e.g., Bloomsmith & Lambeth, 1995; Weller & Bennett, 2001). Whilst such studies have achieved interesting results, it should be noted that with such limited comparisons there is potential for other factors to affect behaviour, such as effects of the time of day. The methodology used in Chapter 6 therefore demonstrated a more rigorous way of comparing observation periods. Whilst in Chapter 6 it was not feasible to record gecko behaviour throughout the day, two different observation sessions (e.g., pre-feed and pre-random, or pre-set time) were used in each feeding condition (predictable and unpredictable) in order to control for any effects of the time of day or of more general effects across the conditions. Hence greater confidence was possible in the effect of predictable feeding.

The current programme of research has therefore addressed the issue of suitable observation lengths for investigating the effect of predictable feeding routines. Suitable sampling techniques were also addressed. For example, the use of one-zero sampling (as discussed in Sections 2.4 and 6.4.2) successfully enabled the determination of clear patterns of multiple tiger behaviours throughout the day (Chapter 3), that showed greater detail than previous work in zoo environments, such as detail on duration and start time of anticipatory behaviour. These patterns were similar to the patterns of data often achieved under laboratory conditions using short interval schedules (Lejeune & Wearden, 2006) (as described in Section 1.2). Similar patterns of data were achieved using the measure of busyness (as discussed below), and hence this programme of research has successfully demonstrated methods by which studies of predictability in zoo environments can produce similar results to laboratory studies, therefore opening up the topic of predictability for study in the ‘field’.

The other advantage of one-zero sampling, relevant to the study of anticipation, is the ability to record multiple behaviours during each sampling interval (Smith, 1985). This was demonstrated in Section 4.3 where a greater number of behaviours were observed in the video clips of tiger behaviour using one-zero than instantaneous sampling. Also, in Chapter 6, one-zero recording of gecko behaviour resulted in a greater number of records of each behaviour than instantaneous sampling, resulting in a record of the pattern of change of each behaviour, and clearer comparisons between predictable and unpredictable conditions.

Whilst the studies described above successfully minimised some of the drawbacks of previous research by using long study and observation lengths, these study lengths still required a high investment of time from a single, or small group of observers, and time was required for training these observers. Therefore, in addition to the traditional data collection methods used throughout this programme of work, investigation took place into the development of a simple, qualitative measure of behaviour that could be used by multiple observers, but required relatively little training. The measure of busyness was developed as one such technique, and was tested for reliability and validity, against traditional measures.

In an initial test of busyness (Chapter 3), results demonstrated good inter-observer reliability between pairs of observers who rated busyness, and patterns of busyness were found to reflect patterns of behaviour observed using traditional methods. Busyness scores were found to positively correlate with locomotion (both tigers) and pacing (female tiger only) and to negatively correlate with inactive alert and sleeping behaviours (both tigers). Hence busyness showed potential as a method to reliably record patterns of behaviour. However, it was necessary to further this testing of busyness, and specifically test the reliability, validity and utility of the measure, in laboratory studies (Chapter 4).

Under laboratory conditions using video footage of tiger behaviour, busyness scores reported by human participants repeatedly showed substantial reliability (Chapter 4). Reliability scores (ICC) of >.80 (consistency) and >.72 (absolute) (and a Kappa score of .75) were achieved in each test of reliability, demonstrating results higher than the recommended levels for good reliability (e.g., Chang, n.d.; Landis & Koch, 1977; Portney & Watkins, 2000), and substantially higher than some scores reported in the literature for previous reliability tests of other, qualitative measures (e.g., King & Figueredo, 1997; King & Landau, 2003; Weiss, et al., 2006). These results, and the reliability scores achieved in Chapter 3, gave evidence for substantial inter-observer reliability of the measure of busyness.

Busyness scores were found to positively correlate with those tiger behaviours related to activity and alertness, such as walking and running, and negatively correlate with inactive or sleeping behaviours (Section 4.3). Greater behavioural diversity was demonstrated in intermediate levels of busyness (2-4), and other behaviours such as social contact and investigation did not correlate significantly with busyness (Section 4.3). Different behaviours were found to be associated with each busyness score, and qualitative differences across the scale were reflected in increases in activity, speed, animation and alertness, as busyness increased (Section 4.3). Daily patterns of busyness were also found to match daily patterns of tiger behaviours (Section 4.5). Also, in a further 'field' test of busyness, patterns of busyness were also found to match daily patterns of meerkat behaviours (Chapter 5). Whilst no clear pattern of change in meerkat behaviour was demonstrated prior to predictable feeding using traditional measures (Chapter 5), ratings of busyness showed agreement, and likewise showed no clear change at the corresponding time of day. Hence it can be concluded that, based on multiple validation tests, busyness is associated with genuine patterns of behaviour and is a valid measure, reflecting both the occurrence of different behaviours associated with different busyness levels, and a continuum of qualitative aspects of behaviour, for example, activity and alertness.

As a measure which reflected patterns in several behaviours, busyness represented a useful 'compound' measure of behaviour (Section 4.6). Therefore busyness shows potential as an easy to use and reliable integrative measure of animal behaviour. However, by virtue of this, busyness is not a suitable measure to be used to obtain such detailed interpretation of results in terms of specific behaviours, as achieved for tiger behaviour in Chapter 3, where a range of behavioural patterns were observed, such as pacing, and enclosure use. However one of the main conclusions of this programme of study is that it is not always possible to carry out such detailed research over time, and busyness is a quick and simple way of getting data, which could then be used to identify areas for further, more detailed study, and could also define what time period needs to be observed.

Busyness is also suitable for use in monitoring patterns of behaviour; participants' scores of busyness from video of tiger behaviour revealed a temporal pattern of tiger busyness, despite the participants being blind to the chronological order of the video that they watched (Section 4.5). Not only does this finding support the applicability of busyness as a measure, but it also supports the results of Chapter 3; the feeding regime was the same in both studies, and participants in Section 4.5 recorded similar patterns of tiger busyness whilst being blind to occurrence of predictable feeding. Tiger busyness can therefore be confirmed (under the current study conditions) to increase in the lead up to a predictable feeding time, such that busyness is a suitable measure of anticipatory behaviour.

In order to develop busyness as a measure, it was necessary to investigate how the level of detail in the instructions given to users of the measure affected their performance. As busyness was designed to be a simple measure requiring little training, detailed definitions of each level of busyness were not given. However the results of Section 4.4 suggest that, as a minimum, example behaviours for the anchor points of the scale should be given to observers in order to improve ease of use of the measure and to encourage full use of the range of the scale, thus reducing left-hand bias (Friedman & Amoo, 1999; Friedman, et al., 1994). All instruction conditions (low, medium and high detail) resulted in data with good inter-observer reliability. However, it should be noted that there were differences in the mean scores recorded by participants in each condition, with greater instruction detail resulting in higher (overall) mean scores, through greater use of the higher end of the busyness scale. Indeed, participants reported fewer difficulties interpreting high busyness scores when given more detailed instructions. Few studies give detail on the instructions provided to observers when using qualitative measures, however the current research has demonstrated that instructions can influence the magnitude of the recorded ratings, and hence should form a careful consideration in study design.

Busyness was recorded using a 1 to 5 scale, however it would be interesting to test the effect of other scales (such as 1 to 3, or 1 to 10), to determine their suitability. It seems likely that other scales would not make much difference as busyness is a subjective measure and each level is not precisely defined. If the anchor points are sufficiently described then one would expect results to be proportionally spread across the difference scale sizes. Indeed, Bendig (1954) compared results of ratings of food preference using 2,3,5,7 and 9 category scales, and concluded that “test reliability [summed ratings for each rater] is independent of the number of scale categories, and that rater reliability is relatively constant” (p40). Other research has produced varying results, with different authors concluding that different scales can produce largely comparable (e.g., Dawes, 2008) or differing (e.g., Preston & Colman, 2000) results. More research is therefore needed on this topic, and on the effect of scale type on busyness ratings. It is possible that even if different scales do not vary in their results, they may vary in their ease of use. Indeed, some participants in Chapter 4, Study 3, in the high detail instruction condition commented that a greater number of scale points would make the measure easier to use. Also, different scales may be beneficial for different species. For example, busyness was not used in Chapter 6 due to the relatively static nature of gecko behaviour (and other reasons, as stated in Section 6.1.5); however a 1-3 scale could potentially have been of use. Therefore the type of scale of busyness may be worthy of further investigation.

The measure of busyness was recorded with substantial reliability and validity against traditional measures. Busyness therefore has the potential to be used by multiple observers and has the benefit of requiring only a small amount of training for use (Section 4.4). Aspects of this thesis have greatly benefitted from the use of multiple observers, and the current work has identified suitable tools to facilitate the use of multiple observers. Those studies that did not use multiple observers required high observation input, and gathering sufficient data was a challenge. For example, Chapter 6 used video footage of gecko behaviour, so multiple observers were not used, however this was a very labour-intensive methodology and long observation periods were not feasible. Therefore, Chapter 6, as with many other published reports of predictability, was restricted to sampling certain periods of the day, and detailed daily patterns of behaviour could not be determined. Whilst statistically significant results were achieved, findings may have benefitted (for example enabling clearer *post hoc* results) from a greater number of observations, and with the inclusion of more study animals.

Increased sample sizes may have also made previous literature more robust. For example, despite trends in this direction, Lyons et al. (1997) found no statistically significant difference (Willcoxon's Matched Pairs) between pre- and post-feed pacing in a sample of 7 felids. There is now scope to use qualitative measures, such as busyness, with multiple observers, in order to increase the number of observations and sample size, for example by monitoring animals in different enclosures or zoos. However, it should be noted that, as discussed in Section 2.4, descriptive analysis of data from individuals or small groups of animals is a valuable tool in the study of predictability, as certain elements of an animal's response to predictability may be specific to their situation (Sections 1.4.3, 1.5 and 8.2). However, long periods of observation are still required in order to obtain detailed patterns of behaviour, for which multiple-observer methods such as busyness, would be useful.

Multi-observer methods such as busyness have potential for use with very large numbers of observers, which leads on to a consideration of citizen science programmes (see Section 2.8). Indeed, the current research demonstrated the application of busyness and simple questions, to a citizen science study in which members of the public were successfully able to use a touch screen computer to record data on fish behaviour (Chapter 7). The touch screen computer used two qualitative research questions regarding busyness and enclosure use, plus two simple quality control measures. These simple questions were sufficient to obtain data from visitors which showed patterns of increasing busyness, and increasing use of the enclosure area near the feeder, in the time leading up to a predictable feeding time. This was consistent with anecdotal evidence of time-place learning in these fish, and with data collected by student observers. However, a greater overall length of observations throughout the day was achieved using the touch screen, compared to the student data, resulting in clearer behavioural patterns, and demonstrating a benefit of the citizen science approach.

The touch screen computer was very easy to use, and could be positioned right beside the enclosure, where the visitors could record live observations of fish behaviour. Once programmed, the touch screen required minimal investment of time from the researcher. Thus, this citizen science approach produced a high output of data, with minimal time input. The equipment was relatively inexpensive, and waterproof versions are available for outdoor exhibits. There is clear potential to use this technology in further predictability research, by setting up touch screen units at other enclosures. This could help to expand the number of species investigated, or increase the number of individuals studied. The high volume of data that could be obtained would address some of the problems of previous predictability studies (see Section 2.2), and (as mentioned above and in Section 7.4) would produce patterns of behaviour throughout the day.

The questions used on the touch screen were suitable for the current study, however there is now potential to develop, and validate a suite of template citizen science research questions which could be used at any zoo enclosure. For example, a series of basic questions could be developed, covering a range of topics such as busyness, enclosure use and enrichment use. These could then be adapted to the specific requirements of any research project and enclosure under investigation. The study of topics such as the effect of environmental enrichment can often use only restricted samples of animal behaviour. For example, Cummings et al. (2007), and Powell (1995) investigated the effect of environmental enrichment on maned wolves (*Chrysocyon brachyurus*) and lions (respectively) using only one or two daily 30-minute observation sessions over a limited number of days. It would seem sensible to suggest that such study would benefit from observations over an extended period of time, indeed Cummings et al. (2007) made this recommendation when evaluating their study. Large volumes of data, over extended periods could be achieved using citizen science, and hence this could be of benefit to a range of animal behaviour topics.

The questions posed to visitors could be employed, not only using a touch screen, but using other technology such as webcams and smart phones. Use of such technology would make the research project accessible to an even greater number of observers. In this way, citizen science has the potential to make use of visitor observations to answer a wide range of questions related to animal behaviour. Visitors could capture behavioural observations that research or keeping staff may not have time to accomplish, thus increasing the volume of data, or highlighting specific areas for further study. It is hoped that the approach outlined in the current thesis will act as a foundation for the development and validation of methods that allow the collection and filtering of large amounts of data, on a range of topic areas in animal behaviour research.

In addition to the benefits of citizen science to research, another aim of citizen science is to benefit the volunteer who is taking part (as discussed in Section

7.4). An important question is whether the experience of being involved in behavioural research has an effect on the visitor that affects their knowledge or attitudes towards the subject animals, provides a deeper understanding of the scientific issues being addressed, or in some other way produces positive effects. Therefore, alongside animal behaviour data collection, there is scope to obtain conservation psychology data regarding the visitors' experience of research participation, and potential effects on their enjoyment and attitudes towards animals, zoos and conservation.

## 8.2. Theoretical issues

The research conducted for this thesis has shown some varied results in terms of a response (or lack of) to predictable feeding times. It is therefore likely that there are factors other than the feeding time which may affect an animal's response to predictability. Such factors may be exogenous or endogenous. For example, in Chapter 5 it was suggested that additional *ad lib* feeding from visitors may have been a factor which influenced meerkat response to the keeper-determined predictable feed. Factors such as this could be termed as 'environmental complexity'. In laboratory studies, animals are likely to be in less complex environments compared to zoo exhibits, hence anticipation of a predictable event may be more 'important' to them. A more complex captive environment may provide more opportunities for control or for successful appetitive behaviour. For example in Chapter 5, the meerkats were able to forage in their enclosure for any interloper insects, or had the opportunity to receive food from visitors, so may have been either satiated, or sufficiently occupied such that anticipation of the predictable feeds was not necessary, and they could adopt a more reactive strategy. Bassett and Buchanan-Smith (2007) describe how predictability and control are linked, and suggest that if providing control is not possible, then predictability may be important to animals. Therefore it may be reasonable to suggest, that if control is possible, then the relative importance of predictability may be decreased, which may explain the lack of a clear anticipatory response in Chapter 5.

The tigers in Chapter 3 were never fed in their enclosure, and hence had no opportunity for successful foraging behaviour during the day. Feeding was therefore an important daily event, for which they may have been highly motivated, and so patterns of anticipatory behaviour were demonstrated. In Jenny and Schmid's (2002) study in which tigers were fed at unpredictable times from automated feeding boxes, the tigers' control over feeding was increased since it was necessary for them to open the boxes themselves (Sections 1.4.2 and 1.4.3). This may have been a reason for the tigers' change in behaviour as, with the addition of control, they were no longer reliant on predictability. However in Jenny and Schmid's study, feeding times were also made unpredictable, hence predictability and controllability could not be separated, and further work would be necessary to determine the relative effects of each.

Another factor which may affect an animal's response to predictability is their motivation for feeding. This may affect the likelihood to anticipate food in relation to their level of hunger, or interest in the feed, (which may in turn be affected by opportunities for appetitive behaviour or other feeds, as mentioned above). For example, hunger created by reduced caloric density of a restricted feed led to greater activity in anticipation of feeding time in mice (Holmes & Mistlberger, 2000). Also, smaller meal size resulted in a greater likelihood of anticipatory behaviour in greenback flounder (Purser & Chen, 2001). In terms of the attractiveness of the feed, mice have been reported to show anticipatory locomotor activity to only certain types of palatable food reward on a predictable daily schedule, when *ad lib* food is also available (Hsu et al., 2010). Also, when given rewards on a fixed interval schedule (5 s), pigeons commenced anticipatory key pecking earlier in anticipation of a higher, than lower reward magnitude (longer duration of access to food) (Ludvig et al., 2011).

Another factor that can affect an animal's response to temporal predictability is that of signalled predictability. In Chapter 3 it was demonstrated that increases in proportions of tiger behaviours and busyness in the lead up to the feeding time commenced in advance of the arrival of the keepers to the enclosure, hence anticipation of the temporal event can be concluded. This is a clearer result than that often achieved in the literature, where signalled predictability can confound the effect of temporal predictability, and the reported analysis often does not separate behaviour prior to and after feeding cues (as discussed in Section 1.4.3). For example, in their studies, Altman et al. (2005) suggested that lion anticipatory pacing was cued by the arrival of the keepers, and this was not separated from the effects of temporal predictability, and Peters et al. (2012) do not report horse anticipatory behaviour prior to the start of feeding cues. The length of each day's observation in the current research allowed examination of behaviour prior to the arrival of keepers (assumed to be a feeding cue). The resulting data demonstrated anticipatory behaviour in tigers, but not clearly in meerkats. However, results of the meerkat observations demonstrated that some patterns in meerkat behaviour were no longer shown when data in which keepers were present were removed from the analysis, suggesting that the meerkats may react to the signalled predictability of the arrival of the keepers, rather than the temporal predictability. The tigers also responded to the arrival of the keepers (in addition to temporal predictability), with further changes in enclosure use and pacing behaviour. In order to determine the effect of temporally predictable feeding, future work must control for signalled predictability, or observations should extend prior to the commencement of any signals.

Bassett and Buchanan-Smith (2007) recommend using a unique, reliable signal to announce feeding, and that this would facilitate feeding at unpredictable times and reduce any frustrating effect of unreliable signals, such as keeper presence at non-feeding times. In terms of the tiger results, providing a unique signal would not be necessary, as the tigers responded to the temporal predictability before a signal would become useful. In terms of the meerkat results, this may also not be necessary as, if unreliable signals were a problem it may be expected that, if the keeper arrival signal was associated with a predictable feeding time, the signal would become reliable once again and the meerkats would anticipate the signal at this time. That this was not the case suggests that an additional signal may be unnecessary. Before making recommendations on the suitability of providing signals for predictable or unpredictable routines, it seems necessary for further research to separate the effects of each, and to determine whether animals prioritise one type of predictability over the other. It should also be noted that some kind of feeding signal will likely always be present for zoo animals, and this may not always be identifiable to the human observer.

Another issue of interest is the question of how irregular an event needs to be in order to be perceived as unpredictable to the animals. As discussed in Section 2.2, previous literature has varied in timings that the authors classify as ‘unpredictable’, with researchers varying feeds by up to 36 hours, or only +/- 1 hour. In the current research, feeding was made as unpredictable as possible, by random assignment of feeding times within a working day or afternoon (geckos and meerkats respectively). Even with this random assignment of feeding times, food was still provided within a portion of the day and was not unpredictable over 24 (or more) hours. It could therefore be argued that food may be predicted within that portion of the day. Future research would benefit by investigating the effects of providing feeds at random intervals over a greater or lesser time period. Indeed, if unpredictable feeding is desired for management, it would be beneficial to establish the minimum time period over which random feeds could occur, and still be considered unpredictable.

Whilst welfare conclusions cannot be drawn based on the varied results and small sample size of this programme of research (indeed the current research was not designed for this purpose), the effect of predictability on welfare is an important consideration. The effects of predictable or unpredictable routines on animal welfare may be site-specific, due to the nature of other factors affecting the animal’s behavioural response. It is necessary for further research to be carried out on a range of species, in order to draw together a meta-analysis of the effects of predictability on animal behaviour and welfare. The occurrence of daily behavioural patterns raises important questions about the implications of such patterns alongside other circadian patterns, and whether they are natural or adaptive, or whether they are caused, or exacerbated by captivity and human routine, and the implications of this. Anticipatory behaviours should be considered in the context of whether they occupy a large part of the day, and might be considered to be stereotypic or abnormal (see Section 1.3). In Chapter 3, an increase in tiger pacing; an often cited welfare indicator under some circumstances (Mench & Mason, 1997), was identified in the time prior to feeding, and further research would be necessary to determine the welfare implications of such pacing in these, and other felids. For example, it could be questioned whether there is a difference between anticipatory and stereotypic pacing, and at what point such a distinction could be made (e.g., Watters, 2012).

Miller et al. (1983) reported that electric shocks that were predictable (signalled predictability) and able to be anticipated, were more aversive to rats than unpredictable shocks (as described in Section 1.4.1), and similarly it has been suggested that anticipation of positive appetitive events can make these events more rewarding to the animals. For example, research (largely related to signalled predictability) has demonstrated that anticipation of an appetitive event can have rewarding effects beyond those of the event alone (e.g., de Jonge, Tilly et al., 2008; Dudink et al., 2006; Spruijt et al., 2001). However, in order to support such a welfare conclusion in terms of routinely predictable daily feeds, such claims require more research, and it should be clearly demonstrated whether anticipation of an event indicates the rewarding nature of that event, or whether this is an indication of frustration, or whether such effects can change over time. Abbot et al. (1984) suggested that over long periods, predictable, rather than unpredictable shocks may be more stressful, and Jenny and Schmidt (2002) suggested that stereotypic pacing in tigers was due to frustrated appetitive behaviour (although the authors do not report whether this behaviour was observed in the pre-feeding period). In the absence of control over an event, the potential for frustration must be considered, as should the potential cumulative effect of rewarding (with the feed) and hence reinforcing food anticipatory behaviour.

## 8.3. Recommendations and future work

Throughout this thesis, several suggestions have been made for future research in order to further develop the findings of the current programme of research. These suggestions and others will be summarised below.

Perhaps the main recommendation for further research is the need to increase the number of studies of predictability (including anticipatory behaviour and the effect of predictable, compared to unpredictable routines) on a range of species, in order that a meta-analysis could be conducted to determine the effects on behaviour and potentially, welfare. In particular, further research is necessary on gecko and reptile timing ability, and effects of predictability, as there is little literature available on this subject, but the current research suggests implications for reptile behaviour. The limited work on these topics may be due to the difficulties in collecting suitable data (Section 2.2); however the methods developed in this thesis would provide a suitable approach for such future research.

It seems sensible to suggest that species' natural history and factors such as their feeding ecology should be considered in an animal's response to predicable feeding in captive environments. This could help to guide decisions on the relative importance of behavioural results and consequent management practise. Indeed the further study and interpretation of predictability in zoo environments is of importance in order to guide management decisions. For example: knowledge of patterns of behaviour such as start and peak times of anticipatory behaviour, and the relationship with daily routines; knowledge of the influence of external cues or other factors on daily patterns of behaviour; and any welfare implications of the effects of routines. Such research may also clarify whether observed animal behaviour agrees with staff assumptions of the effect of routines, as staff may only see the behaviour of the animals when their presence may have acted as a cue for particular behaviours. The above research should then form an important part of husbandry recommendations (evidence-based husbandry).

Future research should investigate the effect of different factors on an animal's response to predictability, such as the environment (including environmental complexity, opportunities for additional feeding or appetitive behaviour, and zoo visitors) and cues or signals. The effect of temporal and signalled predictability should be separated in order to determine their individual effects and importance, as well as their combined effects, or over-riding influence of a particular type of predictability.

To extend the topics of this thesis, research should investigate the extent of feed timing which can be considered to be unpredictable. That is, how regularly can an event occur and be considered to be unpredictable (see Section 8.2). There is also scope to develop predictability research in zoo environments in terms of the effects of other predictable events (such as cleaning or enrichment provision) on patterns of behaviour. The study of daily behaviour patterns may also provide new direction for environmental enrichment research in order to determine optimum times of day to provide enrichment, for example to potentially mitigate any negative effects of predictable feeding routines. Even aside from these temporal properties, the methodologies developed in the current research may be of benefit to the study of environmental enrichment, and indeed other topics, where behavioural observations over an extended period of time would be of benefit. The qualitative (e.g. busyness) and citizen science methods outlined in the current thesis should be developed in a variety of future research projects, to collect data over long periods, in order to determine the full extent of any patterns of behavioural change.

Future work would be beneficial to establish the extent to which simple, subjective measures such as busyness would be appropriate in settings other than those investigated in the current research, for example using different species or sizes of groups, mixed species exhibits, or occasions where different animals in a group show very different behaviours (Section 4.6). Nevertheless, the present work has shown busyness to be useful across a remarkable range of applications, from the study of a pair of tigers (even when the two tigers were behaving differently), to hundreds of fish in a mixed-species tank. As discussed in Section 8.1.2, investigation of the number of scale points used to rate busyness would now be beneficial in order to develop the most useful, easy to use, and reliable design for the measure.

As a result of the current programme of work, a number of recommendations can be made for implementation in future studies of predictability:

1) Studies of predictability should aim for long study durations, giving time for substantial daily repeat observations and for the animals to become accustomed to the routine.

2) Attempts should be made to identify, and control for any potential confounding factors, such as feeding cues. Alternatively, observations should include periods

before, as well as after the commencement of such cues.
3) Observations should cover as much of the day as possible in order to determine daily patterns of behaviour, thus enabling the elucidation of the true extent of pre-feeding patterns. Where observation over a whole day is not possible, a minimum of two observation periods should be conducted each day; immediately prior to feeding and at a random time of the day (if feeding according to a predictable routine) or immediately prior to feeding and at a set time of day equivalent to predictable feeding times (if feeding according to an unpredictable routine), as shown in Chapter 6.
4) If long periods of observation are not possible by a single, or small group of observers, qualitative methods such as busyness should be employed with a larger group of volunteer observers. Such qualitative investigation could be used instead of or alongside individual observation, or as preliminary work in order to identify suitable times of day for more detailed observations.

## 8.4. Overall conclusions

To summarise, the current programme of work has demonstrated the effects of temporally predictable feeding in zoo-housed individuals of three species, and has produced evidence of time-place learning in fish held in a large, public aquarium. It has demonstrated that anticipatory behaviour is not always clearly apparent, even when feeding times are predictable, suggesting that exogenous and endogenous factors can influence the behavioural response.

The literature on predictability is subject to a number of limitations, and there are major challenges to research in the area. The current work developed suitable techniques for overcoming some of these challenges. One such technique is the measure of busyness which was shown to be reliable and valid. The current research has demonstrated the process of development of suitable qualitative measures, and has shown how rigorous testing can provide confidence in the data obtained using a subjective measure. Research using aquarium visitors demonstrated that there is potential to use busyness and other qualitative or simple measures in citizen science projects, in which members of the public contribute to a large dataset.

Predictable and unpredictable feeding times can have an effect on animal behaviour, such that it anticipates the feeding time. There is a lack of consensus in the literature on the effects of such routines, and the current research has gone some way towards furthering knowledge on this subject. Methodological constraints in the study of predictability limited the current development of the topic, however it is believed that the methods developed in this thesis will be of help to overcome some of the challenges to work in this field. Future work can therefore increase the study of predictability on a range of species, in order to fully develop our understanding of animal responses to predictable routines, and indeed the long term effects of a range of factors related to the captive environment. This knowledge could then inform and benefit husbandry decisions.

# Appendices

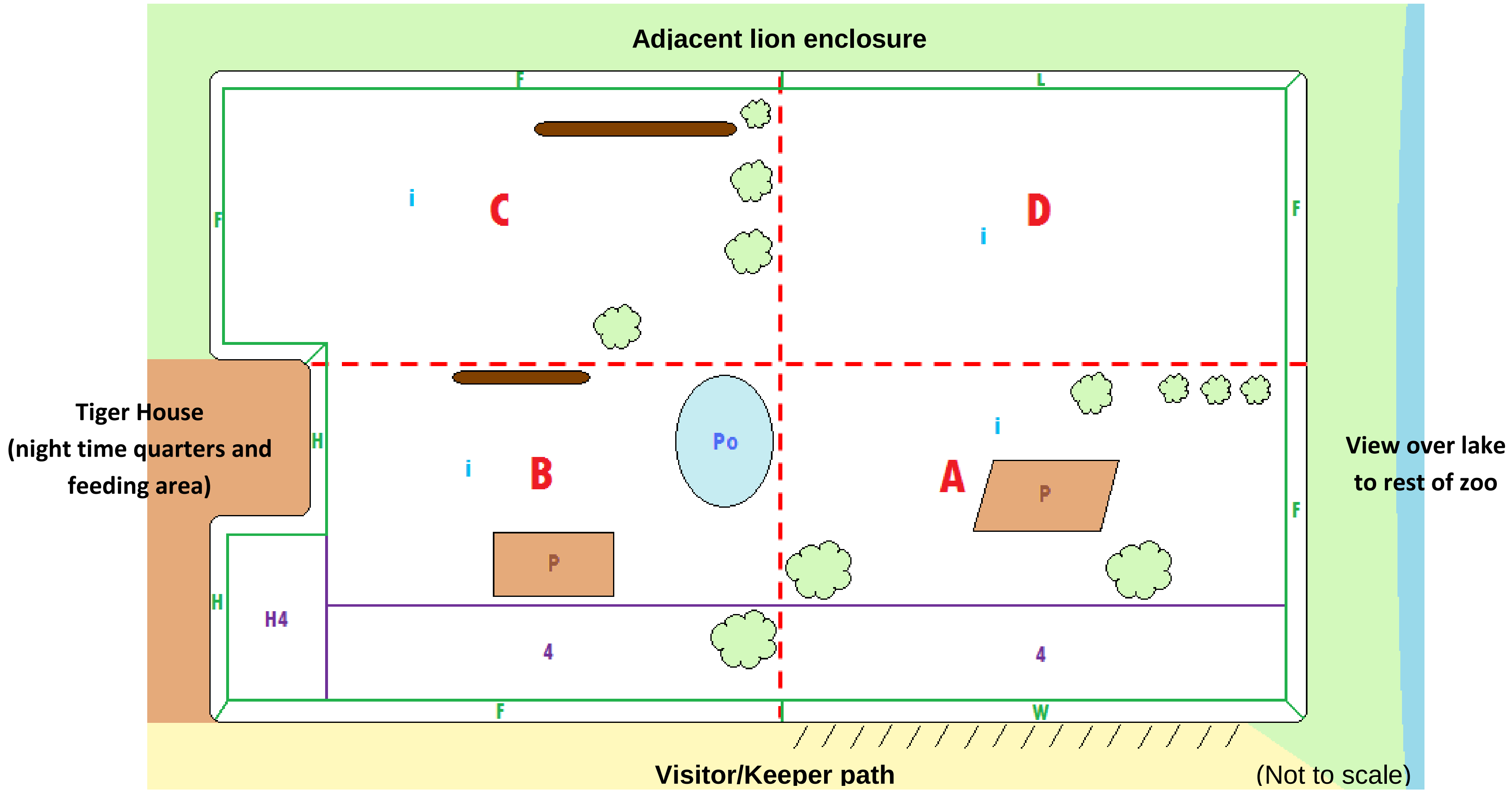
## Appendix 2.1.

*Details of Web of Science search for methodology review.*

| Search number | Search Terms | Search Field* | Refinements |
|---|---|---|---|
| 1 | Predictability OR Predictable | Title | Agricultural Dairy Animal Science, Agriculture Multidisciplinary, Behavioural Sciences, Biology, Fisheries, Marine Freshwater Biology, Psychology Applied, Psychology Biological, Psychology Multidisciplinary, Psychology Experimental, Psychology, Zoology |
| 2 | Unpredictability OR Unpredictable | | |
| 3 | “Feeding time” OR “Feed time” | | |
| 4 | Predictability | Topic | |
| 5 | Temporal Predictability | | |
| 6 | Feeding Schedule | | |
| 7 | “Food anticipatory activity” OR “Food anticipatory behaviour” OR “Food anticipatory behavior” | | |
| 8 | “Feeding regime” OR “Feeding regimes” | | |

*Where possible, searches were carried out for key words in the ‘Topic’ search field, however these key words covered a very large number of research areas across many different scientific disciplines and it was therefore necessary to restrict certain searches to within the ‘Title’ search field. However, as an important keyword for this search it was deemed important to search for “Predictability” within ‘Topic’ as well as in the ‘Title’ to avoid missing relevant studies.

## **Appendix 3.1:** *Enclosure Map*



**A B C D:** Quarters of enclosure

**F:** Area up to 1m from enclosure fence

**W**: Area up to 1m from visitor viewing window

**L**: Area up to 1m from fence next to lion enclosure

**H**: Area up to 1m from fence next to tiger house

**4**: Area 1-4m from fence in areas A and B

**H4**: Area 1-4m from tiger house

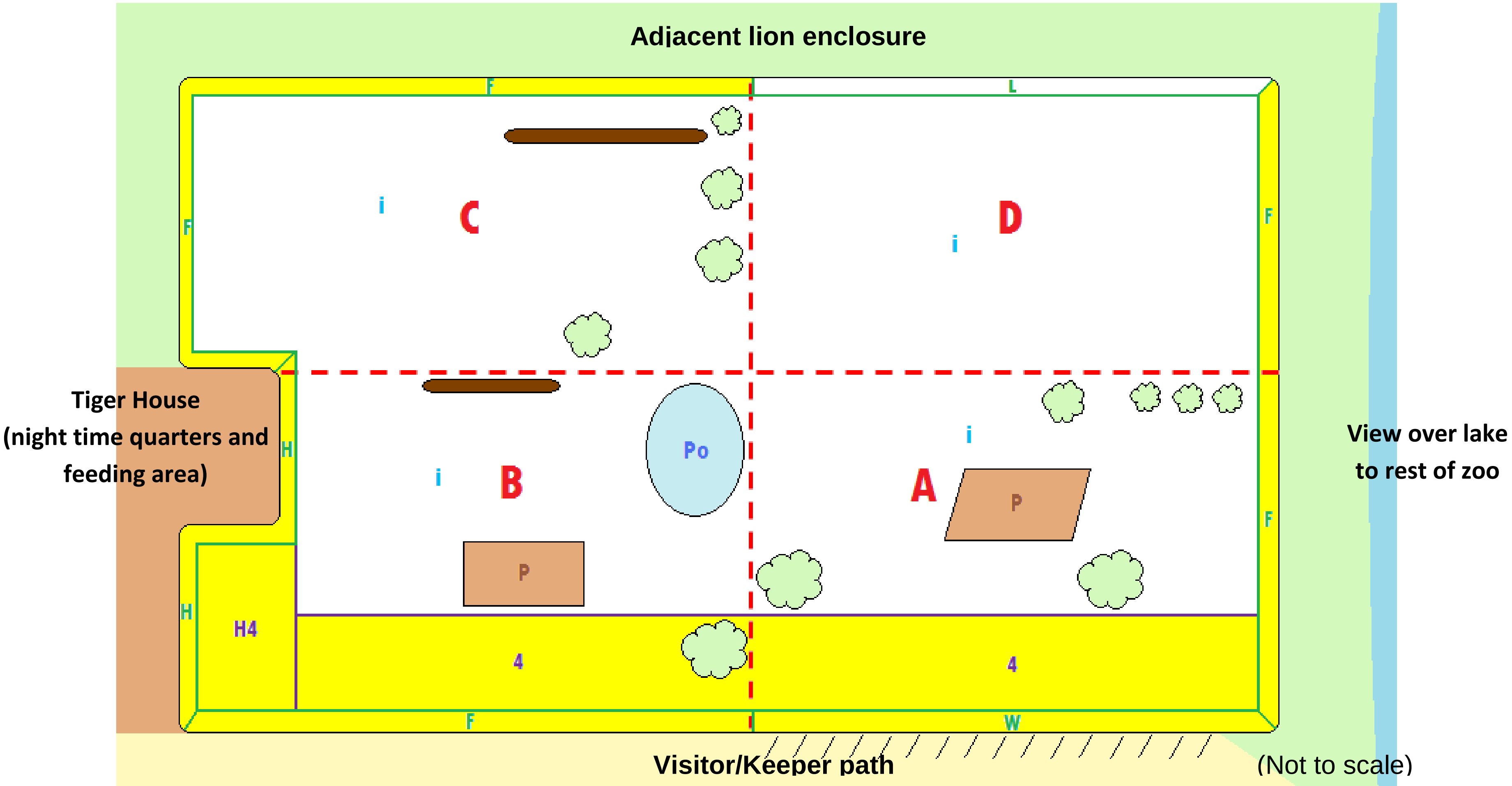
i: Inner area of grass

**Po**: Pond

**P**: Platform

⁄ ⁄ : Visitor viewing window

*Appendix 3.1.1: Map of tiger enclosure showing the enclosure quarters and resource areas.*



**A B C D**: Quarters of enclosure
**F:** Area up to 1m from enclosure fence
**W**: Area up to 1m from visitor viewing window
**L**: Area up to 1m from fence next to lion enclosure

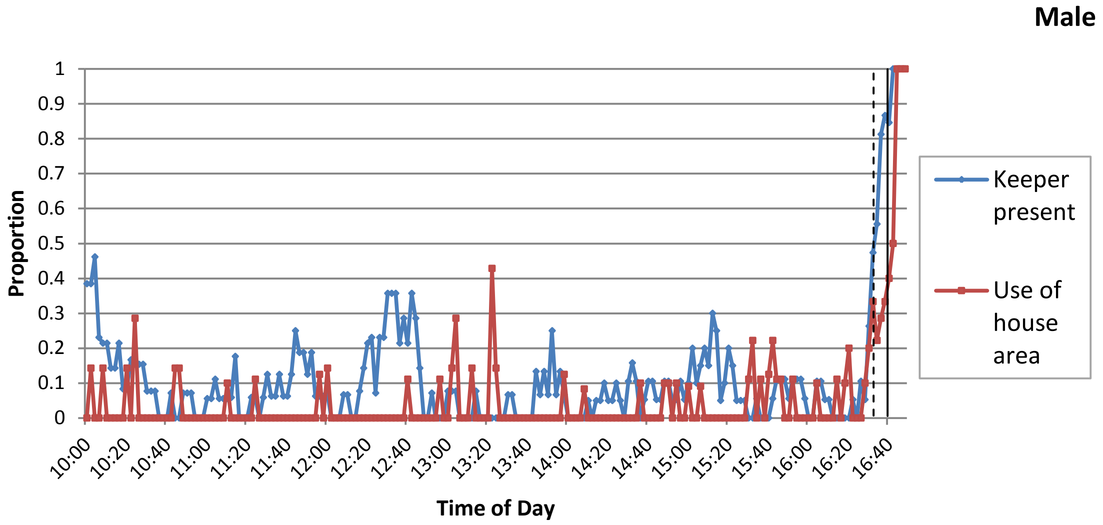
**H**: Area up to 1m from fence next to tiger house
**4**: Area 1-4m from fence in areas A and B
**H4**: Area 1-4m from tiger house
i: Inner area of grass

**Po**: Pond
**P**: Platform
/ / : Visitor viewing window
🟨 : 4m strip of interest

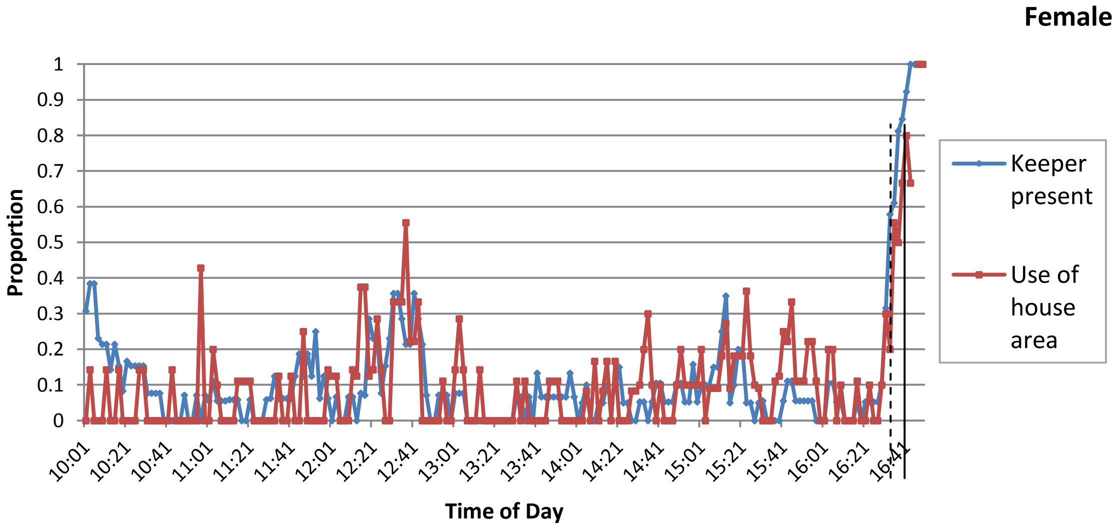
*Appendix 3.1.2: Map of tiger enclosure highlighting the 4m strip running down the fence in areas A and B, which was of particular interest in the analysis. (N.B. due to the nature of the enclosure areas this also includes some other areas of the fence, as highlighted)*

# Appendix 3.2

*Graphs of keeper presence and tiger use of the area up to 4m in front of the indoor quarters, demonstrating an increase in tiger use of this area, on arrival of the keepers at the end of the day.*



*Appendix 3.2.1: Graph to show the proportion of all repeats of each minute of the day in which the male tiger was observed in the area up to 4m in front of the indoor quarters (house area) with corresponding proportions of all repeats of each minute of the day in which keepers were recorded as present at the enclosure. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*



*Appendix 3.2.2: Graph to show the proportion of all repeats of each minute of the day in which the female tiger was observed in the area up to 4m in front of the indoor quarters (house area) with corresponding proportions of all repeats of each minute of the day in which keepers were recorded as present at the enclosure. The solid vertical line shows the mean feeding time, the dashed line represents the mean keeper arrival time (max. n for a data point = 20)*

# Appendix 4.1.

*Instruction sheets given to participants for Development of Busyness Study 1.*

## Testing methods of animal behaviour data collection

Do you have any experience of collecting animal behaviour data? **YES / NO** (please circle)

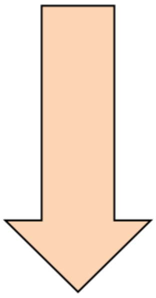
If yes, please give details ………………………………………………………………………..

In this study you will watch a 20 minute video of tigers in their enclosure in a zoo. This video is composed of a series of minute long clips of the tigers which will be played consecutively. Please watch the video, and after each minute clip write down a rating for the tigers' behaviour for that minute in the attached chart (the rating scale will be explained shortly).

You will hear a chiming sound each minute. This will be your cue to rate the behaviour that you have observed for the minute that you have just watched. As the video will continue straight on to the next minute, you will need to return to watching the video as soon as you have written down your rating for that minute. Please consider each minute independently.

**Rating Scale:**

After each minute of video you need to write down how 'busy' you think the tiger enclosure was **over the whole minute of the clip**. This will be your subjective opinion of how much you think is going on in the enclosure. For example, you might like to think about what the tigers are doing and how many of them are doing it. Please give your rating on a scale of 1-5, where 1 is the lowest level of busyness that the enclosure could be (for example with all tigers asleep), and where 5 is the highest level of busyness that the enclosure could be (for example with all tigers very animated and active). Please see the chart below for further advice:



Rating

1 – All or most tigers very quiet e.g., sleeping or inactive.

2

3

4

5 – All or most tigers very busy e.g., highest level of locomotion or activity.

**N.B. There may be a different number of tigers present in each minute of the clips but please consider each minute separately and only rate the behaviour of the tigers that you see in each minute.**

**To summarise**: Please watch the video clips and each time you hear the chiming sound write down your rating of how busy the enclosure was for the minute directly preceding the chime, then, straight away, watch the video again and repeat this process until you reach the end of the video clip. Repeat this process for all of the video clips.

Please tick here to indicate that you have read, and understand these instructions: ☐

If you have any questions please ask the lead researcher, if not, please turn to the next page of this booklet.

# Appendix 4.2.

*Instruction sheets given to participants for Development of Busyness Study 2.*

## Animal Behaviour Methods

Do you have any experience of collecting animal behaviour data? **YES / NO** (please circle)

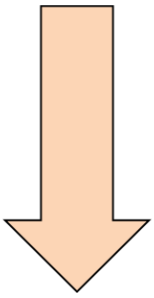
If yes, please give details ………………………………………………………………………..

In this study you will watch a 45 minute video of 2 tigers in their enclosure in a zoo. This video is composed of a series of minute long clips of the tigers which will be played consecutively. Please watch the video, and after each minute clip write down a rating for the tigers' behaviour for that minute in the attached chart (the rating scale will be explained shortly). There will be a practise at the start and a short break, half way through the session where you will be able to pause the video for a few minutes.

You will hear a chiming sound each minute. This marks the end of each minute clip and will be your cue to rate the behaviour that you have observed for the minute that you have just watched. As the video will continue straight on to the next minute, you will need to return to watching the video as soon as you have written down your rating for that minute. This video is made up of a series of different clips, some filmed in continuous sequence, and some edited together from different clips. Please consider each minute independently.

**Rating Scale:**

After each minute of video you need to write down how 'busy' you think the tiger enclosure was **over the whole minute of the clip**. This will be your subjective opinion of how much you think is going on in the enclosure. For example, you might like to think about what the tigers are doing and how many of them are doing it. Please give your rating on a scale of 1-5, where 1 is the lowest level of busyness that the enclosure could be (for example with all tigers asleep), and where 5 is the highest level of busyness that the enclosure could be (for example with all tigers very animated and active). Please see the chart below for further advice:



Rating

1 – All or most tigers very quiet e.g., sleeping or inactive.

2

3

4

5 – All or most tigers very busy e.g., highest level of locomotion or activity.

**N.B. Please consider each minute separately, and please only use the numbers 1,2,3,4 or 5 (i.e. don't use 2.5 etc.)**

**To summarise**: Please watch the video clips and each time you hear the chiming sound write down your rating of how busy the enclosure was for the minute directly before the chime, then, straight away, watch the video again and repeat this process until you reach the end of the video.

Please tick here to indicate that you have read, and understand these instructions: ☐

# Appendix 4.3.

*Instruction sheets given to participants for Development of Busyness Study 3.*

## Low detail instructions

Do you have any experience of collecting animal behaviour data? **YES / NO** (please circle)

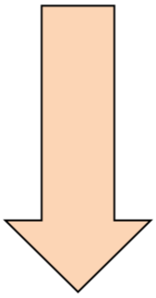
If yes, please give details …………………………………………………………………………..

In this study you will watch a 25 minute video of 2 tigers in their enclosure in a zoo. This video is composed of a series of minute long clips of the tigers which will be played consecutively. Please watch the video, and after each minute clip write down a rating for the tigers' behaviour for that minute in the attached chart (the rating scale will be explained shortly).

You will hear a chiming sound each minute. This marks the end of each minute clip and will be your cue to rate the behaviour that you have observed for the minute that you have just watched. As the video will continue straight on to the next minute, you will need to return to watching the video as soon as you have written down your rating for that minute. This video is made up of a series of different clips, please consider each minute independently.

**Rating Scale:**

After each minute of video you need to write down how 'busy' you think the tiger enclosure was **over the whole minute of the clip**. This will be your subjective opinion of how much you think is going on in the enclosure. For example, you might like to think about what the tigers are doing and how many of them are doing it. Please give your rating on a scale of 1-5, where 1 is the lowest level of busyness that the animals could show, and where 5 is the highest level of busyness that the animals could show. Please see the chart below for further advice:



Rating

1 – Lowest level of busyness

2

3

4

5 – Highest level of busyness

**N.B. Please consider each minute separately, and please only use the numbers 1,2,3,4 or 5 (i.e. don't use 2.5 etc.)**

**To summarise**: Please watch the video clips and each time you hear the chiming sound write down your rating of how busy the enclosure was for the minute directly before the chime, then, straight away, watch the video again and repeat this process until you reach the end of the video.

Please tick here to indicate that you have read, and understand these instructions: ☐

If you have any questions please ask the lead researcher, if not, please turn to the next page of this booklet.

## Medium and high detail instructions (high detail also included video examples for busyness levels 1 and 5)

Do you have any experience of collecting animal behaviour data? **YES / NO** (please circle)

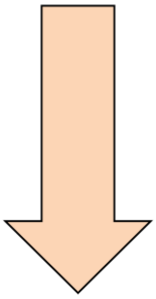
If yes, please give details ………………………………………………………………………..

In this study you will watch a 25 minute video of 2 tigers in their enclosure in a zoo. This video is composed of a series of minute long clips of the tigers which will be played consecutively. Please watch the video, and after each minute clip write down a rating for the tigers' behaviour for that minute in the attached chart (the rating scale will be explained shortly).

You will hear a chiming sound each minute. This marks the end of each minute clip and will be your cue to rate the behaviour that you have observed for the minute that you have just watched. As the video will continue straight on to the next minute, you will need to return to watching the video as soon as you have written down your rating for that minute. This video is made up of a series of different clips, please consider each minute independently.

### Rating Scale:

After each minute of video you need to write down how 'busy' you think the tiger enclosure was **over the whole minute of the clip**. This will be your subjective opinion of how much you think is going on in the enclosure. For example, you might like to think about what the tigers are doing and how many of them are doing it. Please give your rating on a scale of 1-5, where 1 is the lowest level of busyness that the animals could show (for example all tigers might be asleep or inactive), and where 5 is the highest level of busyness that the animals could show (for example all tigers might be running or highly active). Please see the chart below for further advice:



Rating

1 – All or most tigers very quiet e.g., sleeping or inactive.

2

3

4

5 – All or most tigers very busy e.g., running or active.

**N.B. Please consider each minute separately, and please only use the numbers 1,2,3,4 or 5 (i.e. don't use 2.5 etc.)**

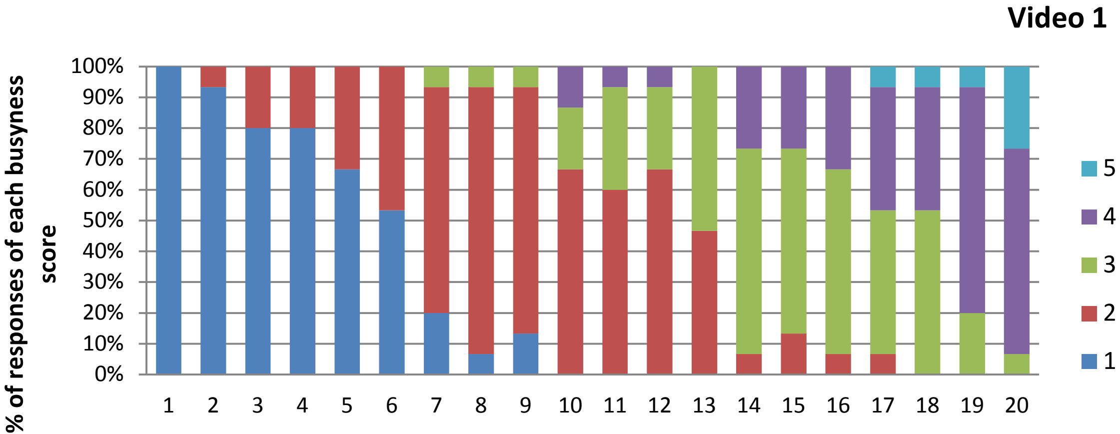
**To summarise**: Please watch the video clips and each time you hear the chiming sound write down your rating of how busy the enclosure was for the minute directly before the chime, then, straight away, watch the video again and repeat this process until you reach the end of the video.

Please tick here to indicate that you have read, and understand these instructions: ☐

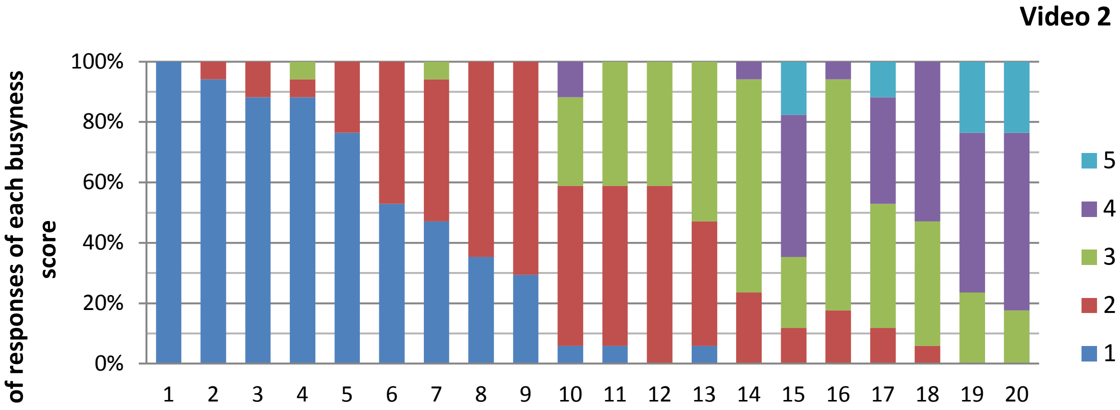
If you have any questions please ask the lead researcher, if not, please turn to the next page of this booklet.

# Appendix 4.4.

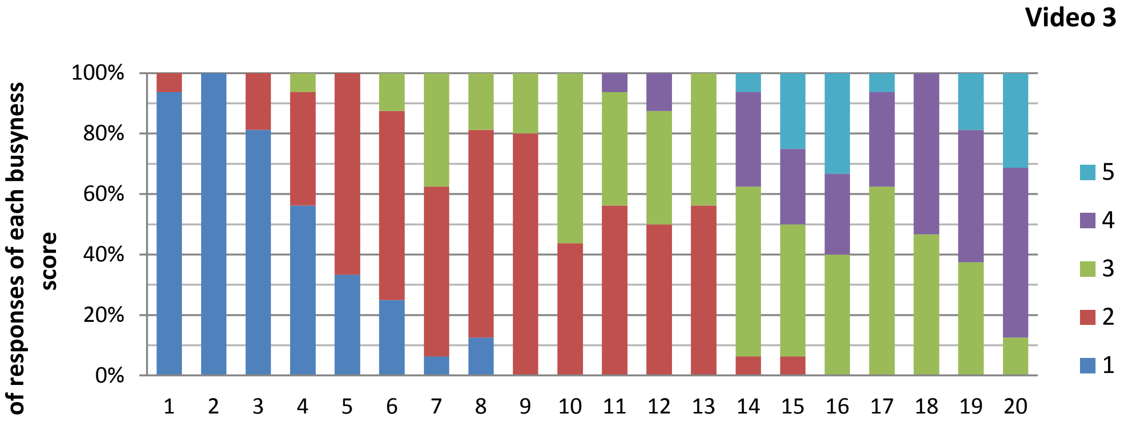
*Graphs to show the participants scores of busyness for each minute video clip, for each video order for Development of Busyness Study 1(Section 4.2).*



*Appendix 4.4.1: Percentage of participants who watched* ***Video order 1*** *rating each video clip at each level of busyness. Colours represent the different busyness levels.*



*Appendix 4.4.2: Percentage of participants who watched* ***Video order 2*** *rating each video clip at each level of busyness. Colours represent the different busyness levels.*



*Appendix 4.4.3: Percentage of participants who watched* ***Video order 3*** *rating each video clip at each level of busyness. Colours represent the different busyness levels.*

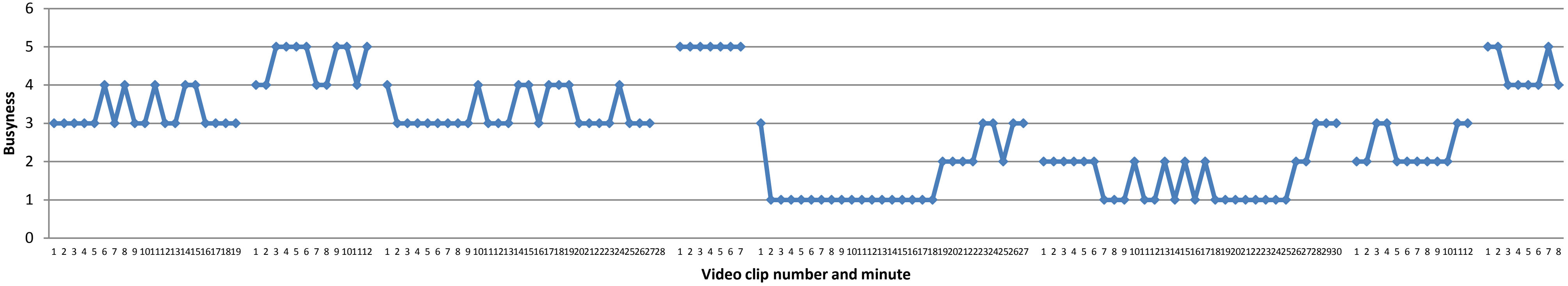
# Appendix 4.5.

*Spearman's rank correlations of experimenter's busyness scores with those of the individual participants, for Development of Busyness Study 1 (Section 4.2).*

| Rater | Mean busyness score over all clips | Correlation with experimenter (Spearman's *r*) | *p*-value |
|---|---|---|---|
| Experimenter | 3.00 | n/a | n/a |
| Participant 1 | 2.00 | 0.85 | <.01 |
| Participant 2 | 2.30 | 0.88 | <.01 |
| Participant 3 | 2.10 | 0.91 | <.01 |
| Participant 4 | 2.10 | 0.88 | <.01 |
| Participant 5 | 2.25 | 0.85 | <.01 |
| Participant 6 | 2.70 | 0.92 | <.01 |
| Participant 7 | 2.30 | 0.91 | <.01 |
| Participant 8 | 2.35 | 0.83 | <.01 |
| Participant 9 | 2.15 | 0.89 | <.01 |
| Participant 10 | 2.25 | 0.92 | <.01 |
| Participant 11 | 2.80 | 0.89 | <.01 |
| Participant 12 | 2.35 | 0.90 | <.01 |
| Participant 13 | 2.65 | 0.85 | <.01 |
| Participant 14 | 2.95 | 0.82 | <.01 |
| Participant 15 | 2.45 | 0.88 | <.01 |
| Participant 16 | 2.50 | 0.90 | <.01 |
| Participant 17 | 2.30 | 0.88 | <.01 |
| Participant 18 | 2.15 | 0.88 | <.01 |
| Participant 19 | 1.60 | 0.83 | <.01 |
| Participant 20 | 2.65 | 0.88 | <.01 |
| Participant 21 | 2.20 | 0.88 | <.01 |
| Participant 22 | 2.10 | 0.89 | <.01 |
| Participant 23 | 2.45 | 0.92 | <.01 |
| Participant 24 | 2.40 | 0.83 | <.01 |
| Participant 25 | 2.10 | 0.88 | <.01 |
| Participant 26 | 2.70 | 0.90 | <.01 |
| Participant 27 | 2.15 | 0.89 | <.01 |
| Participant 28 | 2.10 | 0.78 | <.01 |
| Participant 29 | 2.65 | 0.76 | <.01 |
| Participant 30 | 2.60 | 0.87 | <.01 |
| Participant 31 | 2.50 | 0.88 | <.01 |
| Participant 32 | 2.05 | 0.88 | <.01 |
| Participant 33 | 3.15 | 0.95 | <.01 |
| Participant 34 | 3.00 | 0.92 | <.01 |
| Participant 35 | 2.20 | 0.93 | <.01 |
| Participant 36 | 2.80 | 0.92 | <.01 |
| Participant 37 | 1.90 | 0.86 | <.01 |
| Participant 38 | 3.05 | 0.89 | <.01 |
| Participant 39 | 2.65 | 0.91 | <.01 |
| Participant 40 | 2.35 | 0.93 | <.01 |
| Participant 41 | 2.55 | 0.82 | <.01 |
| Participant 42 | 2.20 | 0.88 | <.01 |
| Participant 43 | 2.60 | 0.87 | <.01 |
| Participant 44 | 3.10 | 0.93 | <.01 |
| Participant 45 | 2.20 | 0.92 | <.01 |
| Participant 46 | 2.55 | 0.88 | <.01 |

# Appendix 4.6.

*Graph of busyness scores for each minute of unedited video for validation of busyness study (Section 4.3)*



Appendix 4.6. Graph to show the busyness scores (as scored by the experimenter) for the original, continuous video before editing. Video presented in this graph is of those recordings of video that were greater than 5 minutes in length, and the sections of lines on the graph, between the breaks, represent busyness scores for different recordings of continuous video. Results show little variation in busyness scores within 5 minute sections of continuous video, it was therefore decided that a mean busyness score for 5 minutes of video would provide a meaningful value of busyness to be used in this study.

# Appendix 4.7.

*List of tiger behaviours used in instantaneous scan, and one-zero sampling of tiger video for validation of busyness study (Section 4.3).*

| **Behaviour** | **Description** |
|---|---|
| *State behaviours* | |
| Not visible | Tiger is out of sight |
| Sleeping | Tiger is lying down. Eyes are closed and the animal's body is relaxed showing little or no response to stimuli. |
| Inactive alert | Tiger is stationary, but is awake and eyes are open.<br>Levels of alertness:<br>Low = tiger is lying down sternally, laterally or on back and shows minimal responses to external stimuli, e.g., looks around with eyes, and ears may move but otherwise little movement.<br>Medium = tiger is usually either lying down sternally or sitting up. Tiger is more responsive to external stimuli and shows steady movements of eyes, ears or head towards certain stimuli.<br>High = tiger is sitting up, standing, or possibly lying down sternally. Tiger is very responsive to external stimuli and turns head quickly towards the source of stimuli. Ear position, eyes and head show that the animal is very alert. May involve a change in position, e.g., from lying down to sitting up. |
| Locomotion (any) | Tiger moves from one location to another over a distance of greater than one body length. Different types of locomotion are listed below. |
| Walking | Slowest type of locomotion. Forward movement, moving opposite limbs with two paws on the ground at any one time |
| Trotting | Medium speed locomotion. Usually two limbs on the ground at a time. Body bobs up and down with the trotting motion. |
| Running | Fastest locomotion. Tiger pushes off ground and moves quickly forward with full extension of limbs. All paws may leave the ground simultaneously at points as tiger runs |
| Stalking | Low crouching advance to a target of an object or other animal with eyes orientating on the target. |
| Wading | Tiger moves through water (also includes swimming) |
| Pacing | Repetitive and unvarying locomotion with the same route taken at least three times. Animal may walk in a figure of eight and turn the same direction. |
| Repetitive walking/anticipatory walking | Walking up and down in the same area of enclosure. Like pacing but route and distance covered may vary. |
| Investigating | Exploration of environment including sniffing or touching objects. May walk with nose to the ground. |
| Self maintenance | Tiger directs behaviour towards themselves e.g., grooming, licking or scratching. Tiger may use its mouth, tongue or paw. |
| Drinking | Ingesting water. |
| Playing with objects | Manipulation of objects such as branches or leaves |
| Social play | Tiger makes contact with, and interacts with another tiger. Includes mock or play fighting with low levels of intensity. |
| *Event behaviours* | |
| Agonistic behaviour | Tiger fights with another tiger. High intensity activity involving contact or attempted contact between tigers such as scratching, wrestling and biting. |
| Social contact | Tiger makes contact with another tiger in a positive way. Includes licking other tiger, head and body rubs. |
| Marking | Including spray marking, scratching objects (eg. trees) with front claws, rubbing objects with head or body, scraping ground with hind legs. |

# Appendix 5.1.

*Photographs of meerkat enclosure.*

*Appendix 5.1.1: Meerkat enclosure section 1, as viewed from the upper visitor viewing area*

*Appendix 5.1.2: Meerkat enclosure section 1, as viewed from the lower visitor viewing area*

*Appendix 5.1.3: Meerkat enclosure section 2, as viewed from the upper visitor viewing area*

*Appendix 5.1.4: Meerkat enclosure section 2, as viewed from the lower visitor viewing area*

# Appendix 6.1.

*Photograph of experimental set-up (a), computer view of camera output (b) and timestamp devices (c), for Chapter 6.*

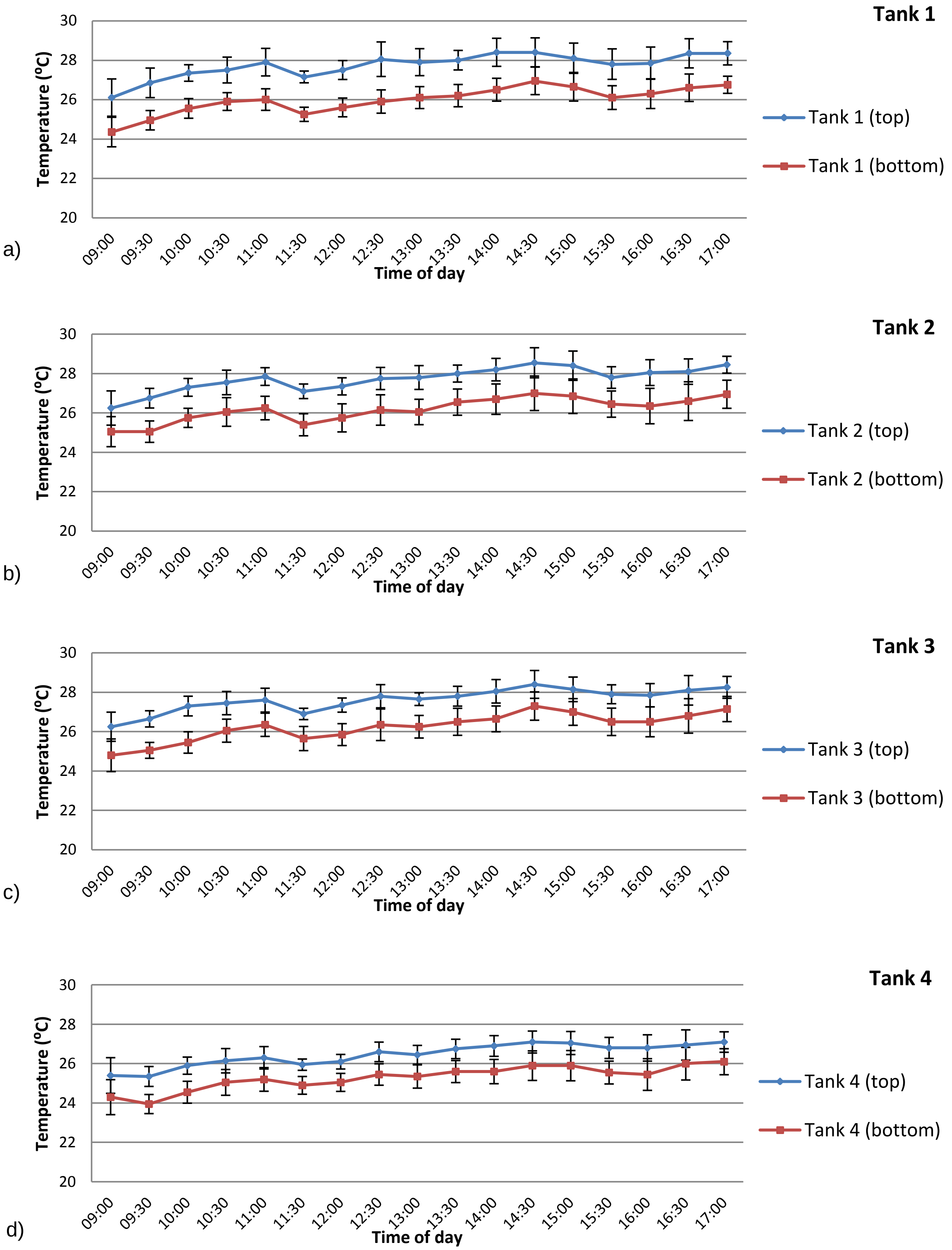
a)

b)

c)

# Appendix 6.2.

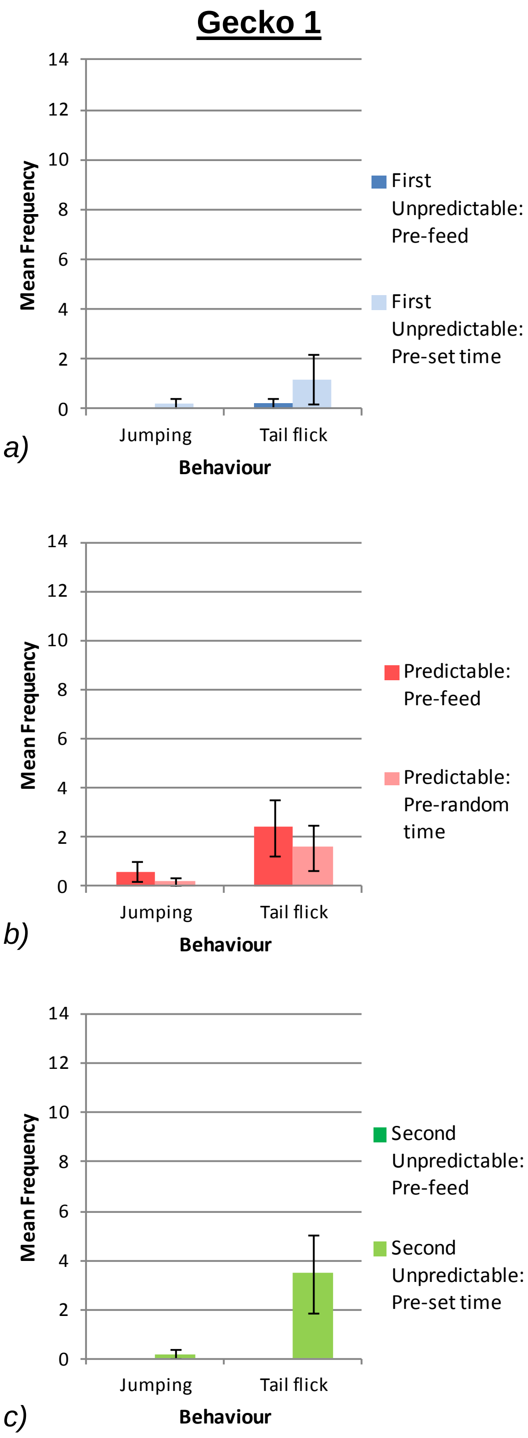
*Graphs to show the mean temperatures throughout the day at the top and bottom of each tank (Tank 1 housed Gecko 1 etc.).*



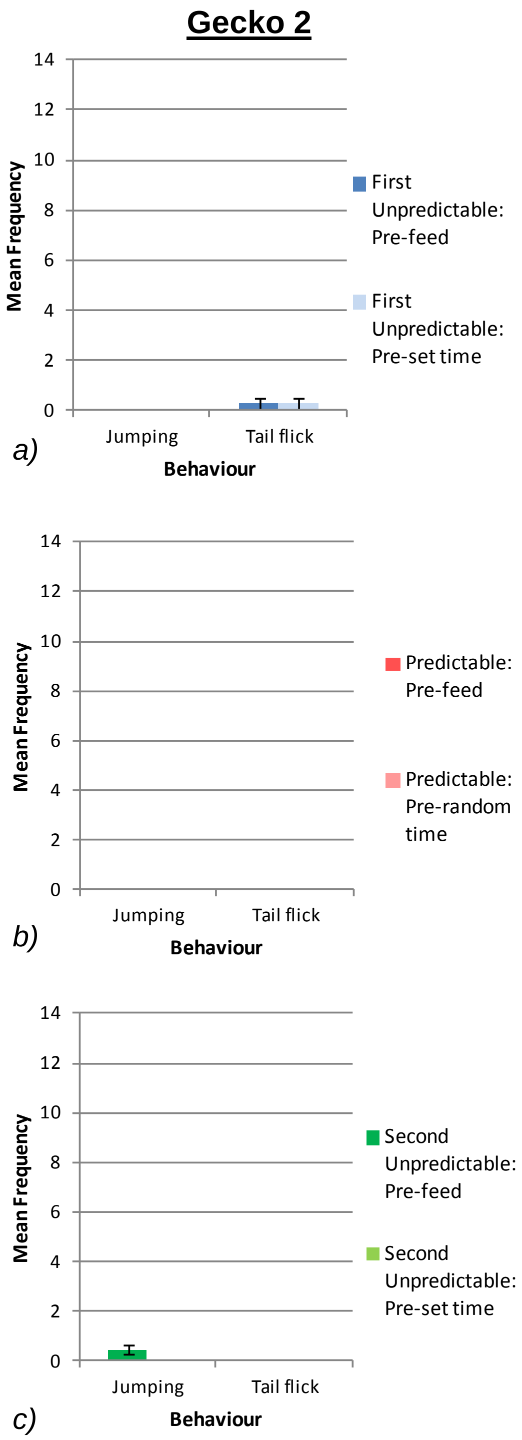
*Appendix 6.2.1. Mean daily temperatures at the top and bottom of a) Tank 1, b) Tank 2, c) Tank 3 and d) Tank 4 (n=4 for each data point). Error bars show SE.*

# Appendix 6.3.

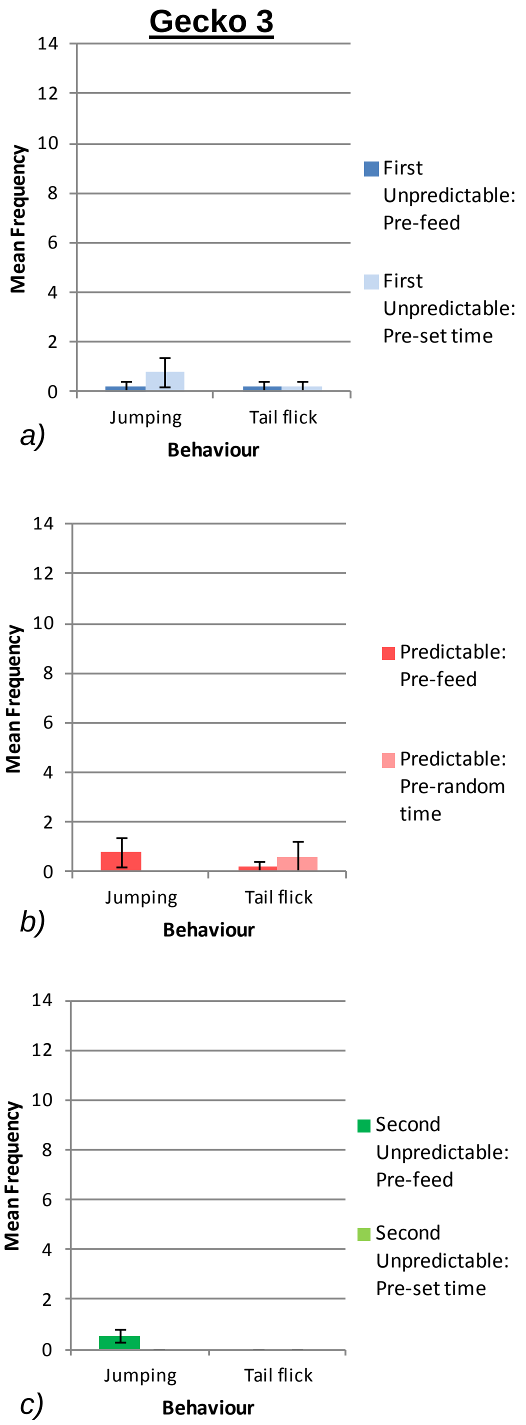
*Graphs to show the proportions of tail flicking and jumping in pre-feed, pre-set time and pre-random time observations in the different feeding conditions for each gecko.*



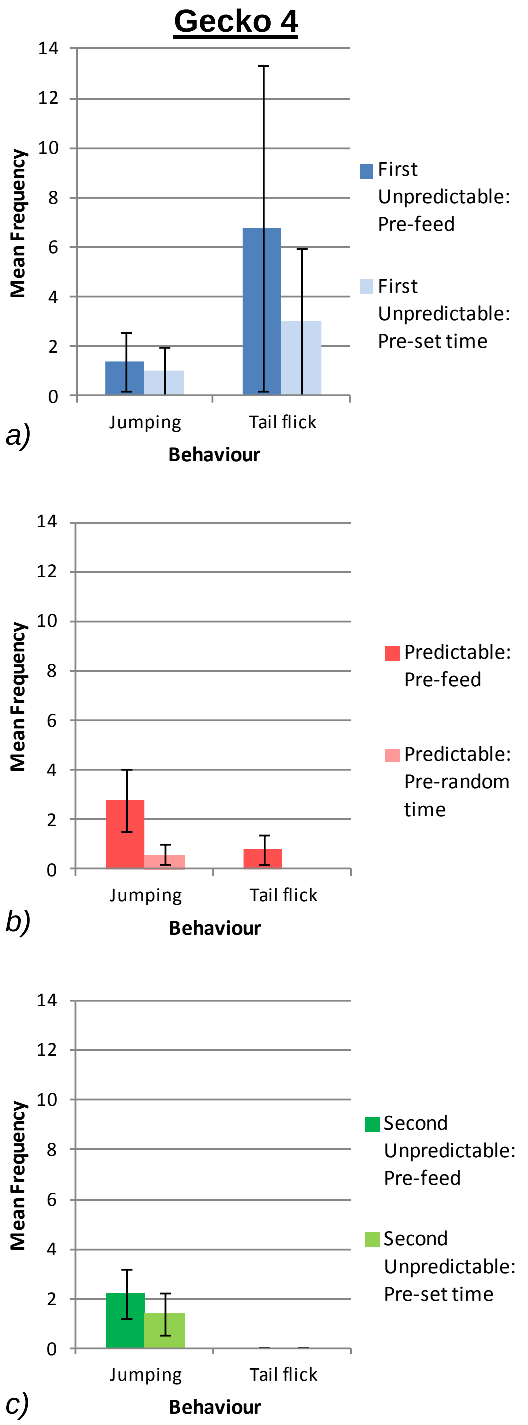
*Appendix 6.3.1: Mean proportions of jumping and tail flicking for Gecko 1 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*



*Appendix 6.3.2: Mean proportions of jumping and tail flicking for Gecko 2 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*



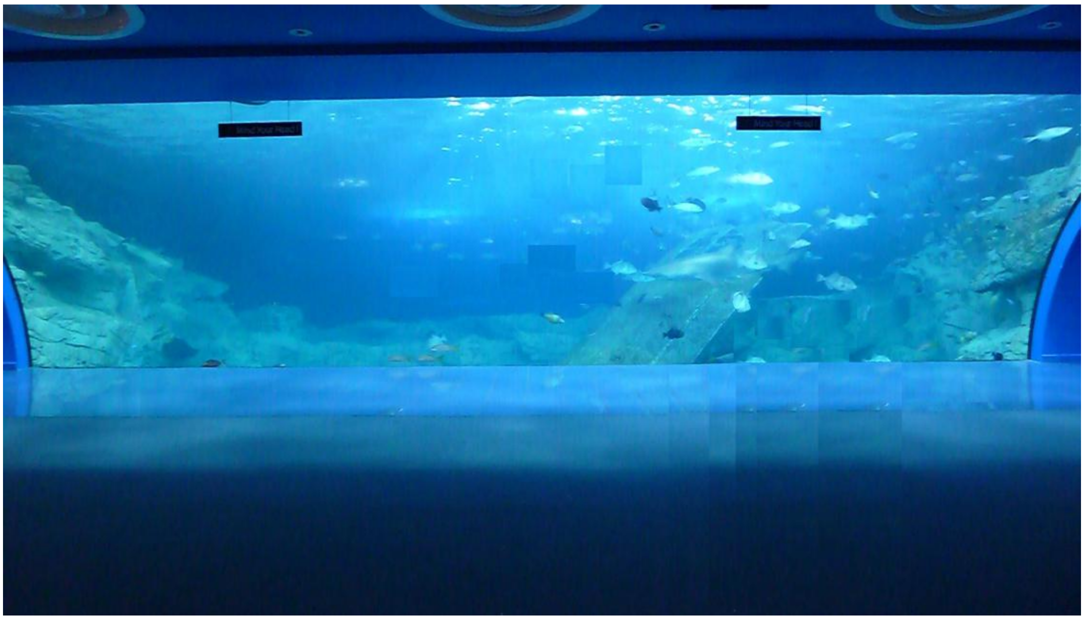
*Appendix 6.3.3: Mean proportions of jumping and tail flicking for Gecko 3 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*



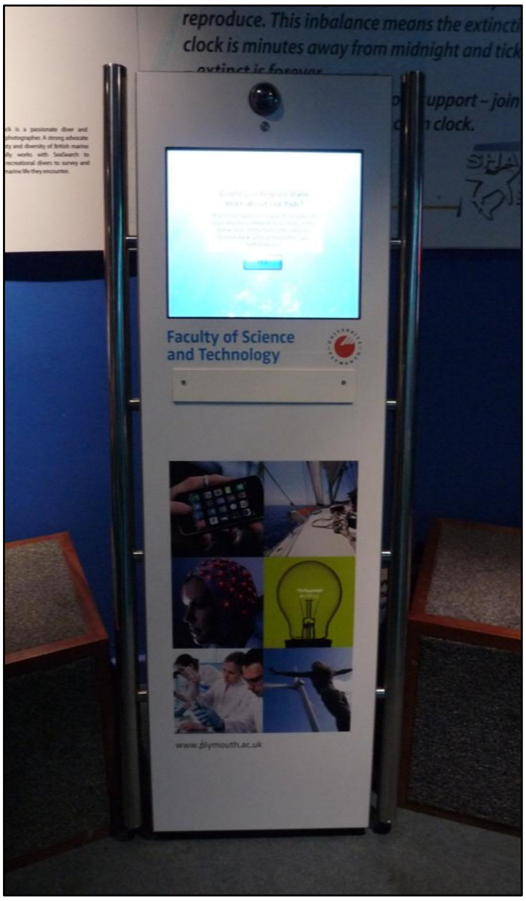
*Appendix 6.3.4: Mean proportions of jumping and tail flicking for Gecko 4 in both observation sessions in the first unpredictable condition (a), predictable condition (b) and the second unpredictable condition (c)*

# Appendix 7.1.

*Photographs showing the Atlantic Ocean tank and visitor viewing area (a) and the touch screen apparatus (b).*



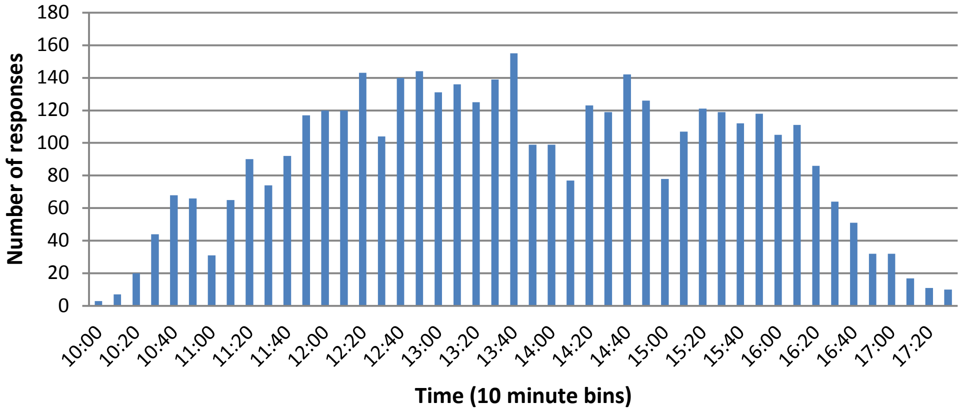
a)



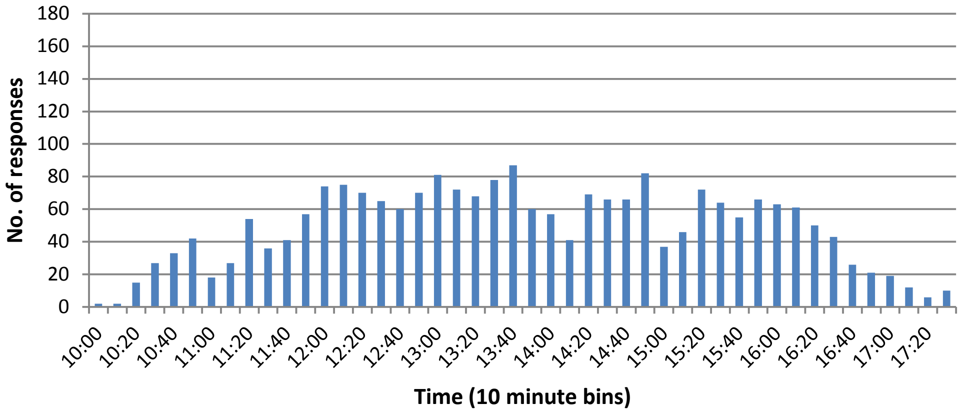
b)

# Appendix 7.2.

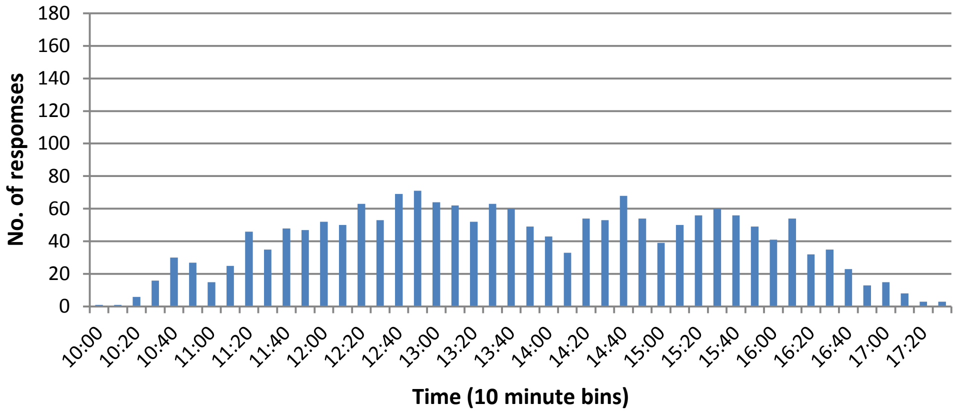
*Graphs showing number of touch screen responses for each 10 minutes of the day.*



*Appendix 7.2.1: Graph to show the number of touch screen responses for research question 1 regarding the tank location of the fish.*



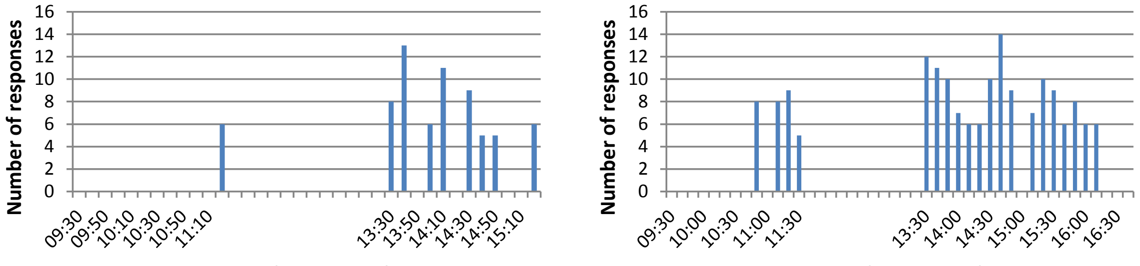
*Appendix 7.2.2: Graph to show the number of touch screen responses for research question 2 regarding the busyness of the fish on the left of the tank.*



*Appendix 7.2.3: Graph to show the number of touch screen responses for research question 2 regarding the busyness of the fish on the right of the tank.*

# Appendix 7.3.

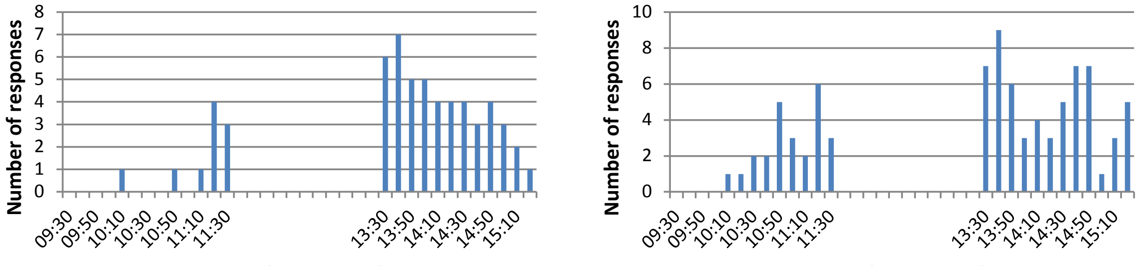
*Graphs showing number of touch screen responses for each 10 minutes of the day (data matched to student baseline phase).*



*i)*

*ii)*

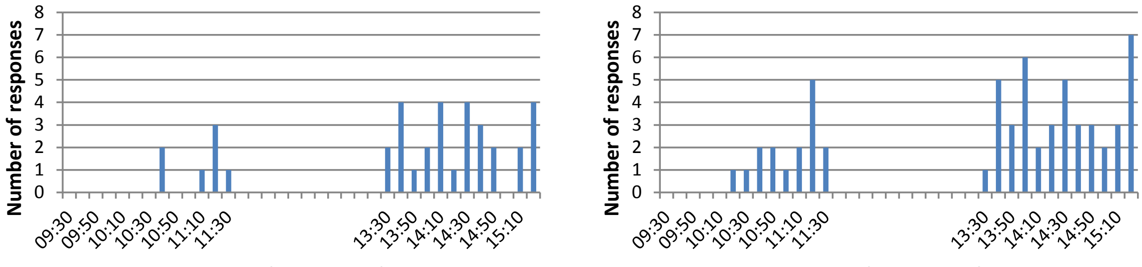
*Appendix 7.3.1: Graph to show the number of touch screen responses for research question 1 regarding the tank location of the fish for dates matched to the student baseline phase (i) and intervention phase (ii).*



*i)*

*ii)*

*Appendix 7.3.2: Graph to show the number of touch screen responses for research question 2 regarding the busyness of the fish on the left of the tank for dates matched to the student baseline phase (i) and intervention phase (ii).*



*i)*

*ii)*

*Appendix 7.3.3: Graph to show the number of touch screen responses for research question 2 regarding the busyness of the fish on the right of the tank for dates matched to the student baseline phase (i) and intervention phase (ii).*

# References

Abbott, B. B., Schoen, L. S., & Badia, P. (1984). Predictable and unpredictable shock: behavioral measures of aversion and physiological measures of stress. *Psychological Bulletin, 96*, 45-71.

Abe, H., & Rusak, B. (1992). Anticipatory activity and entrainment of circadian rhythms in Syrian hamsters exposed to restricted palatable diets. *American Journal of Physiology, 263*, 116-124.

Altmann, J. (1974). Observational study of behaviour: sampling methods. *Behaviour, 49*, 227-266.

Altman, J. D., Gross, K. L., & Lowry, S. R. (2005). Nutritional and behavioral effects of gorge and fast feeding in captive lions. *Journal of Applied Animal Welfare Science, 8*, 47-57.

Aschoff, J., & von Goetz, C. (1986). Effects of feeding cycles on circadian rhythms in squirrel monkeys. *Journal of Biological Rhythms, 1*, 267-276.

Azzaydi, M., Rubio, V. C., Martínez López, F. J., Sánchez-Vázquez, F. J., Zamora, S., & Madrid, J. A. (2007). Effect of restricted feeding schedule on seasonal shifting of daily demand feeding pattern and food anticipatory activity in European sea bass (*Dicentrarchus labrax L.*). *Chronobiology International, 24*, 859-874.

Badia, P., Harsh, J., & Coker, C. C. (1975). Subjects choose fixed time over variable time shock and vice versa: shock distribution and shock-free time as factors. *Learning and Motivation, 6*, 264-272.

Baker, K. C., & Aureli, F. (1997). Behavioural indicators of anxiety: An empirical test in chimpanzees. *Behaviour, 134*, 1031-1050.

Banham Zoo (2009). Giraffe or meekat feeding experience. Retrieved 19th May, 2011, from http://www.banhamzoo.co.uk/general-information/giraffe-feeding-experience.php

Barreto, R. E., Rodrigues, P., Luchiari, A. C., & Delicio, H. C. (2006). Time-place learning in individually reared angelfish, but not in pearl cichlid. *Behavioural Processes, 73*, 367-372.

Bartlett, R. D., & Bartlett, P. B. (2001). *Day geckos*. New York: Barron's Educational Series, Inc.

Bashaw, M. J., Bloomsmith, M. A., Marr, M. J., & Maple, T. L. (2003). To hunt or not to hunt? A feeding enrichment experiment with captive large felids. *Zoo Biology, 22*, 189-198.

Bassett, L., & Buchanan-Smith, H. M. (2007). Effects of predictability on the welfare of captive animals. *Applied Animal Behaviour Science, 102*, 223-245.

Bendig, A. W. (1954). Reliability and the number of rating scale categories. *The Journal of Applied Psychology, 38*, 39-40.

Berger, A. (2011). Activity patterns, chronobiology and the assessment of stress and welfare in zoo and wild animals. *International Zoo Yearbook, 45*, 80-90.

Bishop, J., te Stroete, S., Melfi, V., & Chapman, J. (2007). Can environmental enrichment affect visitor stay time? *Zoo Research News. BIAZA Research Newsletter, 8*, 5.

Bloomsmith, M. A., & Lambeth, S. P. (1995). Effects of predictable versus unpredictable feeding schedules on chimpanzee behavior. *Applied Animal Behaviour Science, 44*, 65-74.

Bohórquez Alonso, M. L., Martínez Cotrina, J., Aguilar Pardo, D., Font, E., & Molina-Borja, M. (2010). Sex differences in antipredator tail-waving displays of the diurnal yellow-headed gecko *Gonatodes albogularis* from tropical forests of Colombia. *Journal of Ethology, 28*, 305-311.

Bonney, R., Cooper, C. B., Dickinson, J., Kelling, S., & Phillips, T. (2009). Citizen science: A developing tool for expanding science knowledge and scientific literacy. *BioScience, 59*, 977-984.

Brännäs, E., Berglund, U., & Eriksson, L.-O. (2005). Time learning and anticipatory activity in groups of Arctic charr. *Ethology, 111*, 681-692.

Broom, D. M., & Johnson, K. G. (1993). *Stress and animal welfare*. London: Chapman & Hall.

Brossard, D., Lewenstein, B., & Bonney, R. (2005). Scientific knowledge and attitude change: The impact of a citizen science project. *International Journal of Science Education, 27*, 1099-1121.

Brotherton, P. N. M., Clutton-Brock, T. H., O'Riain, M. J., Gaynor, D., Sharpe, L., Kansky, R., et al. (2001). Offspring food allocation by parents and helpers in a cooperative mammal. *Behavioral Ecology, 12*, 590-599.

Brown, C., & Partridge, J. (n.d.). *Husbandry guidelines for meerkats*: Bristol Zoo Gardens in conjunction with the U.K. Small Carnivore Taxon Advisory Group.

Cabin, R.J., & Mitchell, R.J. (2000). To Bonferroni or not to Bonferroni: When and how are the questions. *Bulletin of the Ecological Society of America, 81*, 246-248.

Cañon Jones, H. A., Noble, C., Damsgård, B., & Pearce, G. P. (2012). Investigating the influence of predictable and unpredictable feed delivery schedules upon the behaviour and welfare of Atlantic salmon parr (*Salmo salar*) using social network analysis and fin damage. *Applied Animal Behaviour Science 138*, 132-140.

Capitanio, J. P. (1999). Personality dimensions in adult male rhesus macaques: prediction of behaviors across time and situation. *American Journal of Primatology, 47*, 299-320.

Carlstead, K. (1986). Predictability of feeding: its effect on agonistic behaviour and growth in grower pigs. *Applied Animal Behaviour Science, 16*, 25-38.

Carlstead, K. (1998). Determining the causes of stereotypic behaviours in zoo animals: towards developing appropriate enrichment. In D. Shepherdson, J. Mellen & M. Hutchins (Eds.), *Second Nature. Environmental enrichment for captive animals*. Washington, DC: Smithsonian Institution Press.

Carlstead, K., Fraser, J., Bennett, C., & Kleiman, D. G. (1999). Black rhinoceros (*Diceros bicornis*) in U.S. zoos: II. Behavior, breeding success, and mortality in relation to housing facilities. *Zoo Biology, 18*, 35-52.

Carlstead, K., Mellen, J., & Kleiman, D. G. (1999). Black Rhinoceros (Diceros bicornis) in U.S. Zoos: I. Individual Behavior Profiles and Their Relationship to Breeding Success. *Zoo Biology, 18*, 17-34.

Carpenter, A. I., Côté, I. M., & Jones, C. G. (2003). Habitat use, egg laying sites and activity patterns of an endangered Mauritian gecko (*Phelsuma guentheri*). *Herpetological Journal, 13*, 155-157.

Castles, D. L., Whiten, A., & Aureli, F. (1999). Social anxiety, relationships and self-directed behaviour among wild female olive baboons. *Animal Behaviour, 58*, 1207–1215.

Challet, E. (2010). Interactions between light, mealtime and calorie restriction to control daily timing in mammals. *Journal of Comparative Physiology B, 180*, 631-644.

Chamove, A., Hosey, G. R., & Schaetzel, P. (1988). Visitors excite primates in zoos. *Zoo Biology, 7*, 359-369.

Chang, A. (n.d.). Intraclass correlation for parametric data. Introduction and explanation Retrieved 16th September, 2011, from http://www.stattools.net/ICC_Exp.php

Chen, W.-M., & Purser, G. J. (2001). The effect of feeding regime on growth, locomotor activity pattern and the development of food anticipatory activity in greenback flounder. *Journal of Fish Biology, 58*, 177-187.

Chen, W.-M., & Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology, 61*, 1410-1422.

ChimpanZoo (2003). ChimpanZoo: Research, education and enrichment. Data collection. Retrieved 3rd May, 2012, from http://www.chimpanzoo.org/data_collection.html

Chundawat, R. S., Habib, B., Karanth, U., Kawanishi, K., Ahmad Khan, J., Lynam, T., et al. (2011). *Panthera tigris*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. Retrieved 5th January, 2012, from http://www.iucnredlist.org

Clay, A. W., Perdue, B. M., Gaalema, D. E., Dolins, F. L., & Bloomsmith, M. A. (2011). The use of technology to enhance zoological parks. *Zoo Biology, 30*, 487-497.

Clutton-Brock, T. H., O'Riain, M. J., Brotherton, P. N. M., Gaynor, D., Kansky, R., Griffin, A. S., et al. (1999). Selfish sentinels in cooperative mammals. *Science, 284*, 1640-1644.

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educational and Psychological Measurement, 20*, 37-46.

Cohn, J. P. (2008). Citizen science: Can volunteers do real research? *BioScience, 58*, 192-197.

Crawford, J. R., & Henry, J. D. (2004). The Positive and Negative Affect Schedule (PANAS): Construct validity, measurement properties and normative data in a large non-clinical sample. *British Journal of Clinical Psychology, 43*, 245-265.

Cronbach, L. J., & Meehl, P. E. (1955). Construct validity in psychological tests. *Psychological Bulletin, 52*, 281-302.

Crystal, J. D. (2001). Circadian time perception. *Journal of Experimental Psychology, 27*, 68-78.

Crystal, J. D. (2006). Long-interval timing is based on a self-sustaining endogenous oscillator. *Behavioural Processes, 72*, 149-160.
Cummings, D., Brown, J. L., Rodden, M. D., & Songsasen, N. (2007). Behavioral and physiologic responses to environmental enrichment in the maned wolf (*Chrysocyon brachyurus*). *Zoo Biology, 26*, 331-343.

D'Cruze, N., Sabel, J., Dawson, J., & Kumar, S. (2009). The influence of habitat type and structure on the abundance of *Phelsuma madagascariensis grandis* (Gekkoninae) in northern Madagascar. *Herpetological Conservation and Biology, 4*, 55-61.

Davey, G. (2007). Visitors' effects on the welfare of animals in the zoo: A review. *Journal of Applied Animal Welfare Science, 10*, 169-183.

Dawes, J. (2008). Do data characteristics change according to the number of scale points used ? An experiment using 5 point, 7 point and 10 point scales. *International Journal of Market Research, 50*, 1-19.

Dawis, R. V. (1987). Scale construction. *Journal of Counseling Psychology, 34*, 481-489.

de Groot, M. H. M., & Rusak, B. (2004). Housing conditions influence the expression of food-anticipatory activity in mice. *Physiology & Behavior, 83*, 447-457.

de Jonge, F. H., Ooms, M., Kuurman, W. W., Maes, J. H. R., & Spruijt, B. M. (2008). Are pigs sensitive to variability in food rewards? *Applied Animal Behaviour Science, 114*, 93-104.

de Jonge, F. H., Tilly, S.-L., Baars, A. M., & Spruijt, B. M. (2008). On the rewarding nature of appetitive feeding behaviour in pigs (*Sus scrofa*): Do domesticated pigs contrafreeload? *Applied Animal Behaviour Science, 114*, 359-372.

Dews, P. B. (1978). Studies on responding under fixed-interval schedules of reinforcement: II. The scalloped pattern of the cumulative record. *Journal of the Experimental Analysis of Behavior, 29*, 67-75.

Dierenfeld, E. S., Bush, M., Phillips, L., & Montali, R. (1994). Nutrition, food preparation and feeding. In R. Tilson, G. Brady, K. Traylor-Holzer & D. Armstrong (Eds.), *Management and conservation of captive tigers, Panthera tigris*. Apple Valley, Minnesota: Minnesota Zoo.

Doolan, S. P., & Macdonald, D. W. (1997). Band structure and failures of reproductive suppression in a cooperatively breeding carnivore, the slender-tailed meerkat (*Suricata suricatta*). *Behaviour, 134*, 827-848.

Dudink, S., Simonse, H., Marks, I., de Jonge, F. H., & Spruijt, B. M. (2006). Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. *Applied Animal Behaviour Science, 101*, 86-101.

Düpjan, S., Schön, P.-C., Puppe, B., Tuchscherer, A., & Manteuffel, G. (2008). Differential vocal responses to physical and mental stressors in domestic pigs (*Sus scrofa*). *Applied Animal Behaviour Science, 114*, 105-115.

Dunbar, R. I. M. (1976). Some aspects of research design and their implications in the observational study of behaviour. *Behaviour, 58*, 78-98.

Ellis, D. J., Firth, B. T., & Belan, I. (2007). Circadian rhythms of locomotor activity and temperature selection in sleepy lizards, *Tiliqua rugosa*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 193*, 695-701.

Ewer, R. F. (1963). The behaviour of the meerkat, *Suricata suricatta* (Schreber). *Zeitschrift Fur Tierpsychologie, 20*, 570-607.

Feaver, J., Mendl, M., & Bateson, P. (1986). A method for rating the individual distinctiveness of domestic cats. *Animal Behaviour, 34*, 1016-1025.

Fernandez, E. J., Tamborski, M. A., Pickens, S. R., & Timberlake, W. (2009). Animal-visitor interactions in the modern zoo: Conflicts and interventions. *Applied Animal Behaviour Science, 120*, 1-8.

Field, A. P. (2005). Intraclass correlation. In B. Everitt & C. Howell (Eds.), *Encyclopedia of Behavioral Statistics* (Vol. 2, pp. 948-954). New York: Wiley.

Frankenberg, E. (1979). Influence of light and temperature on daily activity patterns of three Israeli forms of *Ptyodactylus* (Reptilia: Gekkoninae) *Journal of Zoology (London), 189*, 21-30.

Freeman, E. W., Schulte, B. A., & Brown, J. L. (2010). Using behavioral observations and keeper questionnaires to assess social relationships among captive female African elephants. *Zoo Biology, 29*, 140-153.

Friedman, H. H., & Amoo, T. (1999). Rating the rating scales. *The Journal of Marketing Management, 9*, 114-123.

Friedman, H. H., Herskovitz, P. J., & Pollack, S. (1994). *The biasing effects of scale-checking styles on response to a Likert scale.* Paper presented at the The American Statistical Association Annual Conference: Survey Research Methods.

Fritz, P., & Fritz, J. (1979). Resocialization of chimpanzees. Ten years of experience at the Primate Foundation of Arizona. *Journal of Medical Primatology, 8*, 202-221.

Furrer, S. C., Jaag, K., von Stockar, S., & Rübel, A. (2006). First experiences with free-ranging giant day geckos (*Phelsuma madagascariensis grandis, Gray*

*1870*) in the Masoala rainforest exhibit in Zurich Zoo, Switzerland. *Zoo Biology, 25*, 409-415.

Galhardo, L., Vital, J., & Oliveira, R. F. (2011). The role of predictability in the stress response of a cichlid fish. *Physiology & Behavior, 102*, 367-372.

Gee, P., Stephenson, D., & Wright, D. E. (1994). Temporal discrimination learning of operant feeding in goldfish (Carassius auratus). *Journal of Experimental Animal Behaviour, 62*, 1-13.

Gerlach, J., Ineich, I., & Vences, M. (2011). *Phelsuma laticauda*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1 Retrieved 1st September, 2012, from http://www.iucnredlist.org

Gibbon, J. (1977). Scalar expectancy theory and Weber's law in animal timing. *Psychological Review, 84*, 279-325

.

Gilbert-Norton, L. B., Leaver, L. A., & Shivik, J. A. (2009). The effect of randomly altering the time and location of feeding on the behaviour of captive coyotes (*Canis latrans*). *Applied Animal Behaviour Science, 120*, 179-185.

Gilbert, A., Churchill, Jr., & Peter, J. P. (1984). Research design effects on the reliability of rating scales: A meta-analysis. *Journal of Marketing Research, 21*, 360-375.

Gliner, J. A. (1972). Predictable vs. unpredictable shock: preference behavior and stomach ulceration. *Physiology & Behavior, 9*, 693-698.

Gopar-Canales, K. L., Miranda-Anaya, M., Moreno-Sáenz, E., & Barrera-Mera, B. (2010). Circadian locomotor activity in the juvenile red eared turtle *Trachemys scripta elegans*: free running and entrainment. *Biological Rhythm Research, 41*, 305-312.

Gosling, S. D. (1998). Personality dimensions in spotted hyenas (*Crocuta crocuta*). *Journal of Comparative Psychology, 112*, 107-118.

Gosling, S. D. (2001). From mice to men: what can we learn about personality from animal research? *Psychological Bulletin, 127*, 45-86.

Gosling, S. D., Kwan, V. S. Y., & John, O. P. (2003). A dog's got personality: A cross-species comparative approach to personality judgments in dogs and humans. *Journal of Personality and Social Psychology, 85*, 1161-1169.

Hansen, S. W., & Møller, S. H. (2008). Diurnal activity patterns of farm mink (*Mustela vison*) subjected to different feeding routines. *Applied Animal Behaviour Science, 111*, 146-157.

Hare, V. J. (1998). Artificial prey that fights back (and other tales of tiger enrichment). *The Shape of Enrichment, 7*, 1-4.

Hatersley, E. (2005). Vigilance and foraging behaviour in a mixed-species exhibit. Unpublished Dissertation. Anglia Polytechnic University.

Hawkes, K., Kaplan, H., Hill, K., & Hurtado, M. (1987). A problem of bias in scan sampling. *Journal of Anthropological Research, 43*, 239-246.

Heckrotte, C. (1975). Temperature and light effects on the circadian rhythm and locomotory activity of the plains garter snake (*Thamnophis radix hayendi*). *Journal of Interdisciplinary Cycle Research, 6*, 279-290.

Herrero, M. J., Pascual, M., Madrid, J. A., & Sánchez-Vázquez, F. J. (2005). Demand-feeding rhythms and feeding-entrainment of locomotor activity rhythms in tench (*Tinca tinca*). *Physiology & Behavior, 84*, 595-605.

Hogan, E., S, Houpt, K. A., & Sweeney, K. (1988). The effect of enclosure size on social interactions and daily activity patterns of the captive Asiatic wild horse (*Equus przewalskii*). *Applied Animal Behaviour Science, 21*, 147-168.

Hollén, L. I., & Manser, M. B. (2007). Persistence of alarm-call behaviour in the absence of predators: A comparison between wild and captive-born meerkats (*Suricata suricatta*). *Ethology, 113*, 1038-1047.

Holmes, M. M., & Mistlberger, R., E (2000). Food anticipatory activity and photic entrainment in food-restricted BALB/c mice. *Physiology & Behavior, 68*, 655-666.

Hosey, G. R. (2000). Zoo animals and their human audiences: what is the visitor effect? *Animal Welfare, 9*, 343-357.

Hosey, G. R. (2005). How does the zoo environment affect the behaviour of captive primates? *Applied Animal Behaviour Science, 90*, 107-129.

Hosey, G. R. (2008). A preliminary model of human-animal relationships in the zoo. *Applied Animal Behaviour Science, 109*, 105-127.

Howard, C. J. (1980). Breeding the Flat-tailed day gecko *Phelsuma laticauda* at Twycross Zoo. *International Zoo Yearbook, 20*, 193-196.

Hsu, C. T., Patton, D. F., Mistlberger, R., E, & Steele, A. D. (2010). Palatable Meal Anticipation in Mice. *PLoS ONE, 5*, 1-13.

Ikeuchi, I., Mori, A., & Hasegawa, M. (2005). Natural history of *Phelsuma madagascariensis kochi* from a dry forest in Madagascar. *Amphibia-Reptilia, 26*, 475-483.

ISIS (2011). International Species Information System. *Panthera tigris sumatrae*. Sumatran tiger Retrieved 5th January, 2012, from http://www.isis.org/Pages/findanimals.aspx

Jenny, S., & Schmid, H. (2002). Effect of feeding boxes on the behaviour of stereotyping Amur tigers (*Panthera tigris altaica*) in the Zurich Zoo, Zurich, Switzerland. *Zoo Biology, 21*, 573-584.

Johannesson, T., & Ladewig, J. (2000). The effect of irregular feeding times on the behaviour and growth of dairy calves. *Applied Animal Behaviour Science, 69*, 103-111.

Johnston, J. M., & Pennypacker, H. S. (2009). *Strategies and tactics of behavioral research* (Third ed.). New York: Routledge.

Jones, A. C., & Gosling, S. D. (2005). Temperament and personality in dogs (*Canis familiaris*): A review and evaluation of past research. *Applied Animal Behaviour Science, 95*, 1-53.

Kasai, M., Yamamoto, T., Kitasako, K., & Kiyohara, S. (2009). Feeding activity rhythm in Japanese sea catfish *Plotosus japonicas*. *Fisheries Science, 75*, 1125-1132.

Kawanishi, K., & Lynam, T. (2008). *Panthera tigris ssp. jacksoni*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. Retrieved 5th January, 2012, from http://www.iucnredlist.org

Kessler, M. R., & Turner, D. C. (1997). Stress and adaptation of cats (*Felis silvestris catus*) housed singly, in pairs and in groups in boarding catteries. *Animal Welfare, 6*, 243-254.

Kessler, M. R., & Turner, D. C. (1999). Effects of density and cage size on stress in domestic cats (*Felis silvestris catus*) housed in animal shelters and boarding catteries. *Animal Welfare, 8*, 259-267.

Killeen, P. R., & Fetterman, J. G. (1988). A Behavioral Theory of Timing. *Psychological Review, 95*, 274-295.

King, J. E., & Figueredo, A. J. (1997). The Five-Factor Model plus dominance in chimpanzee personality. *Journal of Research in Personality, 31*, 257-271.

King, J. E., & Landau, V. I. (2003). Can chimpanzee (*Pan troglodytes*) happiness be estimated by human raters? *Journal of Research in Personality, 37*, 1-15.

Kingdon, J. (1997). *The Kingdon Field Guide to African Mammals*. San Diego: Harcourt Brace.

Krishnamurthy, R. (1994). Vocalizations of captive Francois' langurs linked to stereotypy and possible stress. *Folia Primatologica, 63*, 91-95.

Landis, J. R., & Koch, G. G. (1977). The Measurement of Observer Agreement for Categorical Data *Biometrics, 33*, 159-174.

Lane, J. (2006). Can non-invasive glucocorticoid measures be used as reliable indicators of stress in animals? *Animal Welfare, 15*, 331-342.

Lang, J. W. (1976). Amphibious behavior of *Alligator mississippiensis*: roles of a circadian rhythm and light *Science, 191*, 575-577.

Law, G., MacDonald, A., & Reid, A. (1997). Dispelling some common misconceptions about the keeping of felids in captivity. *International Zoo Yearbook, 35*, 197-207.

Lawler, J. E., Cox, R. H., Barker, G. F., Hubbard, J. W., & Sanders, B. J. (1984). Cardiovascular, plasma catecholamine, and corticosterone responses to alterations in the predictability of electric shock in the rat. *Physiological Psychology, 12*, 227-232.

Lejeune, H. (1990). Timing: differences in continuity or generality beyond differences. In D. E. Blackman & H. Lejeune (Eds.), *Behaviour analysis in theory* (pp. 53-90). Hove, East Sussex: Lawrence Erlbaum Associates.

Lejeune, H., & Wearden, J. H. (1991). The comparative psychology of fixed-interval responding: some quantitative analyses. *Learning and Motivation, 22*, 84-111.

Lejeune, H., & Wearden, J. H. (2006). Scalar properties in animal timing: Conformity and violations. *The Quarterly Journal of Experimental Psychology, 59*, 1975-1908.

Lepczyk, C. A. (2005). Integrating published data and citizen science to describe bird diversity across a landscape. *Journal of Applied Ecology, 42*, 672-677.

Linkie, M., Wibisono, H. T., Martyr, D. J., & Sunarto, S. (2008). *Panthera tigris ssp. sumatrae*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. Retrieved 5th January, 2012, from http://www.iucnredlist.org

Lintott, C. J., Schawinski, K., Slosar, A., Land, K., Bamford, S., Thomas, D., et al. (2008). Galaxy Zoo: Morphologies derived from visual inspection of galaxies from the Sloan Digital Sky Survey. *Monthly Notices of the Royal Astronomical Society, 389*, 1179-1189.

Lloyd, A. S., Martin, J. E., Bornett-Gauci, H. L. I., & Wilkinson, R. G. (2007). Evaluation of a novel method of horse personality assessment: Rater-agreement and links to behaviour. *Applied Animal Behaviour Science, 105*, 205-222.

Lloyd, A. S., Martin, J. E., Bornett-Gauci, H. L. I., & Wilkinson, R. G. (2008). Horse personality: Variation between breeds. *Applied Animal Behaviour Science, 112*, 369-383.

Ludvig, E. A., Balci, F., & Spetch, M. L. (2011). Reward magnitude and timing in pigeons. *Behavioural Processes, 86*, 359-363.

Luo, S.-J., Kim, J.-H., Johnson, W. E., van der Walt, J., Martenson, J., Yuhki, N., et al. (2004). Phylogeography and genetic ancestry of tigers (*Panthera tigris*). *PLoS Biology, 2*, 2275-2293.

Lyons, J., Young, R. J., & Deag, J. M. (1997). The effect of physical characteristics of the environment and feeding regime on the behaviour of captive felids. *Zoo Biology, 16*, 71-83.

Macdonald, D., & Hoffmann, M. (2008). *Suricata suricatta*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2011.2. Retrieved 5th January, 2012, from http://www.iucnredlist.org

Machado, A. (1997). Learning the temporal dynamics of behavior. *Psychological Review, 104*, 241-265.

Maestripieri, D., Schino, G., Aureli, F., & Troisi, A. (1992). A modest proposal: displacement activities as an indicator of emotions in primates. *Animal Behaviour, 44*, 967–979.

Mallapur, A., & Chellam, R. (2002). Environmental influences on stereotypy and the activity budget of Indian leopards (*Panthera pardus*) in four zoos in southern India. *Zoo Biology, 21*, 585-595.

Martin, P., & Bateson, P. (1993). *Measuring behaviour: An introductory guide* (Second ed.). Cambridge: Cambridge University Press.

Martin, P., & Bateson, P. (2007). *Measuring behaviour: An introductory guide* (Third ed. Vol. Cambridge): Cambridge University Press.

Mason, G. J. (1991). Stereotypies: a critical review. *Animal Behaviour, 41*, 1015-1037.

Mason, G. J., Clubb, R., Latham, N., & Vickery, S. (2007). Why and how should we use environmental enrichment to tackle stereotypic behaviour? *Applied Animal Behaviour Science, 102*, 163-188.

Mazák, V. (1981). Panthera tigris. *Mammalian Species, 152*, 1-8.

McCaffrey, R. E. (2005). Using citizen science in urban bird studies. *Urban Habitats, 3*, 70-86. Retrieved from http://www.urbanhabitats.org/v03n01/citizenscience_full.html

McCrae, R. R., & Costa, P. T., Jr. (1987). Validation of the Five-Factor Model of Personality across instruments and observers. *Journal of Personality and Social Psychology, 52*, 81-90.

McGraw, K. O., & Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychological Methods, 1*, 30-46.

Meagher, R. K. (2009). Observer ratings: Validity and value as a tool for animal welfare research. *Applied Animal Behaviour Science, 119*, 1-14.

Mellen, J. D. (1991). Factors influencing reproductive success in small captive exotic felids (*Felis spp.*): a multiple regression analysis. *Zoo Biology, 10*, 95-110.

Mench, J. A., & Mason, G. J. (1997). Behaviour. In M. C. Appleby & B. O. Hughes (Eds.), *Animal welfare* (pp. 127-141). Oxon: CABI Publishing.

Miller, R. R., Greco, C., Vigorito, M., & Marlin, N. A. (1983). Signaled tailshock is perceived as similar to a stronger unsignaled tailshock: implications for a functional analysis of classical conditioning. *Journal of Experimental Psychology: Animal Behavior Processes, 9*, 105–131.

Mineka, S., & Kihlstrom, J. F. (1978). Unpredictable and uncontrollable events: A new perspective on experimental neurosis. *Journal of Abnormal Psychology, 87*, 256-271.

Minero, M., Tosi, M. V., Canali, E., & Wemelsfelder, F. (2009). Quantitative and qualitative assessment of the response of foals to the presence of an unfamiliar human. *Applied Animal Behaviour Science, 116*, 74-81.

Mistlberger, R. E. (1994). Circadian food-anticipatory activity: Formal models and physiological mechanisms. *Neuroscience and Biobehavioral Reviews, 18*, 171-195.

Mistlberger, R. E. (2009). Food-anticipatory circadian rhythms: concepts and methods. *European Journal of Neuroscience, 30*, 1718-1729.

Mistlberger, R. E. (2011). Neurobiology of food anticipatory circadian rhythms. *Physiology & Behavior, 104*, 535-545.

Mistlberger, R. E., & Rusak, B. (1987). Palatable daily meals entrain anticipatory activity rhythms in free-feeding rats: Dependence on meal size and nutrient content. *Physiology & Behavior, 41*, 219-226.

Mistlberger, R. E., & Rusak, B. (2005). Biological rhythms and behaviour. In J. J. Bolhuis & L. A. Giraldeau (Eds.), *The Behaviour of Animals, Mechanisms, Function and Evolution* (pp. 71-96). Malden, MA: Blackwell Publishing Ltd.

Moran, G. (1984). Vigilance behaviour and alarm calls in a captive group of meerkats, *Suricata suricatta*. *Zeitschrift Fur Tierpsychologie, 65*, 228-240.

Morgan, D. L., & Morgan, R. K. (2001). Single-participant research design: Bringing science to managed care. *American Psychologist, 56*, 119-127.

Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science, 102*, 262-302.

Murden, T. (2010). Terry Murden looks at why Comparethemarket.com's cult figurehead has everyone sitting up and taking notice. *Scotland on Sunday*. Retrieved from http://business.scotsman.com/business/-Terry-Murden-looks-at.5988705.jp

National Geographic (2011). Valley of the Khans Project. Retrieved 24th October, 2011, from http://www.nationalgeographic.com/field/projects/valley-khans-project/

Nogueira, S. S. C., Calazans, S. G., Costa, T. S. O., Peregrino, H., & Nogueira-Filho, S. L. G. (2011). Effects of varying feed provision on behavioral patterns of farmed collared peccary (Mammalia, Tayassuidae). *Applied Animal Behaviour Science, 132*, 193-199.

Norris, K. S., & Kavanau, J. L. (1966). The burrowing of the western shovel-nosed snake *Chionactis occipitalis* Hallowell, and the undersand environment. *Copeia, 4*, 650-664.

Nowell, K., & Jackson, P. (1996). *Wild Cats Status Survey and Conservation Action Plan*. Switzerland: IUCN/SSC Cat Specialist Group.

Nunnally, J. C. (1978). *Psychometric theory.* (Second ed.). New York: McGraw-Hill.

O’Brien, T. G., Kinnaird, M. F., & Wibisono, H. T. (2003). Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. *Animal Conservation, 6*, 131-139.

Ogden, J., Lindburg, D. G., & Maple, T. L. (1993). Preference for structural environmental features in captive lowland gorillas (*Gorilla gorilla gorilla*) *Zoo Biology, 12*, 381-395.

Oishi, T., Nagai, K., Harada, Y., Naruse, M., Ohtani, M., Kawano, E., et al. (2004). Circadian Rhythms in Amphibians and Reptiles: Ecological Implications. *Biological Rhythm Research, 35*, 105-120.

Ota, H., & Ineich, I. (2006). Colonization of the gold dust day gecko, *Phelsuma laticauda* (Reptilia: Gekkonidae), in Moorea of the Society Archipelago, French Polynesia. *Current Herpetology, 25*, 97-99.

Paignton Zoo (2011). Paignton Zoo; Experience days; Meet the meerkats. Retrieved 19th May, 2011, from https://shop.paigntonzoo.org.uk/product.php?productid=482

Paradise Wildlife Park (nd). Meet the meerkats Retrieved 19th May, 2011, from http://www.pwpark.com/experiences/animal_experiences/meet_meerkats

Pederson, A. K., King, J. E., & Landau, V. I. (2005). Chimpanzee (*Pan troglodytes*) personality predicts behavior. *Journal of Research in Personality, 39*, 534-549.

Pérez-Padilla, A., Magalhães, P., & Pellón, R. (2010). The effects of food presentation at regular or irregular times on the development of activity-based anorexia in rats. *Behavioural Processes, 84*, 541-545.

Peters, S. M., Bleijenberg, E. H., van Dierendonck, M. C., van der Harst, J. E., & Spruijt, B. M. (2012). Characterization of anticipatory behaviour in domesticated horses (*Equus caballus*). *Applied Animal Behaviour Science, 138*, 60-69.

Piccione, G., Bertolucci, C., Caola, G., & Foà, A. (2007). Effects of restricted feeding on circadian activity rhythms of sheep - A brief report. *Applied Animal Behaviour Science, 107*, 233-238.

Pitman, D. L., Natelson, B. H., Ottenweller, J. E., McCarty, R., Pritzel, T., & Tapp, W. N. (1995). Effects of exposure to stressors of varying predictability on adrenal function in rats. *Behavioral Neuroscience, 109*, 767–776.

Polizzi di Sorrentino, E., Schino, G., Visalberghi, E., & Aureli, F. (2010). What time is it? Coping with expected feeding time in capuchin monkeys. *Animal Behaviour, 80*, 117-123.

Portney, L. G., & Watkins, M. P. (2000). *Foundations of clinical research. Applications to practice*. New Jersey: Prentice Hall Inc.

Powell, D. M. (1995). Preliminary evaluation of environmental enrichment techniques for African lions (*Panthera leo*). *Animal Welfare, 4*, 361-370.

Preston, C. C., & Colman, A. M. (2000). Optimal number of response categories in rating scales: reliability, validity, discriminating power, and respondent preferences. *Acta Psychologica, 104*, 1-15.

Price, K. P. (1972). Predictable and unpredictable shock: their pathological effects on restrained and unrestrained rats. *Psychological Reports, 30*, 419-426.

Prokasy, W. F. (1956). The acquisition of observing responses in the absence of differential external reinforcement. *Journal of Comparative and Physiological Psychology, 49*, 131-134.

Purser, G. J., & Chen, W.-M. (2001). The effect of meal size and meal duration on food anticipatory activity in greenback flounder. *Journal of Fish Biology, 58*, 188-200.

Raddick, M. J., Bracey, G., Gay, P. L., Lintott, C. J., Murray, P., Schawinski, K., et al. (2010). Galaxy Zoo: Exploring the motivations of citizen science volunteers. *Astronomy Education Review, 9*, 010103.

Reebs, S. G. (1996). Time-place learning in golden shiners (Pisces: Cyprinidae). *Behavioural Processes, 36*, 253-262.

Reebs, S. G., & Gallant, B. Y. (1997). Food-anticipatory activity as a cue for local enhancement in golden shiners (Pisces: Cyprinidae, *Notemigonus crysoleucas*). *Ethology, 103*, 1060-1069.

Reebs, S. G., & Lague, M. (2000). Daily food-anticipatory activity in golden shiners: A test of endogenous timing mechanisms. *Physiology & Behavior, 70*, 35-43.

Renner, M., & Renner, C. H. (1993). Expert and novice intuitive judgements about animal behavior. *Bulletin of the Psychonomic Society, 31*, 551-552.

Rhine, R. J., & Linville, A. K. (1980). Properties of one-zero scores in observational studies of primate social behavior: The effect of assumptions on empirical analyses. *Primates, 21*, 111-122.

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution, 43*, 223-225.

Rousing, T., & Wemelsfelder, F. (2006). Qualitative assessment of social behaviour of dairy cows housed in loose housing systems. *Applied Animal Behaviour Science, 101*, 40-53.

RSPB (2011). Big garden birdwatch. Retrieved 24th October, 2011, from http://www.rspb.org.uk/birdwatch/

Sakamoto, W., Naito, Y., Uchida, I., & Kureha, K. (1990). Circadian rhythm on diving motion of the loggerhead turtle *Caretta caretta* during inter-nesting and its fluctuations induced by the oceanic environmental events. *Bulletin of the Japanese Society of Scientific Fisheries, 56*, 263-272.

Salazar-Juárez, A., Aguilar-Roblero, R., Parra, L., & Escobar, C. (2003). Restricted feeding schedules modulate free-running drinking activity in malnourished rats. *Biological Rhythm Research, 34*, 459-474.

Salovey, P., Mayer, J. D., Goldman, S. L., Turvey, C., & Palfai, T. P. (1995). Emotional attention, clarity and repair: Exploring emotional intelligence using the Trait Meta-Mood Scale. In J. W. Pennebaker (Ed.), *Emotion, disclosure and health* (pp. 125-154). Washington, D.C: American Psychological Association.

Sambrook, E. A., & Buchanan-Smith, H. M. (1997). Control and complexity in novel object enrichment. *Animal Welfare, 6*, 207-216.

Sánchez-Vásquez, F. J., Madrid, J. A., Zamora, S., & Tabata, M. (1997). Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *Journal of Comparative Physiology A, 181*, 121-132.

Sánchez, J. A., López-Olmeda, J. F., Blanco-Vives, B., & Sánchez-Vázquez, F. J. (2009). Effects of feeding schedule on locomotor activity rhythms and stress response in sea bream. *Physiology & Behavior, 98*, 125-129.

Sandhaus, E. A. (2004). *Variation of feeding regimes: Effects on giant panda (Ailuropoda melanoleuca) behavior.* Georgia Institute of Technology.

Schaller, G. B. (1967). *The deer and the tiger. A study of wildlife in India*. Chicago: University of Chicago Press.

Schütz, K., & Jensen, P. (1999). *Foraging behaviour and activity in red junglefowl (Gallus gallus) and in domesticated breeds.* Paper presented at the 33rd International Congress of the International Society for Applied Ethology, Lillehammer, Norway.

Seidensticker, J. (2010). Saving wild tigers: A case study in biodiversity loss and challenges to be met for recovery beyond 2010. *Integrative Zoology, 5*, 285-299.

Seidensticker, J., & McDougal, C. (1993). Tiger predatory behaviour, ecology and conservation. *Symposia of the Zoological Society of London, 65*, 105-125.

Seifan, T., Federman, A., Mautz, W. J., Smith, K. J., & Werner, Y. L. (2010). Nocturnal foraging in a diurnal tropical lizard (Squamata: Gekkonidae: *Phelsuma laticauda*) on Hawaii. *Journal of Tropical Ecology, 26*, 243-246.

Seipp, R. (1990). *Basic Management Guidelines for the Maintenance of Phelsuma klemmeri*.

Shankhala, K. (1978). *Tiger! The story of the Indian tiger*. London: Collins.

Shear, M. K., Vander Bilt, J., Rucci, P., Endicott, J., Lydiard, B., Otto, M. W., et al. (2001). Reliability and validity of a structured interview guide for the Hamilton Anxiety Rating Scale (SIGH-A). *Depression and Anxiety, 13*, 166-178.

Shepherdson, D. J., Carlstead, K., Mellen, J. D., & Seidensticker, J. (1993). The influence of food presentation of the behaviour of small cats in confined spaces. *Zoo Biology, 12*, 203-216.

Shoemaker, A. H., Maruska, E. J., & Rockwell, R. (1997). *Minimum Husbandry Guidelines for Mammals: Large Felids*: American Association of Zoos and Aquariums.

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations - uses in assessing rater reliability. *Psychological Bulletin, 86*, 420-428.

Skinner, B. F. (1966). What is the experimental analysis of behavior? *Journal of the Experimental Analysis of Behavior, 9*, 213-218.

Smith, P. K. (1985). The reliability and validity of one-zero sampling: misconceived criticisms and unacknowledged assumptions. *British Educational Research Journal, 11*, 215-220.

Spruijt, B. M., van den Bos, R., & Pijlman, F. T. A. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science, 72*, 145-171.

Steinhauer, G. D. (1984). Preference for predictable small rewards over unpredictable larger rewards. *Psychological Reports, 54*, 467-471.

Stevenson-Hinde, J., & Zunz, M. (1978). Subjective assessment of individual rhesus monkeys. *Primates, 19*, 473-482.

Sulzman, F. M., Fuller, C. A., & Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology, 43*, 795-800.

Sunquist, M. E. (1981). The social organization of tigers (*Panthera tigris)* in Royal Chitawan National Park, Nepal. *Smithsonian Contributions to Zoology, 336*, 1-107.

Tami, G., & Gallagher, A. (2009). Description of the behaviour of domestic dog (*Canis familiaris*) by experienced and inexperienced people. *Applied Animal Behaviour Science, 120*, 159-169.

Terezow, M. G., Nelson, N. J., & Markwell, T. J. (2008). Circadian emergence and movement of captive juvenile tuatara (*Sphenodon* spp.). *New Zealand Journal of Zoology, 35*, 205-216.

Thapar, V. (2004). *Tiger. The ultimate guide*. New York: CDS Books in association with Two Brothers Press.

The Wildlife and Dinosaur Adventure Park Combe Martin (nd). Meet the meerkats Retrieved 19th May, 2011, from http://www.devonthemepark.co.uk/experiences/meet-the-meerkats

Thomson Reuters (2011). Web of Knowledge. Retrieved 24th September, 2011, from http://apps.webofknowledge.com/WOS_GeneralSearch_input.do?SID=P1ecggkhLB8ipcPF7do&product=WOS&search_mode=GeneralSearch&preferencesSaved=

Thornton, A. (2008). Early body condition, time budgets and the acquisition of foraging skills in meerkats. *Animal Behaviour, 75*, 951-962.

Tilson, R., & Nyhus, P. J. (2010). The biology and politics of Sumatran tigers: Crash of the Conservation Garuda. In R. Tilson & P. J. Nyhus (Eds.), *Tigers of the world. The science, politics and conservation of Panthera tigris* (pp. 377-394). London: Elsevier.

Todman, J. B., & Dugard, P. (2001). *Single-case and small-n experimental designs. A practical guide to randomization tests*. Mahwah, New Jersey: Lawrence Erlbaum Associates, Publishers.

Tosini, G., Bertolucci, C., & Foà, A. (2001). The circadian system of reptiles: a multioscillatory and multiphotoreceptive system. *Physiology & Behavior, 72*, 461-471.

Ulm, R. R., Volpicelli, J. R., Cerpa, R., & McIntyre, K. (1987). Weight gain in post-weaned rats as a function of predictable and unpredictable feeding schedules. *Physiology & Behavior, 40*, 287-289.

Ulyan, M. J., Burrows, A. E., Buzzell, C. A., Raghanti, M. A., Marcinkiewicz, J. L., & Phillips, K. A. (2006). The effects of predictable and unpredictable feeding schedules on the behavior and physiology of captive brown capuchins (Cebus apella). *Applied Animal Behaviour Science, 101*, 154-160.

Underwood, H. (1992). Endogenous rhythms. In C. Gans & D. Crews (Eds.), *Hormones, brain, and behavior. Biology of the Reptilia* (Vol. 18, Physiology E). Chicago: The University of Chicago Press.

Van Heygen, E. (2004). The genus *Phelsuma* GRAY, 1825 on the Ampasindava peninsula, Madagascar. *Phelsuma, 12*, 99-117.

Van Metter, J. E., Dana Harriger, M., & Bolen, R. H. (2008). Environmental enrichment utilizing stimulus objects for African lions (*Panthera leo leo*) and Sumatran tigers (*Panthera tigris sumatrae*). *BIOS, 79*, 7-16.

Waitt, C., & Buchanan-Smith, H. M. (2001). What time is feeding? How delays and anticipation of feeding schedules affect stump-tailed macaque behavior. *Applied Animal Behaviour Science, 75*, 75-85.

Wanger, T. C., Motzke, I., Furrer, S. C., & Gruber, B. (2009). Movement patterns and habitat selection of the giant day gecko (*Phelsuma madagascariensis grandis*) in the Masoala rainforest exhibit, Zurich Zoo. *Salamandra, 45*, 147-153.

Wasserman, F. E., & Cruikshank, W. W. (1983). The relationship between time of feeding and aggression in a group of captive hamadryas baboons. *Primates, 24*, 432-435.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS Scales. *Journal of Personality and Social Psychology, 54*, 1063-1070.

Watters, J. (2012). *The importance of correctly classifying behaviour: The case of stereotypic pacing in zoo animals.* Paper presented at the 46th Conference of the International Society for Applied Ethology, Vienna.

Weckel, M. E., Mack, D., Nagy, C., Christie, R., & Wincorn, A. (2010). Using citizen science to map human-coyote interaction in suburban New York, USA. *Journal of Wildlife Management, 74*, 1163-1171.

Weinberg, J., & Levine, S. (1980). Psychobiology of coping in animals: The effects of predictability. In S. Levine & H. Ursin (Eds.), *Coping and health*. New York: Plenum Press.

Weiss, A., King, J. E., & Perkins, L. (2006). Personality and subjective well-being in orangutans (*Pongo pygmaeus* and *Pongo abelii*). *Journal of Personality and Social Psychology, 90*, 501-511.

Weiss, J. M. (1972). Psychological factors in stress and disease. *Scientific American, 226*, 104–113.

Weller, S. H., & Bennett, C. (2001). Twenty-four hour activity budgets and patterns of behavior in captive ocelots (*Leopardus pardalis*). *Applied Animal Behaviour Science, 71*, 67-79.

Wemelsfelder, F. (1997). The scientific validity of subjective concepts in models of animal welfare. *Applied Animal Behaviour Science, 53*, 75-88.

Wemelsfelder, F. (2007). How animals communicate quality of life: the qualitative assessment of behaviour. *Animal Welfare, 16*, 25-31.

Wemelsfelder, F., Batchelor, C., Jarvis, S., Farish, M., & Calvert, S. (2003). *The relationship between qualitative and quantitative assessments of pig behaviour.* Paper presented at the 37th International Congress of the International Society for Applied Ethology, Abano Terme, Italy.

Wemelsfelder, F., & Birke, L. (1997). Environmental challenge. In M. C. Appleby & B. O. Hughes (Eds.), *Animal welfare* (pp. 35-47). Oxon: CABI Publishing.

Wemelsfelder, F., Hunter, E. A., Mendl, M. T., & Lawrence, A. B. (2000). The spontaneous qualitative assessment of behavioural expressions in pigs: first explorations of a novel methodology for integrative animal welfare measurement. *Applied Animal Behaviour Science, 67*, 193-215.

Wemelsfelder, F., Hunter, T. E. A., Mendl, M. T., & Lawrence, A. B. (2001). Assessing the 'whole animal': a free choice profiling approach. *Animal Behaviour, 62*, 209-220.

Wemelsfelder, F., Nevison, I., & Lawrence, A. B. (2009). The effect of perceived environmental background on qualitative assessments of pig behaviour. *Animal Behaviour, 78*, 477-484.

Wemmer, C., & Fleming, M. J. (1975). Management of meerkats. *International Zoo Yearbook, 15*, 73-77.

Werner, Y. L., & Chou, L. M. (2002). Observations on the ecology of the arrhythmic equatorial gecko *Cnemaspis kendallii* in Singapore (Sauria: Gekkoninae). *The Raffles Bulletin of Zoology, 50*, 185-196.

Werner, Y. L., Okada, S., Ota, H., Perry, G., & Tokunaga, S. (1997). Varied and fluctuating foraging modes in nocturnal lizards of the family Gekkonidae. *Asiatic Herpetological Research, 7*, 153-165.

Werner, Y. L., Takahashi, Y., Yasukawa, Y., & Ota, H. (2004). The varied foraging mode of the subtropical eublepharid gecko G*oniurosaurus kuroiwae orientalis*. *Journal of Natural History, 38*, 119-134.

Wheler, C. L., & Fa, J. E. (1995). Enclosure utilization and activity of Round Island geckos (*Phelsuma guentheri*). *Zoo Biology, 14*, 361-369.

White, W., & Timberlake, W. (1995). Two meals promote entrainment of rat food-anticipatory and rest-activity rhythms. *Physiology & Behavior, 57*, 1067-1074.

Whitham, J. C., & Wielebnowski, N. (2009). Animal-based welfare monitoring: using keeper ratings as an assessment tool. *Zoo Biology, 28*, 545-560.

Wielebnowski, N. (1999). Behavioral differences as predictors of breeding status in captive cheetahs. *Zoo Biology, 18*, 335-349.

Wikelski, M., & Hau, M. (1995). Is there an endogenous tidal foraging rhythm in marine iguanas? *Journal of Biological Rhythms, 10*, 335-350.

Willmer, P., Stone, G., & Johnston, I. (2004). *Environmental physiology of animals* (Second ed.). Malden, MA: Blackwell Publishing.

Wilson, W. L., & Wilson, C. C. (1968). *Aggressive interactions of captive chimpanzees living in a semi free-ranging environment*. New Mexico: Aeromedical Research Laboratory.

Winne, C. T., & Keck, M. B. (2004). Daily activity patterns of Whiptail Lizards (Squamata: Teiidae: *Aspidoscelis*): a proximate response to environmental conditions or an endogenous rhythm? *Functional Ecology, 18*, 314-321.

Young, R. J. (2003). *Environmental enrichment for captive animals*. Oxford: Blackwell Science Ltd.

Zeiler, M. D. (1968). Fixed and variable schedules of response-independent reinforcement. *Journal of the Experimental Analysis of Behavior, 11*, 405-414.

ZSL (2012). Help Researchers. Retrieved 8th May, 2012, from http://www.zsl.org/conservation/get-involved/index,292,ZI.html