UNIVERSITY OF DERBY

A Comparison of Methods of Quantifying and Assessing the Behaviour and Welfare of Bornean Orangutans (*Pongo pygmaeus*): a Case Study at Twycross Zoo

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Abbreviations used in the thesis

ACTH – Adrenocorticotropic Hormone
AZA – Association of Zoos and Aquariums
BIAZA – British and Irish Association of Zoos and Aquariums
BSB – Behaviour – Space use Bipartite
BBB – Behaviour – Behaviour Bipartite
CRH – Corticotropin Releasing Hormone
DEE – Daily Energy Expenditure
EAZA – European Association of Zoos and Aquariums
EIA – Enzyme Immunoassay
fGCM – faecal Glucocorticoid Metabolites
GC – Glucocorticoid
GLS – Generalised Least Squares
IUCN – International Union for Conservation of Nature
LASSO – Least Absolute Shrinkage and Selection Operator
LH – Luteinising Hormone
ML – Maximum Likelihood
mtDNA – mitochondrial Deoxyribonucleic Acid
mya – million years ago
nMDS – non-metric Multidimensional Scaling
PCA – Principle Component Analysis
REML – Restricted Maximum Likelihood
SDB – Self-Directed Behaviour
SEAZA – South East Asian Zoos Association
SNA – Social Network Analysis
SSB – Space use – Space use Bipartite
TUC – Time Use Category
UNEP – United Nations Environment Programme
WAZA – World Association of Zoos and Aquariums

Preface

The work contained within this thesis has been solely authored by the doctoral candidate, with only guidance and direction given by the supervisory package. Where work has been submitted for publication and contained within this thesis, the candidate is the primary author, with only guidance given by the co-authors. The programme of research conducted for this thesis, the results obtained, and the wider reading and resulting thoughts and conclusions have been disseminated through various channels and are listed below:

Conferences:

Poster:

Bentley, R. H., Bulling, M. T., Huck, M. & Vahed, K (2014) The Impact of Food-Based Enrichment on the Welfare of Captive Bornean Orangutans (*Pongo pygmaeus*), presented to ASAB Easter Conference, Sheffield, April 7-9, 2014

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Talk:

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Abstract

The maintenance of both the psychological and physiological health of captive animals is a key priority of modern zoos. Recognising that characteristics of the captive environment have the potential to decrease animal welfare, methods for quantifying and assessing welfare have been developed as part of the process for improving animal welfare. Traditionally, observations of animal behaviour and quantifying time budgets in relation to those of the animals' wild counterparts have been utilised to assess animal welfare. Hormonal assays have also been implemented to quantify the physiological stress response of animals in captivity and identify the extent of stress being experienced. Each of these methods focuses on a different indicator of animal welfare, is quantified in different ways and provides a different perspective on the welfare of the animals. Given the limited time and financial budgets available to zoos and animal carers, identifying the most appropriate method of welfare assessment would be advantageous in helping to secure the best possible health of captive animals and to maximise their value in captivity.

This thesis implemented both behavioural observations and hormonal assays to identify the strengths and weaknesses of each methodology, and make recommendations for future research. The study involved a group of four Bornean orangutans (*Pongo pygmaeus*) housed at Twycross Zoo. Behavioural observations involved continuous group sampling and the development of an ethogram to record a comprehensive account of orangutan activity over the course of a 12 week enrichment programme. Simultaneous to these observations, faecal samples were collected from each orangutan and processed via Enzyme Immunoassay (EIA) to quantify levels of faecal glucocorticoid metabolites (fGCM) in each sample.

While recognising the recent developments in ecological analytical methods, the capacity for extending network analysis beyond the application to social networks, and its use as a welfare assessment tool were explored. Behavioural and space-use networks were developed using data from a second study of the orangutans housed at Twycross Zoo. The flexibility of network analysis in visually representing different data types allowed for the intuitive representation of complex behavioural data. Further research investigated the use of network metrics in providing deeper insights into animal behaviour and space use patterns. In addition, bipartite networks were assessed for their potential to detect and show patterns in the relationships between two sets of behavioural data.

Each of the methods used had a number of strengths and weaknesses, but importantly each contributed a different perspective in the assessment of behaviour patterns and welfare, suggesting that an integrated approach to behaviour studies utilising several methods would be ideal. Cost and logistic constraints make this unlikely in most cases. However, the thesis ends with a look to the future and the recognition that the current rapid development of technology for use in animal behaviour studies, coupled with equally rapid development of analytical techniques, may help to dramatically increase the amount of information gained from the average animal behaviour study in the future. Such improvements have never been more urgent, with the requirement for understanding animal behaviour in light of current extinction rates within the context of habitat destruction and climate change. It is hoped that this thesis will make a contribution to improving future animal behaviour and welfare studies by providing an assessment of both traditional methods of study as well as demonstrating the use and potential of new ways of applying network analysis within such studies.

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This is not what I had planned at the start of my university career but somehow I have found myself writing a PhD thesis and there are many people who deserve my thanks for making it possible.

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Chapter ONE: Introduction to the Thesis

1.1 Chapter overview

This chapter sets the context of the thesis. The increasing rate of human impacts on natural habitat leading to the current rapid rates of extinction of species means that conservation strategies and projects need to be as effective as possible. Zoos and aquariums have a long history of involvement with supporting conservation projects, but in more recent years this involvement has increased dramatically on a global scale. Zoos can help to provide valuable knowledge about species ecological and physical needs, help to educate the general public about conservation and the need for it, as well as supporting populations of rare species to supplement current declining species and to provide a valid reservoir of individuals for reintroduction programmes. As such, zoos need to ensure that animal health and welfare is maximized within the constraints of the captive environment. Underlying the ability to do this is a requirement to be able to quantify and assess behaviour patterns in relation to welfare.

This chapter initially gives an overview of the roles zoos play in conservation, highlighting the requirements for high levels of animal welfare. This is followed by a summary of one of the main methods that zoos employ to increase welfare and to reduce stress and frequency of stereotypic behaviours, environmental enrichment. Studies of animal behaviour in zoos tend to utilise similar methodologies and these are briefly summarised. This thesis uses Bornean orangutans (*Pongo pygmaeus*) as a model species around which methods of study are developed and investigated. Thus the chapter details the taxonomy, behaviour in the wild and ecology of orangutans. It outlines the current state of wild orangutan populations and details the threats to their survival. The chapter finishes by bringing the issue back to captivity and draws attention to captive orangutan populations. The importance of positive welfare in captive populations is explored, followed by a justification for using them as a model species for this thesis.

The next chapter will build on this foundation by quantifying the current state of the study of the use of enrichment within captive ape populations, highlighting the relative scarcity of such research on orangutans, providing further justification for their use in this thesis.

1.2 Zoos, their roles and responsibilities

The modern zoo is far removed from the original zoos focusing on using animals to entertain the public, with much greater focus on educating the public and in supporting research and conservation. In this section the evolution of the roles of zoos from their origins is examined along with a brief examination of the main drivers for this change.

1.2.1 Origins of the modern zoo and the associated legislation

Modern day zoos are defined as "permanent establishments where animals of wild species are kept for exhibition to the public for 7 or more days of the year, with the exception of circuses, pet shops". The first modern zoo was established in Vienna in 1752 by the Holy Roman Emperor Francis I (Alexander and Alexander, 2008). The emphasis of zoos during this time was heavily placed on the entertainment value of the animals and the opportunity to view rare and exotic animals, mostly within barred cages and housing that would be considered unsuitable today (Kisling, 2001). The nineteenth century saw a change of interest, focusing on animals as subjects of scientific study and led to the establishment of the Jardins de Plantes in Paris and later, Regent's Park Zoo in London (Hosey *et al*., 2009). During the second half of the nineteenth century, zoos became established worldwide and attitudes towards animal enclosures began to change (Rees, 2011).

In 1907 the animal trader Carl Hagenbeck revolutionized captive animal enclosures, using moats and mountainous-like landscapes to replicate the natural habitats of exotic animals (Alexander and Alexander, 2008; Rees, 2011). This type of enclosure became the blueprint upon which subsequent zoos have been based (Rees, 2011). Chester Zoo, built by George Mottershead opened in 1931 and was based on Hagenbeck's ideas, using moats and ditches to separate the animals and the public (Rees, 2011). The change in direction by Hagenbeck and Mottershead initiated the shift of purpose of zoos form purely entertainment to the conservation and breeding of rare species (Alexander and Alexander, 2008).

Over the following years, an understanding of the biological functioning of animals increased significantly and led to establishment of ethology as a scientific discipline (Broom, 2011). During the 1960s animal welfare research took large strides in establishing the "five freedoms", largely in response to Ruth Harrison's book "Animal Machines" (Broom, 2011;

McCulloch, 2013). From then on, a great increase in animal welfare research and public concern has been recorded (Broom, 2005).

As a result of growing public pressure, Great Britain passed the Zoo Licencing act of 1981 which aims to ensure that animals housed in institutions falling under the definition of ‘zoo’ are provided with a suitable environment and the opportunity to express what is classed as 'normal behaviour' (DEFRA, 2012). This framework has more recently been extended to allow legislation to be shared regionally. The EC Zoos directive (Directive 1999/22/EC, 1999) was a major step in European zoo legislation (Hosey *et al.*, 2009). The Directive lays out requirements for the licencing and inspection of zoos within Member States. Critically, it promotes record keeping, maintaining standards of animal care and encourages participation in education and conservation programmes (Hosey *et al.*, 2009). Article 3 of the directive requires Member States to ensure that animals are provided with appropriate environments which allow them to exhibit their natural behaviours taking into account the specific needs of the species and the individuals. The directive requires that the Member States establish a licensing and inspection system and enforce the requirement of Article 3 (Directive 1999/22/EC, 1999). However, EC directives are not directly binding on Member States and therefore the legislation laid out must be incorporated in the national legislation. In order to accommodate this, the Zoo Licensing Act 1981 was amended to form the Zoo licensing Act 1981 (amendment) (England and Wales) Regulations 2002. The new legislation for England and Wales covers a wide range of institutions, from large zoos to wildlife parks, and incorporates the requirements for zoos to contribute to conservation and education, in line with the EC Zoos Directive. As a result of this, UK zoos must now demonstrate involvement in conservation and research as well as educational programmes for the public awareness of conservation (Hosey *et al.*, 2009).

Most zoos around the world belong to a zoo association (e.g. the Association of Zoos and Aquariums (AZA), the British and Irish Association of Zoos and Aquariums (BIAZA), the World Association of Zoos and Aquariums (WAZA). These do not usually form legislation but they are part of the regulatory framework, generating a set of voluntary standards for zoos to follow. These zoo associations promote and facilitate good practice and professionalism. They set out guidelines and principles that their members are required to follow in order to gain and maintain accreditation, and state that it is their primary goal to ensure the highest standard of welfare to animals in their care. Zoos in Britain and Ireland are members BIAZA. Founded in

1966, BIAZA is a professional organisation which represents almost all significant zoos and aquariums in Great Britain and Ireland, helping to coordinate their efforts in conservation, education and research (BIAZA, 2011).

1.3 Animal welfare in captivity

Welfare has been defined by a wide range of authors and organisations, but most refer to both mental and physical health as well as an animals ability to adapt to its environment (Koknaroglu and Akunal, 2013), reflecting that "animal welfare" encompasses both the physical and psychological well-being of animals and is a state of living with all the basic needs being met. For example, the European Association of Zoos and Aquaria (EAZA) state that welfare refers to the "physical, behavioural and social well-being of animals through the provision of appropriate conditions for the species involved, including but not necessarily limited to, housing, environment, diet, medical care and social contact where applicable" (EAZA, 2008). This makes it clear that animal welfare concerns all elements of an animal's environment and that it is the responsibility of animal keepers to ensure that all of these elements are at an appropriate standard to promote positive welfare. In order to support putting this into practice the Animal Welfare Act (2006) lays out a set of provisions that the persons responsible must follow in order to provide the appropriate care for captive animals (Animal Welfare Act, 2006).

With the clear requirements and obligations to maintain high standards of welfare comes the need to be able to monitor and assess levels of welfare. The species' behaviours in the wild can be used as a benchmark against which to measure the welfare of captive animals, implying that the absence of species-typical behaviour is a consequence of poor welfare. To date, there has been a large focus on using patterns of behaviour in the wild as a measurement of good welfare (Veasey *et al.*, 1996). However, this approach may be regarded as too simplistic and some have suggested that there should be a shift towards providing a suitable environment that allows animals to simply exhibit behavioural freedom rather than focussing on aiming for an imitation of the behavioural patterns observed in the wild (Claxton, 2011). Others suggest an approach which is based on an animal's ability to cope within its captive environment as an indicator of welfare (Hill and Broom, 2009), recognising that animals are perceptive of a number of different factors encountered in their environment and physiological stress may manifest as abnormal behaviours.

1.3.1 The biological response to stress

The habitats of wild animals are dynamic environments with varying levels of unpredictability. Throughout evolution, animals have adapted to the predictable components of their environments using physical, morphological and behavioural modifications (Möstl and Palme, 2002). The unpredictable events represent stressful stimuli for animals and cause the activation of the physiological stress response, a biological defence involving the mobilisation of energy allowing the animal to cope with the stressor (Moberg, 2000; Sapolsky, 2002). Although a well-established indicator of good animal welfare is the absence of stress (Möstl and Palme, 2002), stress is an unavoidable part of everyday life and it is not necessarily detrimental for an animal to experience stress to an extent (Moberg, 2000). When an animal is faced with a stressor (e.g. a predator or rival male), it is perceived as a threat to homeostasis by the brain and Corticotropin Releasing Hormone (CRH) is released from the hypothalamus (Sapolsky, 2002). This triggers adrenocorticotropic hormone (ACTH) release from the anterior pituitary gland (Elder and Menzel, 2001). ACTH targets the adrenal cortex and causes a rise in the production of glucocorticoids (Charmandari *et al.*, 2005). Glucocorticoids (GCs) help to mobilise the energy stored in body fat and this can be transferred to the tissues the animal needs to utilise to cope with the stressor, for example to run away from a predator or participate in a fight (Sapolsky, 2002). Environmental stressors including periods of drought or extreme cold are known to force evolution by selecting for genes which code for an effective coping response, imposing directional selection on a population (Hoffmann and Hercus, 2000; Wright, 2004).

Animals in captivity face a different range of stresses to individuals in the wild. For example, they do not need to escape predation and the regular supply of food reduces the pressure to hunt and forage (Newberry, 1995). However, natural behaviours are motivated by elements of the environment which are absent in the typical zoo environment (Morgan and Tromborg, 2007), and zoo housing often prevents animals from engaging in behaviours that are typical of their species. For example, a young male may have an intrinsic desire to mate but in the absence of a female this behaviour is thwarted. When stressed in captivity, the same stress response is initiated. In the wild, the mobilised energy stores are expended by running away from a predator or towards a prey item or fighting. But in captivity, fixed barriers restrict animals from escaping a stressor and other stressful stimuli remain permanent components of the environment. Therefore, the animal can be exposed to sustained stressors and the stress response is not terminated, or is repeatedly triggered. This prolonged activation of the stress response can have adverse consequences for the animal (Charmandari *et al.*, 2005; Wingfield, 2013) resulting in immunosuppression, reduced growth, halted reproduction and the expression of abnormal behaviours (Moberg, 2000; Charmandari *et al.*, 2005; Muehlenbein *et al.*, 2012). The reasons for these effects lie in the fact that GCs are involved in the immune defence, as part of the feedback system which reduces the inflammatory/immune response. Under chronic stress, the concentration of GCs reach levels which cause suppression of the immune response, leaving the animal susceptible to infectious diseases (Möstl and Palme, 2002). In terms of energy, the most expensive bodily function, particularly for females, is reproduction (Sapolsky, 2002) and it is often interrupted when challenged with stress. The female reproductive system relies on a carefully timed sequence of hormone releases (Moberg, 1985) and any interruption of these endocrine events may endanger reproductive success. Ovulation relies on precise timing between the release of Luteinising hormone (LH) and oestrus behaviour. Therefore the hormonal changes involved in the stress response may result in failure to release an egg, jeopardising the reproductive opportunity (Moberg, 1985). Aside from these deleterious effects of stress, animals may also develop abnormal or stereotypic behaviours as a method of coping with an inadequate environment.

1.3.2 Abnormal and stereotypic behaviours

In the wild, animals are exposed to an array of sensory inputs. Sounds, smells and sights of the wild habitat have a substantial effect on an animal’s physiology and psychology (Wells, 2009a) and hence, behaviour. It is therefore widely accepted that a lack of sensory input in the captive environment is likely to inhibit species' natural behaviours and may lead to the development of abnormal or stereotypical behaviours (Marriner and Drickamer, 1994).

Abnormal behaviours are considered to be behaviours that occur in captivity but not in natural settings, or behaviours that occur much more or less frequently in captivity than in the wild (Erwin *et al.*, 1979). Examples of abnormal behaviour exhibited in captive primates include regurgitation and reingestion (Baker and Easley, 1996), hair pulling (Mason *et al.*, 2005), and excessive aggression (Honess and Marin, 2006). Different abnormal behaviours are thought to result from different causes. Regurgitation and reingestion behaviours are thought to develop from a desire to increase the amount of time spent feeding, due to a lack of foraging opportunities (Akers and Schildkraut, 1985). In contrast, over-grooming, where the animal plucks out fur, hair, or feathers of itself or another individual, leaving bald patches of skin

(Hosey and Skyner, 2007), is a symptom of psychological stress and may be indicative of poor health (Fraser *et al*., 2008; Reinhardt, 2005). Excessive aggression between captive individuals is often a result of tension between individuals which may be generated by the captive environment not being suitable for the species in question or from the demographic group composition differing from those found in the wild (Wells, 2005).

Stereotypic behaviours are a subset of abnormal behaviours, and are considered to be those that are unvarying and repetitive with no apparent function (Mason, 2006). They include bar-biting and gum chewing in sows, tongue rolling by cows (Bergeron *et al*., 2006) and pacing in carnivores (Clubb and Vickery, 2008). Investigation suggests that these behaviours are linked to natural behaviours (Mason and Rushen, 2008). Bar-biting is thought to be triggered by a feeding routine which is not sufficient for the individual's needs, whilst pacing is thought to result from the desire to forage, hunt or patrol a territory but being unable to perform these activities in captivity (Mason and Rushen, 2008). However, these behaviours do not achieve any goal other than acting as a coping mechanism in order to fulfil an internal compulsion (Mason, 1991; Wechsler, 1995). An alternative cause of stereotypic behaviours can be due to the enclosure providing an environment that causes persistent stress or fear (Shyne, 2006). The form of stereotypic behaviour, once adopted, tends to be unvarying and this is thought to be due to the rigidity of the captive environment and the lack of control that captive animals have over their environment (Broom, 1991). Performing repeated patterns of behaviour releases endorphins in the brain which appear to reduce the negative emotions experienced by the animal (Cronin *et al*., 1986). Importantly these behaviours occur in bouts that may be particularly sustained and difficult to erase (Mason and Latham, 2004). These problems can be exacerbated when the stereotypic behaviours are associated with particular times of day or locations within the enclosure, as this can lead to them eventually becoming independent of the original stimulus and a permanent occurrence in the animal’s routine (Mason and Latham, 2004). Endorphins can be addictive, therefore the animal may continue performing stereotypic behaviours even when their environment has been improved (Spedding, 2000).

In addition to the implications for an animal's welfare, allowing animals to develop abnormal behaviours compromises the zoos influence in conservation and education (Honess and Marin, 2006). Animals displaying stereotypic behaviours have little resemblance to their wild counterparts, decreasing their potential in breeding and conservation efforts as well as their value as an educational resource (Mason *et al*., 2007). To combat the problems caused by captive conditions and to provide variable suitable habitat for captive animals, enrichment devices are often utilised (Mason *et al.*, 2007). Enrichment has the potential to reduce the prevalence of abnormal and stereotypic behaviours but also to avoid their occurrence in the first place (Young, 2003).

1.4 Enrichment for captive animals

Zoos and other captive animal institutions have long endeavoured to enhance captive animal environments via modifications to enclosures. Researchers such as Robert Yerkes (1925) were the first to highlight the importance of physical and social environments for captive animals but it is only relatively recently that this technique has been labelled enrichment (Mellen and Sevenich MacPhee, 2001; Shepherdson, 2003). Definitions of enrichment have varied and the goals of enrichment have varied between promoting behaviours seen in the wild and preventing (or reducing) stereotypic or abnormal behaviours (Gilloux *et al.*, 1992). Some authors have viewed enrichment more broadly as a technique designed to improve biological functioning of captive animals using environmental modifications resulting in an improvement in animal welfare (de Azevedo *et al.*, 2007; Newberry, 1995). Similarly, Shepherdson (1998) defined enrichment as, “animal husbandry techniques aimed at enhancing the quality of care to captive animals by providing the necessary stimuli for optimal well-being”.

Generally, there is a strong focus on the need for enrichment to encourage ‘natural behaviours’ (Chamove, 1989; Gilloux *et al.*, 1992; Celli *et al.*, 2003). Natural behaviour may be regarded as a range of behaviours displayed as an adaptation to an animal’s natural environment to increase its fitness (Špinka, 2006). A second potential reference for this latter definition in the case of captive bred animals, is the behaviour of the animal’s wild ancestors before captivity (Newberry, 1995).

Given the range of definitions and views, Mellen and Sevenich MacPhee (2001) proposed that there is no general definition for the objectives of enrichment to suit all techniques that are used. Rather, they suggest that for each species, a plan is devised to ensure that the captive environment meets all the animal’s needs taking into consideration the species’ natural history and constraints of the enclosure. Similarly, Wells (2009a) concluded that enrichment should encourage behaviours seen in the wild, increase an animal’s ability to cope with the challenges of captive life, increase the use of its environment, and reduce or eliminate patterns of abnormal behaviours.

The goals of enrichment for different studies and institutions vary, but if the ultimate goal of containing animals in captivity is for the purposes of research, conservation and ultimately the enhancement or creation of wild populations, the aims of enrichment should be specific to these goals. For effective use of animals as an educational resource or in studies aiming to understand natural behaviour patterns, enrichment should aim to conserve the behavioural and physical repertoires of the animals. The concern is that the captive environment has the potential to induce abnormal and or stereotypical behaviours. Specifically for the purposes of conservation, enrichment should aim to promote or maintain the reproduction of genetically viable and varied captive populations. Animals in captivity should have the freedom to mate naturally and produce viable offspring as part of their natural behavioural repertoire, in order to fulfil their role in zoo conservation. Critically, captive animals should also display typical parental care behaviour (Carlstead and Shepherdson, 1994). Finally, in terms of conservation, enrichment should enhance the survival probabilities of animals that are to be released into the wild (Shepherdson, 1994). If animals are part of a breeding program, they should be fully equipped with the behavioural repertoire of their wild counterparts in order for them to fully integrate and survive in the wild. Enrichment that promotes wild behaviours with minimal direct human training is likely to be most successful at this objective. However, as most animals kept in captivity are not part of a breeding program and will never be released into the wild (ZSL, 2017), their familiarity with an environment resembling a wild habitat may be less important. Some authors advocate focusing on providing an environment that meets the needs of the animals in captivity, ensuring optimum welfare, rather than attempting to replicate a wild habitat (Claxton, 2011). Enrichment should therefore reduce potential sources of stress in captivity and provide animals with the ability to cope with environmental stress (Mellen and Sevenich MacPhee, 2001).

Captive animals are limited to behaviours afforded by their enclosure environment, with limited access to resources and predictable feeding times. The opportunity for captive animals to exercise behavioural freedom is therefore constrained. It is thought that the addition of enrichment items will enhance the environment and provide options for how an animal may spend its time budget. Enrichment may simply require additions or alterations to the animal’s environment that allow it more choices of how to distribute its time, resulting in more wild-like or natural behaviours (Carlstead and Shepherdson, 1994). The idea that captive animals require a behavioural freedom appears in many of the goals of enrichment laid out by researchers (Morimura, 2003).

In summary, the objective of enrichment is ultimately to promote natural behaviours in captive animals and to increase the level of behavioural diversity displayed as well as reducing or eliminating any abnormal or stereotypic behaviours observed (Carrasco *et al*., 2009). Thus for the purposes of this thesis, enrichment is defined as:

C*hanges to an animal's captive environment or husbandry routine that provide behavioural choices, promote the natural behaviours of wild conspecifics and/or reduce or eliminate abnormal and stereotypic behaviours, ultimately improving or enhancing the welfare of the animals involved.*

Shepherdson (1994) highlights the importance of linking enrichment with a positive outcome which should be associated with the natural history of the species concerned. Enrichment can restore a contingency between the performance of a behaviour and the appropriate consequence, such as finding food. This strategy enrichment is often the most successful (Tarou and Bashaw, 2007). It is important that animals have a degree of control over this experience and have the appropriate level of cognitive ability to make the connection between behaviour and consequence. Enrichment that provides extrinsic reinforcement (when the result of the behaviour is external to the behaviour itself, therefore increasing the motivation to perform the behaviour) is likely to produce a prolonged effect as opposed to enrichment that relies on the behaviour itself being enriching (Tarou and Bashaw, 2007).

In order to promote natural behaviours, it follows that the enrichment should mimic the challenges faced in the animal natural habitat, as only then can we be sure that the enrichment will appeal to their cognitive and physical abilities (Csatádi *et al*., 2008). Using enrichment in this way works on the hypothesis that if animals are given the opportunity to perform these natural behaviours, they are less likely to display abnormal or stereotypic behaviours (Mason *et al*., 2007). Therefore it is important that enrichment is used to alter behaviours correctly and so the type of enrichment device used is important.

1.4.1 Categories of enrichment for captive animals, focussing on use with captive primates

Enrichment can utilise tools of a number of different forms, including nutritional, sensory, physical, occupational and social enrichment (Bloomsmith *et al*., 1991). Therefore, animal care staff have many options when designing an enrichment programme.

Nutritional (or feeding) enrichment involves the manipulation of food or the methods of providing the food (Hoy *et al*., 2010). Puzzle feeders, which require individuals to manipulate the enrichment device in some way in order to gain access to food, have the potential to increase the time spent foraging and can be very effective for animals with greater cognitive abilities. For example, termite fishing behaviour, where individuals use a small stick to probe and retrieve termites, has often been observed in wild primates (Brent and Eichberg, 1991) and enrichment devices (e.g. artificial termite mounds) can be used to mimic behaviour in captivity (Nash, 1982). However, simply altering the way in which an animals' diet is presented can be very effective. For example, such a method was used successfully in engaging captive gorillas (*Gorilla gorilla gorilla*) in foraging behaviour, when three different methods of scattering their food was trialled (Ryan *et al*., 2012). Keepers find nutritional enrichment particularly useful as it is easily implemented, can be provided at very little costs and requires very little time to prepare (Hoy *et al*., 2010).

Sensory enrichment is designed to trigger one or more of an animal's senses (Wells, 2009b) and includes visual, auditory and olfactory stimuli (Young, 2003). For example, the playing of classical music had some small benefit to captive gorillas over the playing of sounds similar to those found in their natural habitat (Wells *et al*., 2006). Playing of music also calmed chimpanzees (*Pan troglodytes*) housed in a laboratory (Howell *et al*., 2003). Olfactory enrichment does not appear to be particularly useful with apes (Wells *et al*., 2007), but the use of visual enrichment via videotapes was successful in reducing the display of abnormal behaviour by Japanese macaques (*Macaca fuscata;* Ogura, 2012). The apparent difference in effectiveness of olfactory and auditory enrichment treatments of apes in the literature highlights the importance of using appropriate enrichment treatments.

Physical enrichment involves alterations to the animal enclosure or the addition of accessories to the enclosure (Young, 2003). This form of enrichment can add complexity to the enclosure, providing further sensory stimuli to the animals and enabling a greater choice in behaviour patterns. Adding a selection of permanent or semi-permanent devices provides an even greater range of behavioural options to choose from (Schapiro *et al*., 1991). For primates physical enrichment can be particularly important. For example, the addition of platforms, ropes or poles allows animals to explore forms of locomotion (Videan *et al*., 2005), providing both physical and mental stimulation through natural forms of behaviour. Installing such devices can be involved, time consuming and expensive. However, effective physical enrichment treatments need not be complicated and expensive. For example, adding substrates such as leaf litter and vegetation to an enclosure are very cheap and can be effective in promoting exploratory behaviour (Swaisgood and Shepherdson, 2005).

Occupational enrichment requires animals to complete a task or solve a problem, and includes animal training (Laule and Desmond, 1998; Laule and Whittaker, 2007). There have been many attempts to use computer-based tasks as enrichment for captive apes with some success (Tarou *et al*., 2004; Perdue *et al*., 2012; Mallavarapu *et al*., 2013). For example, artificial termite mounds have been used successfully to stimulate tool-use behaviour in chimpanzees (Nash, 1982), orangutans (Nakamichi, 2004), and bonobos (Boose *et al*., 2013), offering occupational enrichment due to the requirement to manipulate items such as twigs in order to gain access to food. Occupational enrichment has the potential to be particularly effective when used in impoverished enclosures, but devices can require substantial time and financial cost to implement (especially those involving computers), and there has been relatively little research into this form of enrichment (de Azevedo *et al*., 2007).

Social enrichment involves introducing other conspecific individuals, and has been suggested that this form of enrichment is the most beneficial enrichment for reducing abnormal behaviour in social species (Reinhardt *et al*., 1987; Bloomsmith and Lambeth, 2000). However, sourcing new individuals and the potential stress of introducing group members means that it is a complex form of enrichment to provide (Amrein *et al*., 2014). Social enrichment may also involve contact with contraspecifics. For example, a mixed species exhibit housing both orangutans and siamangs (*Symphalangus syndactylus*) was successfully established and shown to be enriching for both species (Pearson *et al*., 2010). Interactions, between animal keepers and captive animals are considered to be enriching as they offer stimulation whilst preserving a level of predictability within the environment, thereby reducing stress in the animals (Claxton, 2011).

With such a range of enrichment strategies available to animal care staff, it is important to understand the reasons for providing enrichment and ways in which their success may be evaluated. Enrichment is used frequently as part of the normal husbandry protocols of many zoos (Young, 2003). In contrast, the scientific evaluation of their effect receives less attention (Hoy *et al*., 2010). In order to provide successful enrichment and to efficiently improve or maintain the positive welfare of captive animals, institutions need to share their experiences and expertise and build up evidence based databases for the different enrichment methodologies. This would allow for a greater more robust knowledge base addressing ways of tackling abnormal behaviours and decreasing time spent in unhealthy inactivity by captive animals (Young, 2003).

Animals with higher cognitive functioning are particularly vulnerable to developing abnormal or stereotypic behaviours (McGrew, 1981). This is particularly important as over the last decade there has been increasing evidence that a wider range of animals have significant cognitive abilities. For example New Caledonian crows (*Corvus moneduloides*) are now known to display innovation in tool use in problem solving tasks (Taylor *et al*., 2010). Such animals are able to use more complex forms of enrichment which target their natural abilities. This has long been recognized as being the case particularly for apes. Constraints imposed by the captive environment may be particularly pronounced for all great apes which have well developed social skills and use more complex approaches to foraging and communication than other primates (Begun, 2004). The captive environment does not always provide apes with the opportunity to engage in these behaviours, leading to boredom, frustration and eventually stress (Celli *et al*., 2003; Csatádi *et al*., 2008).This highlights the necessity to extend our knowledge of great ape enrichment approaches.

Apes have been consistently used as models of human evolution throughout psychological and anthropological research (Sayers *et al*., 2008). Chimpanzees in particular have become the focus of extensive research into our evolutionary past, and their prevalence in captive settings (including laboratories, zoos and rescue centres) has maintained them as a high priority in enrichment programmes (Bloomsmith and Else, 2005). Their natural habitat and behavioural needs have been researched relatively widely over the decades providing much knowledge to support the effective application of enrichment treatment for this species. However, other species of apes have been relatively neglected in such research, and this is particularly true for orangutans (*Pongo* spp.). As the great ape species most distantly related to ourselves (*Homo sapiens;* Stauffer *et al*., 2001), orangutans are given less attention with regards to cognitive research. However, decline in the wild has put them at a high risk of extinction over the coming decades (Marshall *et al*., 2016) and puts added pressure on zoos to maintain viable captive populations (Shepherdson, 1994).

1.5 Orangutans

Among the remaining apes, orangutans (*Pongo* spp.) have received less attention with regards to understanding their housing requirements and enrichment needs, and are often maintained in groups that do not meet their social needs. Naturally solitary animals, orangutans are often housed with other conspecifics in groupings resembling the group structuring of gorillas, immediately providing a potential cause of behavioural problems (Weingrill *et al*., 2011). Coupled with their considerable intelligence as revealed by cognitive testing (Forss *et al*., 2016), there is a strong need for the development of environmental enrichment for captive orangutans. However, the correct employment of enrichment with captive orangutans requires a thorough understanding of their ecology and behaviour in natural environments.

1.5.1 The taxonomy and distribution of the orangutan genus (*Pongo* spp.)

The ape superfamily (Hominoidea) consist of gibbons (family: Hylobatidae), gorillas (*Gorilla* spp.), chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*), orangutans (*Pongo* spp.) as well as humans (*Homo sapiens*). It is a branch of the parvorder Catarrhini, but is separated from the Old World monkeys (which include baboons, macaques and langurs). The great ape family (*Hominidae*) excludes all gibbon species, classed as the 'lesser apes', The lesser apes were the first group to separate from the other apes and are known to have smaller brains relative to body size than all of the other apes (Semendeferi *et al*., 2002).

The divergence of orangutans from the human lineage, has been dated at 11.3±1.3 million years ago (Stauffer *et al*., 2001), making the orangutan grouping the most distant from humans in the hominin lineage. Orangutans are categorised under the subfamily *Ponginae*, which separates them from the other great apes in Homininae, and are placed within the genus *Pongo* (Guy *et al*., 2003).

Fossils found in the Siwaliks of Pakistan have been dated to 12.5 million years ago (mya) and, as no earlier fossils have been found, this is the minimum estimate for the origin of the orangutan lineage and their divergence from the human lineage (Jones *et al*., 1992). Current wild orangutan populations can only be found in Indonesia. However, fossil evidence shows that they once inhabited South East and Mainland Asia (Locke *et al*., 2011; Wich *et al*., 2009). All other great ape species (excluding humans) inhabit only Africa as wild populations (Caldecott and Miles, 2005).

Analysis of mitochondrial DNA (mtDNA) has revealed two distinct species of orangutan which are thought to have speciated 400,000 years ago (Locke *et al*., 2011). The Sumatran orangutan (*Pongo abelii*) and the Bornean orangutan (Xu and Arnason, 1996), inhabiting the islands of Sumatra and Borneo, respectively, the species being isolated by the South China Sea (**Figure 1.1**).

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Figure 1.1 Estimated range and IUCN estimated population status of Bornean (orange) and Sumatran (red) Orangutans (from Stiles *et al.*, 2013).

1.5.2 The morphology of orangutans and their adaptations to the environment

Sumatran orangutans are light orange in colour when young, whilst adults have sandy-red coloured hair that is long on the arms and back, with blonde-yellow hair around the face and in their beards. Bornean orangutans are much darker in colour, the hair of adults being a maroon colour and almost black for older individuals (MacKinnon, 1974).

Both species of orangutan exhibit strong sexual dimorphism (Locke *et al*., 2011). Male orangutans can develop in two ways. Up to 15 years of age, male and female orangutans remain similar in size and weight. However at sexual maturity, some males will remain in arrested development and maintain their body size of around 80kg for a number of years (Dunkel *et al*., 2013). In contrast, dominant males will grow much larger in size, up to 130kg compared to their female counterparts (30-80kg; Payne and Prudente, 2008). Dominant males develop secondary sexual characteristics: a fatty crown, a large throat pouch and fatty cheek pads either side of their face, known as flanges. The throat pouch allows males to create their ‘long call’, a vocalisation which is used by flanged males to determine the boundaries of their home range (Ross and Geissmann, 2007). This long call helps to maintains dominance relationships between male orangutans and is utilised by sexually receptive females to locate eligible sexual partners (Galdikas, 1983). The flanges of the fully developed males are thought to function as parabolic reflectors, giving the face a large surface to collect the sound of long calls (Galdikas, 1983). This allows flanged males to estimate the distances to other males and thus to avoid direct contact. The males in arrested development possess hard rims on their cheeks where their cheek pads may eventually develop if they later transition to flanged status (MacKinnon, 1974).

Orangutans are the largest primates to inhabit the forest canopy and must navigate their body around the flexible and unstable supports of the forest trees. Orangutan morphology differs from the African apes who, whilst some have adapted to a degree of arboreal locomotion, are mainly adapted for terrestrial knuckle-walking (Lovejoy *et al*., 2009). Orangutan shoulder, wrist, knee and hip joints facilitate climbing movements and navigating the arboreal pathways and gaps due to their distinct rotational abilities (Zihlman *et al*., 2011). In addition, they have long arms, longer than their total height, with long fingers and a short pollex (thumb), and their legs are much shorter than their arms, which is not common in primates (Fleagle, 2013). Their feet are similar to those of the other apes, having long finger-like toes with an opposable big toe (hallux), allowing for extensive brachiation (Payne and Prudente, 2008).

Supporting the adaptations shown in body shape, the muscle architecture of the feet is specially adapted to allow prehensility using the flexibility of the joints and heavy musculature to assist movement in a three dimensional environment (Zihlman *et al*., 2011). The forelimbs are extremely strong with exceptional reach, supporting their arboreal lifestyle as they use their upper body strength to pull themselves from tree to tree. This is enhanced by their strong shoulder muscles with a specialised shoulder joint formation providing stability in their movements. Orangutan hands are also well adapted; the digital flexors are heavier than those of ground dwelling primates, allowing easy extension and their large palms for effective grasping (Zihlman *et al.*, 2011).

1.5.3 The habitat of wild orangutans

Orangutans inhabit several types of primary forest, including swamp-forest, lowland dipterocarp and hill and mountain forest, with areas of peat swamps known to support the highest densities (Felton *et al.*, 2003). Living as a solitary animal, adult males occupy large home ranges of approximately 2,000-3,000 ha in area, and which they defend actively (Delgado and van Schaik, 2000). In contrast, females are not territorial and their home ranges are smaller (around 900 ha). Females are generally solitary but do occasionally associate with other females (Delgado and van Schaik, 2000). There is very often overlap between the home ranges of females (Knott *et al.*, 2008; Singleton and van Schaik, 2001) and these areas of overlap usually lie within that of territories held by males. Subadult males are more transient than the adults, travelling through the canopy without a fixed home range (Singleton and van Schaik, 2001).

Wild orangutans avoid descending to the ground, and this impacts on how they gather resources. For example, they do not drink from streams, instead gaining water from the fruits that they eat and from drinking pools of water collected in epiphytes (van Schaik *et al.*, 2003). The inner bark of lianas is also a valuable and rich source of water (MacKinnon, 1974).

1.5.4 The dietary needs of wild orangutans

Orangutans display a “typical ape” foraging strategy in which ripe fruit is preferred and so called ‘fall back foods’ are depended on during times of fruit scarcity (Doran-Sheehy *et al.*, 2009). Orangutans have a very low daily energy expenditure, which suggests that they have adapted to minimise their energy throughput, previously unknown in apes (Pontzer *et al.*, 2010). This is thought to be a strategy developed to minimise the chances of starvation in a highly stochastic environment with a variable and unpredictable food supply, often imposing long periods of food shortage (Pontzer *et al.*, 2010). In general, the diet of orangutans is made up primarily of fruit, followed by vegetable matter, leaves and then bark and invertebrates (Fox *et al.*, 2004; Morrogh-Bernard *et al.*, 2009). Importantly, there are marked differences in the level of unpredictability of fruit availability between Borneo and Sumatra. On Borneo, the fruit supply is so intermittent that orangutans may go for months at a time without fruit in their diet. However, on Sumatra, fruit is a more stable food source due to the greater abundance of fruiting trees (Marshall *et al*., 2009a; Morrogh-Bernard *et al*., 2009). The dipterocarp forests which they generally inhabit are subject to the phenomenon of mass flowering and fruiting, known as ‘masting’ (Curran and Leighton, 2000). During this event, a patch of dipterocarp and canopy flora will flower and fruit simultaneously. This event often occurs at intervals of many years (generally ranging from 2 to 10 years) and lasts between a few weeks to a few months (Appanah, 1993). In addition to the temporal variation there is also spatial variation, as the area of the patches over which this occurs varies in size (Appanah, 1993). Orangutans take advantage of these asynchronous fruiting events, travelling long distances to gorge themselves on the rich yet ephemeral fruit supply (Knott, 1998).

The dietary composition of orangutan populations is highly dependent on the areas of forest inhabited. In general, orangutans’ diet is made up primarily of fruit, followed by vegetable matter, leaves and then bark and invertebrates (Fox *et al*., 2004; Morrogh-Bernard *et al*., 2009). Orangutans are also known to feed on many species of insects including honey bees, termites, ants and wasp galls (MacKinnon, 1974). Regardless of fruit availability, orangutans will spend over six hours of their active day feeding (Fox *et al*., 2004). They are unique among the apes as they regularly experience prolonged periods of negative energy balance which has important effects on their behaviour and socioecology (Harrison *et al*., 2010). Orangutans decrease their activity time when food availability is low and increase social interactions when fruit availability is high (van Schaik, 1999; Pontzer *et al*., 2010). The ability to shift dietary composition is of great benefit to wild orangutans due to the significant variation in their preferred food of fruit (Knott, 1998).

During periods of high fruit availability, orangutans are able to greatly exceed their calorie intake requirement and therefore gain weight (Morrogh-Bernard *et al*., 2009), and during a masting event, they are able to achieve a 0.66kg weight gain per day (Knott, 1998). When fruit is not available, orangutans feed on bark, which has a much lower calorific content and, during these times, orangutans are known to lose weight (Knott, 1998). Observations of a wild orangutan population in an isolated agroforest landscape observed a change from 56% of the diet based on fruit to a bark-based diet (44%), and females of the population resorted to crop raiding when farmers had gone home (Campbell-Smith *et al.*, 2011a). Thus the habitat imposes considerable constraints and variation on the diet of orangutans and this has important consequences for their social structure and patterns of behaviour.

1.5.5 Behavioural ecology of wild orangutans

Living solely in the forest canopy makes orangutans difficult to observe continuously and therefore, knowledge of their behaviour is difficult to gather. The knowledge available is the product of ongoing observations over numbers of years.

1.5.5.1 Daily activity

Starting at dawn with morning foraging bouts broken up by periods of rest, feeding takes up a large proportion of an orangutan’s day (MacKinnon, 1974). Orangutans spend an average of 43% of their day feeding (or in other food-related activities such as foraging), 41.5% resting, 13.5% travelling and the remaining 2% in other activities (e.g. vocalising, nest building; Delgado and van Schaik, 2000). However, these proportions vary depending on the habitat type, reproductive stage and fruit availability (Delgado and van Schaik, 2000). At midday, orangutans often construct a day nest, sometimes revisiting their night nest, to rest. This rest period is extended during the rainy season (MacKinnon, 1974). Night nests are usually built approximately half an hour before sunset.

1.5.5.2 Social organisation

Orangutans exhibit fission-fusion sociality, predominantly living alone (Delgado and van Schaik, 2000). When aggregations do form, they fluctuate in size and composition as individuals move through the environment, lacking any defined community structure (van Schaik, 1999). This social structure is unique among the great apes as their African cousins display closer social bonding and more stable social structures. For example, western gorillas (*Gorilla gorilla*) form stable groups of up to about 10 individuals, but groups of up to 20 have been known. These are usually multi-female groups containing a single male and females exhibit primary and secondary dispersal from the natal group (Robbins *et al*., 2004). Chimpanzees are sometimes known to live alone; however, they usually live in large parties. These can consist of males only (bachelor groups) who patrol their terrestrial boundaries (Sugiyama and Koman, 1979), or a mixture of males and females. Female ranges often overlap and lie within male-defended areas with the most reproductively successful individuals remaining in the core of these areas (Williams *et al*., 2002). It is thought that social groups are developed as a strategy of more efficiently exploiting resources within the habitat (van Schaik, 1983). However, the solitary lifestyle exhibited by orangutans is also thought to be an adaptation in particular to the situation of having scarce resources (Knott, 1999). Clustered patches of fruiting trees scattered across the forests of Borneo and Sumatra mean that there is not enough food in a single area to support large, stable groups (MacKinnon, 1974). However, during the masting events, orangutans form small feeding groups (two or three individuals, that can be males or females or both) where they will deplete the food source and occasionally travel around the area together in search of other fruiting trees (Utami-Atmoko *et al.,* 1997; Knott, 1998). Females are also sociable when rearing offspring, with nursing mothers forming small groups and infants playing together (van Schaik, 1999). This grouping may be a strategy to reduce predation (van Schaik, 1999) and, although short lived, the interactions are likely to play an important role in the development of infants and their social behaviours as well as supporting young mothers (Sugardjito *et al*., 1987). Interestingly, Sumatran orangutans are known to be more social than Bornean orangutans forming parties more often and coordinating movements more regularly than their Bornean counterparts (Delgado and van Schaik, 2000; van Schaik *et al*., 2009). This difference may be due to the more aggregated distribution of resources on Sumatra compared to Borneo, and the increased threat from large predators leading to Sumatran orangutans benefiting from living at higher densities (Husson *et al*., 2009). In contrast, the sparse distribution of resources on Borneo means these animals must maintain a more solitary lifestyle (van Schaik *et al*., 2009).

Although orangutans are promiscuous breeders where males and females have multiple mates over their lifetime, they may mate with the same individual(s) for several months, forming a consortship (Utami-Atmoko *et al*., 2009), described as a short-term polygynous mating system (Marshall *et al*., 2009b). Age of first reproduction is typically 15 years for females and 25 years for males (Marshall *et al*., 2009b). Females are able to reproduce up to approximately 50 years old (Morais, 2013). Mothers give birth to only one infant at a time. There are rare cases of twins, but in such circumstances it is common for one of the infants to be abandoned or to die (Marshall *et al*., 2009b). Gestation is approximately nine months, and offspring are dependent on their mothers for between six and nine years (Delgado and van Schaik, 2000). During this time immatures learn how to survive in the wild by observing their mother's behaviours, copying their feeding routine, and practicing nest building (van Noordwijk *et al*., 2009). One of the important skills passed on from mother to infants, is the use of tools, in both accessing food and building nests. Such skills must be passed from mother to infant as the solitary lifestyle does not allow for social learning later in life.

1.5.5.3 Tool-use in wild orangutans

Tool-use is defined as an activity in which the tool must be “held in the hand, foot or mouth and used to enable the operator to attain an immediate goal” (Boesch and Boesch, 1990). Orangutans have been observed to manufacture tools to access food (Call and Tomasello, 1994; van Schaik *et al*., 1996; Fox *et al*., 1999). They remove the twigs and leaves from branches and using them to extract insects from tree holes and to pry seeds from hard-husked fruit (van Schaik *et al*., 1996; Fox *et al*., 1999).

During particularly rainy days, wild orangutans hold large branches over their heads to create an ‘umbrella’ (MacKinnon, 1974; Fox *et al*., 1999). These shelters are also employed in times of strong sunlight as a sunshade. Orangutans have also been known to use piled up branches and leaves to create a screen between themselves and the observer, or to place leaves over their backs to hide themselves (MacKinnon, 1974). Young orangutans develop these skills by playing with leaves and branches, placing them on their heads and during play-nesting (MacKinnon, 1974). As with orangutans, chimpanzees also display a wide range of tool use using twigs to dip for ants, manufacturing spoons out of leaves and using rocks to open hard fruits (Tomasello *et al*., 1987). In both species it is thought that these behaviours develop as ‘traditions’, with the skills being passed from one generation to another, but this is restricted to the more sociable populations of orangutans and chimpanzees (van Schaik and Pradhan, 2003). Orangutans also exhibit tool use in nest building. Nests are constructed by bending small branches in a circle. This is done using feet, the orangutan bending and twisting the branches to create a concave platform, and then pushing the branches down to create a mattress (van Casteren *et al*., 2012).

Although the environment has shaped the physical form, social structure and behaviour of orangutans, they are currently being challenged by a number of anthropological influences which are changing these environments and are threatening their survival.

1.5.6 Threats to wild orangutan populations

The total population of Sumatran orangutans has previously been estimated to be 6600 wild individuals (Wich *et al*., 2008). However, transect surveys from 2016 have provided an updated estimated population of 14,613 (Wich *et al*., 2016). This increase was mainly due to orangutans being found at higher elevations than they were previously considered to occupy, thus extending their geographical range. In addition, orangutans were also found to be geographically more widely distributed than had previously been thought when new areas were surveyed (Wich *et al.*, 2016). However, even with the discovery of these larger populations, Sumatran orangutans are still listed as 'Critically Endangered' on the IUCN Red List of Threatened Species (IUCN, 2016a).

Populations of Bornean orangutans were initially estimated to be larger (approximately 45,000 individuals; van Solinge, 2008), and consequently they were classified as 'Endangered' on the IUCN Red List (Ancrenaz *et al.*, 2008). However in 2016 the species was re-classified as 'Critically Endangered' by the IUCN, who concluded that between 2-3,000 individuals had been killed each year over the past 40 years (IUCN, 2016b). Although precise numbers of surviving individuals are not available, it has been estimated that the population of Bornean orangutans will continue to decrease over the next decades and that both species will be extinct in the wild within the next 10 years (Johnston, 2016). In total the world orangutan population is currently approximately 60,000 and orangutans are the most at risk ape of all Hominids.

Orangutan remains from the Pleistocene and Holocene epochs have been found in caves in parts of Borneo where modern orangutans are not found (van Schaik *et al.*, 1995). Current wild orangutan populations remain fragmented and isolated (Goossens *et al.*, 2006) and it is estimated that the current habitat of Sumatran and Bornean orangutans is less than 5% of the original *Pongo* distribution range (Meijaard *et al.*, 2012). Records suggest that the sharp decline in numbers leading to their current IUCN classifications has started only recently (Meijaard *et al.*, 2010b). Current factors threatening the survival of both Bornean and Sumatran orangutans include hunting (by the small numbers of traditional hunters), disease, logging (both illegal and legal), fires, forest conversion (to plantations and infrastructure) and the illegal pet trade (IUCN, 2016a, 2016b).

Sumatra suffers from the highest rates of deforestation in the tropics (Campbell-Smith *et al.*, 2011b). Economically, the forests are considered by the government to be most valuable for their contributions to exports, employment and local infrastructure, rather than for their biodiversity (Dudley, 2002). Deforestation in Southeast Asia between 1990 and 1997 was 0.91% pa (per annum), and more recently (2000 to 2010) this had increased to 2.20% pa (Gregory *et al.*, 2012). Between 1990 and 2010 7.54 Mha of primary forest were lost with an additional 2.31 Mha being degraded (Margono *et al*., 2012). In total, 47% of the primary forest in Sumatra was cleared or degraded over this two decade period (Margono *et al*., 2012).

Widespread logging has been the most common and long standing threat to orangutan populations, but a more recent threat has been the conversion of remaining orangutan habitat to oil palm (*Elaeis guineensis*) plantations (Nantha and Tisdell, 2009). Palm oil, the extract of the oil palm fruit, is the world's most consumed vegetable oil and accounts for nearly 60% of all trade of oils and fats by volume (Carter *et al*, 2007). Biodiesel produced from palm oil possesses very similar properties to petroleum derived diesel (Lam *et al*., 2009) and is promoted for mitigating carbon dioxide emissions as it is produced solely from living plants.

Orangutans and oil palm plantations compete directly for the same lowland terrain, and the production of palm oil being one of the most lucrative cash crops puts orangutans at extreme risk (Nantha and Tisdell, 2009). The low cost of setting up and running oil palm plantations in these countries as well as the viable land and lack of government legislation around forest protection make these countries very attractive to large companies (Nantha and Tisdell, 2009). Plantations form breaks within the forest canopy, forcing orangutans to come down to the forest floor. Increasingly, orangutans are relying on these plantations as a food supply resulting in increasing conflict between plantation owners and orangutans (Campbell-Smith *et al*., 2011b).

Conflict between villagers and orangutans accounts for between 1950 and 3100 orangutan deaths each year (Meijaard *et al*., 2011). Orangutans raid plantation crops in search of food and are considered a pest to oil palm, and similarly to rubber tree (*Hevea brasiliensis*) and sugarcane (*Saccharum officinarum*; Campbell-Smith *et al*., 2011b). Whilst many plantation owners scare the animals away, there is a small percentage that will actively kill the orangutan (Meijaard *et al*., 2011). Local communities around Indonesian rainforests are also known to poach primates as a form of subsistence hunting, and their diet includes orangutan meat (Than *et al*., 2011). Some restaurants have also been known to offer orangutan meat as part of a special menu (CITES/GRASP, 2006).

In addition to oil palm plantations, habitat destruction and fragmentation is also being caused by unsustainable timber extraction, tree plantations for pulp and paper, small-scale agriculture and mining (Meijaard *et al*., 2010a; Niningsih *et al*., 2017). The Indonesian government has formally recognised the importance of the forests for biodiversity, economic development and social aspirations and therefore launched the Ecosystem Restoration Concept in 2007 (Gaveau *et al*., 2013) with the aim of allowing the heavily forested areas to recover their potential to produce timber whilst maintaining ecosystem services. However, palm oil plantations give a much greater economic return than does timber harvesting and therefore the objectives of the Ecosystem Restoration Concept is difficult to drive forward when the short-term economic balance is strongly weighted against it (Gaveau *et al*., 2013).

There is also considerable illegal logging occurring. Current Indonesian law aims to protect the environment and the rights of local people through the protection of forest areas and the control of logging concessions (Ravenel *et al*., 2005). However, large companies are able to purchase licences to log areas of forest to build plantations for palm oil, rubber and paper pulp (Smith *et al*., 2003). Indonesia has the globe's largest illegal logging problem (Tacconi, 2012). For example, illegal timber logging accounted for over 73% of the total volume of logged wood in Indonesia in 2003 (UNEP, 2007).

The poor economic and political infrastructure in Indonesia means that illegal logging gangs can easily gain access to logging roads and infiltrate road blocks without resistance (Smith *et al*., 2003). In addition, illegal logging provides larger financial incentives than any benefit gained by the conservation initiatives provided by the government (Jepson *et al*., 2001; Nantha and Tisdell, 2009). Timber companies have been increasingly entering orangutan strongholds over the past few years and UNEP (United Nations Evironment Programme; 2007) reports that, out of 41 Indonesian national parks surveyed, 37 had been subject to illegal logging. The accessible lowland habitat favoured by the orangutans is targeted for its favourable trees (Campbell-Smith *et al*., 2011b). In addition, orangutans display a preference for feeding from trees that are >50 cm diameter at breast height (dbh), the category of choice for loggers (Felton *et al*., 2003), and trees selected by loggers are likely to support valuable food resources for the orangutans.

The lack of sustainable land-use, poor planning and poor forest management has degraded orangutan habitat, leaving them fire prone (Siegert *et al*., 2001). This problem is significant, and in July 2015, forest fires broke out across Indonesia with 358 fire hotspots detected in the Sabangau Forest of Borneo alone (Vidal, 2015). These fires had the potential to render all wild orangutan populations extinct and reduced Indonesia’s economy by US $47 billion (Balch, 2015). Earlier, in September 1997 forest fires in Kalimantan (Borneo) and

Sumatra burned through 0.3-1.7 $x10^6$ ha of forest (Davies and Unam, 1999). The causes of these large forest fires include unsustainable land-use combined with the drought conditions associated with the El Niño southern oscillation event (Davies and Unam, 1999). As a result of the 2015 fires, seven company executives were arrested by Indonesian police as it was determined that pulpwood and palm oil concessions were to blame for the slash and burn practices which initiated and perpetuated the fires (Balch, 2015). As a result large companies such as ‘Wilmar’, an international agribusiness group, and ‘APP Timber’ have signed a zero-deforestation pledge to advocate the “No deforestation movement” in Indonesia (The Forest Dialogue, 2013). It is hoped that movements such as these will promote responsible use of the forests and reduce habitat loss in the south east, but given the current economic incentives this is likely to be difficult to achieve.

The illegal pet trade also poses a significant threat to wild orangutan populations. Young individuals are available for as little as US $100 and can be sold for much more internationally (CITES/GRASP, 2006). Between 1985 and 1990 an estimated 1,000 young orangutans were smuggled from Kalimantan to Taiwan (Payne and Prudente, 2008). The close mother-infant bond in orangutans means that obtaining an infant requires killing the mother (Galdikas, 1995). The young are then kept by poachers or sold to villagers and often smuggled across the borders into Asia (Stiles *et al*., 2013). This threat to orangutan populations and welfare has been recognised by many charitable organisations and a number of teams exist to rescue orangutans held illegally captive. Individuals are then taken to rehabilitation with the eventual aim of releasing them back into the forest when ready.

With the recognition of the poor state of orangutan populations and the extent of the continuing threats to them a number of conservation strategies have been undertaken in order to try and support their populations.

1.5.7 Orangutan conservation

The Sumatran orangutan is currently at a greater threat of extinction than the Bornean orangutan (IUCN, 2016a, 2016b). The lack of suitable habitat and dwindling number of wild individuals is enforced by greater rates forest destruction than are suffered on Borneo. Many remaining Sumatran orangutan populations survive in Aceh, a province of northern Sumatra which has suffered from political unrest as well as environmental catastrophe (Sunderlin, 1999). More recently, the area has seen an improvement in stability, allowing for improvements in conservation efforts. For example, the Ladia Galaska road network project threated to divide one of Sumatra's remaining populations and could result in the loss of 1,384 Sumatran orangutans but was halted when the Central Minister for Home Affairs did not provide consent (Marshall *et al*., 2009b). Although the extended road network planned to connect areas of the forest, making the forest more accessible for the local people, it was heavily conflicted by the biological impact it would have on the Leuseur ecosystem leading to greater fragmentation of the forest and the potential loss of significant proportions of endangered Sumatran wildlife (Clements *et al*., 2014). The abortion of such plans and support from the governments for conservation is a positive step towards conservation and away from development.

Orangutan conservation does not receive the scale of financial support that palm oil and forestry projects do, and orangutans do not provide a direct financial benefit to the Indonesian government (Nantha and Tisdell, 2009). Tourism offers potential for generating financial incentives for orangutan conservation (Wunder, 2000). Orangutan rehabilitation centres offer tourists the opportunity to interact with young orangutans as well as to go out into the forest and see wild orangutans in their natural habitat (Newsome and Rodger, 2012). In addition, legislation means that tourists require permits to enter the forest, the profits of which can be utilised in forest conservation projects. However, it will be difficult to generate significant tourism based solely on viewing wild orangutans, since the solitary and elusive nature of the orangutans makes sightings rare and uncertain (Nantha and Tisdell, 2009).

It will be essential for orangutan conservation to preserve their habitat. Although, previously, it was thought that orangutans were extremely sensitive to forest disturbance, it is now apparent that they are more flexible and able to adjust to disturbance to a degree (Ancrenaz *et al*., 2014). Orangutans are able to use monocultural plantations to survive, but it is not known for how long (Meijaard *et al*., 2012). Orangutans themselves are instrumental in the maintenance of their forest habitat (WWF, 2017). They act as seed dispersers, through eating fruits and passing seeds as they move around the forests. These benefits have consequences for Indonesian forest through their contribution to climate regulation and the maintenance of a healthy forests ecosystem, which in turn supports the provision of clean water for local people (Nantha and Tisdell, 2009), and therefore viewed as an ecosystem service with significant monetary value (Costanza *et al*., 1997).

Indonesian law strengthened its protection for orangutans by setting the maximum penalty for harming or capturing an orangutan to 100 million rupiah (approx. US $7,421) and five years' imprisonment via the 1999 Conservation Law Ministry of Forestry of the Republic of Indonesia, 1990; Centre for Orangutan Protection, 2009). A similar law came into force in Sabah, Malaysian Borneo, in 1997, also with five years' imprisonment but a 50,000-ringgit fine (approx. US $12,000; State of Sabah, 1997). This law also refers to any impact that the conversion of land to plantations has on orangutans, and developers must mitigate forest loss and budget for the cost of translocating orangutans to safer areas of forest (Payne and Prudente, 2008). Related legislation has also been put in place in some countries outside Indonesia. In 1989, Taiwan passed a law making it illegal to keep orangutans as pets (Law of the People's Republic of China on the Protection of Wildlife, 1989). However, such legislation has done little to stem the practice and licences are heavily abused (EIA, 2017).

Large numbers of orphan orangutans have been rescued from the forest and from the pet trade, and a number of centres have been set up with the aim of rehabilitating individuals and releasing them back into the wild. The first rehabilitation centre was set up by Barbara Harrison in Sarawak, Borneo, in 1961, and later the concern for orangutan survival led to the establishment of three rehabilitation centres in Indonesia in the 1970s (Russon, 2009; Orang Utan Republik Foundation, 2017). However, in 1978, a rehabilitation centre in Ketambe, Sumatra was closed due to fears that orangutans had contracted respiratory diseases from humans (Warren and Swan, 2002). Subsequently the remaining two rehabilitation centres were halted due to legislation preventing the release of orangutans back into habitats retaining wild populations. Later, in 1995 the Indonesian Government decided to place regulations on the reintroduction of orangutans, and new rehabilitation centres were established in Indonesia (Warren and Swan, 2002). Since the 1990s the numbers of orangutans housed in rehabilitations centres has rapidly increased (Russon, 2009). For example, in one rehabilitation centre in Borneo, between 60 and 80 orangutans live on the reserve and around 25 orphans are cared for in the nurseries (Orangutan Appeal, 2017). The orangutan Foundation cares for around 300 orangutans in the Tanjung Putting National Park (Orangutan Foundation International, 2017) Reintroduction attempts have had varying success but extensive research into the successes and failures of such practices continues to grow (Keiter *et al*., 1983; Smits *et al*., 1995; Riedler *et al*., 2010; Nayasilana *et al*., 2017).

1.6 Research rationale

In contrast to other apes, the social system of orangutans is not usually replicated in captivity. Appropriate housing conditions are important in supporting good animal welfare but are often difficult to provide. As has been outlined, orangutans are solitary apes and live almost exclusively in the tree canopy of their rainforest habitat (Perkins, 1992). Orangutans rarely form aggregations. However, when such gatherings do occur, they are short-lived and are seen in the context of gorging on the abundance of fruit available from masting trees or when mothers bring their infants together to socialise (van Schaik, 1999). In captivity, however, orangutans are most often maintained in permanent social groups, which more closely resemble gorilla harems, composed of one adult male and a group of females with their infants (Weingrill *et al*., 2011). Also a common characteristic of life in captivity is food that is readily prepared and provided on a regular schedule. In the wild, orangutans spend prolonged periods of their time travelling across the forest canopy in search of suitable fruits (Campbell-Smith *et al*., 2011b). Their rainforest habitat provides a choice of around 400 different fruit species available at varying times of year (Orangutan Foundation, 2017) and fall back foods such as bark, leaves and termites provide sustenance when fruit availability is low.

In captivity, a prepared diet of fruit (e.g. bananas, apples, oranges and grapes) and vegetables (e.g. broccoli, carrots, kale and lettuce) is given to the animals daily (San Diego Zoo, 2017). This reduces the amount of time animals are required to spend searching for, processing and consuming their food (Celli *et al*., 2003; Hosey, 2005). To access their varied diet in the wild, orangutans travel extensively through the forest canopy, therefore using the energy stores gained from their diet. In captivity, orangutans are not often offered large enough areas to expend this energy and they do not experience food shortages as their wild counterparts do. As a result of the diets provided, obesity in captive apes is common (Hosey, 2005; Wilson, 1982). The lack of opportunity to travel long distances can also result in stereotypic behaviour for animals whose physiology requires such activity (Clubb and Mason, 2003).

The disparity between the ecological needs of captive orangutans and those often afforded to them by their environment underlines their eligibility for welfare monitoring and assessment. It is crucial for their successful propagation in captivity that they are provided with habitats that allow individuals to fulfil the behavioural repertoire of their wild counterparts and maintain full psychological and physiological health. Recognising that animal welfare is composed of both psychological and physiological components, it is advised that comprehensive assessment and monitoring of both, is necessary in order to meet the requirements of zoo housed collections.

A range of methods exist to analyse captive animal welfare, focusing on the different components of captive living. But the time and financial cost associated with such methods limits their practicality in captive housing. To justify such investment it is advantageous to compare the utilisation and outcomes of welfare assessment methods and determine their strengths and weaknesses and in so allowing collection managers to make informed decisions when planning welfare assessment and monitoring.

1.7 Research aims and objectives

This thesis implements both behavioural observations and hormone monitoring as methods of quantifying and assessing animal welfare, using Bornean orangutans as a model species. Focusing on each method in turn, the methods of data collection, analysis and interpretation of results are explored. The results of such investigations are discussed extensively to provide understanding of the implications of environmental enrichment on the welfare of animals as well as an assessment of the methods in giving an understanding of animal welfare. Finally the thesis puts forward an additional method of assessment and evaluates its potential use as an additive measure of animal welfare.

The thesis sets out the methods available for quantifying and assessing animal welfare and compares their potential use in research. The desired outcome of this thesis is to provide a comprehensive understanding of the methods and to give recommendations for the assessment of animal welfare for future research.

1.7.1 Primary research aims and supporting objectives

1) To compare methods of quantifying and assessing animal welfare
    a. Implement the established methods of animal welfare assessment using Bornean orangutans as a model
    b. Provide a narrative on the data collection, analysis and interpretation of results.
    c. Determine the presence or absence of a relationship between animal behaviour and the production of stress hormones
2) To provide recommendations for future research

a. Develop and utilise a third potential method of animal welfare assessment
b. Use the narrative produced throughout to discuss the strengths and weaknesses of each method of animal welfare assessment.

1.7.2 Thesis structure

In order to meet the aims of the thesis, four studies were conducted. A systematic review of the great ape enrichment research revealed the gaps in the literature and highlighted areas that require greater attention. The outcome of the systematic review informed the development of an enrichment programme for captive Bornean orangutans and behavioural observations were used to assess the impact of enrichment on animal welfare. The results were then compared to the results of a stress hormone analysis to deduce whether a relationship exists between the two. Finally, the fourth experimental study introduced a novel method of welfare assessment and combined behavioural observations with patterns of space use within the enclosure.

Chapter TWO: Chapter two presents a systematic review of the literature which reports studies that have utilised enrichment to influence the behaviour of captive apes. The chapter will present the reader with the data extracted from the literature in the form of graphs as visualisations of the strengths and weaknesses of the current enrichment research. This chapter identifies gaps in the research and provides a rationale for the following chapter.

Chapter THREE: The third chapter in this thesis is the first of three experimental chapters. It recounts the implementation of frozen fruit cups and an artificial termite mound as enrichment devices as part of a 12 week enrichment programme. Continuous group sampling and a species ethogram were used to collect behavioural observation data. General patterns in orangutan behaviour were identified using the ordination method of principle component analysis (PCA) and patterns were interpreted with respect to the individual orangutans. Linear regression analysis with generalised least squares (GLS) extension, where appropriate, was conducted to illustrate significant changes in orangutan behaviour over the course of the 12 weeks. The results of both analyses are discussed and interpreted with reference to wild orangutan behaviour to assess the effect of the enrichment on orangutan welfare.

Chapter FOUR: During the course of data collection for chapter three, faecal samples were collected from the orangutan enclosure on a daily basis, and chapter four presents the investigation into the use of stress hormones in the assessment of animal welfare. The faecal samples underwent an enzyme immune assay (EIA) to identify the concentration of faecal glucocorticoid metabolites (fGCM) in each sample. These results were then matched with behaviour data on the corresponding days. A lag time of 2 days was added to behaviour data to account for the 48 hour excretion lag of fGCM in orangutans as established by Weingrill *et al.* (2011). fGCM levels were plotted against time over the 12 week study to identify any peaks in fGCM and determine if these were related to the implementation or removal of environmental enrichment. Regression analyses were then conducted on the fGCM and the behavioural data to determine if any of the TUCs were able to act as predictors of fGCM production. The implementation of least absolute shrinkage and selection operator (LASSO) allowed for further analysis which supported the results of regression analysis. The relationship between behaviour and fGCM production are discussed in this chapter as well as a reflection on the method as an assessment tool in animal welfare monitoring.

Chapter FIVE: The methods of monitoring psychological and physiological health have been explained, implemented and discussed, and chapter five introduces an alternative method of monitoring animal welfare in a captive environment. The chapter introduces how network analysis has been used in the study of animal behaviour. Using both behaviour and space use data collected on the orangutans at Twycross Zoo, this chapter demonstrates how a wider range of behavioural data can be converted into network form. A range of visualisations are produced owing to the flexibility of the method. However, the chapter outlines the need to quantify network structure to extend its application.

Chapter SIX: Expanding on the work of the previous chapter, chapter six focuses on the quantitative metrics of network structure. By exploring the metrics used to quantify networks, including individual measures, group measures and intermediate measures, the chapter demonstrates how the use of networks for the study of animal behaviour can go beyond the utility of visualisations of behaviour and space use pattern. These metrics facilitate a deeper understanding of animal behaviour and illustrate their potential in animal welfare assessment.

Chapter SEVEN: The final study chapter of this thesis focuses on a particular type of network, bipartite networks, and explains how they are constructed. Here, the orangutan data utilised in the previous two chapters are used to demonstrate the application of bipartite networks to behaviour and space use data. Example networks are constructed and various quantification measures of network structure are calculated. These are critically discussed in the context of their potential use in studies of animal behaviour and welfare. The ability of the networks to examine patterns of links between two related data sets is identified as a key strength. However, the method meets difficulties in the interpretation of the metrics and highlights the need for further research before the method is applied elsewhere.

Chapter EIGHT: The final chapter of this thesis reflects on the journey taken to achieve the overall aims to compare the methods for quantifying and assessing animal welfare. The findings of each of the studies are discussed and the strengths and weaknesses of the two existing methods of assessment are identified. The potential use of the third method is also considered, the strengths and weaknesses are also reviewed. A summary of the overall program of research is provided and novel contributions of the thesis are reviewed. Finally, the future directions of the research are considered as the thesis explores the potential application of the methods in growing fields of animal behaviour and welfare research.

Chapter TWO: Systematic Review of Research on the Effects of Enrichment on the Welfare of Apes, and Recommendations for the Future

2.1 Chapter overview

The value of captive ape groups is increasing as global species extinctions pose a significant risk to wild populations. However, behavioural problems associated with captivity threaten their value as representatives of the species. The ape super-family (Hominoidae) are recognised as being particularly vulnerable to developing behavioural abnormalities, and environmental enrichment is recognised as a strategy for restoring animal behaviour to that of the species' wild counterparts. The use of enrichment has been discussed in depth in the previous chapter and this chapter will explore how enrichment has been studied in apes whilst highlighting areas in need of more investigation. A literature search produced a catalogue of research papers and each was reviewed to extract data, which were then processed to build an understanding of our current shared knowledge and awareness of the use of environmental enrichment with great apes. The review uncovered a lack of research using orangutans and bonobos as the subject species, and the literature revealed the limited range of enrichment devices trialled with great apes and the relatively short-term investigation of the effect of the enrichment devices on ape behaviour as a measure of welfare.

2.2 Introduction

Globally, species extinction rates are accelerating (Pimm *et al*., 2014; Urban, 2015). The pressures of climate change, habitat conversion and illegal trade have meant that 25% of all mammals (UNEP, 2000), and more than 12% of bird species (Stattersfield *et al*., 2005) are at substantial risk of extinction. Notably, more than half of all primate species are known to be threatened with extinction due to habitat degradation and hunting (ICUN, 2012; Rovero *et al*., 2012). Amongst these, apes are under severe threat. To give an example, numbers of the Eastern lowland gorilla (*Gorilla beringei graueri*) are estimated to have declined from approximately 17,000 individuals in 1995 to as few as 2,000 in 2012 (Stiles *et al*., 2013), and those of the Sumatran orangutan have fallen by 92% since 1900, with the expectation that this will be the first great ape species to be lost completely in the wild (Wich *et al*., 2011). The 17 species of gibbon are also under threat: the Javan gibbon (*Hylobates moloch*) is endangered with less than 4500 individuals recorded in 2002 (Nijman, 2006), the eastern hoolock gibbon

(*Hoolock leuconedys*) consists of less than 200 individuals (Peng-Fei *et al*., 2011), and the Hanain gibbon (*Homascus hainanus*) is the world's rarest ape with only 20 animals remaining (Fellowes *et al*., 2008; Deng *et al*., 2017).

The extent and rate of wild species decline have led to the recognition that the captive breeding of nearly 3,000 bird and mammal taxa is likely to be the only method of avoiding their complete extinction, at least in the short term (Ebenhard, 1995). In order to successfully maintain captive breeding populations it is vital that individuals display a range of natural behaviours, including those leading to reproduction (Carlstead and Shepherdson, 2000). These captive populations also represent valuable sources of behavioural diversity for the purposes of reintroduction programmes and so it follows that providing animals with opportunities to develop natural behaviours is vital for the success of these programmes (Reading *et al*., 2013). However, captive environments are very often deficient in the sensory stimuli that are found in wild habitats (Wells, 2009), leading to the inability of animals to express a full range of natural behaviours (Brent and Eichberg, 1991; Celli *et al*., 2003). When animals are prevented from engaging in natural behaviours (e.g. foraging) they often suffer from stress (Csatádi *et al*., 2008). The psychological response to stress leads to the development of abnormal behaviours or even stereotypic behaviours, such as pacing and head rolling (see **1.3.2 Abnormal and stereotypic behaviours**). The physiological response to long term stress initiates a cascade of hormones that results in the suppression of the immune response (Möstl and Palme, 2002) and a reduction in ovulation by females (Moberg, 1985), which poses a considerable risk to the welfare and reproductive success of captive populations (Shepherdson, 1994).

The use of enrichment is now a well-recognised method of promoting welfare in captive animals (Maki and Bloomsmith, 1989; Carlstead *et al*., 1991; Carlstead and Shepherdson, 2000). Within the literature, there is a range of proposed aims and definitions of enrichment. Many authors have defined enrichment as "techniques that involve making modifications to an animal's environment with the intention of improving the animal's biological functioning" (Newberry, 1995; Shyne, 2006). Others simply define enrichment as "the provision of behavioural choices to captive animals" (de Azevedo *et al*., 2007). However, throughout the literature there is a common theme that enrichment should promote behaviours that are naturally exhibited by the animals' wild counterparts (Chamove, 1989; Honess and Marin, 2006). A popular definition that broadly captures the key criteria of most definitions of enrichment in the literature was offered by Shepherdson (1998), stating that enrichment "enhances the quality of care by providing the stimuli necessary for optimal psychological and physiological well-being" (Tarou and Bashaw, 2007; Hoy *et al*., 2010; Clark, 2011; Reading *et al*., 2013).

Enrichment has been shown to reduce abnormal behaviours (Brent and Eichberg, 1991; Caws *et al*., 2008; Csatádi *et al*., 2008) and increase natural behaviours (see Tripp, 1985; Bloomsmith and Lambeth, 2000; Morimura, 2003) in captive apes. This provides evidence that enrichment can be used successfully to achieve all the proposed goals of Shepherson's (1998) definition. Thus, enrichment is a potentially powerful methodology for promoting the diversity of behaviours necessary for successful captive breeding, and also to retain the natural behavioural repertoire necessary for possible future re-introductions into the wild. However, across the ape species exists a range of social structures and group dynamics, and more recently, research has revealed that there are genuine differences in personality within a group of individuals of the same species (Weiss *et al*., 2012). Consequently, for any particular enrichment device, there is the potential to evoke a negative response in some individuals. When designing and implementing an enrichment treatment, zoos regularly use the published literature for guidance in the process (Hoy *et al*., 2010). This literature, therefore, acts as a critical resource in assessing the effectiveness of enrichment devices across species and under a range of conditions. It has also been recognised that, in order to improve the effectiveness of enrichment methods, it is necessary to quantifiably measure the current use of enrichment (Hoy *et al*., 2010).

The intelligence of apes and their similarities to humans means that their psychological requirement for appropriate environmental stimulation is considerable and they are likely to suffer with poor welfare as a result of the captive environment (Weiss *et al*., 2012; Kurtycz *et al*., 2014). Accordingly, primates are considered to receive enrichment most regularly (Hoy *et al*., 2010). However, in terms of the published literature, most of the studies which assess the use of enrichment focus on the felids and rodents (Swaisgood and Shepherdson, 2005; de Azevedo *et al*., 2007). This provides evidence that, although primates are regularly provided with enrichment, these practices are not scientifically evaluated as frequently as would be expected, or at least that the evaluations conducted do not reach publication. In addition, the survey by Hoy *et al*. (2010) noted that almost half of the 25 zoos questioned reported that there was never any evaluation or formal recording of the effects of enrichment. A primary goal for zoos is to ensure good captive animal welfare (EAZA, 2008). Disseminating the results of the evaluation of enrichment items plays a vital role in co-ordinating enrichment efforts across all zoos, to help achieve the best possible welfare for captive animals. Many enrichment treatments are assessed for the purposes of undergraduate or master's degree projects. The results of these studies may be available to the institution in which the research was conducted, however in most cases, authors do not go as far as to publish the research for access by the scientific community. Therefore the research has little impact on the maintenance of captive populations as a whole. The aims of the current study are to review the published studies which scientifically evaluate the effects of enrichment on the behaviour or welfare of apes, to identify the strengths and weaknesses in the research, and to make recommendations for future research.

2.3 Methodology

2.3.1 Inclusion criteria for published literature

To begin the review, a literature search was performed between January and October 2014 using the databases *Web of Science*, *Elsevier Science Direct* and *Google Scholar*. The search terms used to locate potential literature for this study were *Ape enrichment, Zoo enrichment, Laboratory enrichment, Orangutan enrichment, Orang-utan enrichment, Gorilla enrichment, Chimpanzee enrichment, Bonobo enrichment* and *Gibbon enrichment*. It was important to review all ape enrichment studies in order to evaluate how researchers are conducting and reporting enrichment research. A year range was not set, to ensure that every published study available was included in the review. Peer reviewed articles reporting the findings of a study, in which any form of enrichment was provided to any number of ape individuals or species in captivity and in which behavioural observations were recorded, were included in the sample. Also, to avoid any potential issues with translation, only those articles published in English were selected. Further assessment of this original set of studies was then conducted to check eligibility for this study. Finally, the reference lists of each eligible publication were scanned for other relevant sources to be included in the study and these were also reviewed as described above.

A total of 18 studies were rejected from the sample due to the fact that only a conference abstract for the research was available. As these abstracts do not provide a full methodology to be analysed and are likely to be altered between the conference presentation and final publication, it was not deemed appropriate to include these publications. This selection process resulted in a total of 53 eligible research articles.

2.3.2 Reviewing the literature

Data were extracted from each published study in order to examine the trends in ape enrichment research, the extent of the research produced, and to identify any areas of the research that require further investigation. Details of the categories of data collected from the published literature and how the data were organised are described below.

Year of publication: The year stated on the cover of the paper as the publication date of the research article.

Country of origin: The country in which the research was conducted, i.e. where the subject animals were housed. This country usually coincided with the institution with which the lead author was affiliated.

Type of housing and ape subjects: The type of establishment (zoo or laboratory) in which the research was conducted and the ape subjects upon which the research is based (chimpanzee, bonobo, gorilla, orangutan or gibbon).

Single- or multi-genus: Where the research focused on only one ape genus, it was identified as a ‘single genus study’ whereas cases in which enrichment was provided for two or more separate ape genera were identified as ‘multi-genus’ studies.

Research aim: The aim of the research as stated by the authors of the publication were organised into the following categories:

a) Increase natural behaviour: this included aims such as ‘to encourage tool use behaviour’ and ‘to increase activity’.
b) Reduce abnormal or undesirable behaviour: this included aims such as ‘to reduce coprophagy’ and ‘to reduce aggression’.
c) Reduce abnormal behaviour and increase natural behaviour: these studies aimed to achieve both of the above.
d) Evaluate the effect of enrichment: these authors set out to evaluate the effect of enrichment without addressing any particular behaviour.

Enrichment category: The authors of the research used various definitions to describe the type of enrichment that was provided for the ape group outside of their normal husbandry routine. For the present study, the categories provided by Bloomsmith *et al.* (1991) were adapted to organise the different types of enrichment provided (**Table 2.1**).

Table 2.1. The categories used to organise the types of enrichment provided by the authors of the research. Adapted from Bloomsmith *et al*. (1991)

Type of enrichment	Definition
Social	Direct or indirect interaction with conspecifics or contraspecifics additional to the current group Direct or indirect contact with conspecifics
Physical	Altering the size or complexity of the enclosure or adding objects such as novel toys
Nutritional	Alterations to the delivery (frequency, schedule or presentation) or type of food The presentation of food or the types of food given
Occupational	Allowing animals to exercise some control (puzzles, games or mechanical devices)
Sensory	Additional visual, auditory or olfactory stimuli (e.g. videotapes, music or scented rags)
Multiple	Any two or more of the enrichment types

Number of subjects: The number of individuals included in the study. This information was provided by authors in the methods section of the publication.

Life stage of subjects: Some studies stated the number of juveniles and adults used in the research. Others stated the ages of the subjects and therefore those individuals (from all species) that were younger than 10 years of age were classed as juveniles and all individuals stated as being older than 10 years were classed as adults. This loosely coincides with the age stated by authors at which apes cross from juveniles to adults (or adolescence) generally dictated by age of first reproduction (Morbeck *et al.*, 1996).

Sampling and recording method: The methods of behavioural observation are divided into to two levels, the type of sampling used and the method of recording behavioural observations. These levels were then subcategorised following Martin and Bateson (2007):

A. Sampling method
    I. *Focal:* Observing one individual or group of individuals for a specific amount of time and recording all instances of behaviour.
    II. *Scan:* A whole group of subjects is rapidly scanned at regular intervals and the behaviour of each individual at that instant is recorded.
    III. *Ad libitum:* No systematic constraints are placed on what is recorded or when.
B. Recording method
    I. *Continuous:* Each occurrence of the behaviour pattern is recorded with information about its time of occurrence.
    II. *Instantaneous:* The observation period is divided into sample intervals. On the instant of each sample point, a record is made of the individual's/each individual's behaviour.
    III. *One-Zero:* On the instant of each sample point a record is made of whether or not the behaviour pattern has occurred during the preceding sample interval.

Temporal length of study: The number of days over which observations took place. This information was provided. In some cases, authors did not state specifically how many days the research spanned but may have stated the number of weeks or months. In such cases, the number of days was calculated from this information.

Experimental design: The timeline to which the research was planned. When implanting enrichment, it may be advantageous to conduct baseline observations with which to compare the enrichment condition. It may then be appropriate to follow observations once enrichment has been removed. A number of studies carried out baseline observations of the apes before implementing enrichment, others implemented an ABA design. This involves taking observations during a baseline (A), during a period of enrichment (B) and for a period of time after enrichment (A; Young, 2003). Some researchers only carried out observations during the period in which enrichment was provided.

Not all of the research papers included in the in this review provided all of the information stated above. In these cases the data were noted as ‘unspecified’ and these cases were included in the following results.

2.4 Results

The data collected were recorded in a spreadsheet using Microsoft Excel and analysis were conducted by transforming the data into graphs as follows and identifying key trends.

2.4.1 Year of publication

The published literature spanned 32 years from 1982 to 2014. The greatest number of studies were published from 1996-2000 (n=8-10/year; **Figure 2.1**). In each of the 10-year periods of 1996-2005 and 2006-2015 more articles were published than in the 15-year period from 1980-1995 (**Figure 2.1**). In 2003 five articles detailing an ape enrichment study were published and this was the maximum number of studies published in a single year throughout the period. There were seven different years (1983, 1984, 1987, 1994, 1995, 2001 and 2010) in which there were no studies published that were eligible for this review.

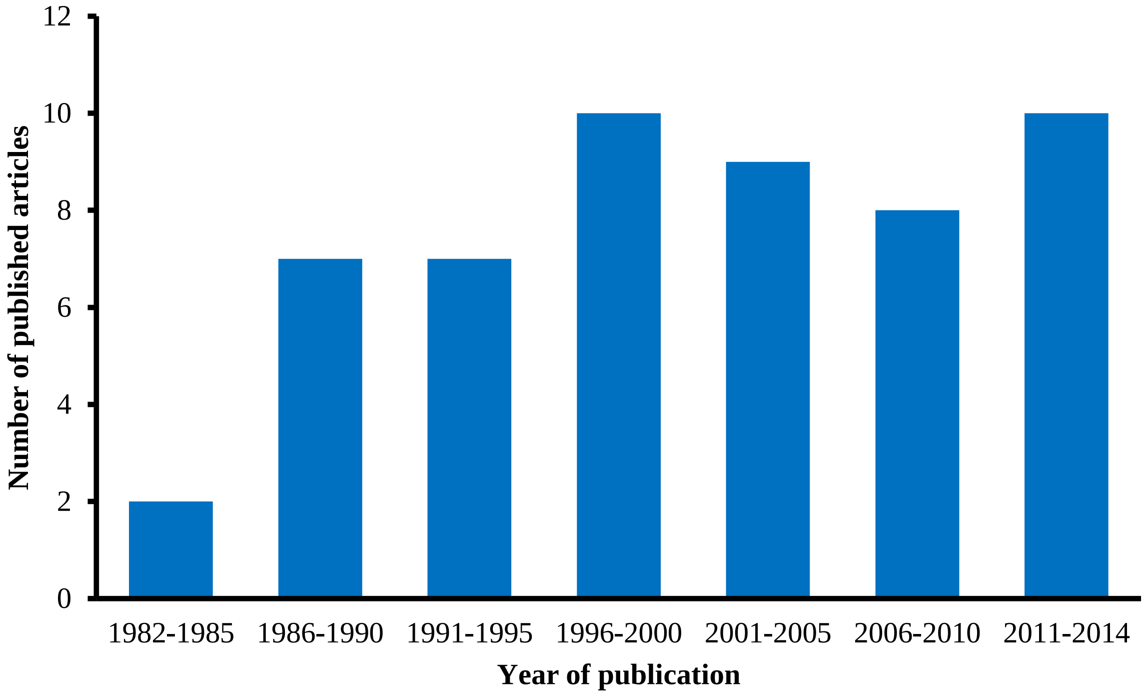


Figure 2.1 Number of publications in each five year period from 1982 to 2014

2.4.2 Country of origin

Published research was conducted in only seven countries (**Figure 2.2**). A clear majority (62%, n=33) of research was conducted in the USA. The second largest contributor to the research was the UK (17%, n=9) and the third largest contributor was Japan (8%, n=4).

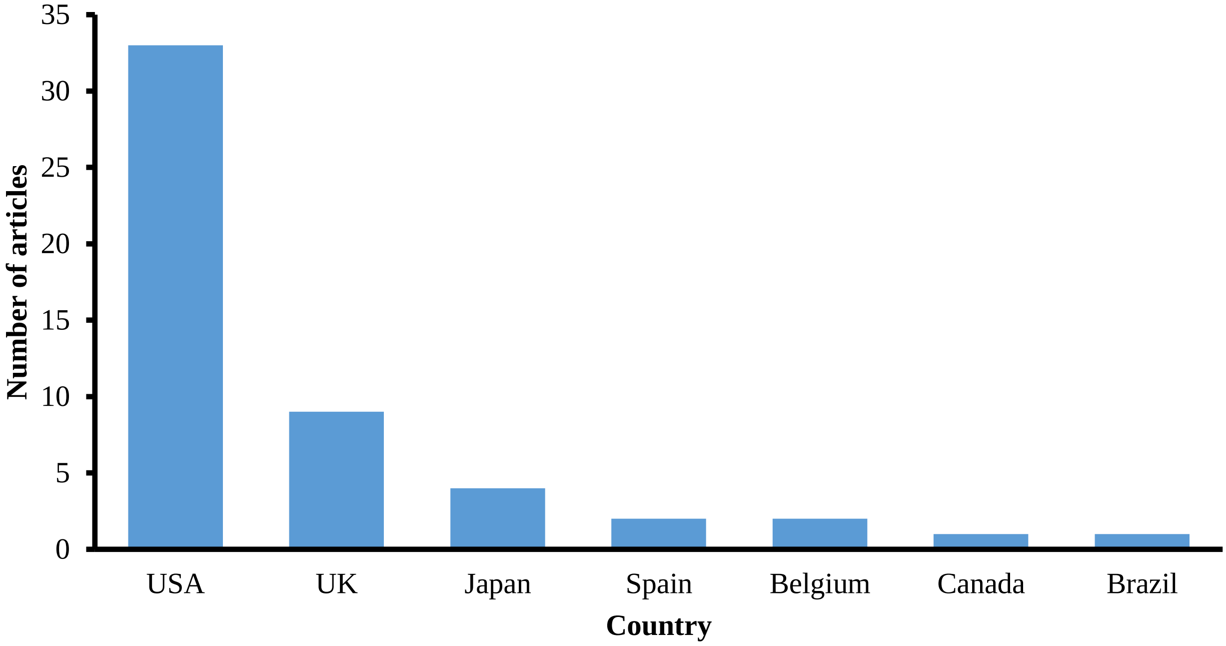


Figure 2.2 Number of research articles originating from each country

2.4.3 Type of housing and ape subjects

The zoo based research covered all ape species, whereas laboratory research concentrated only on chimpanzees (**Figure 2.3**). The majority (70%, n=37) of ape enrichment research was conducted within a zoo setting but a large proportion of the research was also conducted within laboratories. A greater number of chimpanzee studies were conducted in a laboratory (n=22) rather than a zoo (n=9).

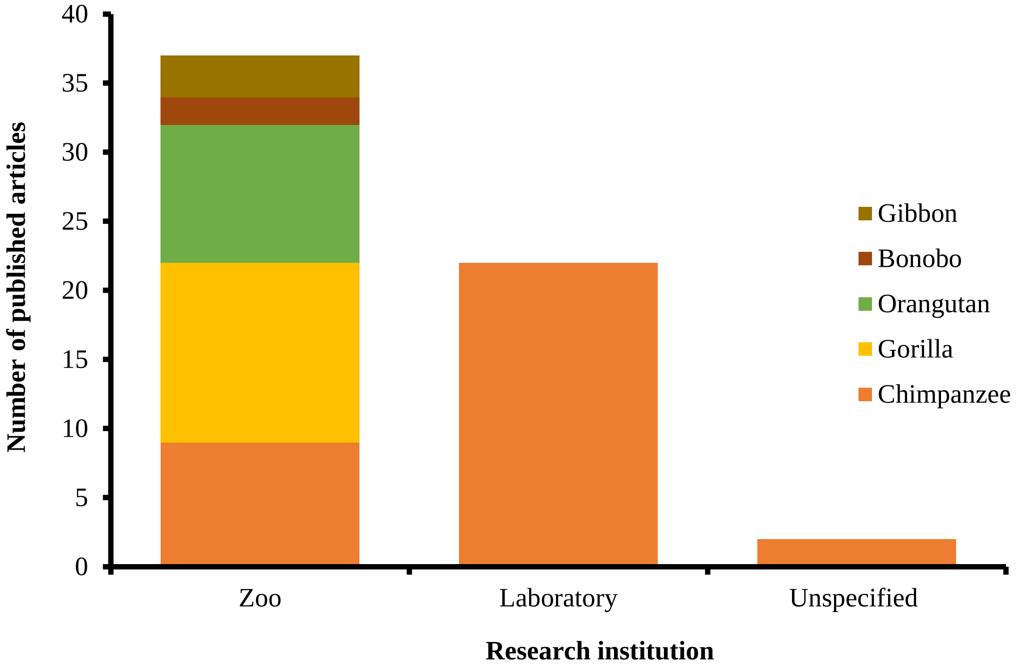


Figure 2.3 Number of articles based in each type of establishment and the species used in the research

2.4.4 Single- or multi-genus

A total of 49 of the published studies concentrated on using enrichment with a single ape species and only four studies used any more than one species in their research (Gilloux *et al.*, 1992; Zaragoza *et al.*, 2011; Schreiner *et al.*, 2012; Kurtycz *et al.*, 2014).

2.4.5 Research aim

Studies that aimed to evaluate the effect of an enrichment device account for 53% (n=28) of those reviewed here, 11% (n=6) of the studies aimed to reduce abnormal behaviours observed in captive apes and 13% (n=7) stated that enrichment was implemented to increase natural behaviours in the apes (**Figure 2.4**). Only 6% (n=3) of the studies combined the aims of reducing abnormal behaviour and increasing natural behaviour. In the sample of articles, one study did not state an aim for the research.

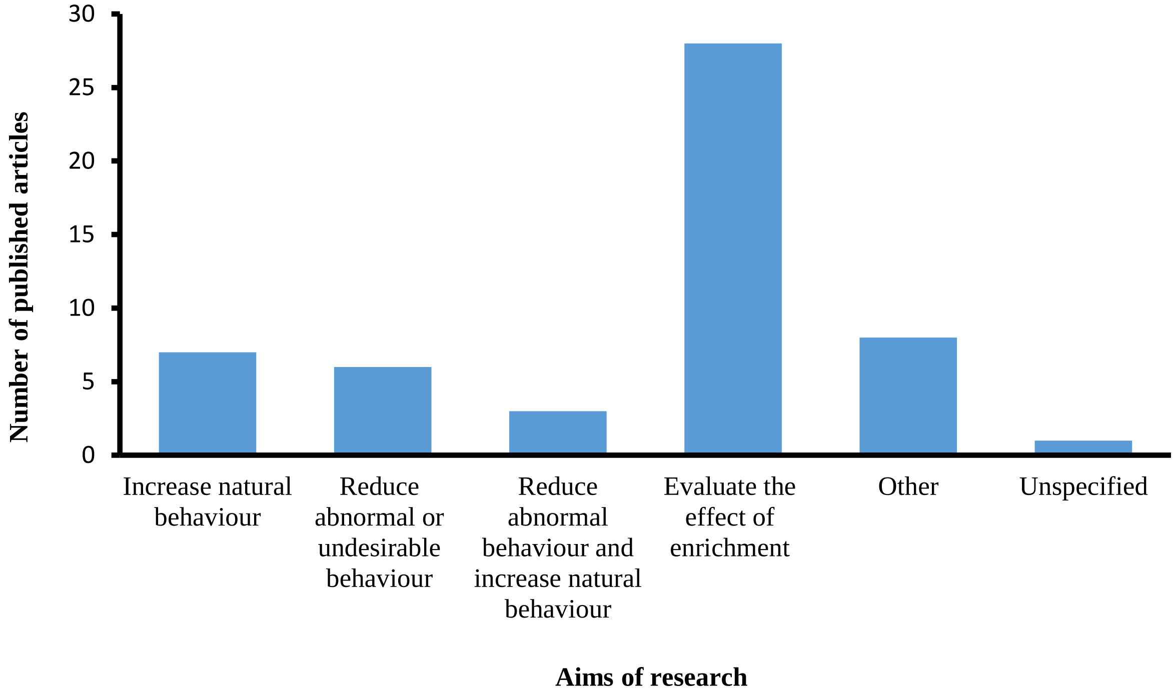


Figure 2.4 Aims of the enrichment studies

2.4.6 Enrichment category

A range of enrichment types were covered in the literature although the use of social enrichment appears to have been explored the least (n=2; **Figure 2.5**). Although only 25% (n=13) of the 53 studies explored the implementation of physical enrichment it was the most common form of enrichment studied. Occupational enrichment was employed in five of the publications and sensory enrichment used in eight. In 11 of the studies, the researchers implemented multiple enrichment items from a number of enrichment categories. These ranged from using browse, videotapes and ice-blocks (Wood, 1998) to Kong toys and wrapping paper (Pruetz and Bloomsmith, 1992).

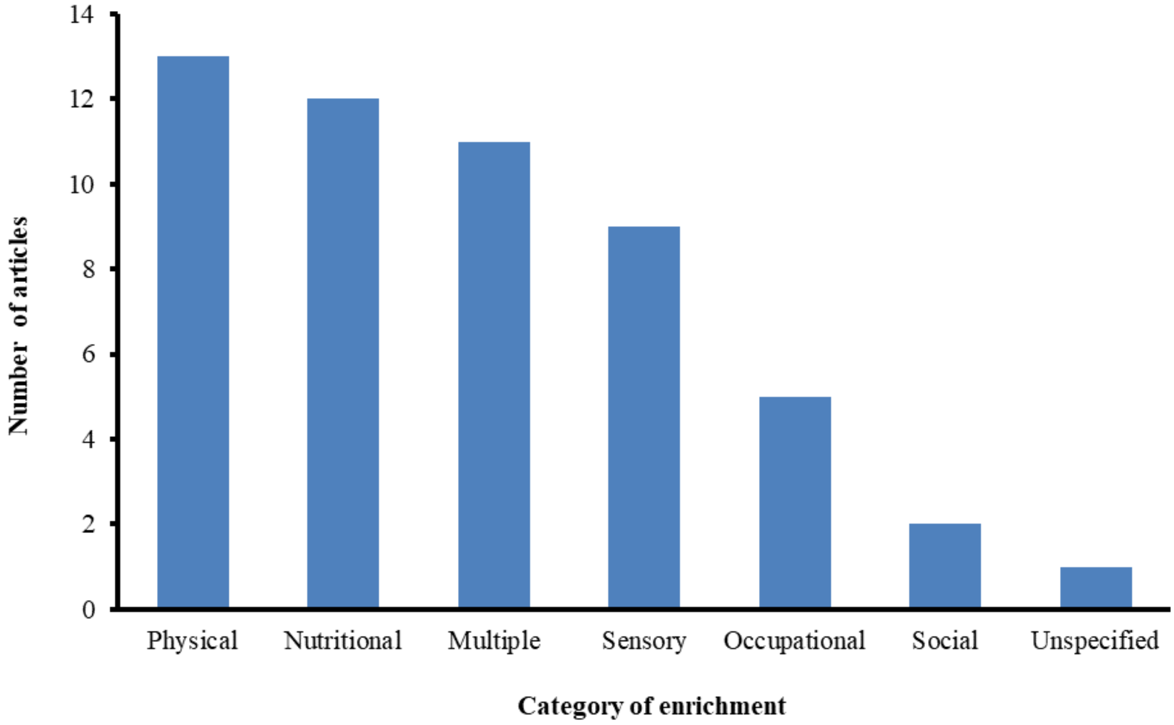


Figure 2.5 Category of enrichment utilised in the research articles.

2.4.7 Number of subjects

The smallest sample utilised in the research was a single orangutan (Pizzutto *et al*., 2008) and the largest number of subjects used was a total of 75 chimpanzees (Videan *et al*., 2005; **Figure 2.6**). The most common group size used in the research is a total of four individuals. All of the studies which involved groups of 13 individuals or more were chimpanzee studies. Two studies did not specify how many subjects were included in the research, one used lar gibbons (*Hylobates lar*) and the other used bonobos (Daman, 1990; Shepherdson *et al*., 1989).

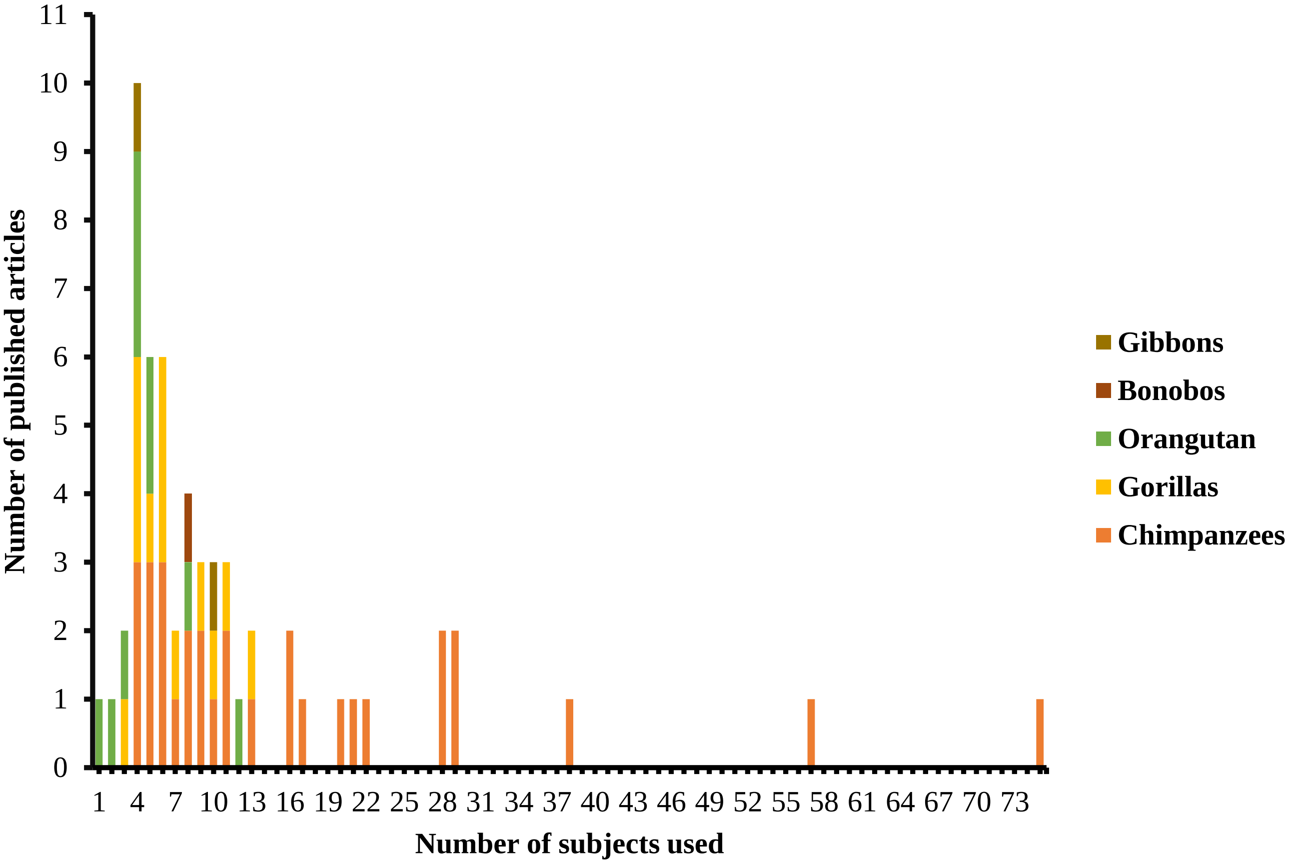


Figure 2.6 Number of subjects of each ape genus used in the published research

2.4.8 Life stage of subjects

The research articles included a mean of 17 adult apes (Median = 15, S.D = 11.1, S.E = 2.54) with n=49 being the most adults studied and n=1 being the least. With regards to juvenile apes a lower mean of 7 (Median = 7, S.D = 5.4, S.E = 1.34) individuals below the age of 10 years old were utilised in the research. A number of studies utilised no juveniles and one study provided enrichment to 21 juvenile apes.

2.4.9 Sampling and recording method

Sampling method: Scan sampling was employed by 53% (n=28) of the studies and focal sampling was used in 40% (n=21; **Figure 2.7**). *Ad libitum* sampling was used by only one author of the enrichment research and three authors did not state how they sampled the behaviour.

Recording method: The use of instantaneous sampling was most popular among authors (60%, n=32) and continuous sampling was employed in 21% (n=11) of studies (**Figure 2.7**). One-zero sampling was employed by only 11% (n=6) authors and four authors did not specify their recording method.

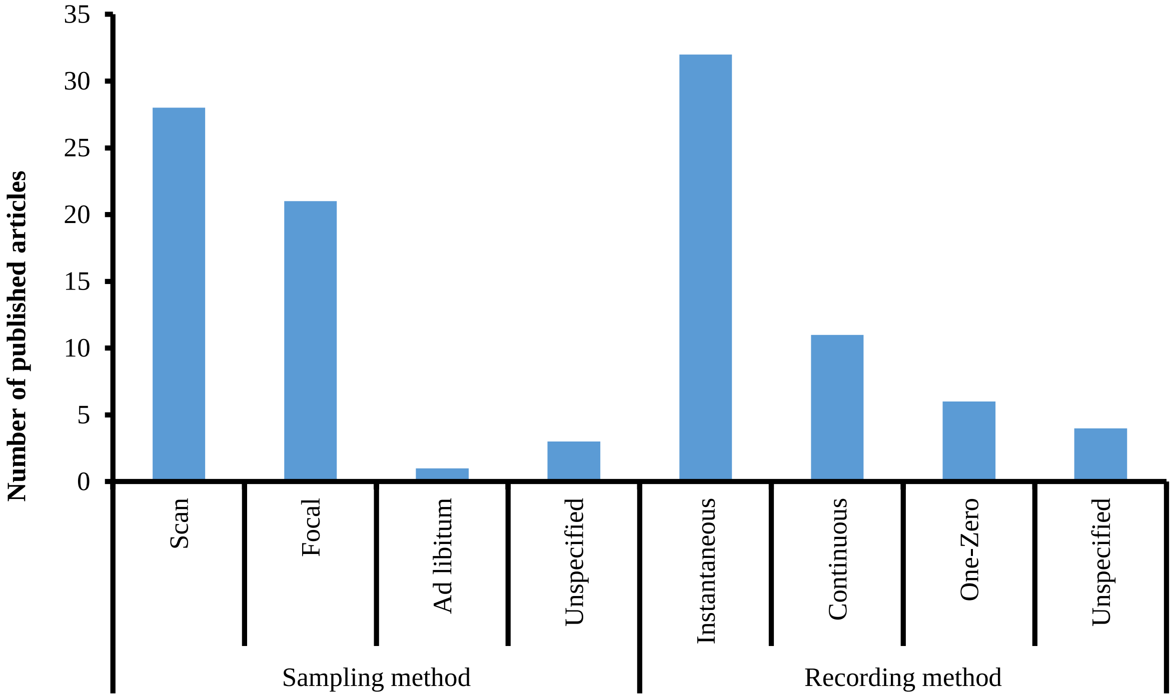


Figure 2.7 Sampling and recording methods utilised by the authors.

2.4.10 Temporal length of study

Out of the 53 studies reviewed, 26% (n= 14) did not specify how many days it took to gather the data used to assess the enrichment (**Figure 2.8**). An equal number of studies observed behaviour for 30 days or less. Fewer than 50% of the studies (n=25) were continued for more than 30 days and only half of these studies (n=12) continued for any longer than 90 days in total.

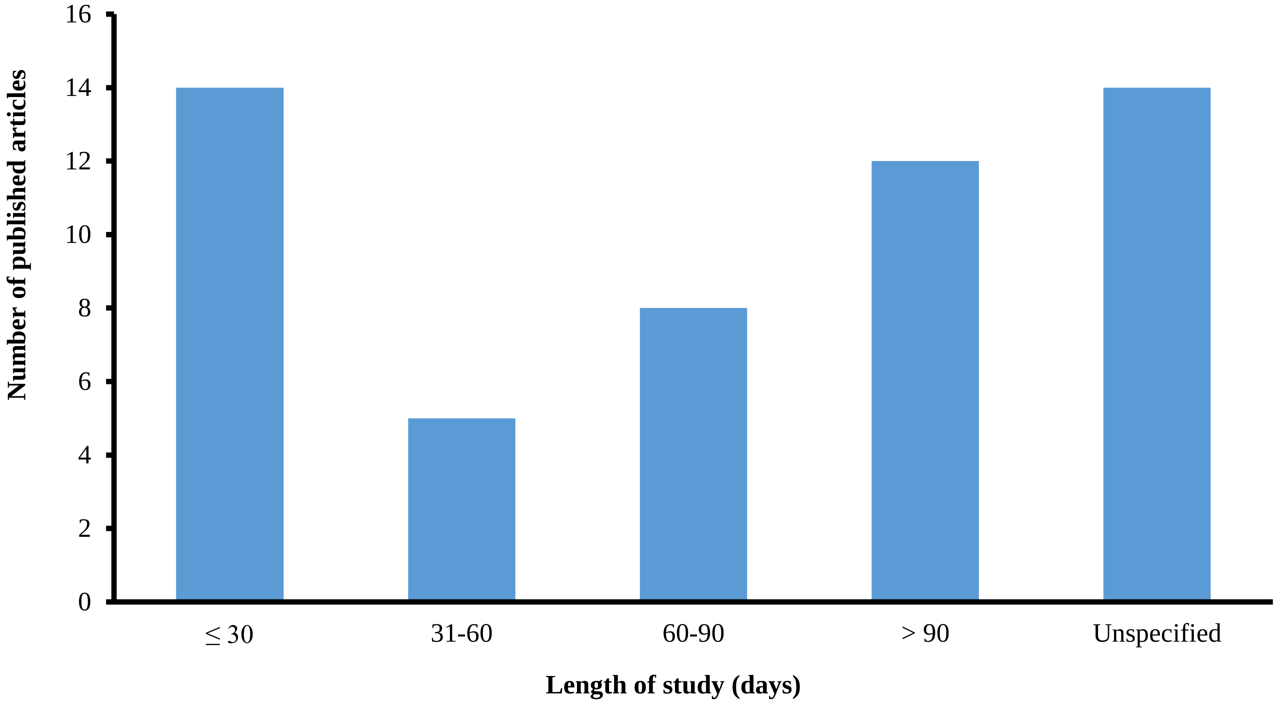


Figure 2.8 Total length, in days, of the enrichment studies, as specified by the authors

2.4.11 Experimental design

Nearly half (47% n=25) of the ape enrichment studies reviewed included observations of animals' baseline behaviours before the implementation of enrichment. Only five (9%, n= 5) of the studies implemented an ABA design.

2.5 Discussion

2.5.1 Year of publication

There was no clear pattern in the number of articles published each year from 1982 to 2014, although there was a slight increase from 1990 onwards, as was predicted by Bloomsmith *et al*. (1991). The increase coincides with the publication of the Universal Declaration of Animal Rights in 1978 which was updated by the Association of Animal Rights in 1989 and presented to the public (Koknaroglu and Akunal, 2013). Since then, there has been exponential growth in overall enrichment publications (de Azevedo *et al*., 2007). However, the maximum five articles on ape enrichment for one year (2003) found in this study is inconsistent with the 'exponential' growth reported for the field of environmental enrichment. In fact, during some years there was no research published reporting ape enrichment studies. It is clear that the field of ape enrichment in terms of the published research does not match up with the rest of the field and research does not appear to be growing at the same rate as it is for other taxa. A gap analysis which included enrichment studies, published between 1985 and 2004, for all taxa discovered that of all animals involved in enrichment research rodents make up 52.0% and primates made up only 16.74% of the research, followed by pigs and chickens (de Azevedo *et al*., 2007).

The wild populations of apes are declining rapidly (van Schaik *et al*., 2001; Huijbregts *et al*., 2003; André *et al*., 2008; Xue *et al*., 2015), adding further pressure on captive animal institutions to ensure the positive welfare of captive groups. Maintenance of behavioural diversity in captive populations is recognised as being important to species conservation and contribution to captive breeding and reintroduction in ensuring survival in the natural habitat (Shepherdson, 1994; Rabin, 2003). The general increase over the three decades matches the growing concern in great ape enrichment, and organisations appear to prioritise apes in enrichment programmes, but the literature, thus far, does not support such efforts. The changing attitudes towards animal welfare began in western countries and this may go some way into explaining the results associated the countries producing the research.

2.5.2 Country of origin

The United States appeared to be ahead of the rest of the world in terms of publishing ape enrichment research. It has been revealed previously that North America as well as Western Europe are the primary producers of all animal behaviour research (Ord *et al*., 2005) and analysis of environmental enrichment literature highlighted that the western countries dominate the field (de Azevedo *et al*., 2007). This may be associated with the recognition of animal welfare in western countries and the level of priority afforded to welfare research. Of all the ape enrichment research to come out of USA, 48% (n=16) studies were based in laboratories, the use of apes in research laboratories is discussed later in this discussion.

Differences in zoo legislation between the continents means that the standards required to keep and maintain captive populations differ. Zoos in Southeast Asia, for example, have a poor reputation with regards to their acquisition and handling of animals (Agoramoorthy, 2004). In particular, Surabaya Zoo, Indonesia has been dubbed “the zoo of death” due to the recent rise in animal deaths thought to be caused by neglect (Budisatrijo, 2014). Safari World, Bangkok (part of the largest orangutan collection in the world) were found to be housing around 70 orangutans without legal registration documentation in 2004 (Agoramoorthy, 2004) and Taiping Zoo in China purchased four gorillas (*Gorilla* spp.) in 2002 that had been captured illegally from the wild (Newman, 2013). It appears that these zoos have been less concerned with the welfare of their captive animals and more with making a profit. These zoos are governed by the South East Asian Zoos Association (SEAZA) who put an emphasis on promoting tourism in Southeast Asia (Agoramoorthy, 2004) and do not follow the same guidelines laid out by western zoos, who follow a strict approach to captive welfare and research (see DEFRA, 2012). The lack of stringent legislation in these zoos means that apes are likely to suffer poor welfare that goes undetected by the authorities, and therefore research into animal welfare and enrichment is also lacking.

2.5.3 Type of housing and ape subjects

The majority of ape enrichment studies reviewed here were conducted in a zoo setting; these articles also covered all captive housed ape species. Yet, all of the research conducted in laboratories only used chimpanzees as research subjects. This is accounted for by the use of chimpanzees within biomedical research in the USA. Since the 1920’s chimpanzees have been studied for their similarity to humans (Brent, 2004) and bred in captivity to aid the biomedical research industry. Between the years 2000 and 2006 grants were provided by the USA to use chimpanzees in both hepatitis and HIV research (Conlee, 2007). However, chimpanzees have a high potential to suffer with stress in laboratory housing (Knight, 2011). The experimental procedures that animals are subjected to can cause significant distress to chimpanzees which leads to the development of abnormal behaviours (Bloomsmith *et al*., 2006). The caregivers at the Save the Chimps (STC) sanctuary, Florida, USA, reported that 60 of the 253 chimpanzees rescued from biomedical research, exhibited abnormal behaviour or the symptoms of psychological distress (Lopresti-Goodman *et al*., 2015). Therefore implementing enrichment for chimpanzees housed in laboratories can be a valuable in preserving the mental health of these captive apes. The use of chimpanzees in biomedical research is now legal only in the

USA and Gabon (Conlee, 2007). Great Britain announced an end to licensing for research on apes in 1997 and Japan abandoned the use of chimpanzees in biomedical research in 2006 (Conlee, 2007).

The use of chimpanzees in research explains why a majority of the ape enrichment research has been produced in the USA. However, there has been a change in attitude towards the use of chimpanzees in research. With the Chimpanzee Health Improvement Maintenance and Protection (CHIMP) Act (see Brent, 2004) came restrictions on their use in medical research in the USA and hundreds of chimpanzees have since been retired from laboratories into sanctuaries. The reduction in chimpanzee use in the USA in research has the potential to trigger a decline in this field of research in the future.

The third largest contributor to the research in this review is Japan, where the studies utilised laboratory housed chimpanzees and further investigation revealed that the three facilities were part of Kyoto University's Primate Research Institute (Primate Research Institute, 2017). The research conducted covers evolutionary morphology, language and intelligence, and cognitive neuroscience. Considering the scale of research performed by the organisation, there is a considerable chance that, over the period covered by this review, more than three enrichment based studies have been published, however, these may well have been published in Japanese only and therefore would not have been included within the sample.

The dwindling numbers of chimpanzees in the wild (Walsh *et al*., 2003) means that the zoo housed populations represent a large proportion of the species' genetic and behavioural diversity, and may be called upon for rehabilitation and reintroduction in the wild (Reading *et al*., 2013). Of the six great ape species involved in zoo based enrichment research, it is the gorillas that appear to be studied the most. Currently there are around 340 gorillas housed in AZA accredited institutions in the USA, but only 262 chimpanzees, 215 orangutans and 80 bonobos (Lincoln Park Zoo, 2001; Gorilla SSP, 2008; BCBI, 2013; Orangutan SPP, 2015). The numbers for these species held in zoos worldwide are not available. Western Gorillas (*Gorilla gorilla*) are listed as 'Critically Endangered' on the IUCN Red List of Endangered Species (IUCN, 2014a) and a subspecies of Eastern Gorilla (*Gorilla beringei graueri*) is currently listed on The World's 25 Most Endangered Primates 2012–2014 (Schwitzer *et al*., 2014).

The only other great ape species currently classed as ‘critically endangered’ by IUCN is the Sumatran orangutan (Singleton *et al.*, 2008), but enrichment research on orangutans accounts for only 18.87% of the total research examined here. Orangutans are naturally solitary animals, but in captivity they are often housed in groups that resemble those of Gorillas. Studies have shown that this group composition found in many zoos can cause unnecessary stress to orangutans, especially the Bornean orangutans (Weingrill *et al.*, 2011). Orangutans are notoriously difficult to observe in the wild (Galdikas, 1985) and therefore researchers depend on captive populations to understand much of their behaviour and biology.

The bonobos are the least studied of the great apes and there are many fewer individuals held in zoos in North America compared to the other ape species (BCBI, 2013), however they are classed on the IUCN Red List as ‘Endangered’(IUCN, 2014b), so it is likely that these captive populations will be more valuable in the near future. Greater investment in the understanding and conservation of bonobo behaviour is essential to their successful propagation in zoos and should be reflected in the research (BCBI, 2013).

The total number of gibbons housed in zoos could not be determined from the literature, however, the Cao-vit gibbon (*Nomascus nasutus*) is the worlds’ rarest ape with less than 150 individuals remaining in the wild and no individuals housed in captivity (Fan, 2017). Although there are 17 species of gibbon, only three enrichment studies were found for this review. Given the large number of gibbon species, it is likely that there will be some variation in social structures and demographic requirements. Therefore, it is paramount that a greater emphasis is placed on sharing enrichment experiences with these apes. This diversity between gibbon species is not represented in the enrichment literature, meaning that animal keepers have very little information available with regards to enrichment guidance. As habitat degradation and complete loss is an increasing concern for all gibbon species (IUCN, 2017), the captive populations are the remaining resources available for future re-introduction and further research. Given the variation within and between ape species, it would be helpful if enrichment devices were trialled with members of multiple species. This information could save time and money for those institutions housing numerous ape groups.

2.5.4 Single- or multi-genus

There is evidence to suggest that different ape species react differently to similar enrichment programmes. When chimpanzees were exposed to a cognitive challenge in the form of a joystick task, rates of self-directed behaviours increased in relation to task difficulty (Leavens *et al*., 2001). However, when orangutans were provided with a similar task, also involving a joystick task with increasing difficulty, there was no increase in any undesirable behaviours and authors advocate the use of such systems as enrichment for captive orangutans (Mallavarapu *et al*., 2013). The use of social enrichment is said to be highly effective in captive apes in reducing behavioural abnormalities (Bloomsmith and Lambeth, 2000). The use of animal training and social play as enrichment for captive gorillas was successful in reducing abnormal behaviours (Carrasco *et al*., 2009). Yet orangutans are naturally solitary animals and group housing of Bornean orangutans has been associated with elevated stress levels (Weingrill *et al*., 2011). Chimpanzees live in large communities with a male dominance hierarchy (Lonsdorf *et al*., 2009), and high ranking males have been known to monopolise enrichment devices (Bloomsmith *et al*., 1990).Therefore, enrichment that is not well distributed can encourage aggression and stress (Honess and Marin, 2006).

Of the 53 papers reviewed here, four studies provided enrichment to two or more ape species. Whilst all four studies determined that the enrichment devices were successful in achieving their aim, species differences were described in all cases (Gilloux *et al*., 1992; Zaragoza *et al*., 2011; Schreiner *et al*., 2012; Kurtycz *et al*., 2014). Three of the four studies asserted that gorillas displayed less activity than other ape species when provided with enrichment (Gilloux *et al*., 1992; Zaragoza *et al*., 2011; Kurtycz *et al*., 2014). In one case, silverback gorillas were considered to monopolise enrichment and prevent access to enrichment by other group members (Gilloux *et al*., 1992) and in another, both feeding and locomotory behaviours decreased during enrichment treatments (Zaragoza *et al*., 2011).

The findings of these multi-genus studies reinforce the need to consider species differences in their response to enrichment. It is of particular importance for organisations housing two or more ape groups and could considerably affect their enrichment strategy. Determining enrichment that is successful for all groups could reduce the time and cost associated with planning and implementing novel enrichments for each group. On the other hand, understanding intra-genus differences in the effect of enrichment can prevent potential deleterious consequences of providing inappropriate enrichment.

2.5.5 Research aim

A range of reasons for studying the use of enrichment are given in the literature, yet there are five distinct categories. In the research reviewed here, almost the same proportion of studies aimed to reduce abnormal behaviours as did aim to increase the exhibition of the species' natural behaviours. However, the majority of the literature covers enrichment studies that were carried out to understand what effect the enrichment may have on behaviour rather than to target specific behaviours. This may reflect a shift in attitudes towards animals’ desires, as described by Young (2003). Previously, the tendency has been towards determining the causes of animal welfare problems and then establishing measures to address such problems; this is considered to be the reactive approach to animal welfare (Young, 2003). This approach has been criticised and it is reported that the use of environmental enrichment to tackle abnormal behaviours only ever has partial success, with no research managing to abolish abnormal behaviours altogether (Mason *et al.*, 2007). However, a shift in focus came with an animal centred approach which focused on what an animal wants and allowing animals to rate the relative value of resources (Dawkins, 1983; Young, 2003). This approach allows animal carers to prioritise animals needs and focus efforts towards the features of the environment which are important for animal welfare. This is a proactive approach to environmental enrichment which identifies those individuals most at risk of welfare problems (Mason *et al.*, 2007).

In the great ape literature reviewed here, those studies which set out to address behavioural problems all reported significant reduction in undesirable, abnormal or stereotypic behaviours as a result of environmental enrichment, however none of the authors were able to report a complete elimination of such behaviours (Brent and Eichberg, 1991; Howell *et al.*, 2003; Csatádi *et al.*, 2008; Robbins and Margulis, 2014). These findings support the notion that enrichment should be implemented as a way of ensuring good animal welfare and not as a reaction to welfare problems. In order to determine what an animal finds valuable or pleasurable in the environment, they must be provided with choice. The large proportion of research which explores the use of enrichment to understand how animals respond, illustrates the shift in attention towards proactive research. Of the 53 studies included in this research, eight stated aims that did not fit into either of the four categories; these included ‘to compare enrichment devices’ (Shefferly *et al.*, 1993), ‘to reduce habituation’ (Tarou *et al.*, 2004) and ‘to test the relationship between controllability and frequency of use’ (Videan *et al.*, 2005).

These studies add greater depth to the enrichment research and the methods used and develop an understanding of the effects of enrichment that do not relate to specific behaviours.

The great ape enrichment research is gathering a wealth of knowledge to understand what apes need and want in their captive environments. This reflects the priorities of zoos and laboratories in providing environments which cater to the species' requirements. Once the aims of the research have been determined, it is the type of enrichment that is the next priority.

2.5.6 Enrichment category

Enrichment from each category described was implemented in the literature reviewed here. Previous research that has studied the use of enrichment with mammals noted that nutritional enrichment was considered by animal care staff to be the most important, because it is easy to implement and does not take up a significant amount of their time (Hoy *et al*., 2010). Other authors advocate the use of social enrichment in reducing abnormal behaviours (Bloomsmith and Lambeth, 2000), however it is observed that additional conspecifics are not easily sourced in zoos and the addition of new group members has the potential to cause stress, especially in ape groups (Amrein *et al*., 2014). Male chimpanzees have been known to fatally injure other male group members both in captivity and in the wild (de Waal, 1986; Mitani *et al*., 2010), yet introduction of new males to already established groups is common practice in captive populations. Thus social enrichment should be implemented with extreme caution, and rigorous monitoring is necessary to avoid negative consequences. The problems associated with introducing new group members explains the limited use of such exercise as enrichment.

As has been discussed, different enrichment items appeal more or less so to different species; the age of group members also influences the behavioural response. Juvenile chimpanzees prefer destructible toys over indestructible ones (Shefferly *et al*., 1993), infant orangutans are less able to use tools than juveniles (Nakamichi, 2004), dominant chimpanzees are more likely to monopolise enrichment and therefore gain more value from it (Celli *et al*., 2003), and nutritional enrichment caused significant changes in different behaviours in different orangutans within a single group (personal observation). It is possible that enrichment which has a positive effect on the welfare of some group members may have no benefit or even cause stress to other group members. Hence the study of multiple enrichment items with a single group may be fundamental in finding the most appropriate enrichment for comparable groups. Some enrichment items hold the attention of apes for only short amounts of time, for example, a tool use task failed to maintain the attention of captive chimpanzees (Celli *et al*., 2003) as did the use of videotapes (Bloomsmith and Lambeth, 2000). Therefore understanding the effect of a number of enrichment items means that care staff may have a tool box of enrichment items at their disposal to use in rotation to maintain the attention of captive apes of various backgrounds and reduce habituation.

Research that explores the use of multiple enrichment items is valuable in developing a programme consisting of enrichment devices that are known to be effective in enhancing the welfare of captive apes. In terms of using the published literature to determine a suitable enrichment item for an ape group, the spread of research across all types of enrichment means that very few studies focus on one single category of enrichment. A more detailed review of the literature determined that in only very few cases was the same device (e.g. videotapes) used in more than one study. The volume of data available on any single enrichment item is not enough for animal care staff to base any decisions for suitable enrichment on. It is evident that there should be a focus in the literature to thoroughly assess the use of those successful enrichment devices in repeatable studies to ensure larger volumes of data are available on specific enrichment items.

2.5.7 Number of subjects

The research in which the largest groups were utilised used chimpanzees as the focal species. The largest group comprised 75 individuals. However, these were housed in smaller sub-groups of between two and seven individuals (Videan *et al*., 2005). The groups also varied in composition with all-male social groups, all-female social groups and harem social groups.

Large samples sizes are necessary to be able to take into account the individual variation that is observed in apes and their response to environmental change. Research has investigated personality traits in apes (Uher and Asendorpf, 2008; Uher *et al*., 2008) and these differences will undoubtedly have an effect on an individual's response to enrichment. Ape social groups usually consist of dominant individuals and subordinates, as well as infants, juveniles and adults, and each will have their own preferences within the captive environment. For example, in all apes studied, mature females displayed the most interest in a puzzle feeder within mixed groups (Gilloux *et al*., 1992) and immature males displayed higher levels of solitary play than females when provided with manipulable objects and the same objects caused distress to an adult male in the group (Pruetz and Bloomsmith, 1992). Therefore the greater number of individuals exposed to enrichment in research, the more likely it is that animal care staff will be able to cater for each ape within the group they care for.

With regards to robust scientific investigation, many of the statistical tests utilised in animal behaviour research require a minimum number of subjects in order to agree with their assumptions. Efforts to overcome the challenges associated with small sample sizes may result in pseudoreplication, violation of the assumptions and Type II errors (Plowman, 2008) and can cause problems of ecological validity when trying to extrapolate to the whole population (Hosey *et al.*, 2009). It is suggested that a minimum of eight individuals is required for standard parametric and non-parametric tests, but smaller samples will require the use of randomization tests (Hosey *et al.*, 2009). Of the ape groups studied, 49.12% (n=28, **Figure 2.6**) contained less than eight individuals and the remaining 50.88% (n=29) studies used eight or more individuals. However, large groups do not necessarily solve the problem, as a single group comprising 20 individuals still leaves researchers at risk of pseudoreplication, in that the whole group represents only a single sample still. Fortunately those studies in which larger numbers of individuals were provided with enrichment, the apes were housed in smaller subset groups which meant that samples comprised of between one and five individuals and avoided potential problems with pseudoreplication (Maki and Bloomsmith, 1989; Lambeth and Bloomsmith, 1992; Pruetz and Bloomsmith, 1992; Ostrower and Brent, 1997; Howell *et al.*, 2003).

In the absence of multiple sub-groups of apes it may be necessary to investigate the use of enrichment with ape groups housed in multiple organisations. Multi-zoo studies allow research to considerably increase their sample size as well as understand the effects of a number of variables (husbandry routine etc.; Hosey *et al.*, 2009). A multi-zoo study was successful in understanding the factors that affect activity in orangutans in nine different zoos (Perkins, 1992). Large samples sizes are also more likely to contain group members at different life stages and thus capture more valuable data.

2.5.8 Life stage of subjects

Within the ape enrichment literature, a greater number of adults are studied than juveniles. This makes sense considering female apes only carry one or two offspring at a time, and males do not often provide paternal care, meaning there is a limited number of infants that a group can contain at once. A total of 14 of the enrichment studies that reported the number of adults or juveniles studied adults only, presumably because no juveniles were available, and four of the studies used only juveniles. As was mentioned earlier, it is vital to understand the effects of enrichment on all group members, of all age and sex classifications to ensure that the optimal enrichment can be employed for the specific group.

There should be greater effort to understand the effect of enrichment on juveniles. Juvenile apes tend to exhibit play behaviour more often than adults and are generally more active (Hemphill and McGrew, 1998). Juveniles also do not have the same necessity to forage as their adult counterparts. Very young apes will not need to forage at all, as their nutrients will be gained from their mother, and so enrichment that is geared towards increasing foraging time, will have little direct effect on the behaviour of juveniles. Yet novel objects that promote play and exploration are likely to hold the attention of juveniles more so than adults (Ramsey and McGrew, 2005). For example, young chimpanzees exhibited increasing levels of interaction with mirrors over time, but adults in the group did not (Lambeth and Bloomsmith, 1992). Nevertheless, infant and juvenile apes learn most of their behaviours from older group members, not necessarily their parents (Biro *et al.*, 2003; Hirata and Celli, 2003). Juvenile orangutans are more likely to use tools that have been previously used by adults (Nakamichi, 2004) and hence learn tool use behaviours from older group members.

Enrichment should be provided for all group members, but there needs to be a greater focus on the effect that enrichment may have on infant and juvenile apes, because it may be possible to prevent abnormal behaviours all together and achieve a behavioural repertoire that matches wild apes from an early age in these group members. As previously discussed, no enrichment studies thus far have been successful in completely abolishing abnormal or stereotypic behaviour and therefore preventing juveniles from experiencing poor welfare should be heavily prioritised. The ability for young apes to learn from other group members means that they can gain an indirect benefit from enrichment that is targeted towards older individuals (Biro *et al.*, 2003; Hirata and Celli, 2003).

2.5.9 Sampling and recording method

When taking behavioural observations, researchers must decide on both the sampling method (which animals to observe and when) and recording method (how to record the observations; Martin and Bateson, 2007). The sampling method used by 52.8% of the researchers in this research was scan sampling. Focal sampling, utilised in 39.6% of the enrichment research, involves taking observations of a particular individual, pair or group of animals at a specified sample points or over a sample period (Martin and Bateson, 2007; Altmann, 1974).

Both scan and focal sampling are widely used to sample animal behaviour in both captive and wild habitats and the decision to use either method is often based on the number of individuals being observed and how quickly they change behaviour. In the research reviewed here, scan sampling was most regularly used to observe chimpanzee groups (Maki and Bloomsmith, 1989; Brent and Eichberg, 1991; Howell *et al*., 2003; Videan *et al*., 2005), whilst focal sampling was used to observer gorilla and orangutan groups (Tripp, 1985; Pizzutto *et al*., 2008; Carrasco *et al*., 2009). The slower nature of gorilla and orangutan activity allows observers to follow groups or individuals and focus on specific individuals. Chimpanzees, however, are generally more active and difficult to distinguish as individuals when most active (personal observation).

One of the enrichment studies employed *ad libitum* sampling to observe animal behaviour (Caws *et al*., 2008). Here relevant behaviours are noted when it is deemed appropriate, without systematic constraints (Martin and Bateson, 2007). This method tends to be biased towards the recording of more notable behaviours and is most useful for preliminary observations to understand the range of behaviours before conducting a scientific evaluation.

The recording methods utilised in the enrichment research included instantaneous recording (60.4%). The score produced gives the proportion of sample points at which the behaviour or pattern of behaviours occurred (Martin and Bateson, 2007) and can provide information on the behavioural synchrony of a group (Altmann, 1974). However, instantaneous sampling is not appropriate for recording rare behaviours or events of short duration, in particular if the data collection period is relatively short (Martin and Bateson, 2007).

An alternative to instantaneous sampling is continuous sampling, utilised in 20.8% (n=11) of the studies. Continuous sampling allows the observer to produce an exact record of the animal's or animals' behaviour, the times at which events occurred and how long events lasted (Martin and Bateson, 2007). Observing animals for continuous periods of time can be advantageous when observing small numbers of animals and those that are slow moving or do not change activity quickly, allowing observers to understand components of behaviours rather than snapshots of behaviours (Hosey *et al*., 2009). However, continuous sampling requires continuous concentration and the ability to identify all individuals at all times, therefore with larger groups or particularly active animals, continuous sampling is less favourable and can reduce the quality of the data collected.

One-zero sampling was used by only 11.3% of the researchers; the method was used to sample all behaviours as described by ethograms (Rooney and Sleeman, 1998; Ryan *et al*., 2012). The score notes the proportion of sample periods in which a certain behaviour occurred at least once (Altmann, 1974; Kraemer, 1979). Although some authors assert that one-zero sampling is not a justified method of observation (Altmann, 1974), when neither instantaneous or continuous sampling are appropriate, one-zero sampling may be useful in recording intermittent behaviours or behaviour patterns such as play behaviour (Martin and Bateson, 2007).

It appears that the selection of both sampling and recording method relies heavily on the aims of the enrichment study and the size of the group involved. Therefore it is important that researchers understand the methods available and that they choose the most appropriate methods in order to collect a sizeable dataset.

2.5.10 Temporal length of study

The length of enrichment studies may be constrained by a variety of factors such as availability of staff time, zoo husbandry routines and the health of individuals. But it is important to consider the variables that may directly affect animal behaviour when designing an enrichment study. For examples, observations of wild orangutans have noted that the display of feeding, travelling and resting was correlated with changes in weather, with rain resulting in increased resting in day nests (MacKinnon, 1974; Mitani, 1989). The hormonal changes associated with the menstrual cycle in female apes is also known to have an effect on affiliative behaviour (Nadler *et al*., 1983). Of the 53 ape enrichment studies analysed in this study 26.4% did not specify how long the study lasted so it is not possible to understand if the effects of weather or season were taken into account. Another 26.4% of the studies did not continue observations for any longer than one month. Animal behaviour is influenced by many variables which fluctuate over time, for example primates are known to alter their thermoregulatory behaviour in response to temperature change (Hill *et al*., 2004). Primates housed in zoo environments are also affected by the change in visitor numbers which coincide with weather change and visitor numbers are known to significantly alter primate behaviour (Hosey and

Druck, 1987). These findings suggest that behavioural observations should be conducted over extended periods of time where possible to allow researchers to take such variables into account.

The effect of these variables on animal behaviour should be determined before implementing enrichment to ensure that that changes in behaviour can be reliably associated with the implementation of the enrichment. Carrying out a period of behavioural observations before introducing enrichment can provide a baseline of data with which to compare the enriched condition. This sort of experimental design has been suggested by Young (2003) and has been implemented in a number of the studies reviewed here.

2.5.11 Experimental design

Of the 53 ape enrichment studies considered in this research, a total of 23 studies observed baseline behaviours before the implementation of enrichment. This means that statistical analyses were able to determine the effect of enrichment in comparison with the non-enriched condition. In studies that aim to combat the incidence of abnormal behaviours in apes, it is important to determine the stability of the abnormal behaviour within focal individuals to be sure of the effect of the enrichment once implemented (Shyne, 2006).

The ABA study design, used in five of the research articles, allows the researcher to consider the effects of implementing enrichment on baseline behaviours as well as the effect of removing enrichment. In cases where the researcher is attempting to combat abnormal behaviours, the return of the abnormal behaviour to baseline levels gives the researcher confidence that "regression to the mean" is not solely responsible for the observed changes (Shyne, 2006). This is also true when the aim of enrichment is to increase the display of a species' natural behaviours. It is also possible that the removal of enrichment items causes stress for captive animals. Individuals may come to rely on enrichment as a way to pass time or to disperse tension and reduce aggression within the group (Honess and Marin, 2006). Primates have been observed to guard enrichment and prevent researchers removing items (Bayne, 1989). Observations of behaviours post-enrichment are valuable in assessing any negative consequences associated with removing enrichment and ensure that the experimental approach is systematic and hypothesis driven (Honess and Marin, 2006).

To develop the evidence collected by enrichment research the use of a cyclical ABAB design would be most enlightening (Swaisgood and Shepherdson, 2005). In terms of the effect of enrichment before and after implementation as it also allows an assessment of habituation and dishabituation, when new enrichment or the re-introduction of enrichment encourages a return of the behavioural response to the original enrichment (Tarou and Bashaw, 2007). These studies would also allow for the variables previously discussed to be taken into account and ensure a more robust exploration of the hypothesis. However, the financial and time constraints on zoo and laboratory behaviour research limit the possibilities of such extended research.

2.6 Conclusion

Zoo populations represent a large proportion of the genetic and behavioural diversity for all ape species, and maintenance of this diversity in captivity is likely to be valuable in future reintroduction programmes. The proportion of enrichment studies conducted on laboratory chimpanzees distorts the picture, illustrating that laboratory chimpanzees are a priority in enrichment research. However, the decline in chimpanzee use in biomedical research over recent decades does not support this. Orangutans and bonobos are largely ignored in the research suggesting that they are a lower priority, yet their wild populations are at great risk of extinction, leaving the captive individuals representing a great deal of their species' diversity. Therefore it would be a great benefit to institutions housing more than a single ape species to be able to implement the same enrichment devices with all groups. Unfortunately, research that looks into the use of enrichment across different species is lacking in quantity.

Evaluating the use of enrichment that addresses specific abnormal behaviours in a small number of individuals is of little use to other institutions but the amount of research conducted to understand the general effects of enrichment is promising. Institutions should prioritise enrichment for all animals vulnerable to developing behavioural abnormalities rather that to treat symptoms of poor welfare once they develop. The enrichment devices used in the research cover a range of those available to animal keepers, yet the volume of studies focusing on each category does not provide a large enough data set on which to draw conclusions regarding their effectiveness and success. Future research should focus on implementing efficient enrichment, available to all animal care staff and carrying out a scientific evaluation with multiple, if not, all ape species where possible. The number of individuals provided with enrichment also needs to be greater, including a wider age range of individuals. This will allow animal care takers to deduct, from the research, how the enrichment might affect each individual ape in the group and make provisions for particular group members.

The methods used to scientifically evaluate enrichment also require more attention. The sampling and recording methods used will be dependent on the ape group studied, but it is important to assess the effects of enrichment over an extended period of time to understand how the apes will use enrichment in all weathers and times throughout hormonal cycles. Providing a greater volume of research to the zoo community will provide a valuable pool of information from which animal care takers can select the enrichment device that will suit their animals and encourage the behavioural diversity that is essential in maintaining successful captive populations. Thus, the following chapter in this thesis implements two forms of enrichment for a group of captive orangutans over an extended time frame, to address the concerns raised in this chapter and determine the effectiveness of behavioural observations as a tool for monitoring and quantifying animal welfare.

Chapter THREE: Behavioural Observations to Understand the Impact of an Enrichment Programme on the Behaviour of Captive Bornean Orangutans (*Pongo pygmaeus*)

3.1 Chapter overview

The systematic review of great ape enrichment literature outlined gaps within the research, one of which being the lack of focus on the enrichment of captive orangutans. This chapter briefly reviews the orangutan enrichment literature and reinforces the findings of the previous study. Secondly, the chapter follows the implementation of two enrichment devices with a group of captive orangutans housed at Twycross Zoo. Behavioural observations were employed as a method for assessing and quantifying the welfare of the group following the introduction of enrichment. Patterns of behaviour have been analysed revealing two distinct dyads in the group. Linear regressions were employed to determine the effect of enrichment of the behaviours of each group member. Using an increase in natural behaviours as an indicator of an improvement of animal welfare, the data support the use of both enrichment devices with captive orangutans. Behavioural observations were an effective method of gathering a detailed account of orangutan activity, and analysis explored each stage in the enrichment programme. However, the difficulties associated with continuous sampling, the variability in day length, and the amount of time consumed by data collection and input limited the potential of the method.

3.2 Introduction

Given that positive animal welfare is associated with the display of the species' natural behaviours, it follows that observations of animal behaviour are instrumental in the welfare of captive animals. So far, the utilisation of behavioural observations following the implementation of enrichment has been reviewed and it was highlighted that very little information exists for the enrichment of captive orangutans (chapter two). The research which has been produced so far has had mixed results. There have been successful attempts to provide orangutans with manipulable objects to encourage desirable activity (Tripp, 1985; Wright, 1995). However, a computer joystick system was found to increase aggressive behaviours and reduce social interactions as well as feeding behaviour (Tarou *et al.*, 2004). In contrast, the use of touchscreen computers did not result in any increases in aggression or distress-related behaviours (Perdue *et al.*, 2012) and another joystick system produced variable results within the orangutan group (Mallavarapu *et al.*, 2013). Various genres of music, based on those preferred by humans, failed to interest a group of orangutans, who generally preferred not to be exposed to the enrichment by demonstrating a preference for silence (Ritvo and MacDonald, 2016). These studies represent the extent of orangutan enrichment research and most notably, those techniques which do not result in welfare improvement.

The review of great ape enrichment research (Chapter two) also highlighted a disparity in the time over which enrichment was provided and monitored, with less than 50% of studies continuing for any more than 30 days. Furthermore few studies have implemented more than a single device over the course of a study and therefore are unable to report any potential comparison between enrichment types. The research concludes that the most successful forms of enrichment are either those which are specifically designed to encourage a species' natural behaviour or to combat the incidence of abnormal or stereotypic behaviour (Wells, 2009b; Claxton, 2011). As a result of these deductions from the literature, the following study investigates the use of two enrichment devices with a group of four captive orangutans. The first enrichment device, frozen fruit cups, was intended to increase the amount of time the individual orangutans devoted to foraging and feeding activities. As discussed in chapter one, feeding and foraging behaviours make up a large proportion of a wild orangutan's daily activity budget and therefore an increase in these behaviours would indicate positive welfare. The second enrichment, an artificial termite mound, was implemented to encourage both tool use and tool manipulation behaviours in each member of the group. Orangutans do not only use tools to access food but also use sticks as part of their grooming and parasite control. Wild orangutans have been observed using branches to scratch themselves as well as to swat away wasps (Galdikas, 1989). Therefore, it is determined that behaviours related to the making and use of tools are important to encourage within captive orangutans cognitive stimulation (Celli *et al.*, 2003).

To address shortcomings of great ape enrichment research, this chapter aims to determine the effect of an enrichment programme, involving two enrichment devices, on the welfare of captive orangutans and to assess the use of behavioural observations as a means of measuring welfare. The utilisation of behaviour observations as a means of assessing animal welfare will also be discussed and its strengths and weaknesses identified.

3.3 Methodology

3.3.1 Subjects and housing

The subjects were a group of four Bornean orangutans housed at Twycross Zoo, Leicestershire, England. One adult male named Batu (25 y/o), two adult females named Kibriah (37 y/o) and Maliku (20 y/o) and one infant female named Molly (3 y/o). Batu was born in Münster, Germany, and transferred to Twycross zoo in 1995. Kibriah was born at Twycross zoo but was loaned to Chester zoo on multiple occasions, where she gave birth to Maliku in 1994. The pair were then returned to Twycross in 1996 and Maliku gave birth to Molly in 2011 (after having a previous daughter, Miri, who now resides in a German zoo); both of Maliku's daughters were fathered by Batu. The group resided in an enclosure comprising of sleeping quarters, an internal enclosure and an external enclosure. The external area of their enclosure measured 372m^2 and contained a large wooden frame for the orangutans to climb and rest on. The internal area comprised of two day-time play areas, separated by a metal wall, and the animals' sleeping quarters extended out of this area. The animals had access to 158.7m^2 of the internal area (**Figure 3.1**).

3.3.2 Husbandry routine

Throughout the study the keepers maintained their usual husbandry routine with the animals. The orangutans were released from their sleeping quarters between 8.30 and 9.30am each morning where they were given free access to the internal day area and external area of their enclosure. The floor of the internal area was covered with a breakfast scatter feed, usually consisting of cabbages, onions, green beans, celery and pellets. Throughout the rest of the day the orangutans had free access to both internal and external areas of the enclosure with the exception of their sleeping quarters, which were closed off for cleaning and re-bedding. Each day the orangutans were given a scatter feed of vegetables between 11.30am and 12.30pm; this was tossed over the wall of the external area to encourage the display of foraging and travelling behaviour in each group member. Between 3.00 and 4.00pm (usually dependent on the weather and the compliance of the orangutans) the animals were locked in the internal area of their enclosure in preparation for their evening meal in their sleeping quarters.

3.3.3 Enrichment treatments

The study involved the use of two types of enrichment separated by periods of baseline observations. In order to assess the use and effect of each type of enrichment the study was divided into phases each focusing on either one type of enrichment or a period of baseline observations (**Table 3.1**). A total three days of observations over the period of study were not completed due to observer sickness.

3.3.3.1 Baseline

Behavioural observations were carried out from 30th June until 19th September 2014 for a total of 12 weeks and divided into four treatments each lasting three weeks (**Table 3.1**). During the first three weeks, the orangutans were observed in their normal husbandry routine with no enrichment. This was to determine a baseline of behaviour levels under normal conditions, to which we can compare the effect of enrichment treatments.

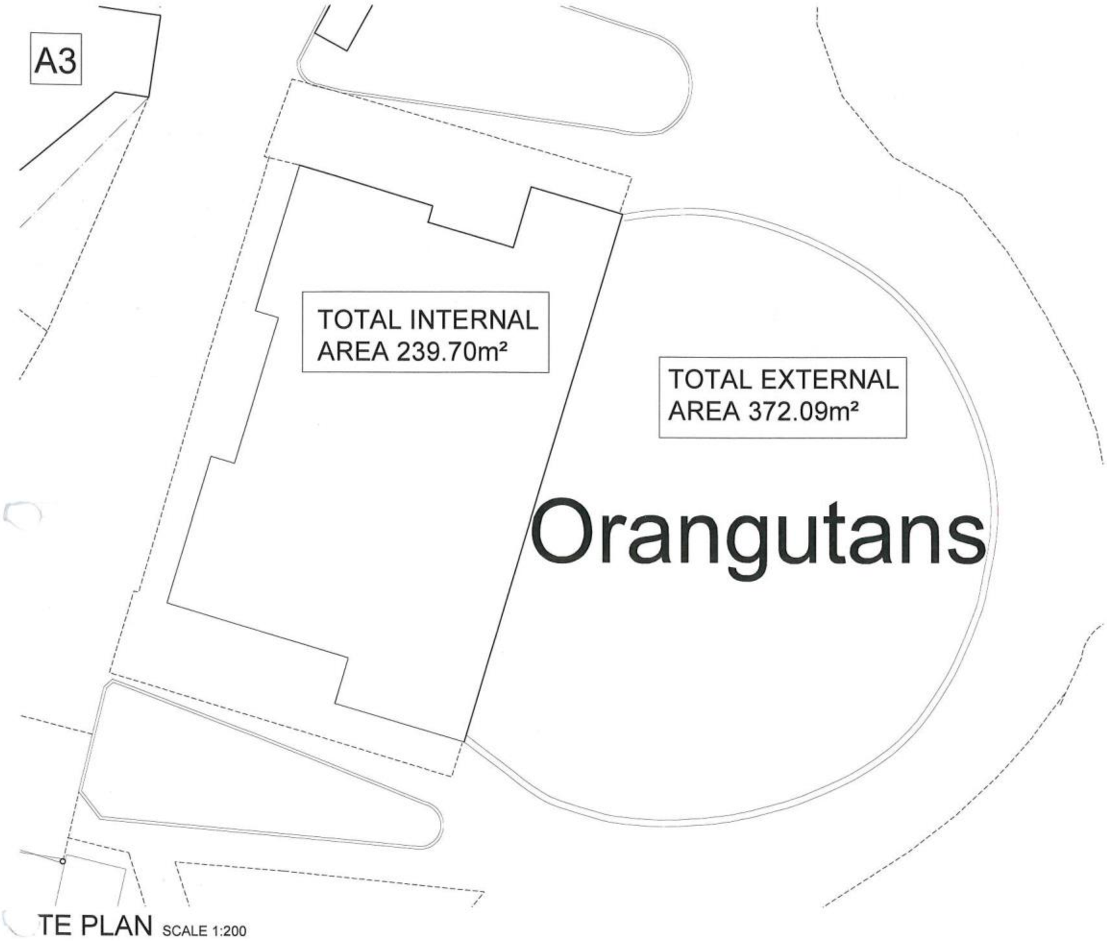


Figure 3.1 Floor plan of the orangutan enclosure at Twycross Zoo.

3.3.3.2 Feeding enrichment

The second enrichment treatment involved continuing baseline observations without enrichment for one week (week 4) and then providing the frozen food enrichment each day for the following week (week 5; Monday-Friday). This was then followed by a week of no enrichment again. This plan followed an ABA experimental design (see Young, 2003), allowing for analysis of potential continuing effects of enrichment once it had been removed or changes in behaviour as a result of removing the enrichment. The enrichment employed in this stage was frozen fruit cups (therefore this period of the study is known as the 'Food' treatment) thrown over the wall of the external enclosure in coordination with normal scatter feed. The fruit cups contained berries and other small fruits, were filled with diluted fruit juice and then frozen. During the scatter feed, eight of these cups were thrown over the external enclosure wall in such a way that each orangutan had an opportunity to collect a number of cups each. The male orangutan, Batu, was well practiced at collecting a lot of food for himself during a scatter feed. Therefore eight, rather than four, cups were provided to provision for the potential monopolisation of resources. These frozen food devices provided a further opportunity for foraging as well as increasing both food handling and feeding time, given that the animals had to tear the plastic cup away and manipulate the ice to get to the fruit inside.

During the third period of the study (week 7-9; **Table 3.1**), the researcher planned to implement an artificial termite mound for tool use enrichment but due to circumstances beyond the control of the researcher and the zoo this phase had to be delayed and instead, the third stage consisted of a further three weeks containing no enrichment. This provided a second set of baseline observations for the study, indicated as 'Baseline2' in the results and analysis.

3.3.3.3 Tool use enrichment

During this final stage of the study, the termite mound (known as the 'Termite' treatment) was implemented as the tool use enrichment in the first week (week 10; **Table 3.1**). The termite mound was built by the ape section leader and maintenance crew at Twycross zoo using a welded steel piping frame as a base. To this frame, plastic pipes were attached to create holes in which yogurt and porridge could be placed, screw caps over the end of the pipes meant that the food would not fall into the mound. This structure was then covered with cement to create a naturalistic looking termite mound (**Figure 3.2**). A small door at the back of the mound allowed keepers to access the pipes and to deposit the food. The termite mound was installed over a weekend (29th-31st August) and the orangutans were given access to the device for a single day before observations resumed. A day of exposure to the mound allowed the orangutans to familiarise themselves with the environmental change.

When behaviour observations resumed, the orangutans were provided with sticks covered in leaves to use as tools. The sticks provided were collected from the zoo grounds and had leaves and twigs branching out from the main stick. Therefore, the orangutans had to manipulate the sticks, by removing the leaves and twigs in order to fit them in the holes. The orangutans inserted the sticks into holes created by the plastic pipes to scoop out the yogurt or porridge.

The following week (week 11) of the study involved the implementation of both devices, the food and termite mound enrichment (as described above) simultaneously. This stage is noted as the ‘TermiteFood’ (**Table 3.1**) enrichment treatment. The final week (week 12) comprised behavioural observations in the absence of enrichment. This also allowed an analysis of whether the removal of the enrichment had an impact on behaviour. The termite mound was not removed, but neither food nor sticks were placed in the mound.

Table 3.1. The timetable of enrichment provided for the orangutans. Weeks 1-3 and 7-9 comprised baseline observations. Weeks 4-6 involved the frozen food enrichment and weeks 10-12 involved the termite mound and the frozen fruit enrichment.

Week	**1**	**2**	**3**	**4**	**5**	**6**
Treatment	None			None	Food	None
Week	**7**	**8**	**9**	**10**	**11**	**12**
Treatment	None			Termite	TermiteFood	None

Figure 3.2 Maliku using a stick to access food in the artificial termite mound.

3.3.4 Behavioural observations

Observations only took place on weekdays to exclude the effect that additional weekend visitors may have on the orangutans' behaviour (Choo *et al.*, 2011)**.** Continuous group sampling began at 9.30 am each day for 1 hour, with 20 minute interval breaks. This gave a total of four hours of observations each day. As the time at which the orangutans were locked away for the night varied from day to day, there was often an incomplete fifth hour of observation.

Due to the availability of only a single observer, only the orangutans occupying the external area of the enclosure would be recorded even if only one orangutan occupied this space. From personal observations prior to data collection, it was noted that Kibriah spent most of her day inside and when doing so she was most often "resting" therefore it was judged fair to assume that orangutans occupying the indoor enclosure were "resting" most of the time. Also, it was very unlikely that Maliku and Molly would occupy separate enclosures for any prolonged time, due to the close maternal bond between them. Therefore at any one time at least two of the four orangutans were being observed. When all orangutans were in the internal enclosure, observations focused on the indoor viewing area. If the orangutans were taken off of public display for any reason, e.g. for enclosure maintenance or veterinary treatment, observations ceased, and continued when orangutans were given free range of the enclosure.

The behavioural ethogram used by Wood (1998), originally designed to monitor chimpanzee behaviour, was adapted for use with orangutans (**Table 3.2)**. The ethogram classifies behaviours into 12 individual Time Use Categories (TUCs). A single observer recorded the TUC for each individual at the start of an observation period, and when an individual changed TUC, the time, name of the orangutan and the new TUC. Additional field notes were taken to provide a supplementary commentary (e.g. husbandry related events, weather events).

Table 3.2 Ethogram of Time Use Categories (TUCs) of captive orangutans. This has been adapted from Wood (1998).

Time Use Category (TUC)	**Description**
Care giving and receiving	. Interactions between mothers and infants
Displaying / fighting	. Aggressive displays . Physical fighting between individuals
Exploring	. Individuals obtaining information about the qualities of the environment or an object
Foraging	. Active and concentrated search for food using the hands and/or the mouth
Feeding	. Ingestion of food . Begging for food
Grooming	. Social or solitary grooming
Object using	. Manipulating objects for use as tools to achieve a goal (nest building, foraging)
Playing	. Social or solitary play . With or without other objects . Playful behaviours without an obvious motive
Sex	. Mating . Self-stimulation
Travelling	. Locomotion to another area of the enclosure . Does not include foraging or exploring
Idle	. Resting . Sleeping . Watching
Object manipulation	. Learning the properties of a tool or object

3.4 Analysis

3.4.1 Differences in behaviour patterns between individuals

Before determining the effect of the enrichment treatments on orangutan behaviour, it is advantageous to look for overall patterns of behaviour within and between individuals. This allows us to determine which behaviours are influential in an individual's daily behavioural repertoire as well as comparing behavioural patterns between individuals to highlight potential intra-group relationships. The ordination method of principal component analysis **(**PCA; Legendre & Legendre, 2003) was used to identify patterns of behaviour across the study period for each individual. Commonly used for high-dimensional data, PCA transfers the data into fewer dimensions whilst retaining the trends and patterns present. All behaviour variables were scaled prior to the application of the PCA, and PCA was conducted for each individual. A scree plot and the Kaiser-Guttman criterion were used to assess the extent to which behaviour dimensions could be robustly reduced. The Kaiser-Guttman criterion produced a histogram of the eigenvalues associated with each principle component, and the rules state that those greater than 1 should be retained as these represent above average components (Lambert *et al*., 1990).

To establish the extent of differences in behaviour patterns between individuals, the distance based method of non-metric multidimensional scaling was used (nMDS; Legendre & Legendre, 2003). This method summarises the data for each individual on each day and plots them according to their similarity. It then provides a visual output in which the distance between data points is directly related to the similarity of the two data points. Hence, it is possible to visually assess relationships between the behaviour patterns of individuals and identify outlying days. These anomalous data points may indicate days of significant behaviour change and warrant further investigation. Behaviour for all individuals was incorporated into a single analysis, with individual data points being matched to particular individuals. The Bray-Curtis dissimilarity metric was used to establish the distance matrix, as well as a combination of a Shepard stress plot, and calculation of the stress metric to assess how robust the nMDS was.

3.4.2 The effect of enrichment on orangutan behaviour

Each phase was analysed separately, as individual studies and the days within each week of the study were treated as replicates of the treatment. A series of general linear models were performed to analyse the effect on the individual behaviours of each orangutan, using the proportion of time the orangutan spent exhibiting each behaviour each day as the dependent variable and the week as the independent variable. The assumptions of the model were tested using residual diagnostic plots. Normal Quantile-Quantile plots were used to assess normality, the residuals were plotted against the fitted values to assess homogeneity of variance, and the residuals were plotted against the weeks to assess non-linearity. The influential data points were identified using Cook's distance (Quinn and Keough, 2002). In cases where heterogeneity of variance was detected, a generalised least squares (GLS) extension was modelled (Pinheiro and Bates, 2006) and the appropriateness of the GLS extension was assessed using both the residual plots and the Akaike Information Criterion. Estimates of model parameters were obtained using restricted maximum likelihood estimation (REML). Those models appearing to be statistically significant highlight week-to-week differences in behaviour and the associated boxplots confirm whether behaviours increased or decreased in proportion. All analyses were performed using the 'nlme' package (v. 3.1; Pinheiro *et al.*, 2012) in the '"R" statistical programming environment (R Development Core Team, 2012).

3.5 Results

3.5.1 Differences in behaviour patterns between individuals

The PCA was generally consistent with the general composition of the proportions of time spent exhibiting each TUC (**Figure 3.4**). For each of the adult orangutans, the vector for the TUC resting was a large contributor to the first principle component (PC1) and foraging tended to point in the opposite direction. This indicates that there is a negative correlation between the behaviours resting and foraging. However, for the youngest member of the group, Molly, the vector for resting was less isolated and instead, object using and travelling were key contributors to PC1. These two vectors were generally balanced in length and direction, indicating a more even distribution of time spent exhibiting these two TUCs. This pattern is apparent with Maliku, but to a lesser extent than is seen with Molly. The greater complexity in the patterns of both Molly's and Maliku's behaviour, suggested by the PCA, are supported by the Kaiser-Guttmann criterion. This suggested that the reduction to three dimensions was suitable for both Batu and Kibriah's data but a reduction to five dimensions was suitable for Maliku and Molly (**Figure 3.3**).

The plots using scaling 1 (not shown) were very similar to those shown in **Figure 3.4**, in which scaling 2 (PC2) was used, in that it is the reverse of scaling 2. The x-axes represented the second principle component dimension and the y-axis represented the first principle component dimension. In the plots the relative positioning of the days was robust but the relative positioning of the vectors was less so. Kibriah showed a distinct clustering of a large number of days associated with considerable amounts of time spent resting. This was also true of Batu, but to a lesser extent, whilst Maliku and Molly showed very little clustering in their daily behaviour patterns. For all individuals there were no clear patterns of clustering associated with sequential days of the study, indicating that, within the clear biases towards particular behaviours, there was significant change in behaviour compositions from day to day for all individuals.

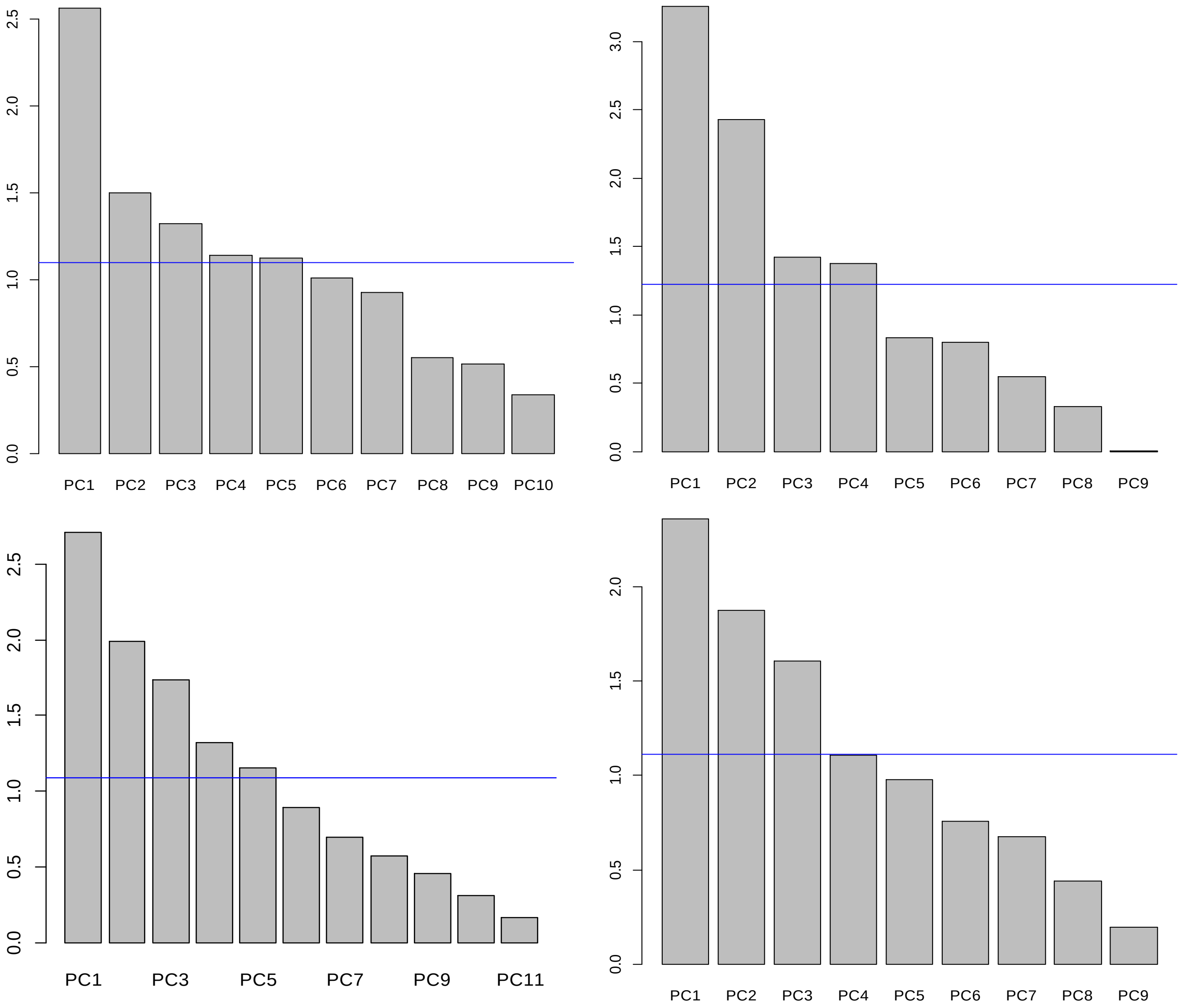


Figure 3.3 Scree plots with the Kaiser-Guttman criterion marked with horizontal blue line. Top left = Batu, top right = Kibriah, bottom left = Maliku, bottom right = Molly.

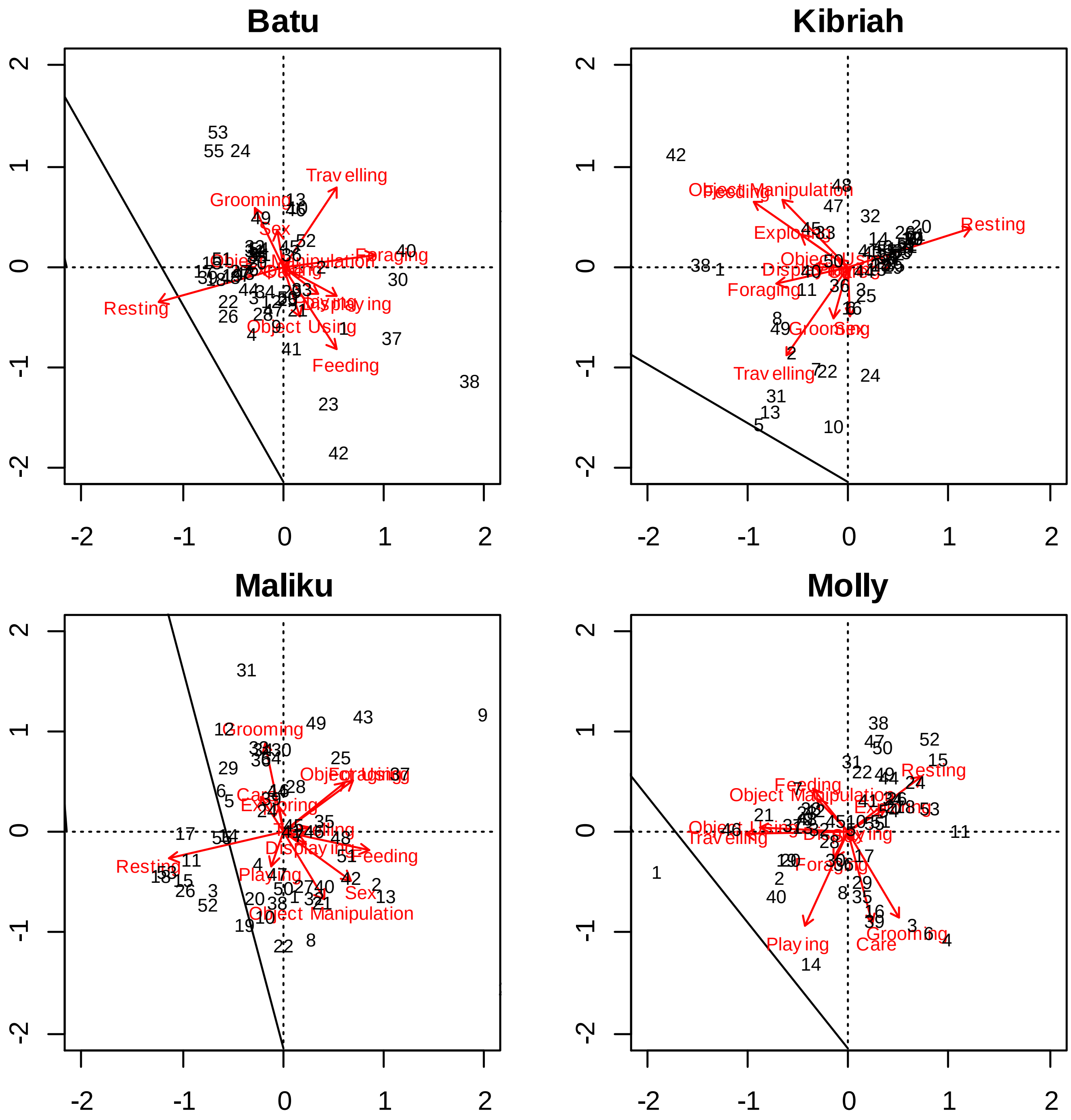


Figure 3.4 Biplots of the PCAs for each individual. The x-axes represent the first principal component dimension, and the y-axes represent the second principle component dimension. Scaling 2 representations were used for each plot, making the relative positioning of the vectors robust, but the relative positioning of the days less robust.

The plot (**Figure 3.5**) produced by the nMDS is considered to be a reliable representation of the multiple behaviour dimensions, indicated by the stress metric of 0.1019. The clustering of points associated with Kibriah suggests considerable similarity in daily behaviour patterns for this individual, similar to that seen in **Figure 3.4**. The data representing Batu show similar clustering but to a lesser extent. There were some points for Batu (day 8 and day 16) which were considerably removed from the rest of the points, and there is a suggestion of a few points (days 30, 37, 38 and 40) forming a small group removed from the main cluster.

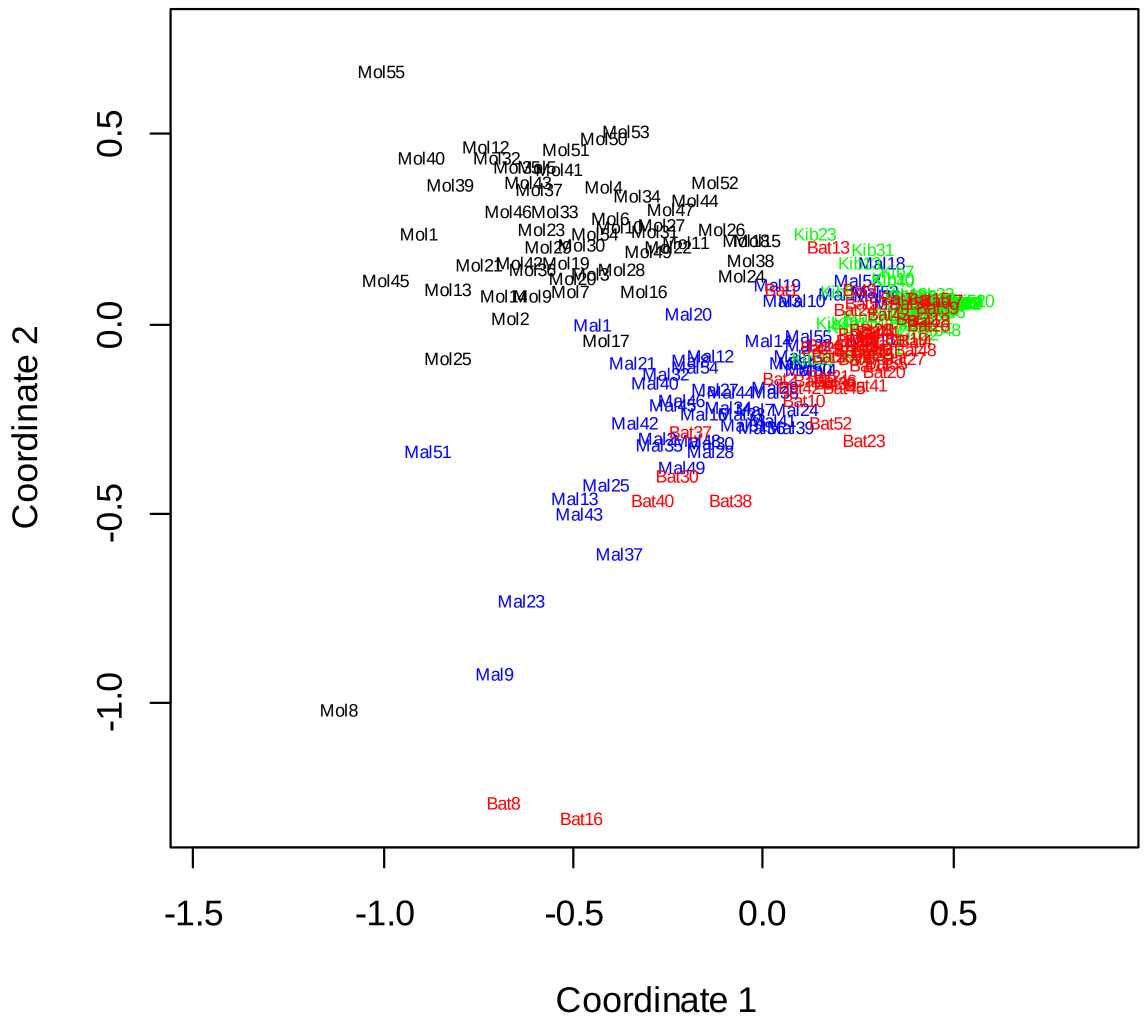


Figure 3.5 non-metric multi-dimensional scaling (nMDS) plot for the daily behaviour patterns of all four individuals. Individual points are identified and colour-coded with the name of the individual (Bat = Batu, red; Kib = Kibriah, green; Mal = Maliku, blue; Mol = Molly, black), plus a number indicating the day of the study that the point corresponds with.

The data points associated with Maliku were considerably spread out, whereas those for Molly were grouped, but not tightly clustered, with the exception of day 8, which sat outside of this group. Clusters of points associated with each group member were easily identified but contained considerable overlap with one another for the adults. The data points for Molly were contained in a distinct grouping for the most part. The separation of the cluster and lack of overlap for Molly identifies a difference in her behaviour pattern from the other three group members.

3.5.2 The effect of enrichment on orangutan behaviour

The results of both the linear models and those with which the GLS was applied are supplied in the appendix of this thesis (**appendix 3.2**) in tables respective to the enrichment treatment analysed. Each table is referred to within the text where appropriate.

3.5.2.1 Baseline

There were no significant p-values in component 1 (no enrichment), thus the changing in weeks (1, 2 and 3) had no significant effect on orangutan behaviour (**appendix 3.2, Table 9.1**).

3.5.2.2 Feeding enrichment

Maliku exhibited significantly more feeding behaviour when provided with the food-based enrichment device in comparison to the weeks before and after exposure to the enrichment (L-ratio = 6.14 df = 2, P = 0.046; **appendix 3.2, Table 9.2**; **Figure 3.6a**). This was also true for Molly (L-ratio = 6.10, df = 2, P = 0.048; **appendix 3.2, Table 9.2**; **Figure 3.6b**). An increased display of object manipulation was also evident in Molly's behaviour during the enrichment treatment (F-statistic = 13.36, df = 9, P = 0.002; **appendix 3.2, Table 9.2**; **Figure 3.6c**). The models run on all other behaviours in each individual did not yield significant results, indicating that the other individuals were not significantly affected by the enrichment during the observations.

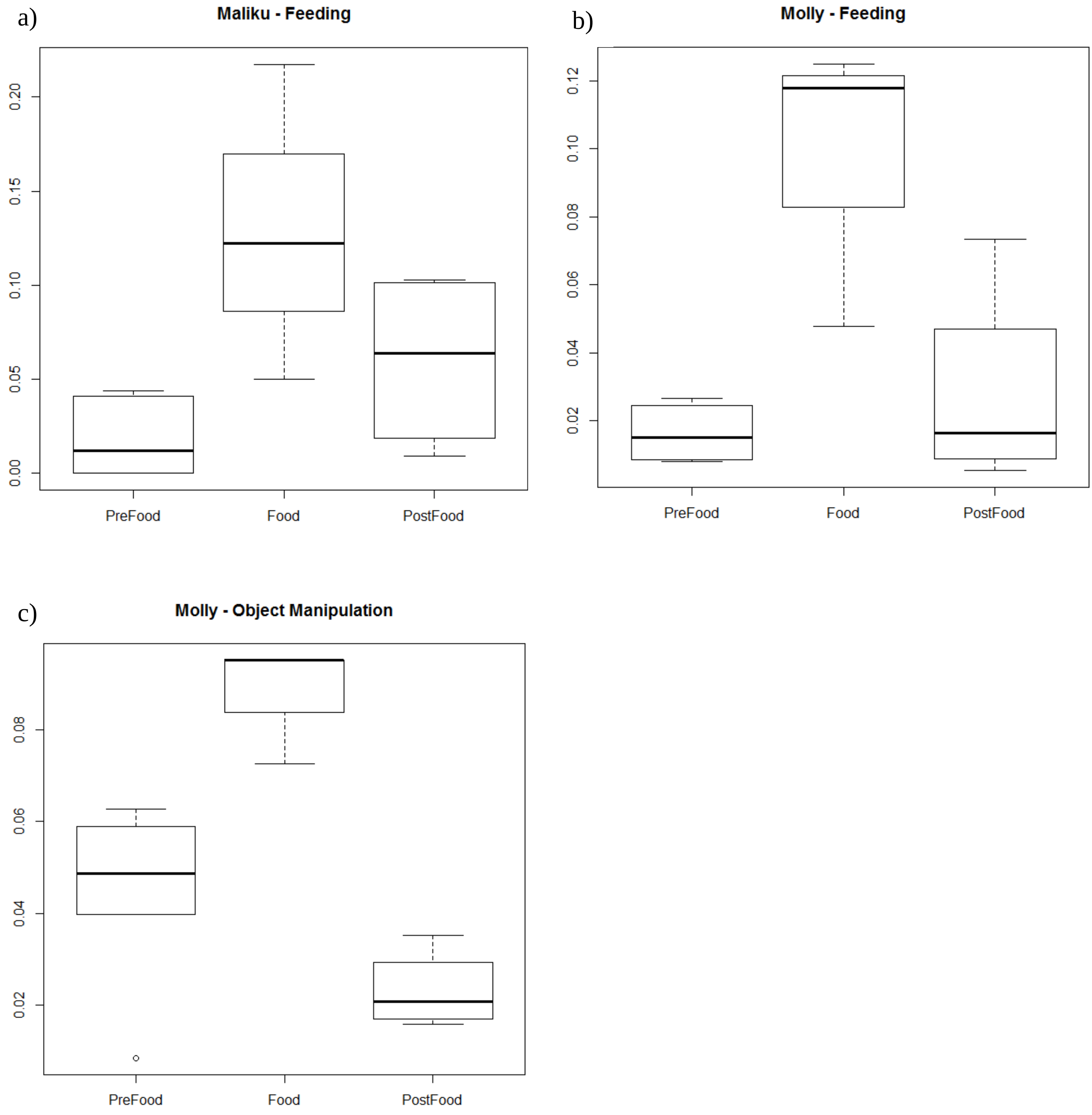


Figure 3.6 Proportion of time orangutans spent displaying respective behaviours during the food-based treatments (a) mean = 0.061, median = 0.042, SD = 0.065; b) mean = 0.048, median = 0.022, SD = 0.042; c) mean = 0.048, median 0.443, SD = 0.030, R^2 adj = 0.692). The horizontal black lines represent the median values, and the boundaries of the boxes represent the upper and lower quartiles.

3.5.2.3 Baseline 2

During the second period of baseline observations Maliku exhibited significantly more travelling behaviour during the first and second week of the treatment in comparison to the third week (L-ratio = 6.428, df = 2, P = 0.040; **appendix 3.2, Table 9.3; Figure 3.7**). The models run on all other behaviours in each individual did not yield significant results.

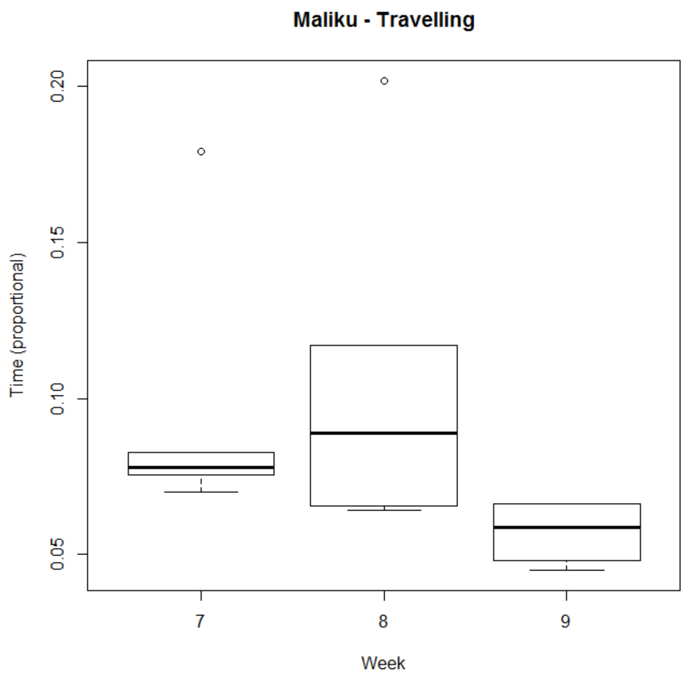


Figure 3.7 Proportion of time Maliku spent travelling during the second round of baseline observations (mean = 0.089, median = 0.073, SD = 0.046). The horizontal black lines represent the median values, and the boundaries of the boxes represent the upper and lower quartiles. The circles represent outlying data. During all three weeks of this treatment, no enrichment wass provided.

3.5.2.4 Tool use enrichment

During the final three weeks of the study, the orangutans were provided with an artificial termite mound for one week and then both the termite mound and frozen fruit cups, and the final week involved no enrichment. The analysis included the average data from the baseline observations in order to compare the effect of enrichment with behavioural observations in the absence of enrichment (see **Figure 3.8**). A number of behaviours appear to have been influenced by the presence of the enrichment. The data illustrate significant changes in the times spent feeding by Batu (L-ratio = 6.802, df = 2, P = 0.033; **appendix 3.2, Table 9.4**; **Figure 3.8a**). Batu's feeding behaviour decreased, from the baseline, when provided with the tool use enrichment but increased when given access to a combination of the two enrichments. This behaviour then decreased again with the removal of both enrichment devices.

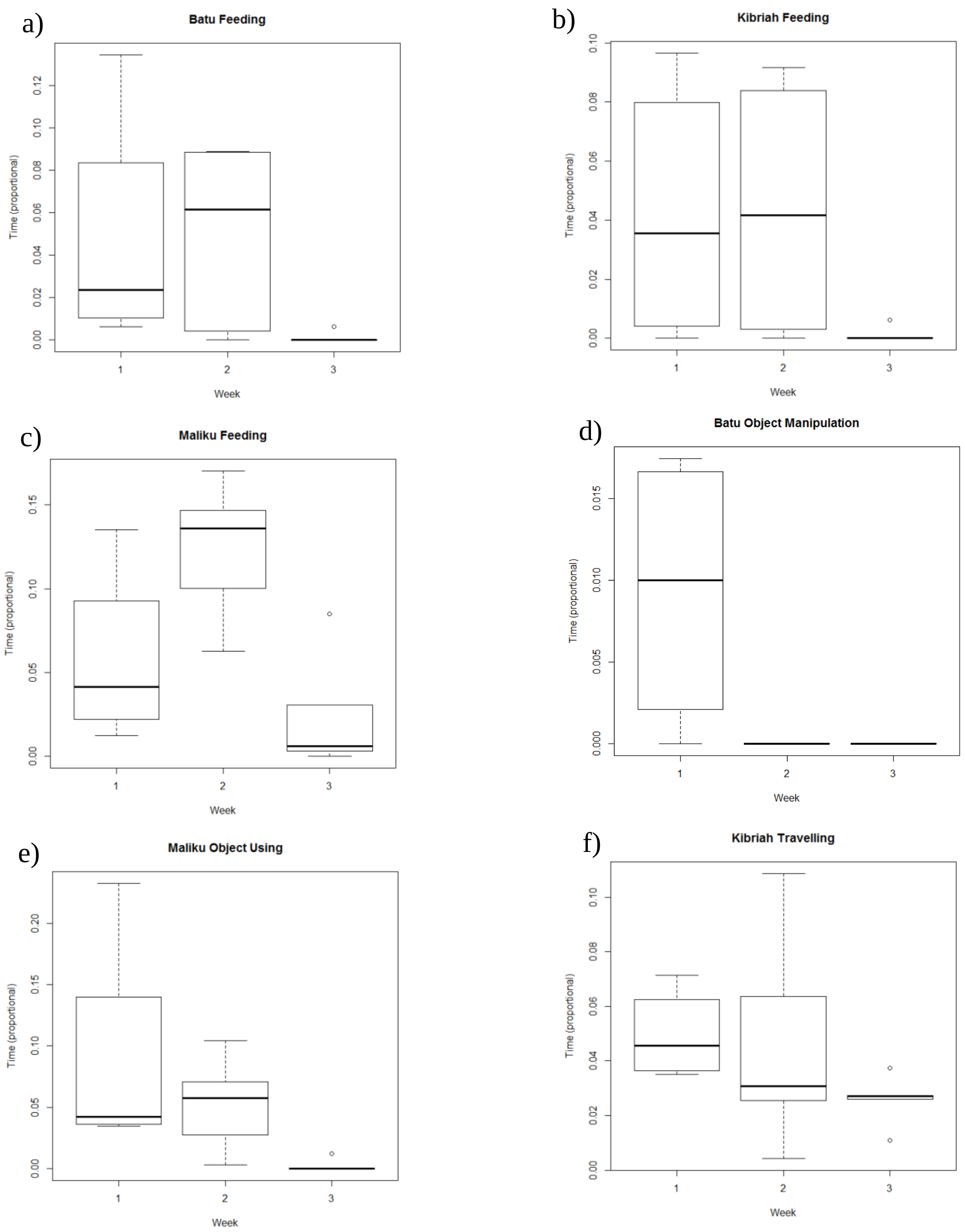
The data illustrate an increase in feeding behaviour by Kibriah when exposed to the tool use enrichment in relation to the baseline and this continued when both enrichments were provided. The proportion of time spent feeding by Kibriah then decreased when both enrichment devices were removed (L-ratio = 6.847, df = 2, P = 0.033; **appendix 3.2, Table 9.4**; **Figure 3.8b**).

There was an increase in the proportion of time Maliku spent feeding when during the tool use enrichment treatment but this increased significantly in the presence of the two enrichments combined. As is seen on both Batu and Kibriah, feeding behaviour decreased when the enrichment devices were removed (F-statistic = 6.525, df = 11, P = 0.014; **appendix 3.2, Table 9.4**; **Figure 3.8c**).

There were also significant changes in the time Batu spent in object manipulation with an increase from baseline on the introduction of the termite mound. This behaviour then disappeared during the following weeks (F-statistic = 6.231, df = 11, P = 0.016; **appendix 3.2, Table 9.4**; **Figure 3.8d**).

A reduction in travelling by Kibriah was observed upon the removal of enrichment (L-ratio = 6.144, df = 2, P = 0.046; **appendix 3.2, Table 9.4**; **Figure 3.8f**). However, this was contrasted by an increase in time spent resting (L-ratio = 12.702, df = 2, P = 0.002; **appendix 3.2, Table 9.4; Figure 3.8g**). The time that Maliku afforded to object using also significantly changed in this final component (L-ratio = 8.444, df = 2, P = 0.015; **appendix 3.2, Table 9.4; Figure 3.8e**).

The data representing Molly showed heterogeneity of variance and therefore required a GLS model to be performed. However singular matrices meant that the model could not be conducted. The preliminary linear models run on the data did not yield any significant results (**appendix 3.2, Table 9.4**).



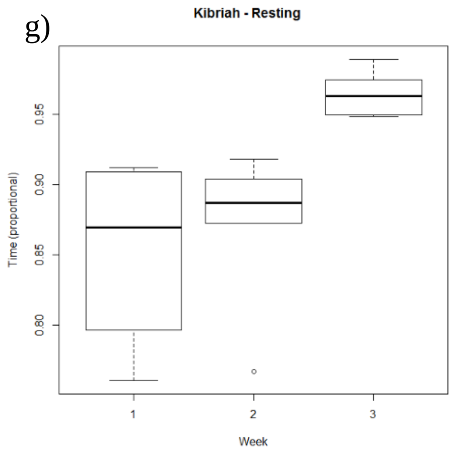


Figure 3.8 Proportion of time orangutans spent displaying respective behaviours during the tool use treatments (a) mean = 0.046, median = 0.032, SD = 0.052; b) mean = 0.023, median = 0.003, SD = 0.037; c) mean = 0.057, median = 0.035, SD = 0.051, R^2 adj = 0.344; d) mean = 0.003, median 0.000, SD = 0.007, R^2 adj = 0.446; e) mean = 0.027, median = 0.011, SD = 0.047; g) mean = 0.898, median = 0.919, SD = 0.076). The horizontal black lines represent the median values, and the boundaries of the boxes represent the upper and lower quartiles. The week variable corresponds to the enrichment treatments as follows: week 1 = termite mound enrichment, week 2 = termite mound and food enrichment, week 3 = no enrichment.

3.6 Discussion

3.6.1 Differences in behaviour patterns between individuals

The behaviour patterns of individuals varied considerably, however the behaviour of Batu and Kibriah was more similar than any other dyad of individuals. Throughout this study, Batu appeared to pay particular attention to Kibriah, often following her around the enclosure, and on occasion he would also maintain physical contact by holding his hand on her head. This behaviour is likely to correlate with Kibriah's receptivity for copulation. In the wild, it has been observed that during courtship males and females travel together and display coordination in their behaviours, and flanged males are known to be preferred by females (Fox, 2002; Knott *et al.*, 2010). Females are known to direct their proceptive mating behaviour towards the male inhabiting her home range (Fox, 2002). In this scenario, with Batu being the sole male within this female's habitat, it would follow that her receptive behaviour be directed towards this individual and therefore support the observations of consistent sociality between these two individuals. It is known that orangutans are more sociable during high food abundance (Mitani *et al.*, 1991) and then move through the forest in travel bands to explore nearby food sources (Utami-Atmoko *et al.*, 1997; Utami-Atmoko *et al.*, 2009). This provides further support for the coordination between these two orangutans. However, the data do not clarify whether these individuals were observed carrying out the recorded behaviours within the same area of the enclosure and therefore at this time, it can only be concluded that their behaviours were coordinated but not necessarily their movements in space.

For all of the adult orangutans, resting occurs most often in their daily activity budget. This appears to be true across the whole study period. Orangutans are slow moving apes and it has been discovered that these apes, relative to body mass, express the lowest daily energy expenditure (DEE) than any other eutherian mammal with the only exception being sloths (Family Bradypodidae*;* Pontzer *et al.*, 2010). More specifically, infant orangutans have been observed to have higher DEE and the eldest maintained the lowest DEE of the orangutan group studied by Pontzer *et al.*, (2010). This evidence supports the observation of higher resting rates in Kibriah (the eldest member of the group) over the course of the study. It is thought that this energy preservation strategy has developed in response to variable food availability (see **1.5.4 The dietary needs of wild orangutans**) and therefore when food availability is low, orangutans reduce their energy expenditure to avoid starvation (Pontzer *et al.*, 2010). This supports the negative correlation between resting and foraging, which is indicated by the PCA

(**Figure 3.4**) and suggests that the decrease in time spent resting has resulted in an increase in the proportion of time the orangutans have devoted to foraging in the presence of a new food source (enrichment). In the wild, when food availability is high, orangutans will gorge on food sources until these are depleted (Knott, 1998). In this case, the enrichments may have mimicked the scenario of fruit masting events and therefore caused orangutans to replace their resting behaviour with foraging to increase their energy intake.

The data for Maliku is more consistently spread out than that of Batu and Kibriah, indicating that this individual explored a broader range of behaviours over the study. This could be linked to the behaviour of her young infant Molly. Molly demonstrated object using and travelling to a greater extent than the other individuals, which illustrates her more inquisitive characteristics. Infant orangutans are known to obtain their skills from older individuals, most notably those skills related to food processing (Jaeggi *et al.*, 2008, 2010) and the extended inter-birth interval of orangutans has been attributed to the need to care for offspring until they are able to range independently, and the high energetic cost of association (van Noordwijk and van Schaik, 2005). The similarity in behaviour patterns for these individuals can be explained by this infant's dependence on the maternal relationship. However, although wild infant orangutans tend to remain close by their mothers, the zoo environment affords infants a safe environment in which to explore and mothers may be aware of the lack of predation and other potential threats. Young orangutans take the opportunity to play with other infants which has known to be important in animal development (van Noordwijk *et al.*, 2012). However, here, in the absence of other juveniles, Molly appeared to use her time appropriately to learn the skills that will be essential to her success as an adult. Further support for these conclusions would come from determining whether Maliku and Molly occupy the same areas of their enclosure and are therefore within the same space whilst carrying out the similar behaviour patterns.

This method of analysing behaviour patterns has allowed for the creation of visual representations of both individual and group behaviour patterns. Without needing to analyse specific behaviours in turn, and calculate changes in behaviour over time, the PCA and nMDS outputs allow researchers to determine which behaviours are influential in an individual's repertoire as well as compare patterns between individuals to determine whether relationships may exist in the group. PCA may become very useful when abnormal or stereotypic behaviours are observed, and determining the extent of the influence of this behaviour on the individual's activity will help to understand its impact on the animal's overall welfare. Detecting similarities and differences in behaviour patterns within groups will be the first step in establishing how individuals may influence one another's behaviour patterns.

3.6.2 The effect of enrichment on orangutan behaviour

3.6.2.1 Baseline

There were no significant changes in any behaviours of any of the four orangutans throughout the first baseline phase of the study. This suggests that there is an absence of any significant week-to-week variation on the behaviour of the orangutans.

During the second phase of baseline observations the only significant change in behaviour observed was an increase in travelling by Maliku. However, the boxplots indicate that this is due to one single data point during the second week of this phase. Therefore it would be unmerited to assume that this signifies an increase in travelling across the entire week and is more likely to be explained by a single event. For this reason, it is deduced that this second baseline is in agreement with the first weeks of baseline observations in that no orangutan behaviours were significantly influenced by week-to-week variations, therefore any significant changes in behaviours during the enrichment treatments can be reliably attributed to the presence of the enrichment device(s).

3.6.2.2 Feeding enrichment

As was discussed previously, the enrichment provided the orangutans with the opportunity to forage and therefore decreased the proportion of time individuals spent resting. The enrichment encouraged behaviour that is linked to a fruit masting event in which orangutans feed on the variable food source until it has depleted (Knott, 1998), which is evident in the significant increase in feeding behaviour by Maliku and Molly. The enrichment itself, fruits frozen in plastic cups, were designed to encourage feeding behaviour in that the orangutans had to spend more time processing the individual pieces of fruit (or in this case each cup), peeling back the plastic and accessing the fruits inside. These findings support those of Morimura (2007) in that food based enrichment has encouraged feeding behaviour in captive apes. In the wild, orangutans spend 43% of their active day feeding when suitable food sources are available (Delgado and van Schaik, 2000). Although such large proportions of daily activity devoted to feeding have not been reached here, the increase in feeding when provided with the food based enrichment supports their use in encouraging species behaviour. In order to substantiate these speculations, it would be necessary to clarify whether the observed increased feeding behaviour was directed towards enrichment items and thus confirming the role of the frozen fruit cups in this observed change.

As part of the processing required to access the frozen food enrichment, the plastic cups were discarded, much like the skin of fruit. The three adult orangutans focused their attention then on consuming the food. Molly, however, used the new material as an opportunity to perform object manipulation. Molly’s interest in the cups appeared to be in chewing, and tearing the plastic, and ultimately destroying the cups until they were no longer engaging (determined from field notes). A preference for destructible toys was determined when providing enrichment for captive chimpanzees (Pruetz and Bloomsmith, 1992; Brent and Stone, 1998). It is thought that the variation in shape and size, as a result of playing with these destructible items, presents a more interactive enrichment and therefore can maintain animal interest as manipulable toys are also shown to be successful in enriching captive primates (Kessel and Brent, 1998). Research also shows that it is younger primates that show the most interest in manipulabe items (Brent *et al.*, 1989). Although this enrichment was designed specifically to encourage feeding and foraging behaviour, the secondary effect on this infant’s other behaviour further support the use of frozen food cups as a strategy to encourage species natural behaviours.

3.6.2.3 Tool use enrichment

Both the artificial termite mound and the frozen fruit cups provided an opportunity for the orangutans to increase the proportion of their day that they devote to feeding. The frozen fruit required increased manipulation of food through chewing and gnawing at the ice and once the appropriate tool had been fashioned, orangutans foraged for the food from the termite mound and feed on the porridge and yogurt that they had extracted.

The presence and utilisation of the termite mound resulted in three of the four individuals displaying significantly more feeding behaviour than during the week after its removal. What is more, these individuals devoted a larger proportion of their day to feeding when both the termite mound and the frozen food enrichment were available. Previous research has focused on the use of artificial termite mounds to promote tool use behaviour (Nash, 1982; Nakamichi, 2004) but to date there has been no known research to explore the use of termite mounds to encourage feeding in captive primates. The larger increase in feeding displayed by

Maliku during the combined treatment suggests that both enrichments were utilised for feeding, and that the addition of the food enrichment provided additional opportunities for feeding rather than an either/or situation. The sticks used to access the mound were covered in leaves and twigs, as described earlier, and the observer noted that these leaves were often eaten by the individuals before the sticks were used as tools. Orangutans regularly eat leaves in the absence of fruit as part of their normal diet (Morrogh-Bernard *et al*., 2009) and therefore this enrichment may have provided feeding opportunities that were not initially planned. However, more detailed observations, to associate tool use and feeding behaviour with the termite mound and the sticks would make greater steps towards confirming this particular discussion.

The significant decrease in feeding behaviour by all adult orangutans upon the removal of the two enrichment devices illustrates the effect of removing enrichment which is targeted towards specific behaviours. As was discussed in chapter two, it is not commonplace to empirically monitor behaviour following the removal of enrichment despite recommendations from the literature (Young, 2003; Swaisgood and Shepherdson, 2005). Here we have important evidence to suggest that animal managers should consider the effect of withdrawing enrichment. During this post-enrichment treatment, individuals were still provided with their normal daily diets as described above (**3.3.2 Husbandry routine**) and therefore orangutans were at no risk of starvation on the removal of food based enrichment. However it appears that the withdrawal of enrichment has significantly affected their motivation to feed for extended periods.

The effect of the termite mound on object manipulation behaviour is clear in Batu. The first week of exposure to this enrichment encouraged tool use behaviour which significantly decreased to no object manipulation behaviour at all during the following weeks. However, in the second week, the termite mound was still in use as an enrichment device. Field notes documented that, during the first days of the termite mound treatment, Batu spent much time stripping leaves and twigs from the sticks provided before using them to access the termite mound. However, on the days that followed (approximately after day 2 of this treatment) Batu appeared to lose interest in the termite mound and no longer gathered sticks to use as tools. This change in behaviour could potentially be explained by optimal foraging theory, in that the food provided by this enrichment was not considered to be a rich enough food source to warrant the energy input needed to access it. Batu may have chosen to wait for the more predictable scatter feed at midday and make the most of this, easily accessible food source, rather than spending time and energy creating a tool to access a relatively small amount of food. The continued increase in feeding behaviour during the combined enrichment treatment may suggest a preference towards frozen food as a food source given its accessibility. However Clay *et al.* (2009) determined that food preferences in orangutans change over time and therefore this observation should not be used to assume an continued preference for the frozen food by Batu. Anecdotal evidence also supports this in that Batu was observed actively utilising the termite mound within the 12 months following this research.

In contrast to the behaviour of Batu, Maliku's display of object using, observed here as using the sticks to access the food from termite mound, was maintained over both weeks of exposure to this enrichment. This suggests that, when the keepers ceased to fill the mound with food, Maliku then ceased her tool use behaviour, indicating that the enrichment was implicit in encouraging this behaviour. Similar termite mounds have shown to be successful in their use with captive chimpanzees (Nash, 1982) as well as orangutans (Nakamichi, 2004) and also with bonobos, although this was not empirically measured (personal observation). It is now known that tool use is not limited to humans, nor is it limited to mammals (Seed and Byrne, 2010) and that it is an expression of cognitive function (Sanz *et al*., 2013). Orangutans do use tools in the wild not only to access food but also to forage, build nests etc. (Galdikas, 1989). These findings enforce the importance of providing orangutans with opportunities to use tools in captivity.

Withdrawal of both enrichment devices corresponded with a significant decrease in travelling behaviour displayed by Kibriah as well as a significant increase in resting. These findings suggest that the enrichment devices were important in encouraging Kibriah to travel through the enclosure. The enrichment also encouraged a range of other observed behaviours (object using, object manipulation, foraging and feeding) but each of these did not show a significant increase in occurrence in comparison with the pre-enrichment trials. The redistribution of daily activity reduced the proportion of time Kibriah could dedicate to resting. The withdrawal of the enrichment reduced the motivation to display food related behaviours and therefore Kibriah returned to the resting behaviour. It was previously discussed that adopting long periods of rest is a strategy adopted by orangutans to preserve energy when food resources are low. In the absence of enrichment, the predictable feeding schedule reduced the need to actively seek food. Although, resting may be considered a natural behaviour, inactivity is undesirable in captive animals resulting in low visitor engagement and weight gain (Fernandez *et al*., 2009; D'Eath *et al*., 2009).

The behaviour patterns of the two orangutan dyads in this group of captive orangutans are consistent with observations of wild orangutan. However, data on the individuals' positioning within the enclosure, to determine their use of the enclosure space in comparison with one another, would make these findings more robust.

The data suggest that the food based enrichment successfully encouraged feeding behaviour as well as object manipulation as a secondary effect of the enrichment design. The termite mound was instrumental in the display of feeding, object using, object manipulation and travelling, all of which are behaviours observed regularly in wild orangutans. Combining both enrichment devices further encouraged these behavioural changes, and the withdrawal of enrichment resulted in significant reductions in species typical behaviours. The changes in orangutan behaviour are consistent with those that would be displayed by their wild counterparts during fruit masting. However, lack of food or the perception of lack of food may cause some physiological stress for captive animals and could also explain a reduction in species natural behaviours.

The use of behavioural observations in this assessment of animal welfare was successful for many reasons. The slow moving nature of the orangutans and the relatively small enclosure meant that continuous group sampling was an appropriate way of providing a detailed account of orangutan activity. In the field, the use of only a pencil and paper as well as an ethogram, required no technical knowledge and thus is a very repeatable method of gathering behavioural data. The ethogram provided a comprehensive list of identifiable behaviours which allowed all behaviours observed to be differentiated. Once the data had been inputted onto a digital spreadsheet, simple data exploration resulted in a range of visualisations, which acted as valuable contributions to the understanding of behavioural patterns as well as quantitative assessment which allowed the identification of significant changes in behaviour. Given that positive animal welfare is regularly defined as the display of natural behaviours, the linear regression analysis facilitated a comparison in behaviours across the enrichment programme and yielded both quantitative results alongside visual representations of behavioural change.

Being able to observe and analyse animal behaviour in this way is beneficial for both zoo staff as well as the animals concerned. The simple method of collecting data requires no specialist knowledge, only a basic background of orangutan behaviour, which would be gained by simple working with the animals. No specialist equipment is required and there is no direct contact with the animals themselves. The methods of analysis allow for both a generally overview of animal behaviour patterns, as well as a detailed picture of changes in activity. This can therefore be used to monitor animals on a long term basis, detecting significant changes, or to observe the effect of specific events on animal behaviour. Using the ABA design also ensures that animal welfare is monitored after an event, so that the long-term consequences may be taken into account. Being able to detect changes in behaviour notifies animal carers that something within the environment may be causing such a change, the resulting activity can be used to determine whether a positive or negative change has taken place, and the use of daily summaries allows for the determination of a specific day of that change. This can be used to keep records of animal activity and ensure that negative changes are reduced and positive changes are not only encouraged, but shared within the zoo community for the benefit of conspecifics elsewhere.

Although the observations of this study were made possible by the rate of orangutan activity and enclosure design, many occasions arose in which the behaviour of orangutans had to be assumed (as explained in the methodology) and thus truly comprehensive accounts of orangutan activity were not possible. The method for recording behaviours was simple, but data input into a digital spreadsheet was found to be time consuming and required lengthy cross checking to avoid errors. The length of time orangutans were allowed access to their entire enclosure was dictated by the keeper routine and compliance of the orangutans. This led to differences in day length and subsequently the need to view behaviours of proportions of time rather than a total sum of time. The time needed to observe the orangutans over the whole 12 weeks meant that over one summer, only this group could be observed, making a multi-zoo study less feasible. Observations reported how orangutan behaviour was affected by the enrichment and the absence of abnormal behaviours suggests that orangutans' welfare did not suffer as a consequence of enrichment. However, the reduction in natural behaviours, coinciding with the removal of enrichment, suggests that orangutans may have suffered some adverse effects. Behavioural observations have implied positive animal welfare but do not, at this time, provide enough evidence to confirm poor animal welfare. To draw such conclusions, exploration of the physiological response to the enrichment could be valuable.

3.7 Conclusion

Both the frozen fruit cups and the artificial termite mound stimulated the display of behaviours comparable to that of wild orangutans and may have mimicked the natural phenomenon of fruit masting. Assessment of behavioural patterns identified two dyads within the group. However, further investigation of the locations of individuals in space would be necessary to confirm the pairings. Behavioural observations, and the small group size meant that the behavioural reaction to enrichment by each individual could be determined and highlighted the within group variation in both daily activity as well as behavioural response to enrichment.

The methods utilised to collect and analyse data were simple and required no specialist equipment. The knowledge necessary to draw conclusions may come from keeper experience and the results allow for varying levels of welfare assessment which can be altered depending on the circumstances.

Although time consuming and limited in the ability to explore physiological effect of enrichment, behavioural observations are a repeatable method of welfare assessment and yield the results necessary to determine the effect of enrichment on animal welfare. The potential for enrichment to induce physiological problems can be explored using the analysis of stress hormone production by the adrenal cortex. Therefore, the next chapter describes the implementation of such methods and outlines the strengths and weaknesses of the methods.

Chapter FOUR: Faecal Glucocorticoid Concentrations to Monitor the Welfare of Captive Bornean orangutans (*Pongo pygmaeus*)

4.1 Chapter overview

Thus far, the necessity to monitor and quantify the welfare of captive animals has been outlined and the use of behavioural observations to achieve such aims has been explored. Behavioural observations have allowed for quantification of the levels of species natural behaviours in response to environmental enrichment and thus an understanding of the orangutans' psychological welfare. However, as discussed, it is imperative for a comprehensive welfare assessment that an animals' physiological welfare is explored. Hence, adrenal cortex activity was monitored via faecal glucocorticoid metabolite (fGCM) assays in conjunction with the data collection of the previous chapter. The fGCM levels determined from the samples did not indicate that the orangutans perceived enrichment as a stressful stimulus. A correlation between fGCM and both resting and playing determined that these behaviours are indicative of low adrenal cortex activity. The strengths and weaknesses of this method of welfare monitoring are also discussed.

4.2 Introduction

Stress is an inevitable component of life for all animals both in captivity and in the wild. Stress allows animals to adapt to the unpredictable components of their environment (Boonstra, 2005; Morgan and Tromborg, 2007). However, animals housed in captivity are not exposed to these same stressors, as the captive environment is wholly diverse from that which most species have evolved to live in (Tennessen, 1989). The regular supply of food reduces the pressure to hunt and forage and the absence of predators means that captive animals do not need to be vigilant (Newberry, 1995). Nevertheless, this environment introduces a distinct range of new stressors, not encountered by wild animals. Life in captivity is characterised by restricted space, regular feeding routines and the near constant presence of humans (Hosey, 2005). Animals in captivity are constantly faced with a range of stimuli not experienced by their wild counterparts, which have the potential to cause significant stress. For instance, in rainforest habitats ambient noise is known to range from 27 to 40 decibels (dB), with wind and leaves being the largest contributors to the noise (Waser and Brown, 1986). In captivity however, the conversations of visitors, noise of maintenance machinery and urban traffic mean that animals in zoos are exposed to sound pressure levels of over 72dB (Tromborg and Coss, 1995). It is well known that loud noise causes stress in many species, resulting stereotypic behaviours and health problems (Powell *et al*., 2006) and has even been suggested to cause deformities in growing foetuses and alter sex ratios in populations (Kight and Swaddle, 2011). Female white-crowned sparrows (*Zonotrichia leucophrys*) produced more female embryos when implanted with stress hormones (Bonier *et al*., 2007) and the noxious effect of loud noise on the immune system of developing foetuses has been highlighted (Sobrian *et al*., 1997).

The restrictive walls and predictable feeding schedules that distinguish life in captivity also induce stress in animals by hampering their ability to carry out internally motivated behaviours such as foraging, mating and migrating. Animals with naturally large home ranges are particularly restricted in captivity, and the lack of opportunity to carry out migrating or foraging behaviours often leads to the development of abnormal behaviours such as pacing (Clubb and Mason, 2003; Clubb and Vickery, 2006). For example, American Black Bears (*Ursus americanus*) spend up to 18 h of their day foraging in the wild, but in captivity only 12-20% of their time was spent exploring or foraging, and 40-60% of time was spent pacing (Carlstead *et al*., 1991). It has been suggested that the pacing behaviour in American Black Bears has developed from thwarted attempts to forage and search for potential mates (McPhee and Carlstead, 2010).

Likewise, wild primates are known to spend the majority of their day foraging (Maple and Finlay, 1989; Delgado and van Schaik, 2000). Their high cognitive ability means that they are able to solve complex tasks and make tools to access food (Beck, 1975; van Schaik *et al*., 1999; Ottoni and De Oliveira, 2004). In captivity, however, food is provided on a regular basis and this often leads to food anticipatory behaviours in primates, such as aggression and heightened vocalisation (Bassett and Buchanan-Smith, 2007). Food is also usually prepared, or at least cut up into manageable pieces, removing the necessity for primates to use tools or otherwise to manipulate food as they would in the wild (Honess and Marin, 2006). The absence of complex social challenges and opportunities to exercise their intelligence means that primates are highly susceptible to stress in captivity (Honess and Marin, 2006).

The static nature of life in captivity means that these stressful stimuli are often permanent components of an animal's life. Each time the animal is faced with a stressor, the stress response is activated to enable the animal to escape, or cope with the stressor and to increase survival. However, the restricted environment thwarts the animal's ability to escape and therefore the stress response is repeated continuously, until the stressor is removed or the animal finds a way of coping with the stress (see **1.3.1 The biological response to stress**).

It is now commonplace to use the activity of the biological stress response as a technique to monitor and assess captive animal welfare (Moberg, 1985, 2000; Mason, 2010). The hormones released during the stress response are the steroids hormones Glucocorticoids (GCs), the most abundant of which is cortisol, in primates (Sapolsky, 2002). Although GCs mobilise existing energy stores, re-diverting all resources to the muscles and tissues required, prolonged activation of the stress response can decrease an animal's health and longevity (Sapolsky, 2002; Charmandari *et al*., 2005; Wingfield, 2013). If all energy has been diverted to the stress response, other vital processes in the body are neglected. This can then result in immunosuppression, reduced growth, halted reproduction and in many cases, abnormal behaviours (Moberg, 2000; Charmandari *et al*., 2005; Muehlenbein *et al*., 2012). The biological stress response was correlated with stereotypic behaviour in giant pandas (*Ailuropoda melanoleuca*) with increased cortisol preceding the display of behaviours such as pacing, head-bobbing and rocking (Liu *et al*., 2006). The release of hormones in response to stress interferes with the process of reproduction by obstructing the release of the egg from the ovary (Dobson and Smith, 2000). Therefore, the timing of stressful events may well result in a missed opportunity for reproduction, representing a significant threat to captive breeding.

In order for zoos to maintain animals in good psychological and physiological health, and therefore permitting their good welfare, it is paramount that they are able to monitor and if necessary, limit the stress that captive animals are exposed to. As a rule of thumb, observations of animal behaviour are very often used by zoo staff and researchers to determine whether animals are suffering with stress (Mason and Mench, 1997; Dawkins, 2004). Self-directed behaviours such as autogrooming and scratching, when displayed at abnormal rates, are interpreted as displacement behaviours, used by animals to cope with stress (Honess and Marin, 2006). Abnormal behaviours are understood to arise from the inability to perform species natural behaviours in the restricted environment, and it is thought that they develop as mechanism for coping with stress (Wechsler, 1995). Hence, the absence of these behaviours and the display of an animals' natural behaviour is often used as an indicator of good welfare (Maki and Bloomsmith, 1989; Carrasco *et al*., 2009; Koknaroglu and Akunal, 2013). However, more recently, it has been highlighted that multiple measures may be necessary to determine the welfare of captive animals (Maple and Perdue, 2013). For example, two animals may receive the same food-based enrichment. The more dominant of the two may perceive the enrichment as an enriching experience, an opportunity to carry out foraging behaviour. The submissive individual may consider the enrichment as a source of stress, a need to gather food before it is monopolised by the dominant individual. Hence, although their behavioural responses are to increase foraging behaviour, their physiological responses may be very different; the subordinate individual experiences higher stress than the other. It has also been discussed that behavioural measures of stress are often misinterpreted and may have multiple underlying motivations (Rushen, 2000). Therefore, a combination of both behavioural observations and monitoring of stress hormones could provide a comprehensive assessment of animal welfare.

The previous chapter involved the implementation of an enrichment programme. Enrichment is a commonly employed method to tackle behavioural abnormalities and reduce stress in captive animal (see **1.4 Enrichment for captive animals**). Enrichment provides behavioural opportunities for animals with the aim of encouraging a wider range of behaviours which resemble those of their wild counterparts (Carrasco *et al*., 2009; Claxton, 2011). Enrichment is designed to be an unpredictable component of captive animal life, to provide novelty in a static environment, therefore providing opportunities to increase the diversity of behaviours displayed and thus enhancing welfare (e.g. unpredictable feeding for sun bears (*Helarctos malayanus*; Schneider *et al*., 2014). However, predictability in the environment has been shown to be beneficial for animals allowing them to anticipate potentially stressful events (Bassett and Buchanan-Smith, 2007; Gottlieb *et al*., 2013). Thus enrichment, in introducing unpredictability in the environment has the potential to induce stress. Therefore the following study will involve the monitoring of glucocorticoids over the course of an enrichment programme to determine whether enrichment may be perceived as a stressful stimulus by the captive orangutans.

As the need to determine levels of physiological stress has become more important in welfare monitoring, researchers have developed methods for determining the levels of cortisol produced in response to stress. Many studies have employed regular blood sampling of individuals to measure cortisol concentrations over time, over the course of a stressful event (Pankhurst and Sharples, 1992; Widmaier and Kunz, 1993). Although it is the most reliable marker of the endocrine response to stress (Wallner *et al.,* 1999), blood plasma cortisol is subject to the natural circadian rhythm and therefore frequent sampling is necessary (Möstl and

Palme, 2002). Taking blood samples also requires handling animals and the use of needles. These methods alone are likely to cause elevated blood cortisol levels and therefore alter the physiological parameters being measured (Touma and Palme, 2005). The difficulties associated with this form of sampling procedure have led to the establishment of non-invasive forms of investigation (Wallner *et al.*, 1999).

Circulating hormones are metabolised by the liver and excreted as metabolites, along with unmetabolised forms, via the kidney and removed in the urine, or via the gut, in the faeces (Touma and Palme, 2005). It is therefore possible to measure physiological stress by monitoring the cortisol (or metabolite) levels in the urine and faeces. As the urine samples contain cortisol that has been circulating since the previous sample was taken, the concentrations reflect the adrenocortical activity over a period of hours rather than in response to specific events (Novak *et al.*, 2013). Collection of urine samples is non-invasive and so there is no additional stress caused to the animal during sampling. However, with zoo housed animals urine is difficult to collect without animals being trained to produce samples. Faecal samples can be collected easily when enclosures are being cleaned and are easier to store than liquid urine. Faecal samples contain glucocorticoid metabolites, the remaining product of hormone metabolism, as well as unmetabolised cortisol. There are species differences in the adrenocortical response to stressor and the metabolism of GCs (Möstl and Palme, 2002). Faeces also contain numerous substances such as unmetabolised cortisol, corticosterone and reduced metabolites (Novak *et al.*, 2013). Therefore researchers must be careful in choosing which substance is measured. Faecal samples are often favoured as they are easy to collect and completely non-invasive and so do not cause any additional stress to the animals (Möstl and Palme, 2002). Faecal samples are produced in discrete periods and reflect the cumulative production and metabolism of hormones over longer periods (i.e. several hours) than in urine or blood, and therefore reflect the overall production of glucocorticoids during both high and low production periods (Touma and Palme, 2005; Cavigelli and Caruso, 2015). In zoos, the husbandry routine of keepers means that enclosures are cleaned regularly and usually at the beginning of the working day (personal observation) giving a daily opportunity to collect animals samples. It has been observed that there is no significant diurnal variation in fGCM levels in orangutans therefore a single sample taken each morning is considered to be sufficient to determine adrenal activity in orangutans (Weingrill *et al.*, 2011; Amrein *et al.*, 2014).

As described earlier, the stress response results in increased levels of glucocorticoids circulating in the body, which is regularly used as an indication of stress (Möstl and Palme, 2002). The glucocorticoid metabolites are then excreted in the faeces and can be measured to determine stress levels. Faecal glucocorticoid metabolites (fGCM) are now widely used to investigate physiology due to the non-invasive nature of collection and stability of results over a period of hours. The methods have been validated for use with chimpanzees (Whitten *et al*., 1998), gorillas (Shutt *et al*., 2012) and orangutans (Weingrill *et al*., 2011). FGCM analysis has been used previously to understand the association between stereotypic behaviour and circulating cortisol levels in polar bears (*Ursus maritimus;* Shepherdson *et al*., 2013), and to identify what kind of events lead to increases in cortisol production in grizzly bears (*Ursus arctos horribilis*) and polar bears (White *et al*., 2015). It has also been used to determine the effect of visitors on the welfare of black-capped capuchins (*Cebus apella;* Sherwen *et al*., 2015). Previous studies into the stress response of orangutans have examined levels of frustration during a computerised task (Elder and Menzel, 2001), stress levels after human visitation in the wild (Muehlenbein *et al*., 2012) and the effect of fission-fusion housing systems in captivity (Amrein *et al*., 2014).

Faecal samples from primates can be analysed using a group-specific enzyme immunoassay (EIA) for 11β-hydroxyetiocholanolone. This assay has been previously verified for the analysis of orangutan faecal glucocorticoids in a study which tested the use of a native (i.e. basal) cortisol assay and two metabolite assays (11β-hydroxyetiocholanolone and 11-oxoetiocholanolone; Weingrill *et al*., 2011). In that study, faecal samples were collected from two female orangutans before and after transportation to another zoo, because transportation is a known stressful stimulus for captive animals. Faecal cortisol levels were low in both orangutans, with only a moderate response from one individual. However, the levels of the cortisol metabolites showed an 8-60 fold increase within the first 48h of the transportation. This indicates the reliability of metabolite assays over native cortisol assays and determines that there is an excretion lag of approximately 48h of cortisol metabolites in orangutans. The choice of the 11β-hydroxyetiocholanolone assay is also supported in an earlier investigation in which high-performance liquid chromatography (HPLC) was used to assess the validity of four EIAs (Heistermann *et al*., 2006). Weingrill *et al*., (2011) also established baseline fGCM concentrations in captive Bornean orangutans (males, mean ± SD: 1243 ± 623 ng/g; females, mean ± SD: 1138 ± 775 ng/g) by monitoring 45 orangutans over four years. These results provide a benchmark with which to compare future investigations into GC production of these animals.

This study aims to establish if fGCM concentrations can be used as predictors of animal behaviour. If heightened stress levels do cause changes in behaviour patterns and elevated fGCM then it would follow that behaviour patterns would be able to act as predictors for fGCM levels, given that there is consistency in the effects on behaviour and physiology. Establishing or refuting consistency in these two indicators is valuable for zoos, allowing for a comprehensive assessment of animal welfare and reducing the work load and financial cost involved in welfare monitoring. By matching behavioural observations with faecal sample collection and applying a lag of 2 days (as per the methods of Weingrill *et al.*, 2011) it is possible to test for correlations between the two measures. The study then goes on to discuss the strengths and weaknesses of using fGCM analysis as tool for monitoring and assessing captive animal welfare in light of the results.

4.3 Methodology

4.3.1 Behavioural observations

To determine the relationship between observed behaviours and fGCM concentrations produced, behavioural observations and faecal sample collection took place simultaneously between 31st July and 19th September 2014. The behavioural data utilised in this study were those collected for behavioural observation analysis in the previous chapter (see **3.3.4 Behavioural observations**) and therefore the same sampling protocol applied. The husbandry routine and study structure employed in the previous study also applied here.

4.3.2 Faecal sample collection

Each morning of the study, zoo keepers collected faecal samples from the orangutans' sleeping quarters. Taking single, daily faecal samples is an established method known to reliably determine daily cortisol production (Weingrill *et al.*, 2011; Amrein *et al.*, 2014). Kibriah and Batu occupied their own quarters at night, and Maliku and Molly were housed together. This allowed keepers to reliably determine which orangutan provided each sample when collected, as Molly produced smaller faecal samples. The orangutans did not provide a sample every morning and therefore there are some days missing from this study. Between 3-10g of faeces were collected and placed in a small plastic sample container with a screw cap.

A label with the orangutan name and a unique ID number was attached to the outer surface of the container and the sample was placed in a freezer (-18°C) within 2 hours of sample collection. At the end of the 12 week data collection period, all of the samples were placed in a polystyrene box, packed with frozen cooler packs and shipped to the Endocrinology Laboratory at the German Primate Centre (DPZ) in Göttingen, Germany.

4.3.3 Enzyme immunoassay

The EIA was conducted following the procedure detailed in Heistermann *et al*. (2006). The faecal samples were freeze dried and an aliquot (0.05-0.07g) of the faecal powder was extracted with 80% methanol (3ml) as per Heistermann *et al*. (1995). The fGCM concentrations were then determined using the group specific enzyme 11b-hydroxyetiocholanolone which indicated the presence of 3α,11b-dihydroxylated GCMs.

4.4 Analysis

To detect any relationships between specific patterns of behaviour performed by individuals and the fGCM concentrations produced by that individual over the course of the enrichment study, regression analyses were performed for each individual, where the proportions of observed behaviours were used as predictor variables for fGCM concentrations. By separating the data into each treatment (as in Chapter three) it was also possible to determine the effect of the enrichment treatment on fGCM. A starting general linear regression model was established using all behaviour categories observed for that individual as potential 'explanatory' variables and in the fGCM as the dependent variable. However, if any behaviour variables were correlated (R> 0.70), only the one most strongly correlated with fGCM levels was included, in order to avoid collinearity amongst predictor variables (Gelman, 2007; Zuur *et al*., 2007, 2011).

Before constructing the linear models, model assumptions were tested using residual diagnostics (Pinheiro & Bates, 2000). Quantile-Quantile plots were used to assess normality, and homogeneity of variance was evaluated by plotting residuals versus fitted values, whilst overly influential data points were tested for using Cook's distance. Where heterogeneity of variance was detected a generalised least squares (GLS; Pinheiro & Bates, 2000; Zuur *et al*., 2007) framework was applied. GLS adds a random structure to the model, explicitly modelling the heterogeneity of variance (Pinheiro & Bates, 2000). The most appropriate random structure was determined using a combination of the Akaike Information Criterion (AIC; Akaike, 1973) and plots of fitted values versus residuals (following Zuur *et al*., 2007) based on a full (fixed) model specification using the restricted maximum likelihood estimation (REML; West *et al*., 2007). The minimum adequate model was then determined using backwards stepwise selection using maximum likelihood methods (following Zuur *et al*., 2011).

Individual models were developed for each orangutan. A mixed model approach could have been applied, treating individual as a random variable (e.g. see Amrein *et al*., 2014). However, having only four orangutans as well as a range of life-stages, this approach would not be appropriate, as the individuals were all very different in their demographics and the mixed framework would have been attempting to derive generally applicable statistical parameters (random component of the model) across just four individuals (Gelman & Hill, 2007). Additionally, the focus of this research was on each individual rather than wanting to explicitly model orangutans as a species.

In order to temporally match up behaviour data with fGCM concentrations a lag of 2 d was applied; that is, behaviour from 2 d prior to the day of a faecal sample being collected were matched to the fGCM level from that faecal sample. This is consistent with the method applied in other primate endocrinology research (Higham *et al*., 2009; Amrein *et al*., 2014). Behavioural observations were conducted on weekdays only and so some faecal samples could not be matched with behaviour data in this way. Following this data processing, the following number of data points remained for the individuals: Batu (34), Kibriah (13), Maliku (36), Molly (27).

Some behaviours, such as resting, were very common, whilst others, such as object use were rare. However, even though some behaviours were rare when looking across the whole study period, it is possible that they would become more frequent during times of greater or lesser stress. This would be illustrated by a correlation between fGCM concentration and the proportion of time spent performing a behaviour. This imbalance in the distribution of common and rare behaviours makes backward stepwise regression less robust (Quinn & Keough, 2002). Therefore a second selection framework was utilised to model fGCM levels on behaviour data in order to check for consistency in variable selection using contrasting methodologies: least absolute shrinkage and selection operator (LASSO; Tibshirani, 1996). The LASSO method is a shrinkage method of selection whereby beta coefficients are constrained leading to some (those associated with the independent variables regarded as having least impact on the dependent variable) being reduced to zero and therefore excluded from the model (Hastie *et al*., 2004). The extent of the constraint can be increased sequentially, allowing for beta coefficients to decrease to zero sequentially, allowing for assessment of the relative importance of the independent variables on the dependent variable. Before applying the LASSO method, the values in each behaviour category were centred by subtracting the mean for that behaviour category from each value (following Hastie *et al*., 2004). This standardises the data so that the different behaviours are equally weighted as the LASSO method proceeds. In some cases, the occurrences of some behaviours were so rare that they effectively provided no information and this caused the LASSO method to crash. When this occurred, these variables were removed and the LASSO method was rerun on the reduced set of potential behaviours. Following the application of the LASSO method, the subset of predictor variables judged to be of most importance were used as the starting point for general linear regression, which was applied as described above in this section. The resulting output highlights behavioural categories that are significantly influenced by fGCM concentration, allowing for identification of behavioural indicators of physiological stress.

Consistency in results between methods of selection (backwards stepwise and LASSO followed by backwards stepwise) would increase confidence in the results, whereas a lack of consistency would lead to greater caution.

In all regression methods applied, only additive terms were applied as the data set was not large enough to allow the inclusion of interaction terms and there were no prior expectations of particular interactions being biologically important.

4.5 Results

4.5.1 Descriptive statistics

The fGCM concentrations of the four orangutans, throughout the study remained within the range that has been determined for other captive housed orangutans (Weingrill *et al*., 2011). Hence, the concentrations measured were not substantially higher or lower than have been measured in orangutans living in other captive environments. The adult male, Batu, maintained the highest fGCM concentrations in the group and the infant female Molly, had the lowest.

Table 4.1 Descriptive statistics for fGCM concentrations over the 12 weeks of study. Treatment titles correspond with those outlined in Table 3.1.

Orangutan	**min**	**median**	mean	**max**	**SD**	SE
None						
Batu	702.2	1311.0	**13355.1**	1987.3	412.73	**119.14**
Kibriah	148.8	1015.2	**901.4**	1076.6	333.41	**126.02**
Maliku	266.4	994.9	**948.1**	1772.2	426.03	**110.00**
Molly	322.9	555.9	**551.2**	748.8	139.73	**46.58**
Food						
Batu	918	957	**1228.1**	1770.2	471.06	**271.97**
Kibriah	716.4	716.4	**716.4**	716.4	203.13	**NA**
Maliku	519.2	856.9	**829.6**	1085.5	297.45	**148.72**
Molly	708.3	849.7	**849.7**	991.1	199.97	**141.4**
None						
Batu	568.1	901.7	**960.6**	1786.5	312.96	**86.80**
Kibriah	116.7	701.8	**785.1**	1491.8	507.13	**226.79**
Maliku	577.7	860.6	**920.5**	1413.6	255.85	**77.14**
Molly	297.4	615.3	**639.8**	960.8	247.61	**71.48**
Termite						
Batu	1206	1415	**1630**	2484	582.86	**291.43**
Kibriah	787.7	856.9	**856.9**	926.1	97.86	**69.2**
Maliku	732.4	1165.5	**1173.7**	1631.1	367.64	**183.82**
Molly	433.4	479.3	**654.9**	952.0	267.44	**154.41**
TermiteFood						
Batu	705.6	982.0	**1088.7**	1695.2	409.29	**204.65**
Kibriah	544.3	647.5	**647.5**	750.8	146.02	**103.25**
Maliku	947.0	973.4	**1028.6**	1165.1	118.96	**68.68**
Molly	598.9	913.2	**913.2**	1227.6	444.56	**314.35**
None						
Batu	1200	1440	**1450**	1814	259.73	**116.15**
Kibriah	789.8	898.9	**898.9**	1008.0	154.29	**109.1**
Maliku	800.8	1725.0	**1726.1**	1910.9	374.54	**152.90**
Molly	483.7	774.0	**795.7**	1111.9	253.26	**113.26**

A number of peaks in fGCM concentration were detected at various points throughout the study within each orangutan (**Figure 4.1**). The maximum recording (2484.3 ng/g) for Batu corresponds with day 44 of the behavioural observations, a closer look at the notes recorded throughout the study indicate no obvious stimuli that Batu may have found stressful (i.e. no changes in husbandry routine etc.). What is more, the peaks in fGCM also do not match with days specifically associated with the introduction or use of either the frozen fruit cups or artificial termite mound.

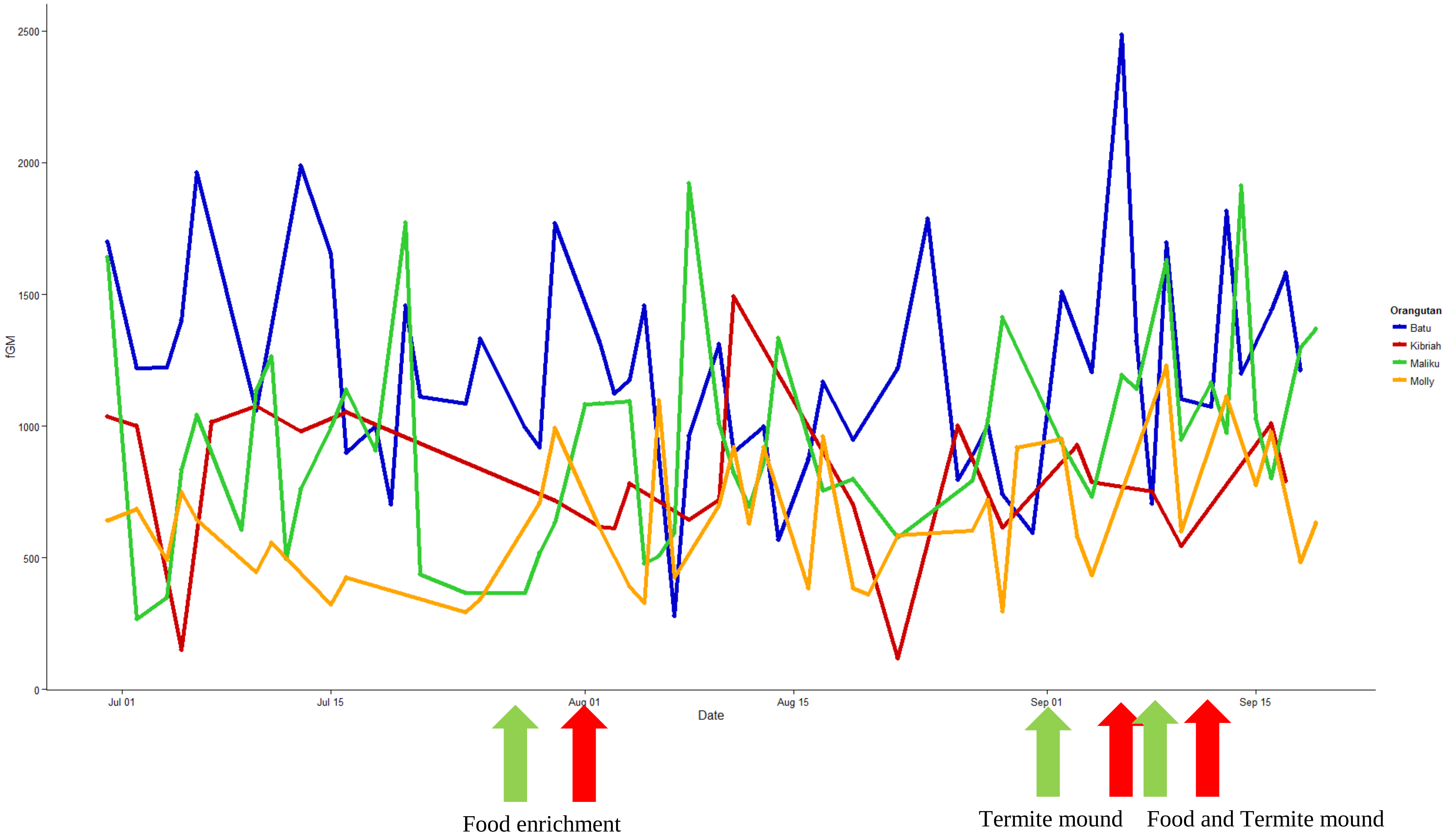


Figure 4.1 fGCM concentrations produced by the orangutans over the 12 week enrichment programme. The time points at which the enrichment treatments began are indicated by green arrows. The red arrows indicate the time points at whicht the enrichment treatment ended.

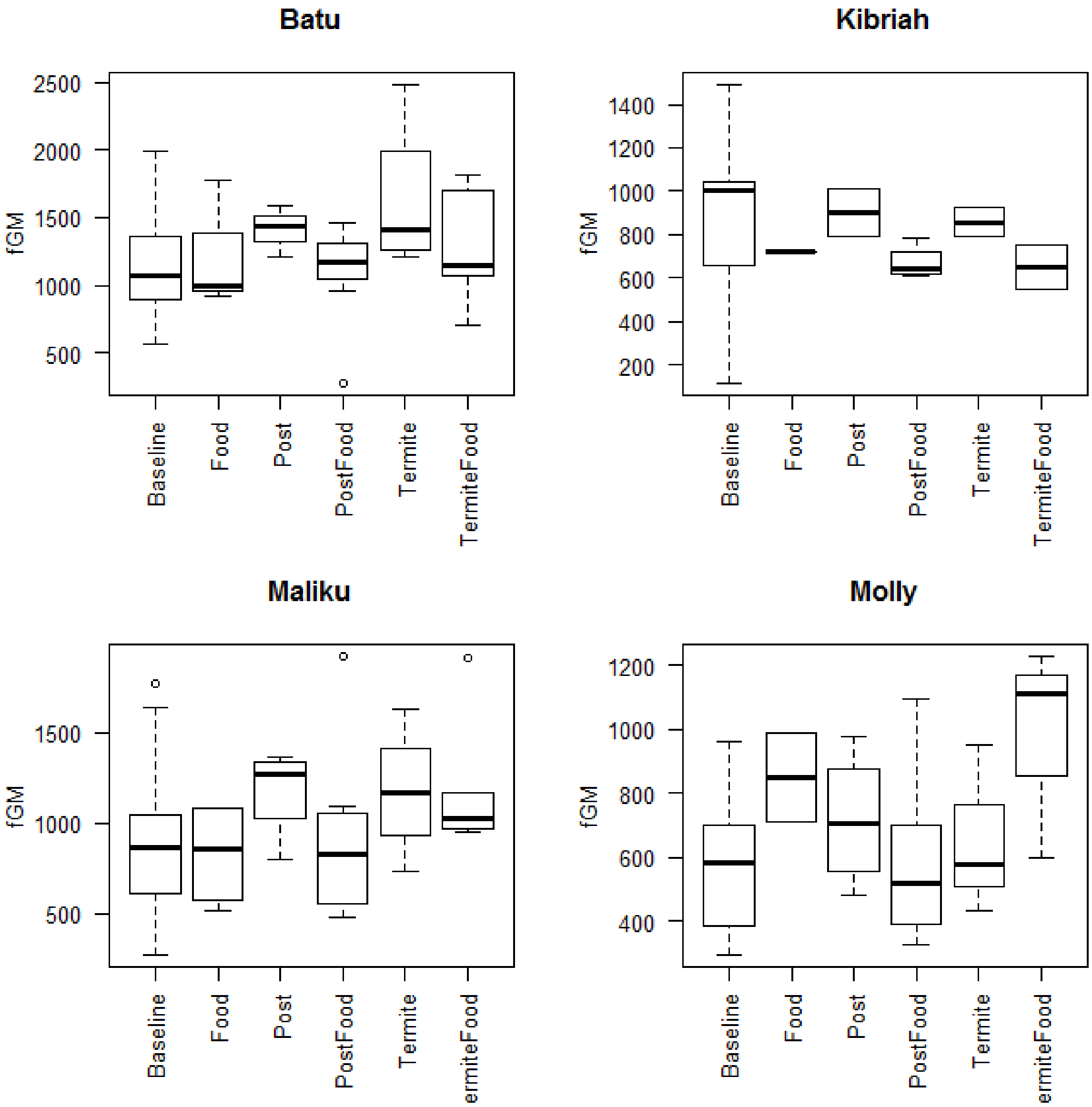


Figure 4.2 Relationship between enrichment treatment and fGCM production throughout the study for each orangutan. The horizontal black lines represent the median values, and the boundaries of the boxes represent the upper and lower quartiles.

Establishing if behaviour patterns can predict fGCM concentrations

The backwards stepwise regression analysis determined that for both Batu and Molly, none of the behaviours recorded were significant predictors of fGCM concentrations throughout the 12 week study. However, in the case of Kibriah, the behaviour 'resting' was a significant predictor of fGCM concentration (*L*-ratio = 5.74, d.f.=1, p=0.0166, **Figure 4.3a**). In the case of Maliku, 'playing' behaviour was a significant predictor of fGCM concentration (*L*-ratio = 9.46, d.f=1, p<0.01 **Figure 4.3b**).

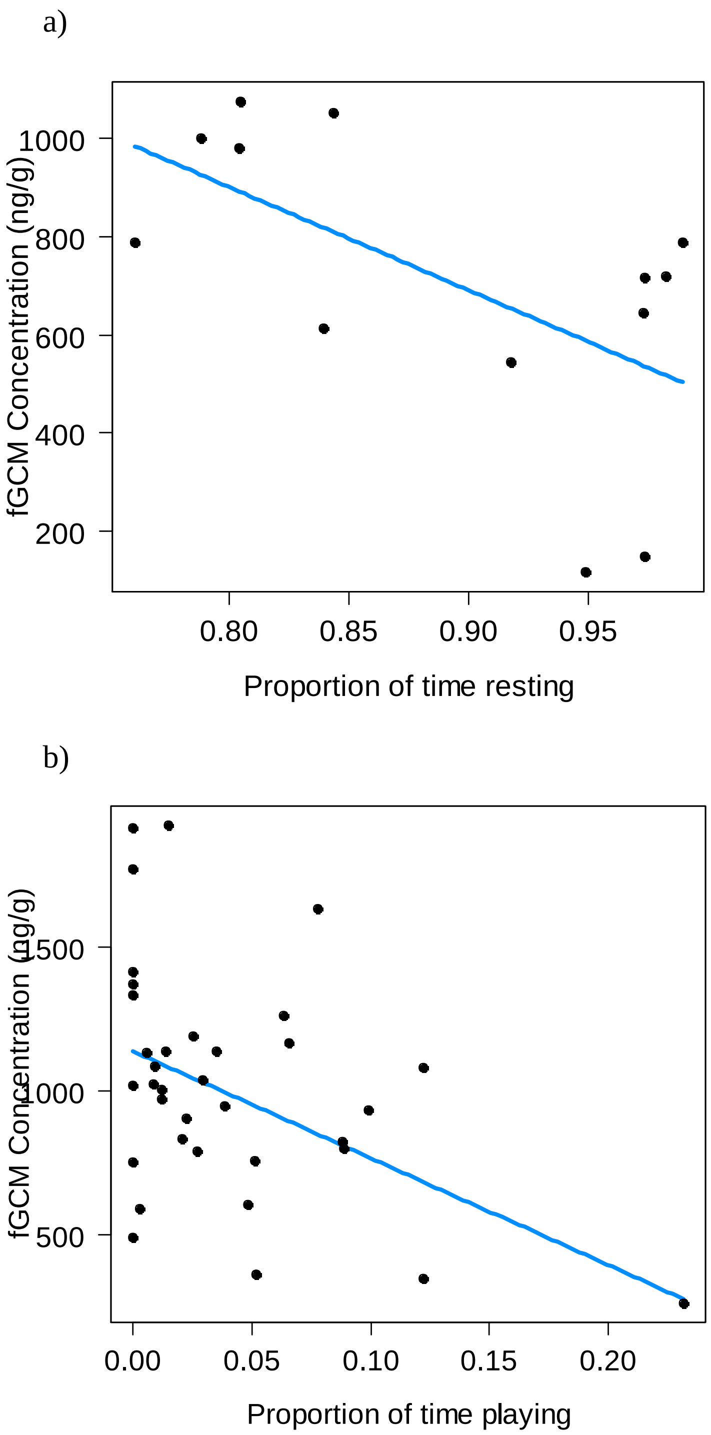


Figure 4.3 Relationship between fGCM concentration and the proportion of time spent a) resting (Kibriah) and b) play behaviour (Maliku). The blue lines represent model predictions.

These results from the backwards stepwise regression were generally supported by the output from the LASSO method (**Figure 4.4**). Each line on the graphs represent a different behaviour category, lines are also different in pattern for further distinction. For both Batu and Molly, there were no behaviours which had coefficients shrinking to zero much later than the other behaviours, although for Molly, feeding and object-use did have slight delays in their patterns of shrinkage. In contrast, playing and resting showed marked delays in shrinkage for Maliku and Kibriah, respectively, matching the results from the stepwise selection procedures.

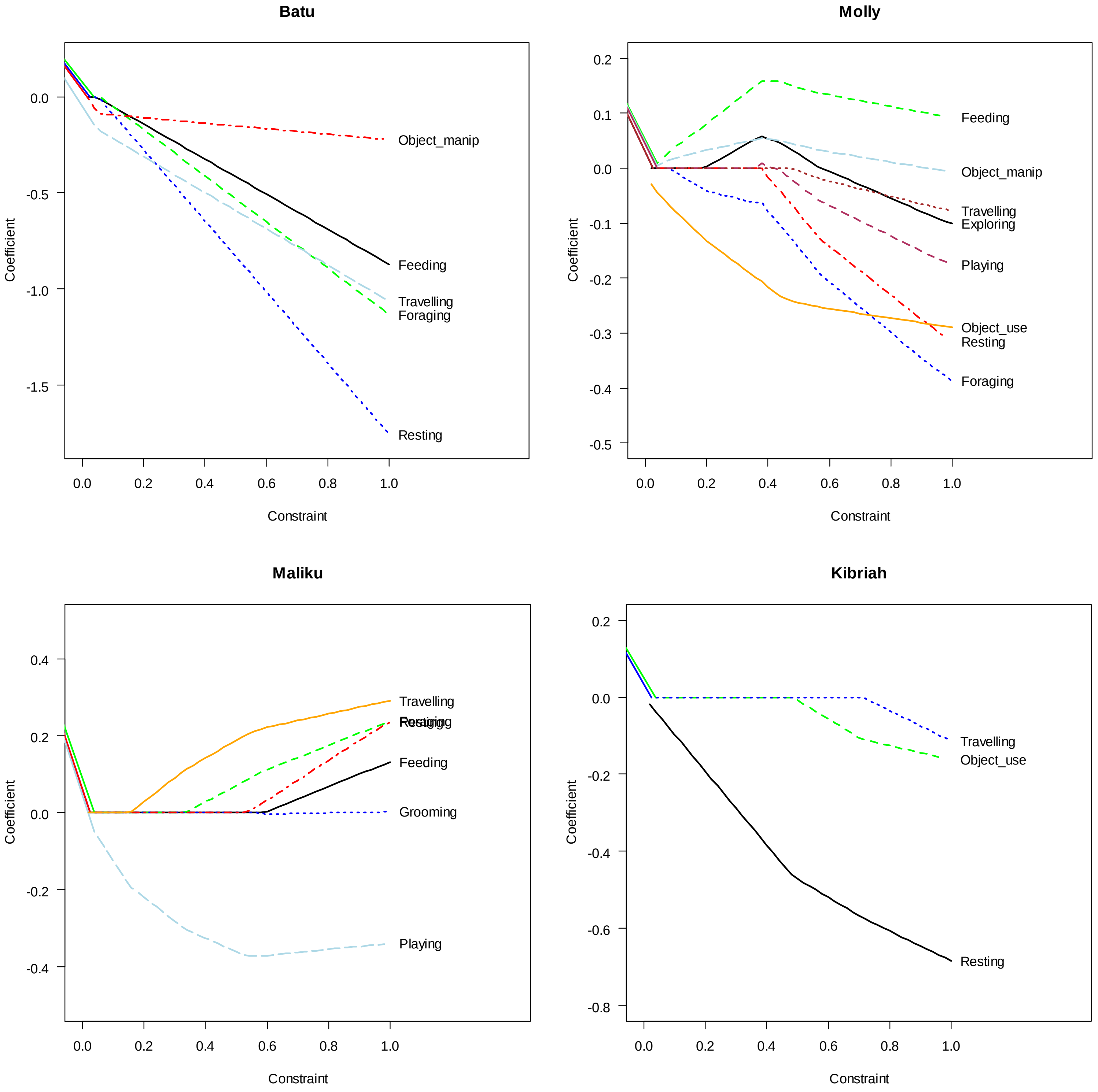


Figure 4.4 LASSO plots for each orangutan. The x-axis shows the level of the constraint on the beta coefficients, 0 representing the greatest constraint, and 1 the least constraint. The y-axis is the estimated beta coefficient value, within the current constraint. Thus, those variables which only shrink to zero very close to a constraint of 0, are regarded as being the variables which are most likely to be influencing the dependent variable.

4.6 Discussion

4.6.1 Individual variations in fGCM concentrations

There was considerable variation in the fGCM concentrations between individuals. These results are consistent with those found in a previous investigation of Bornean orangutan fGCM. The maximum fGCM levels detected in this study do not reach those determined in challenge tests nor are they outside the established baseline range (Weingrill *et al*., 2011). Thus no biologically relevant stress response was detected over the 12 weeks. This supports the use of enrichment as an implement for improving welfare and suggests that there is no disadvantageous stress associated with introducing enrichment devices into this group.

The adult male, Batu, maintained fGCM levels that were consistently higher than that of the females throughout the study with only a few exceptions. Higher levels of cortisol excretion in males have also been found in humans (Kirschbaum *et al*., 1992) and higher cortisol levels have been associated with increased testosterone levels in male savannah baboons (*Papio cynocephalus cynocephalus*), particularly high ranking males (Gesquiere *et al*., 2011). There is a positive correlation between dominance rank and urinary cortisol in wild chimpanzees, which is thought to be a product of their unstable fission-fusion social system, and it is thought that social dominance may be associated with increased energetic expenditure and therefore incurs increased physiological stress on the animal (Muller and Wrangham, 2004). Orangutans do not maintain social groups in the wild, but flanged males are known to defend their territory and dominate over unflanged males, therefore taking on the physiological cost associated with social dominance in other species (Maggioncalda *et al*., 2002). Weingrill *et al*., (2011) also determined that there is a more pronounced stress response in orangutans with increasing group size, perhaps owing to the fact that orangutans are naturally solitary. This trend is known to be more apparent in Bornean orangutans as they are naturally more solitary in the wild than Sumatran orangutans (van Schaik *et al*., 2009).

Molly produced the lowest concentrations. To the best of our knowledge there are no existing measures of infant orangutan fGCM concentration with which to compare these results. However, research suggests that young primates maintain low

HPA activity, resulting in low cortisol production as a result of social buffering (Kikusui *et al*., 2006). Low cortisol levels in young primates might be explained by the phenomenon called “social buffering”, which describes the circumstances when the stress response of an individual is reduced or eliminated by the presence of a familiar individual (Hennessy *et al*., 2009) and has been demonstrated in rhesus monkeys (*Macaca mulatta*). With reference to the mother-infant relationship, the presence of the caregiver can regulate infant physiology and buffer the stress response (Kikusui *et al*., 2006). It is though that the maternal behaviours performed by the mother as part of her care giving responsibility, such as providing protection and comfort, moderate an infants’ exposure to stressful stimuli, and may regulate emotional maturity (Sanchez *et al*., 2015).

Although this study did not appear to induce any physiological stress response in these individuals, the consistently low fGCM levels produced by this infant may indicate the importance of maintaining mother-infant bonds in primate groups until infants reach full maturity. The mother-infant bond in squirrely monkeys was such that the plasma cortisol levels of both mothers and infants was significantly elevated following separation and reduced when pairs were reunited (Coe *et al*., 1978). It has also been suggested that the early separation from their mother impairs orangutans’ ability to acquire the necessary skills for survival providing further support for the maintenance of this bond (Mendonça *et al*., 2016).

4.6.2 Establishing if behaviour patterns can predict fGCM concentrations

For both Batu and Molly, not a single one of the recorded behaviours acted as a significant predictor of fGCM concentration. Therefore, for these individuals, it is potentially not possible to detect levels of physiological stress through behavioural observations alone. Previous research has focused on the potential for a correlation between abnormal behaviours and fGCM; for orangutans this was confirmed with the correlation between fGCM and scratching (Amrein *et al*., 2014). However, this is has not been supported in other research. In a study of Self Directed Behaviours (SDBs) and female baboons (*Papio hamadryas anubis*) within two troops, it was speculated that SDBs could act as behavioural indication of anxiety, and therefore indicate low levels of psychological stress but the data could not confirm this (Higham *et al*., 2009). It was thought that the absence of a relationship between SDBs and adrenal cortex activity suggests that measurement of fGCM may not be sensitive enough to detect an acute cortisol response that would be associated with SDBs (Higham *et al*., 2009). It is possible that SDBs are a method of behavioural coping strategy, used by primates to manage psychological stress in the absence of a more effective behavioural response (Wechsler, 1995). In the absence of stress related behaviour and fGCM levels remaining within the normal range for captive Bornean orangutans, there is no evidence to suggest that these orangutans suffered from stress at any point during this study.

With regards to the two adult female orangutans, Maliku and Kibriah, single behaviours appeared to be indicative of lower fGCM concentrations. The inverse relationship between resting behaviour and fGCM concentration suggests that Kibriah exhibited greater levels of resting behaviour when she was in a state of least physiological stress. This observation contradicts that found by Mendonça *et al*. (2016) who found that a juvenile orangutan exhibited a high proportion of resting and yet elevated cortisol levels when re-released after rescue. Kibriah's resting behaviour was explored in chapter three where it was noted that in the wild, orangutans reduce their activity levels in response to reduced food sources as a strategy for conserving energy (Pontzer *et al*., 2010). It is apparent that this strategy is not associated with any physiological stress and that the removal of the food enrichment, although reducing the desire to forage, allows this individual to engage in behaviour correlated with low stress. Information provided by Twycross Zoo confirms that Kibriah was hand reared from a young age and has an affiliation with humans. Additional observations during the study noted that this individual spent extended amount of her time watching zoo visitors, and keepers thought that this behaviour is a result of her close human contact during early development. Therefore, resting behaviour might be indicative of reduced stress in this individual and thus a positive welfare state. Unfortunately the limited samples contributing to this data set restricts the ability to draw such conclusions.

The inverse relationship of fGCM with a behaviour was also evident in Maliku, with periods of playing appearing to indicate lower fGCM levels. Field notes indicate that these bouts of play behaviour were social and always with the infant, Molly. The analysis of behavioural patterns confirm that these two orangutans exhibited comparable behaviours over the course of the study (see chapter three). Play behaviour is an important part of infant development and when possible, mothers will bring infants together to play and learn important skills (van Noordwijk *et al*., 2009). Research suggests that play behaviour is associated with increased brain size and vital for the development of cognitively complex behaviours (Montgomery, 2014; Poirier and Smith, 1974). The extended juvenile period of orangutans also enables the development of play to maximise learning opportunities (Lewis, 2005). In the wild, orangutan mothers are observed to forego opportunities to forage to allow their infant to play with that of another female (van Noordwijk *et al*., 2016). This illustrates the investment that orangutan mothers are willing to make for the development of their offspring. It has been suggested in various studies that play behaviour occurs when animals are in favourable environments, a behaviour afforded only when the environment is void of threats (Klein *et al*., 2010; Norscia and Palagi, 2011). Therefore the correlation between play behaviour and fGCM levels in Maliku suggests that she does not perceive any environmental stressors during play and therefore has reduced adrenal cortex activity as a result. Play behaviour has also in itself been suggested to be an indicator of positive welfare as it suggests a lack of stressful stimuli (Held and Špinka, 2011).

Here the samples collected did not yield fGCM concentrations consistent with physiological stress in the four captive orangutans. Therefore the enrichment does not appear to induce a stress response in this group. Elevated fGCM levels have been detected in river otters (*Lontra canadensis*) after veterinary examination (Rothschild *et al*., 2008), in African elephants (*Loxodonta africana*) after injury (Ganswindt *et al*., 2010), and in the Mexican wolf (*Canis lupus baileyi*) during high visitor numbers (Pifarré *et al*., 2012). This suggests that the implementation of environmental enrichment does not present a biologically relevant stressful stimulus and supports its use in the husbandry routine of captive orangutans and that fGCM analysis may be more useful to assess the welfare of animals after significant events such as veterinary attention or transportation.

The ability to use observable behaviours as indicators of physiological stress reduces the time commitment necessary to monitor animal welfare. However, as the relationship between fGCM and behaviour is only evident in two of four orangutans, behavioural observations cannot be recommended as a sole monitoring tool. Therefore, fGCM provides an insight into animal physiology which cannot be detected through other established methods. Although no notable elevations in fGCM were detected here, the baseline that has been established through 12 weeks of observation will serve as a benchmark with which to compare future samples collected at times of potential significant stress. The number of samples collected and the large variation illustrated by the data has allowed an indication of the upper and lower limits of fGCM concentrations of an adult male, infant female and two adult female orangutans under normal conditions in the absence of significant stress. A baseline of Bornean orangutan fGCM levels has previously been established (Weingrill *et al*., 2011). However the present study provides data over a period of time three times that of this previous research. Thus, the study presented here extends the current database of Bornean orangutan fGCM levels and highlights the necessity to take group demographics into account when assessing the stress response.

All of the orangutans in this study presented fGCM levels that are consistent with their age and sex as determined in other captive and wild counterparts. Research in the field suggests that elevated fGCM levels are associated with agonistic behaviours, particularly within dominance hierarchies (Cavigelli and Caruso, 2015). During the data collection for the present study, the orangutans did not display any agonistic behaviour, and as a species, orangutans do not adopt a hierarchy such as that of chimpanzees (Muller and Wrangham, 2004) or ring-tailed lemurs (*Lemur catta*; Tennenhouse *et al*., 2017). Similarly, the orangutans did not produce fGCM levels that would imply any significant physiological stress. Instead, two of the orangutans produced reduced fGCM when also displaying behaviours that are consistent with positive welfare. Orangutans are highly adaptive animals and the introduction of enrichment devices and other potentially stressful stimuli are not uncommon within their environment and therefore their fGCM production mirrors their ability to cope in such a captive setting. However, the correlation of reduced fGCM and resting and playing behaviour suggest that behavioural indicators of positive welfare may be reflective of positive psychological states. If the same relationship is detected in other captive species, it may be possible to report good welfare, with more confidence than has previously been possible. Given that current animal welfare research is increasingly trying to identify indicators of positive welfare, this would give greater support for the maintenance of zoo housed populations.

Data collection for this study relied on each orangutan providing a faecal sample each day and accurate identification of each sample. The orangutans did not reliably produce daily faecal samples, although at the time of data collection the orangutans were kept separately overnight. A more recent development of the enclosure has allowed staff to alter their housing conditions and allow the orangutans to remain together through the day and night. This would make positive identification of samples substantially more difficult. Potential alterations to the methods include taking saliva samples to monitor adrenal cortex activity. Such a method has recently been utilised to monitor the effect of positive reinforcement training on the short-term stress response of captive orangutans (Behringer *et al.*, 2014). Whilst orangutans took part in training for medical examination, researchers were able to take saliva samples directly from each individual and thus full account of cortisol production could be gathered. The short five minute delay (as opposed to 2 d for fGCM) allows for monitoring the short-term stress response in response to substantial stressors and sampling can be repeated multiple times a day. However, as has been discovered with the use of faecal samples, saliva samples yield highly variable results and authors stress the necessity to collect many samples over an extended period in order to gather a high resolution data set (Behringer *et al.*, 2014). Saliva sampling would also involve a greater proportion of an animal keepers' time and require positive reinforcement training of the orangutans to obtain the samples. The benefit to using physiological assessments of animal welfare is not only that they directly quantify physiological stress but also that the methods used to detect the relevant steroid hormone can be specifically designed to ensure the most reliable results without compromising animal welfare in the process.

The analysis of faecal samples contributed to the assessment of animal welfare by confirming the absence of physiological response to enrichment and identifying both play and resting behaviour as indicators of reduced fGCM production. The collection of samples was uninvasive and was integrated into the routine of the orangutan care staff. The methods for analysing the faecal samples had been validated previously for use with orangutans, allowing for greater confidence in the results, and the collaboration with the German Primate Centre meant that the necessary laboratory skills and equipment were not required of the researcher. However, this method came with some flaws which limit its use in welfare assessment. The collection of samples relied on each orangutan producing a sample in their bed area every morning but this was often not the case, causing variation in the total number of samples analysed for each orangutans and thus reducing confidence in the statistical analysis performed on the resulting data. Also, the EIA can only quantify the levels of fGCM produced since the last defecation and therefore do not identify the point at which the stress response was initiated. This made it difficult to attribute peaks in fGCM levels to specific events.

4.7 Conclusion

Although stress must be encountered by all animals, both wild and captive, it is the stress which cannot be escaped nor avoided, which can lead to deleterious physiological consequences. In order to avoid or treat the behavioural abnormalities brought about by captivity and restore species natural behaviours within individuals, the use of enrichment is commonplace, with many recorded successes. However, the enrichment itself has the potential to be perceived as a stressor, much like those which elicit the indicators of stress originally.

Observations of animal behaviour provide insight into the use of enrichment to encourage behaviours indicative of good welfare. However the physiological indicators of welfare are not evident in this method. Therefore it was suggested that hormonal assays would increase the lucidity to our understanding of captive animal welfare. The orangutans studied did not indicate, via EIA of faecal samples, that they experienced any significant stress throughout the study. There was no correlation between the implementation of the enrichment devices and the hormonal indicators of stress from each individual, indicating that enrichment itself does not pose a physiological threat to captive orangutans. The investigation into a possible link between fGCM concentrations and observed behaviours concluded that there were no behavioural indicators of heightened stress nor decreased cortisol for both the adult male and infant female of the group. However the correlation between play and resting behaviours and decreased cortisol, suggests that orangutans display indicators of positive welfare when experiencing the least stressful stimuli. This supports the use of behaviour as a welfare monitoring tool but also highlights the need to ensure the correct method of steroid collection, assay and interpretation before drawing conclusions.

This study has highlighted the shortcomings of using fGCM as a welfare monitoring tool in the absence of adverse events. However, the expressed behaviour of animals depends to a large extent on possibilities provided by their environment. Therefore, in a captive environment, designed in itself to encourage species natural behaviours, the use of the space available to the animals, and the appropriateness of the enclosure design may also shed light on the welfare status of its inhabitants. The following chapters explore new methods for using orangutan behavioural patterns and enclosure usage to determine the effectiveness of a captive environment for facilitating positive welfare.

Chapther FIVE: Extending the Use of Network Analysis in Animal Behaviour Research

5.1 Chapter overview

Over the last two decades there has been a rapid expansion in the use of network analyses in diverse areas ranging from studies of the structure and vulnerabilities of the World Wide Web to studies of the microbial community structures in the human gut. A particularly prolific area of research has been the study of the structure and dynamics of social networks. Although social network analysis (SNA) has been primarily applied to human networks, it has also been widely applied to the study of animal social networks. However, network analysis is founded on mathematical graph theory which is abstract in nature, focussing on the patterns of connection and ignoring the nature of the units that are connected. The application of network analysis requires only that information and relationships between units existing in the real world can be transformed into the abstract form of networks. Thus there is extensive potential for widening the use of network analysis in the study of animal behaviour, extending its use beyond that of SNA. This step requires that animal behaviour data other than patterns of social interaction be transformed into network frameworks. Once this is done the extensive and rapidly developing suite of network analytical tools can be applied to a wider range of animal behaviour studies.

This chapter briefly outlines the main characteristics of networks and introduces how network analysis has been used in the study of animal social networks. It then goes on to demonstrate how a wider range of behavioural data can be converted into network form. As an example, these methods are applied to the behaviour and space use data collected from the captive orangutans at Twycross Zoo. The development and visualisation of the networks in relation to behaviour and space use data are illustrated. The chapter ends with a discussion of the development of these networks and the strengths and weaknesses of their visualisations. This links to the next chapter which builds on the work of this chapter by introducing quantitative measures of network structure and examining how these can be related to understanding the implications for animal behaviour and space use.

5.2 Introduction

The study of networks dates back to at least 1736 when Leonard Euler solved the famous Königsberg Bridge Problem. The solution today is regarded as fairly trivial, but Euler's solution is regarded as immensely important as it required the conversion of properties of real world objects (bridges) and their relationships (which islands they were connected to) into an abstract form: a graph. The analysis of the graph produced the solution which could then be translated back into a solution in the real world.

Mathematically, a graph is defined as follows:

"*A* ***graph*** **G** *consists of a non-empty set of elements, called vertices, and a list of unordered pairs of elements, called edges. The set of vertices of the graph* **G** *is called the* **vertex-set** *of* **G***, denoted by* **V(G)***, and the list of edges is called the* **edge-list** *of* **G***, denoted by* **E(G)***. If* **v** *and* **w** *are vertices of* **G***, then an edge of the form* **vw** *or* **wv** *is said to* **join v** *and* **w**" (from Wilson and Watkins, 1990).

This definition is expanded upon below, as it is a critical foundation of the argument in this chapter that networks can be used more extensively in studies of animal behaviour. At this point it is important to note that 'graph' and 'network' are often used interchangeably in the literature. However, 'graph' has a strict mathematical definition (see above), whereas 'network' has a much more general definition: "a collection of interconnected things" (Kolaczyk & Csárdi, 2009). The distinction between the two relates to 'network' being more frequently used when discussing real world objects and concepts, whereas 'graph' is the term used by mathematicians and those developing mathematical metrics and trying to understand the properties of graphs. For the purposes of this chapter it is important to recognise that patterns of behaviour (real world) are being converted into abstract graph form so that the analytical tools developed for graphs can be applied in order to help us understand patterns and dynamics of the behaviour of animals. As long as this is realised, the subtleties of the differences in definition between 'graph' and 'network' are not important. Therefore, in line with the majority of the animal behaviour literature in this area, the term 'network' will be used.

5.2.1 Types of network

The simplest form of a network is a set of vertices joined by edges, as given in the definition of a graph above. This is exemplified in the form of a basic social network, where the vertices represent individuals and the edges represent friendships or some form of pairwise interaction between individuals (**Figure 5.1a**). However, networks can include further characteristics, expanding the level of information that they contain (**Figure 5.1**).

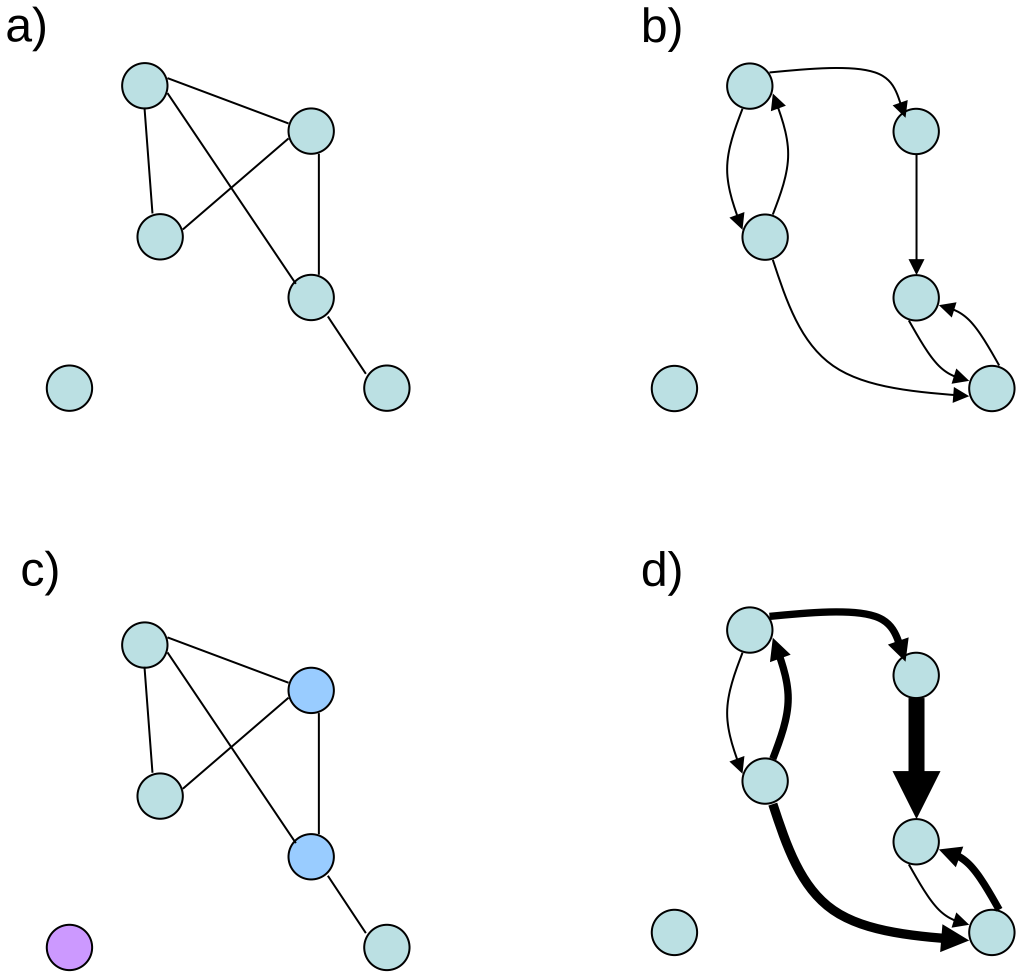


Figure 5.1 Examples of different types of network. a) is a basic network with the vertices (circles) potentially connected by edges (lines), whilst b) is a similar network but with the added complexity of edges being directed (a directed network). c) is a network where a particular quality of a vertex is indicated by the different colours associated with the vertices, and d) shows a network where the directed edges are also weighted to illustrate their relative strengths or frequencies of occurrence (a weighted directed network).

In situations where the relationship between vertices is asymmetrical e.g. one individual grooms another individual, the edges can be directed (**Figure 5.1b**) in order to reflect this asymmetry. The vertices can also have properties. For example, individuals belonging to the same demographic grouping could have the same colour associated with their vertex representation in the network (**Figure 5.1c**). And networks can contain combinations of these additional characteristics. For example, **Figure 5.1d** shows a weighted directed network which could represent patterns of individuals grooming other individuals (directed), where the edges are weighted according to the relative frequencies of who groomed whom over the time of the study. Therefore one of the advantages of using networks is the flexibility in being able to adjust the level of information contained in the network.

5.2.2 Applications of network analysis

Network analysis has been widely applied in the research community. Topics range from the mathematical characteristics of random graphs (Erdös and Rényi, 1960; Rapoport & Horvath, 1961), to the structure of human societies (Travers & Milgram, 1969), to the patterns of citations in research journal articles (Price, 1965), to the structure of the World Wide Web (Huberman, 2001), to the structure of power grids (Watts & Strogatz, 1998; Watts, 1999), and to metabolic pathways (Jeong *et al.*, 2000; Podani, 2001). The ability of networks to represent extremely complex systems has been particularly useful in recent studies of patterns of gene expression (Guelzim *et al.*, 2002; Shen-Orr, 2002; Farkas *et al.,* 2003).

Network analysis has been used in a number of animal behaviour studies and the range of species observed in these studies is broad. For example, Fewell (2003) studied how social structure in social insects could affect foraging behaviour, whilst Croft *et al*. (2005) determined patterns of interactions in wild populations of fish, and Wittemyer *et al*. (2005) studied hierarchical social structures in African elephants (*Loxodonta africana*). However, the animal behavioural literature utilising network analysis is heavily biased towards research on primates (Croft *et al*., 2008). Importantly, although there is this bias, within the research on primates, network analysis has been used to investigate an extensive range of aspects of behaviour (Croft *et al.*, 2008). Some have focussed on determining and describing social structures (e.g. Chepko-Sade *et al.*, 1989; Berman *et al.*, 1997; Bezanson *et al.*, 2002). Others chose to focus on particular behaviours. For example, Pastor-Nieto (2001) examined patterns of food sharing in spider monkeys (A*teles geoffroyi*) and Knox & Sade (1991) examined agonistic behaviour in emperor tamarins (*Sanguinus imperator*). Others investigated potential causes of social network structure and dynamics. For example Kudo and Dunbar (2001) did a comparative study of primates examining the relationship between the extent of social network sizes and the size of species' neocortexes, and Flack & de Waal (2007) looked at how social structure could be stabilized within pigtailed macaques (*Macaca nemestrina*).

Animal behaviour studies have therefore utilised the power of networks in a wide range of situations. However, there are two important biases in the literature. Firstly, the vast majority of studies have focussed on social networks and, secondly, relatively few of these SNA studies have been done in zoo settings (Clark, 2011).

Recently, there has been the recognition that network analysis of social structure is a promising framework for linking behaviour patterns to population dynamics, and therefore could be a powerful tool for effective conservation management (Snijders *et al.*, 2017). This marks an important step forward in extending the use of SNA into addressing more fundamental ecological questions related to social structure. This chapter proposes that the scope of this step forward could be much more extensive if the use of networks in behaviour studies was extended beyond that of SNAs. This would provide a broader base of network frameworks to move forward with, and is likely to be of particular importance in the context of zoos.

This chapter examines how data collected as part of standard behaviour studies can be converted into networks, and how these networks can be utilised to provide a wider range of analytical tools currently available for the study of captive animals. It argues for extending network analysis in behaviour studies beyond social networks, in order to facilitate a more complete understanding of patterns of behaviour and welfare in captive animals. It demonstrates how behaviour networks and space-use networks can be derived, and how they can be utilised within a zoo environment to enhance understanding of animal behaviour and welfare. This sets the foundation for the next chapter in which the framework is extended by showing how combining behaviour and space-use networks in novel ways can provide a deeper level of information in behaviour studies.

5.3 Methodology

5.3.1 Subjects and housing

The four orangutans housed at Twycross Zoo and studied in the previous chapters were used in this study. Observations of behaviour and space use (see below for details) were made from 31st August until 2nd October 2015 (5 weeks) on weekdays only (justified in chapter three).

The study focussed on the four orangutans for a number of reasons. Firstly, due to the previous studies, the researcher was already very familiar with the individuals, the enclosure and the daily patterns of work by the zookeepers. Secondly, the orangutans offered a good test system for this work as the number of individuals was small, they are relatively slow moving and there was good general visibility of the different areas of the enclosure. Therefore, the data collected was likely to be very reliable and relatively complete. Thirdly, during the summer prior to this study, the orangutan enclosure at Twycross Zoo was extended, and the zookeepers were interested in how the orangutans were using the enclosure and how the changes may have affected their behaviour.

The extension to the enclosure included the building of a Termite Wall within the inside section, and an artificial tree and a network of ropes in the outside section (**Appendix 5.1** – Partitioning of orangutan enclosure **Figure 9.6** and **Figure 9.4**, TW and T respectively). In addition, mesh ceiling was added to the inside section, through which the orangutans were provided with their daily feed (**Appendix 5.1 –** Partitioning of orangutan enclosure **Figure 9.6**, EC). An additional change to the way that the food was presented was also made in that the vegetables were no longer cut up into manageable pieces. Instead they were left whole for the orangutans to manipulate through the mesh caging, therefore providing a form of enrichment. These meals were provided each morning, midday and in the early evening.

5.3.2 Data collection

Both behavioural and space use data were collected in order to enable the development of the behavioural and space use networks.

The orangutan enclosure was mentally partitioned into zones (n=38; **Appendix 5.1** – Partitioning of orangutan enclosure). The zones were chosen in a way to balance a need for a meaningful resolution in the spatial data, whilst still being practically feasible to record locations (zones) of the individuals with minimum probability of errors. The zones were also chosen so that they could include at least one individual, and were focussed on the behaviour perspective based on what was already known about the behaviour of the orangutans. For example, some zones were related to activity (climbing apparatus) whilst other zones provided locations for feeding (e.g. termite wall) or resting (e.g. ground zones or the top of the tree structure). In addition, large areas were divided up into smaller zones in order to provide greater detail about how they were being utilised by the orangutans. For example, the backroom floor and extension floor left zones were adjacent floor zones which made up a continuous area, but might be utilised differently. For recording purposes, each zone was designated a unique alphabet based code representing the location and feature within that zone (**Appendix 5.2** – Zone key for partitioning of orangutan enclosure).

During the end of the fourth week of observations, the zoo staff installed four new nesting sites (one on the left hand wall, one on the right hand wall and two on the back wall of the extension). These were added to areas previously not designated as zones due to the orangutans having no access to this space. Following their addition, these nest sites were assigned their own unique zone codes (EPL, EPR and EPB). The orangutans therefore only had access to these zones over the final five days (week 5) of the study.

Data were collected by recording the zone which each individual was occupying in addition to the behaviour that they were conducting at the time. In previous chapters, behavioural observations have been based on continuous scan sampling. However, the changes made to the enclosure prior to this study made it significantly more difficult for a single observer to track the movements of each orangutan continuously. Therefore, instantaneous scan sampling was adopted for this study. In order to try and minimise any loss in information by switching to instantaneous scan sampling, the behavioural data used in the previous chapters was utilised to derive the optimal time period between scans. Simulations of scan sampling of the continuous data collection were conducted utilising different times between scans (from one to ten minutes, at 1 minute increments). That is, the actual continuous behaviour data for each individual was sub-sampled at each of these different frequencies. The patterns of behaviour that were derived from the sub-sampled data were compared against those patterns derived from the complete continuously monitored behaviour data. It was found that for all individuals a time period of three minutes between scans resulted in no significant difference in ethogram structure between the simulated scan sampling and the full continuous sampling. Therefore, in this study scans were taken every three minutes as this would enable the observer to move around the enclosure to be able to maximise the viewing area in relation to the locations of the individual orangutans, whilst minimising the potential loss of information due to not recording data continuously.

Behaviour categories were recorded as in chapters three and four, but with the addition of 'food processing' and 'out of sight' to the list of TUCs. The behaviour of processing food (manipulation of food sources in preparation for ingestion) was a notable species behaviour that had been observed within the previous behavioural observations but had not been designated a category at the time. The extension of the enclosure prior to this study resulted in an area of the enclosure being cut off from public view (the left side of the indoor enclosure, as seen in **Appendix 5.1; Figure 9.4 Right hand view of the indoor area of the orangutan enclosure at Twycross Zoo with zones marked in red.**). This meant that when an individual occupied this area, they could not be seen and therefore the behaviour and zone recorded were ‘out of sight’ (OOS).

Observations began each day at 09:30am (GMT) at which point, the TUC and occupied zone of each orangutan was recorded. This was then repeated every three minutes for one hour. A 20 minute break was then taken by the observer before conducting another hour of scan sampling. This was repeated through the day until 15:47. This was a little earlier than the ideal stopping time of 16:00, but reflected the needs of the zoo staff and the closing of the zoo.

5.3.3 Construction of networks

5.3.3.1 Behavioural networks

Social networks treat individuals as vertices, and there are edges linking pairs of these vertices if the vertices represent individuals which are associated with each other in the particular social context of interest. Behavioural networks can be constructed in a similar way, mapping behaviour characteristics to vertices and edges (**Figure 5.2a**). Here they were constructed for each individual orangutan by mapping behaviours (TUC categories) to the vertices and edges representing transitions between two behaviours. Transitions were identified by examining the behaviour displayed by the individual at time t, and the behaviour exhibited by the individual at time $t + 1$ (i.e. at the next scan 3 minutes later). A directed edge was formed running from the vertex representing the behaviour at time t, to the vertex representing the behaviour at time $t + 1$ (**Figure 5.2b**). On occasions where the individual was recorded displaying the same behaviour in consecutive observations (e.g. feeding at t and feeding again at $t + 1$), a loop was created to illustrate the continued behaviour (**Figure 5.2c**). To create these transitions, an additional column was created in the data set.

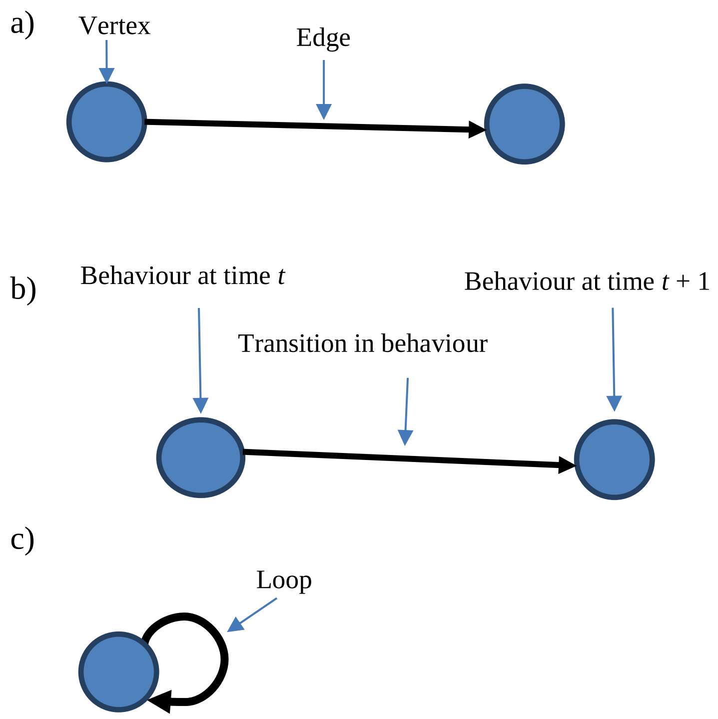


Figure 5.2 Construction of a behaviour network. a) Visual representation of nodes and edges, b) showing the relationship between behaviour sequence and network structure, c) a loop resulting from the continuation of the particular behaviour from one scan sampling to the next (three minutes later).

The behaviour observed in the first scan following a break was treated as a new start to recording, i.e. the last behaviour observed prior to a break and the first behaviour following the break were treated as independent of each other and were not linked in the behaviour networks.

These basic networks could be enhanced following the methods described in the introduction of this chapter. The edges could be weighted based on the relative frequency of the transitions over the time period that the network was representing. In addition the vertices could be weighted (i.e. their relative size) according to the frequency with which the corresponding behaviour was observed over the time period that the network was representing.

5.3.3.2 Space use networks

The same approach was used to generate the space use networks. The vertices of the networks represented the spatial zone that the individual occupied at the time of a scan. The directed edges represented movement from one zone to another between an observational scan and the sequential scan. Again, vertices could be weighted (size adjusted), in this case according to the proportion of time that the individual spent within the corresponding spatial zone over the time period represented by the network. Edges could also be weighted (represented by the relative thickness of the edges) according to the relative frequency that that particular spatial transition was observed over the period of time represented by the network.

Both behavioural and spatial networks were constructed for different periods of time: the total time of the study, weekly, and daily. This enabled an assessment of patterns of behaviour and space use over different time periods and gave the potential for identifying levels of variation in patterns on a daily and weekly timescale.

All data preparation, and visualisation and analysis of networks was done using the R statistical programming language (R Core Team, 2016). Additional R packages used were igraph (Csardi & Nepusz, 2006) and corrplot (Wei & Simko, 2016).

5.4 Results

Observations of orangutan behaviour (TUC) and location in the enclosure (zone) were implemented to construct both behavioural and space use networks. Both network visualisations and the associated network statistics are provided in the following sections with accompanying explanations as to what can be deduced from such analyses.

5.4.1 Behaviour networks

5.4.1.1 Networks over the whole time period of the study

The behaviour network for each individual over the whole of the study period is shown in **Figure 5.3**

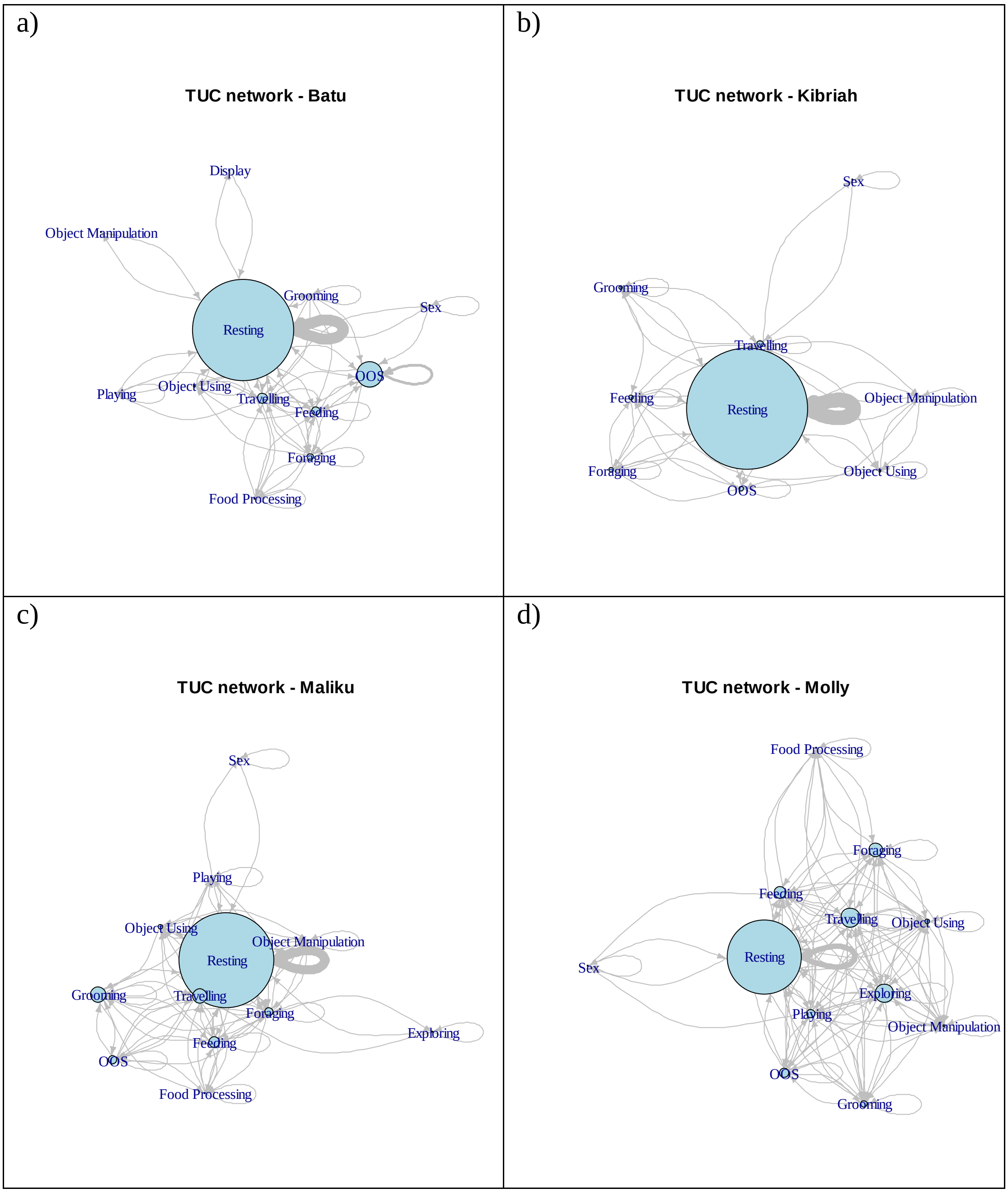


Figure 5.3 Behaviour networks for a) Batu, b) Kibriah, c) Maliku and d) Molly, representing behaviour transitions over the whole of the 5 week study period. Vertices are weighted (sized) according to the relative frequency of the particular behaviour being displayed by the individual over the whole of the study period. Edges are weighted (thickness) according to the relative frequency of transition between the two behaviours by the individual over the whole of the study period. As observations for each individual were recorded at the same time, relative frequencies of behaviours and transitions between behaviours can be legitimately compared between individuals.

The space use network for each individual over the whole of the study period is shown in **Figure 5.4**.

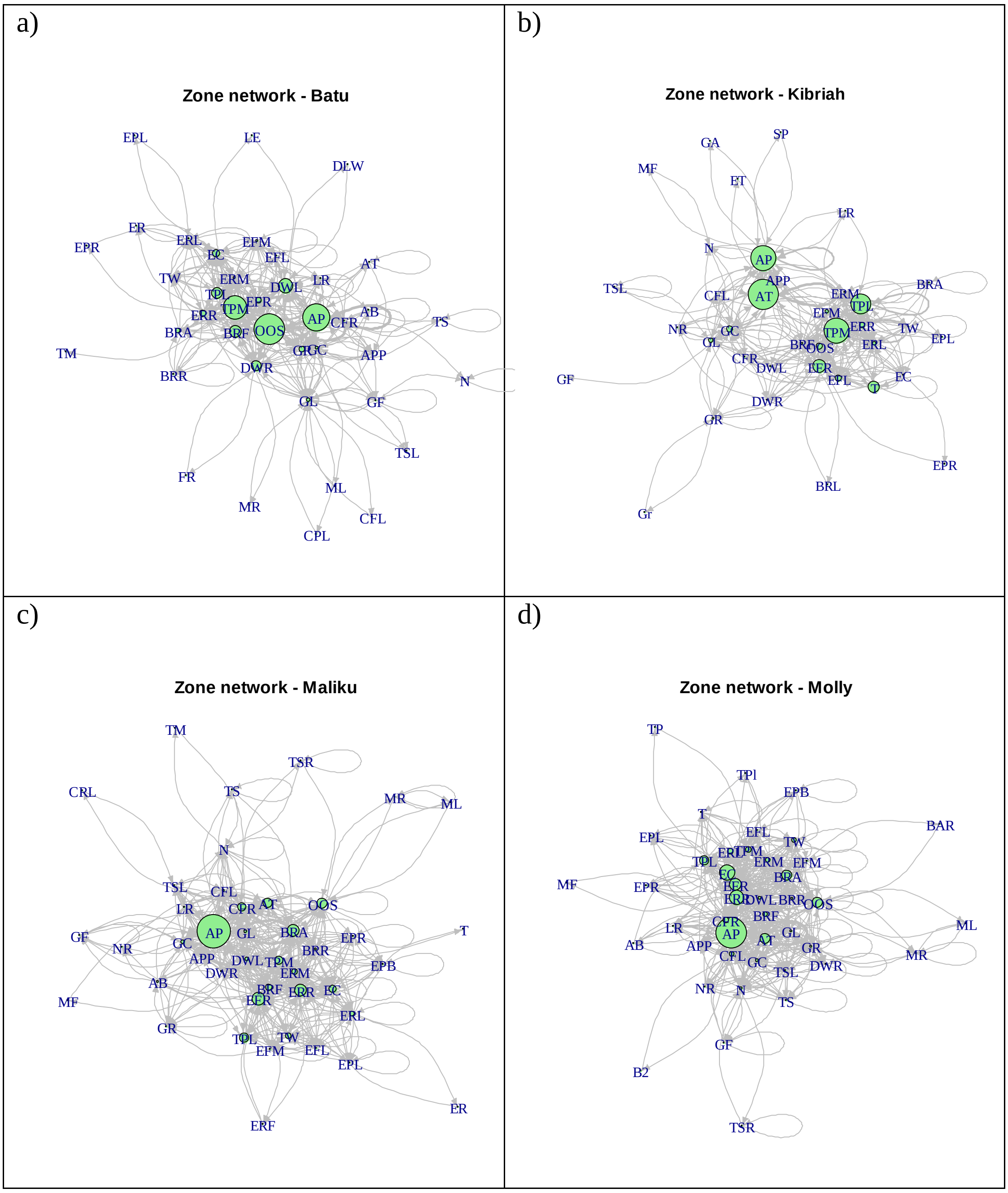


Figure 5.4 Space use networks for a) Batu, b) Kibriah, c) Maliku and d) Molly, representing spatial transitions between zones over the whole of the 5 week study period. Vertices are weighted (sized) according to the relative frequency of the individual being observed in that zone over the whole of the study period. Edges are weighted (thickness) according to the relative frequency of transition between the two spatial zones by the individual over the whole of the study period. As observations for each individual were recorded at the same time, relative frequencies of occupation of zones and transitions between spatial zones can be legitimately compared between individuals. Note that the relative position of the vertices (zones) in the diagrams are designed to try and maximise the clarity of the diagrams, and do not represent the relative spatial positions of the zones in the enclosure.

5.4.1.2 Weekly networks

The behaviour networks for the data collected in each of the five weeks of the study are shown in **Figure 5.5** to **Figure 5.8**. Variation between weeks can be detected by observing the change in structure of the networks. By analysing the number of edges in each successive networks, the complexity of the network, and therefore the diversity in behaviour pattern of the individual, can be tracked over time.

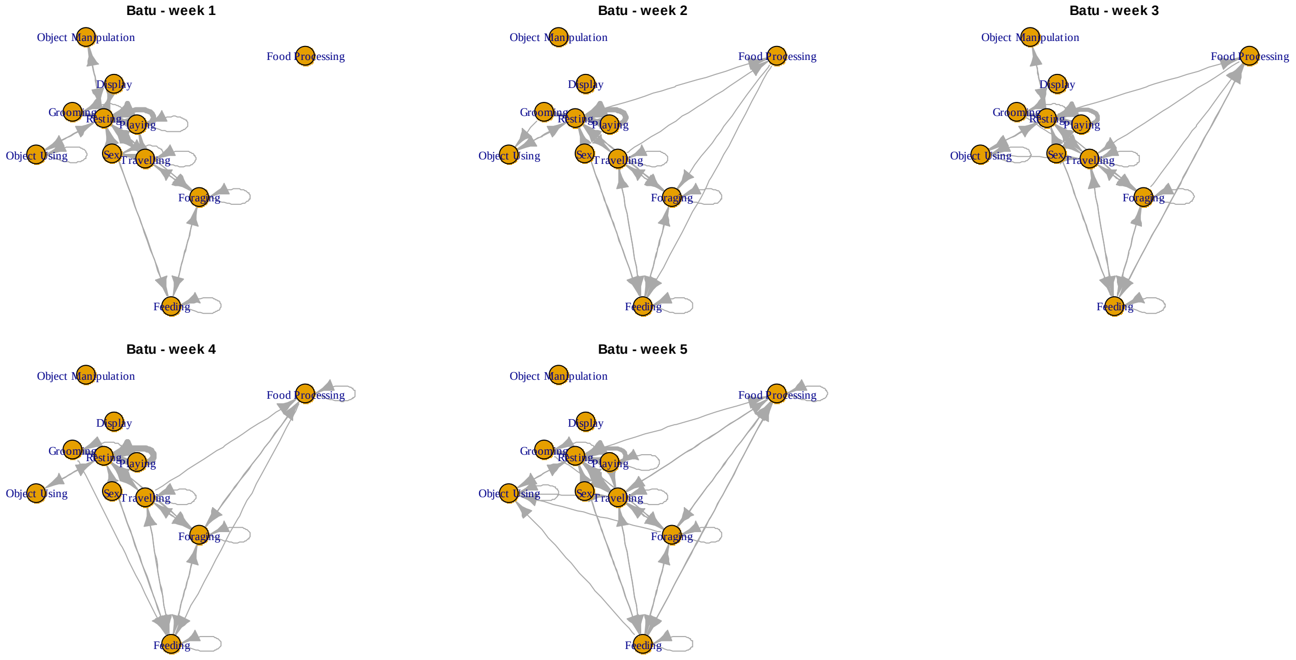


Figure 5.5 Weekly behaviour networks for Batu. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between weeks.

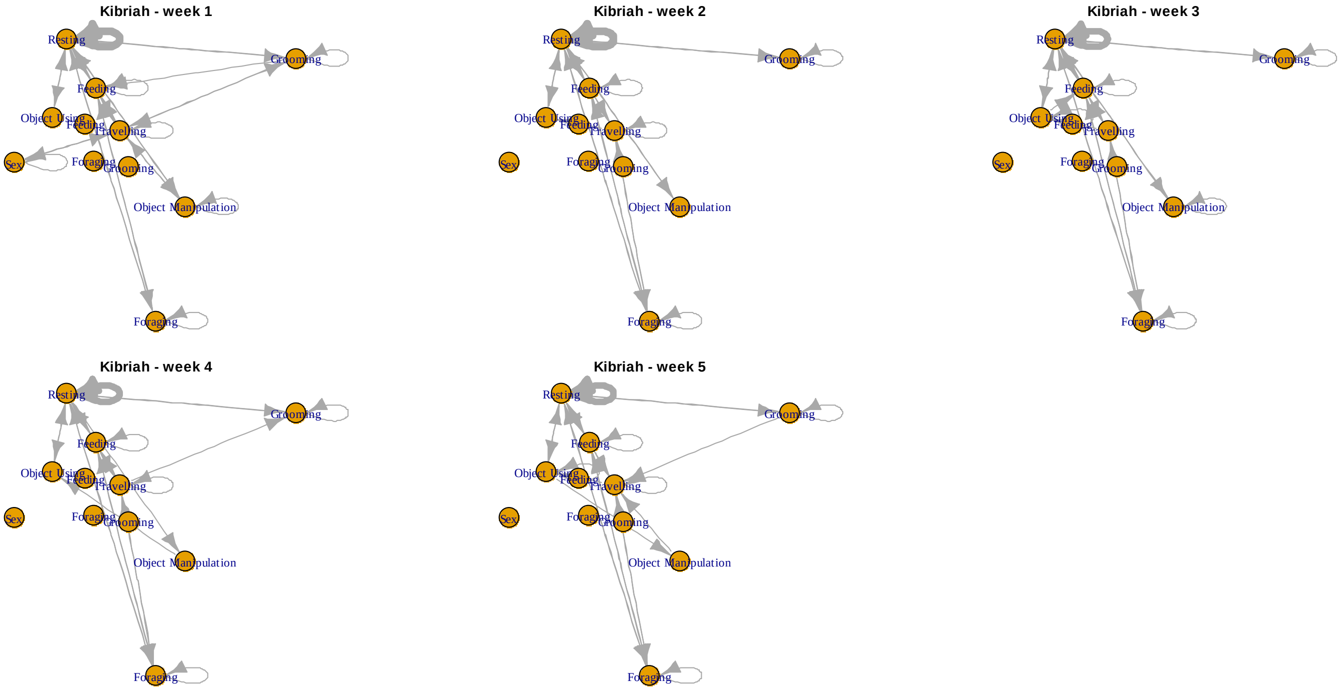


Figure 5.6 Weekly behaviour networks for Kibriah. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between weeks.

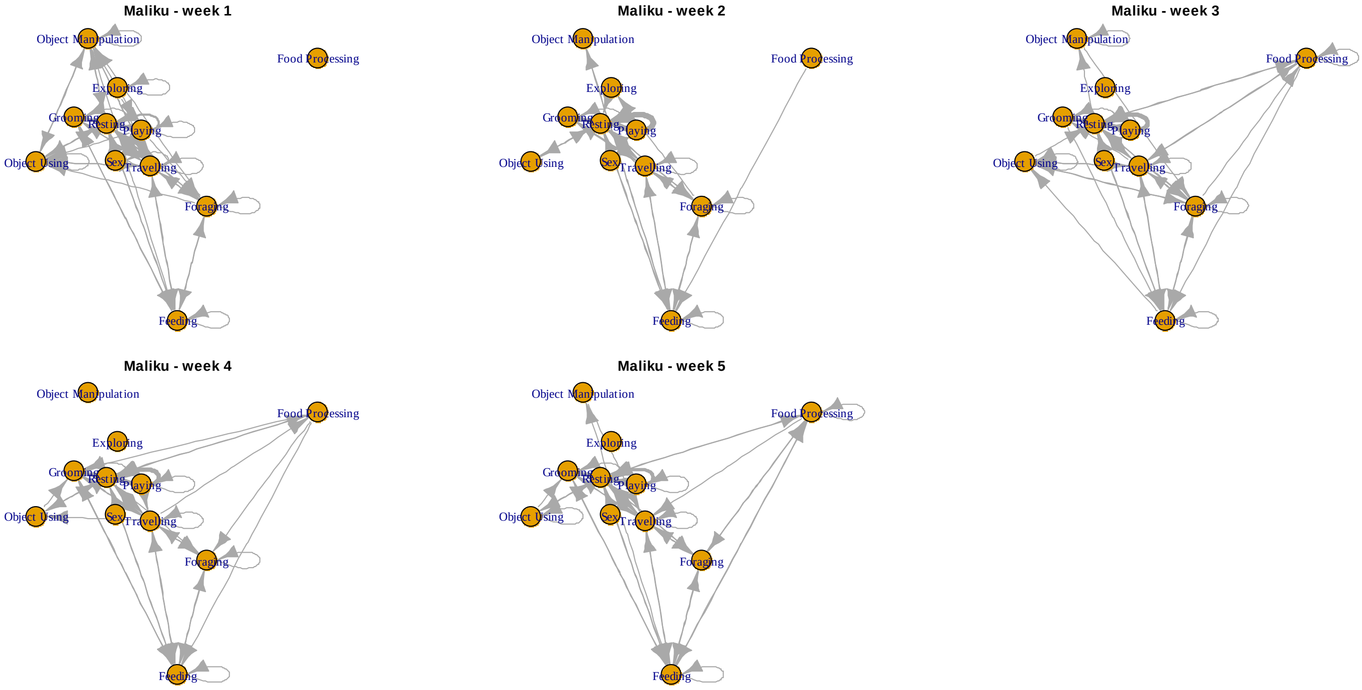


Figure 5.7 Weekly behaviour networks for Maliku. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between weeks.

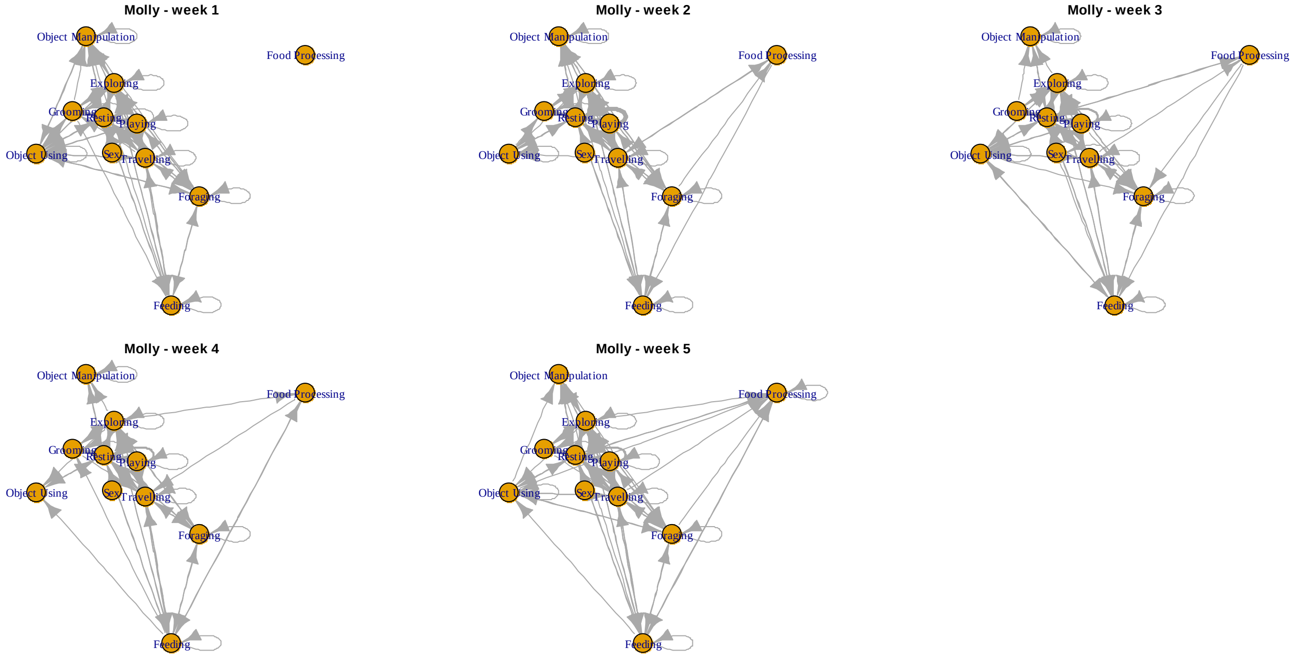


Figure 5.8 Weekly behaviour networks for Molly. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between weeks.

The space use networks for the data collected in each of the five weeks of the study are shown in **Figure 5.9 to Figure 5.12.** By observation alone, it appears that there is greater variation in the space use patterns of individuals than in the behavioural patterns. Methods for quantifying this variation are introduced in the next chapter.

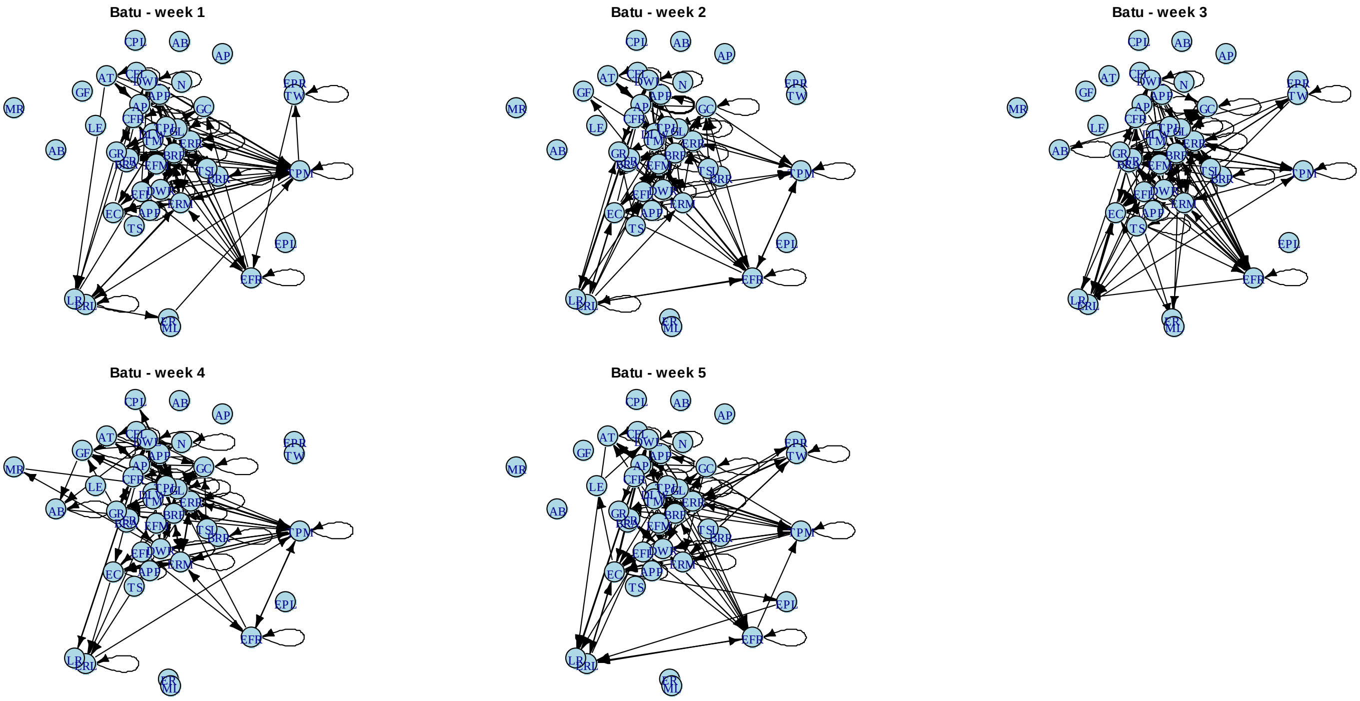


Figure 5.9 Weekly space use networks for Batu. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between weeks.

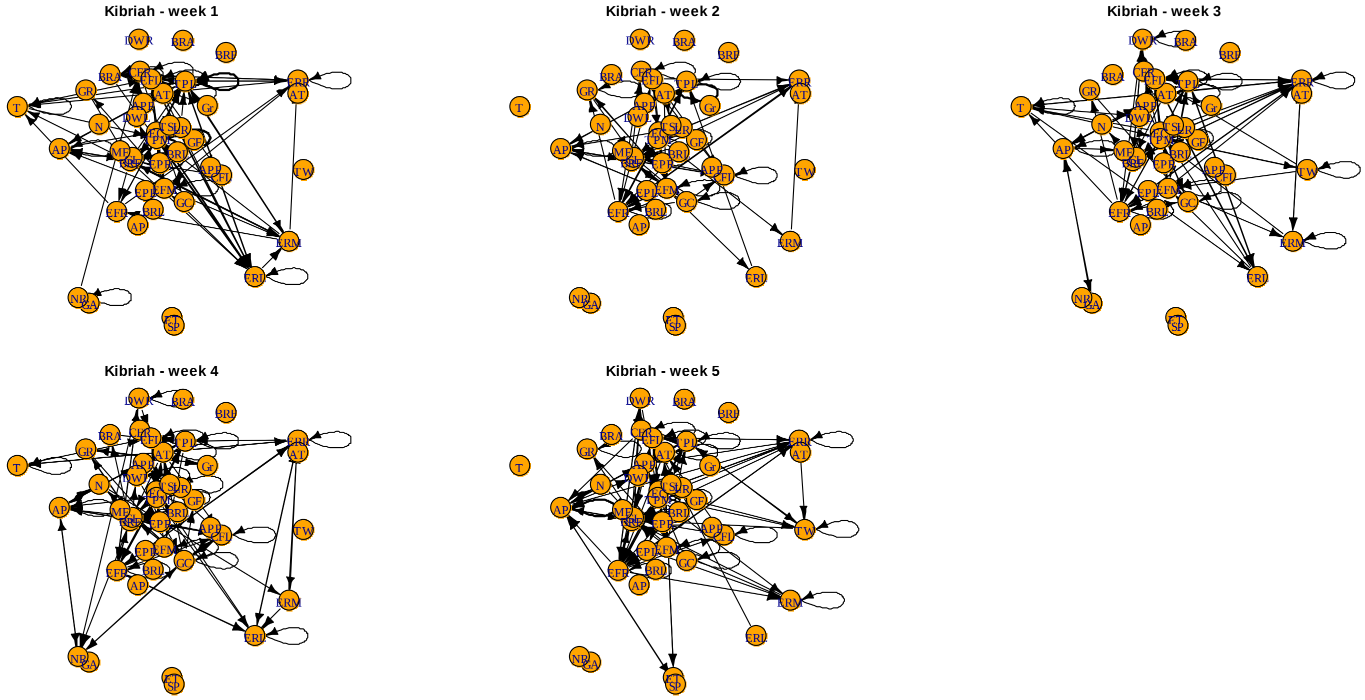


Figure 5.10 Weekly space use networks for Kibriah. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between weeks.

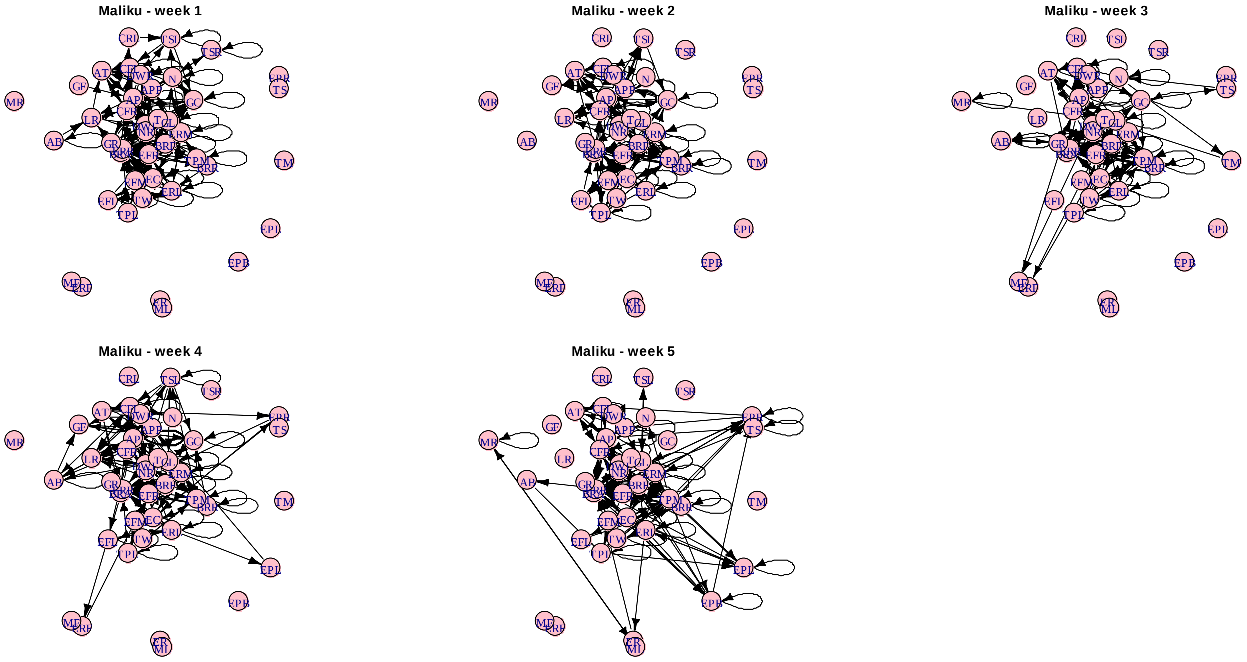


Figure 5.11 Weekly space use networks for Maliku. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between weeks.

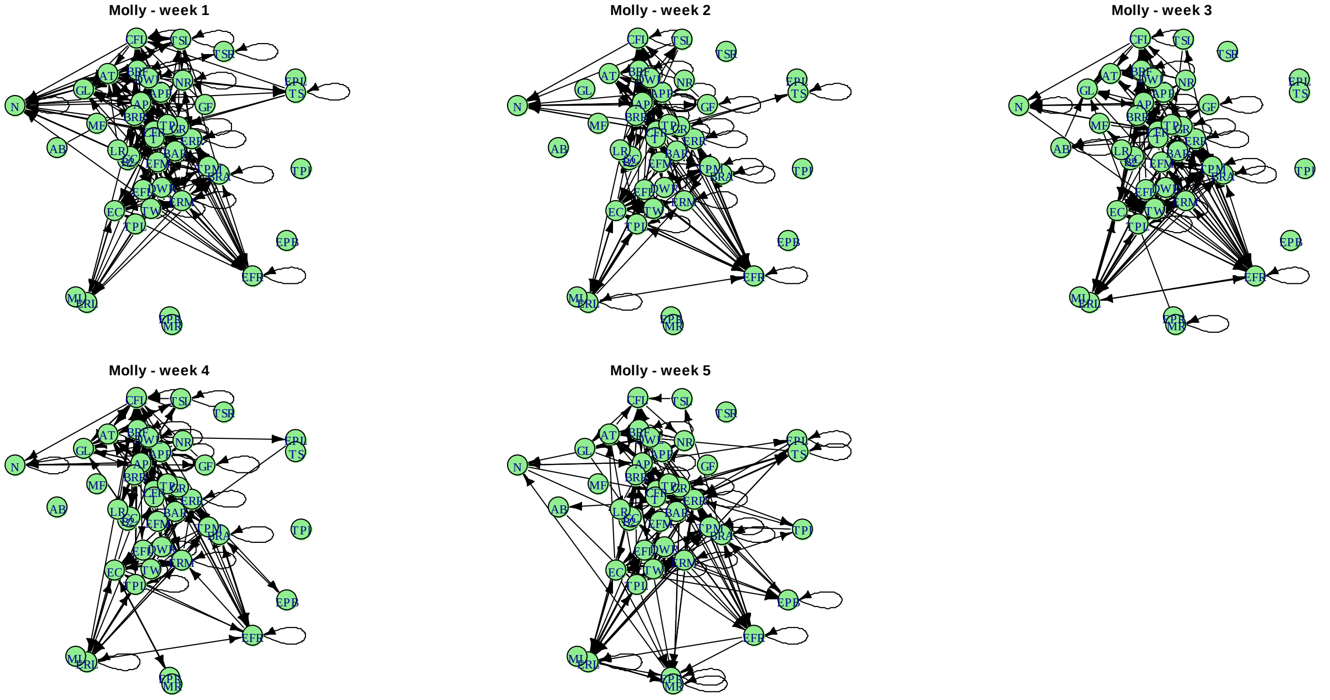


Figure 5.12 Weekly space use networks for Molly. In order to increase clarity, vertices, and edges were not weighted. In addition, the relative layout of the

vertices were fixed in order to allow for easier comparison of networks between weeks.

5.4.1.3 Daily networks

The behaviour networks for the data collected over the first six days of the study are shown in **Figure 5.13** to **Figure 5.16**. Variation between days can be observed by changes in the arrangement or number of edges within the network. A notable change in the number of edges between days is observed in **Figure 5.14.**

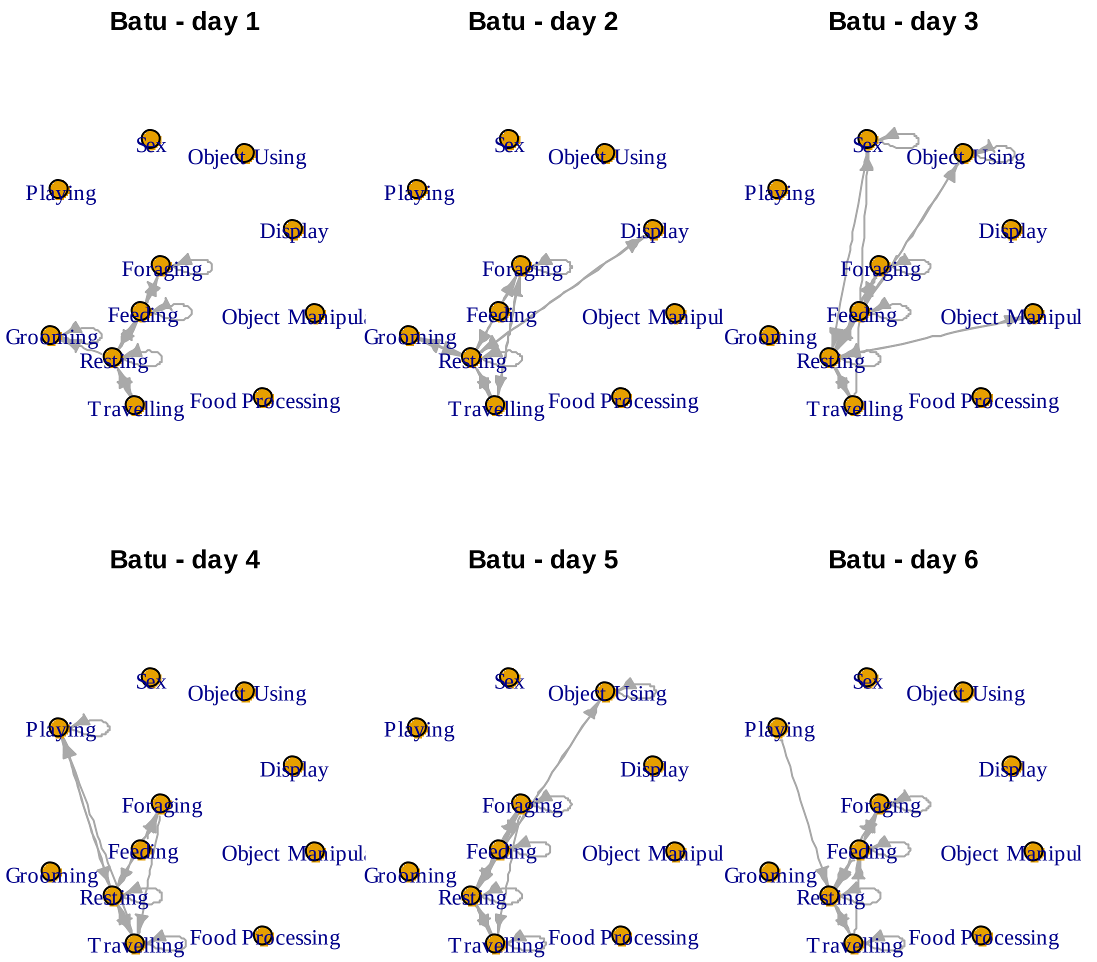


Figure 5.13 Daily behaviour networks for Batu. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between days.

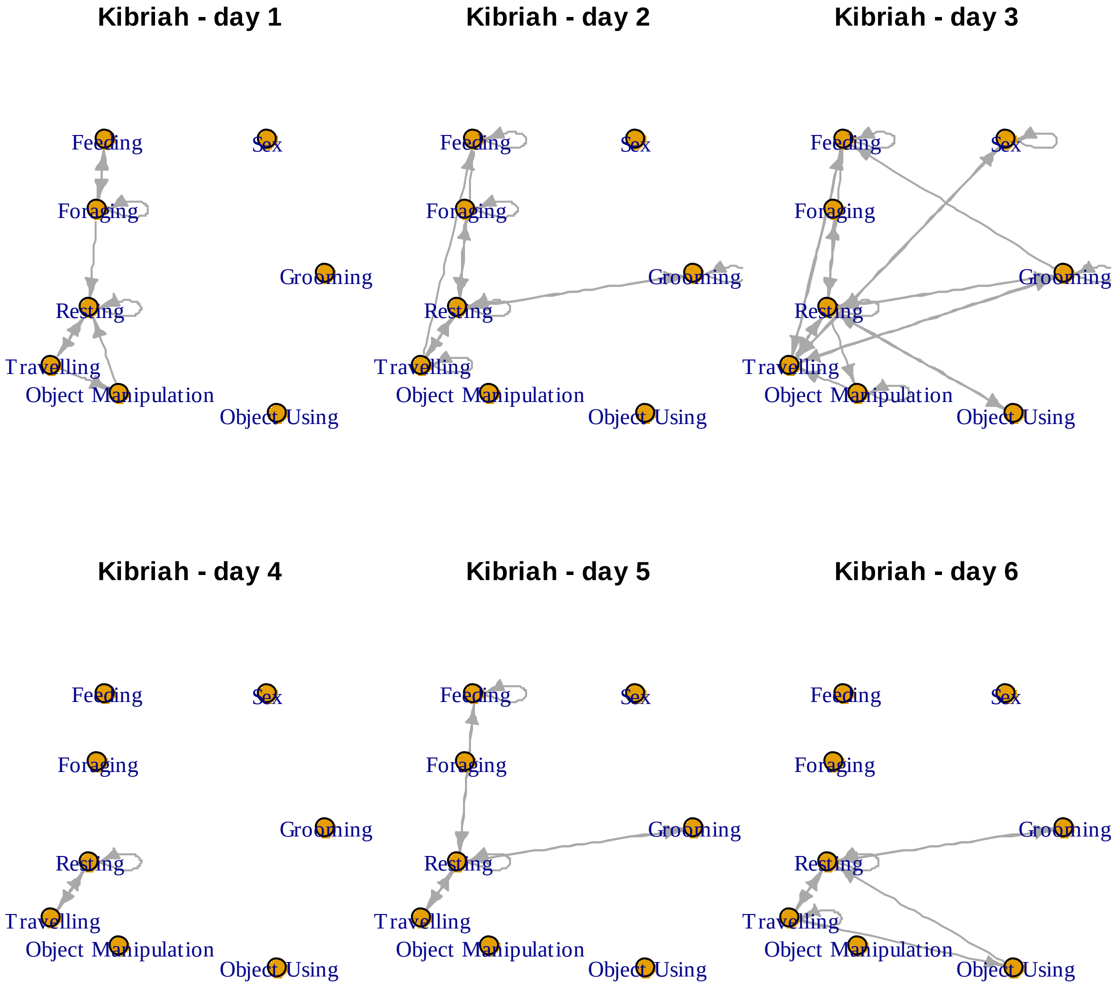
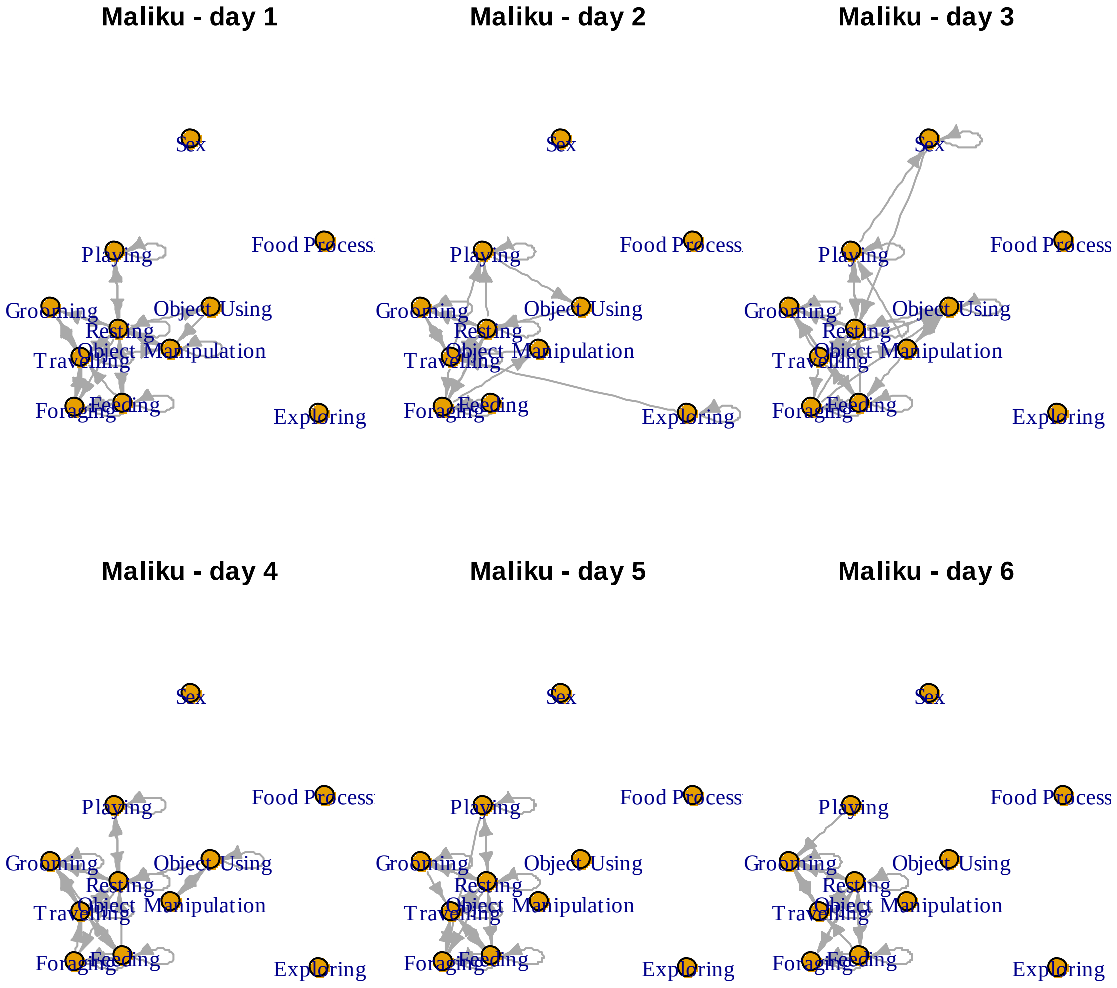


Figure 5.14 Daily behaviour networks for Kibriah. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between days.


Maliku - day 1

Maliku - day 2

Maliku - day 3

Maliku - day 4

Maliku - day 5

Maliku - day 6

Figure 5.15 Daily behaviour networks for Maliku. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between days.

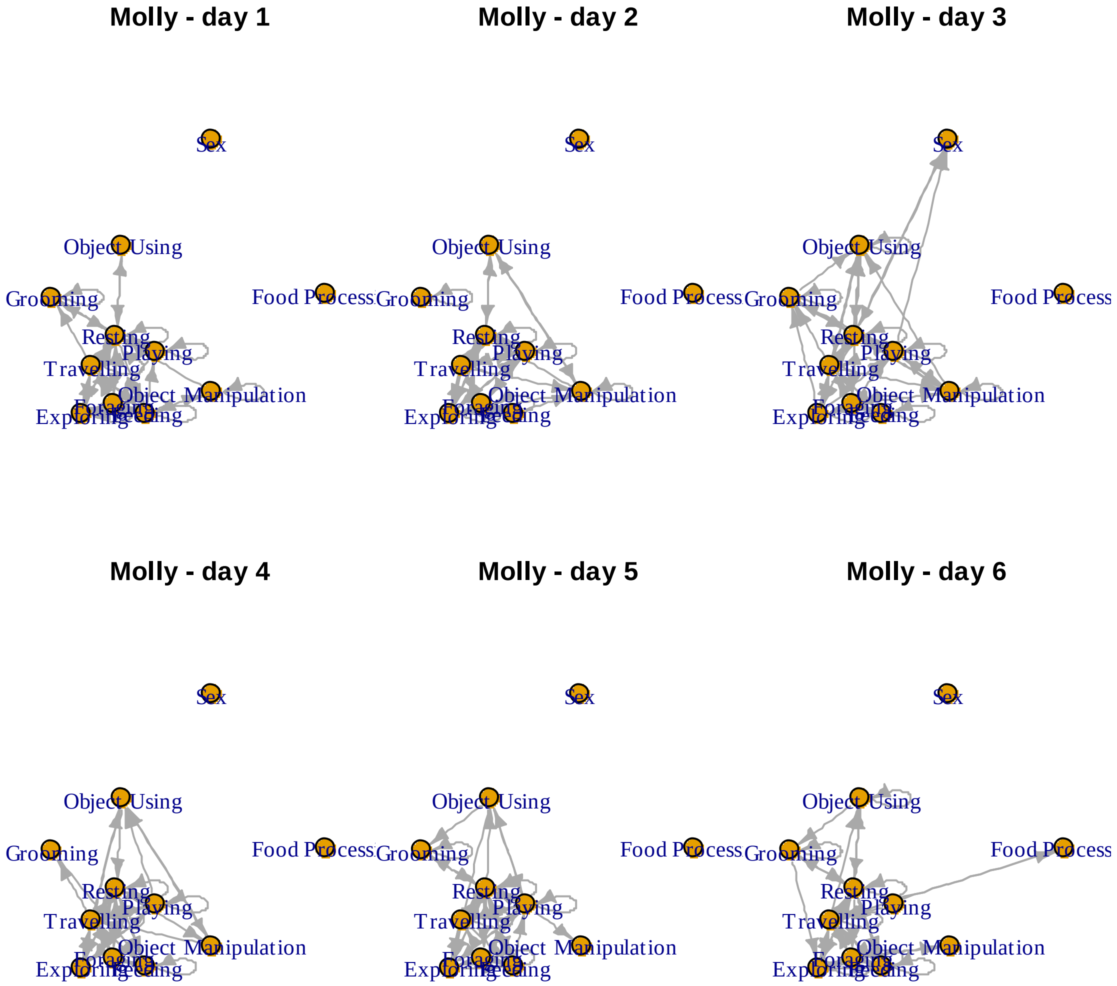


Figure 5.16 Daily behaviour networks for Molly. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between days.

The space use networks for the data collected over the first six days of the study are shown in **Figure 5.17** to **Figure 5.20.** The increased number of edges between zone vertices infers a greater level of complexity which can be quantified (discussed in the next chapter). Note that the layout of the vertices is fixed within each figure, but not between figures. Therefore the spatial activity between individuals is not directly comparable. Notable variation between days is observed in the following networks**.**

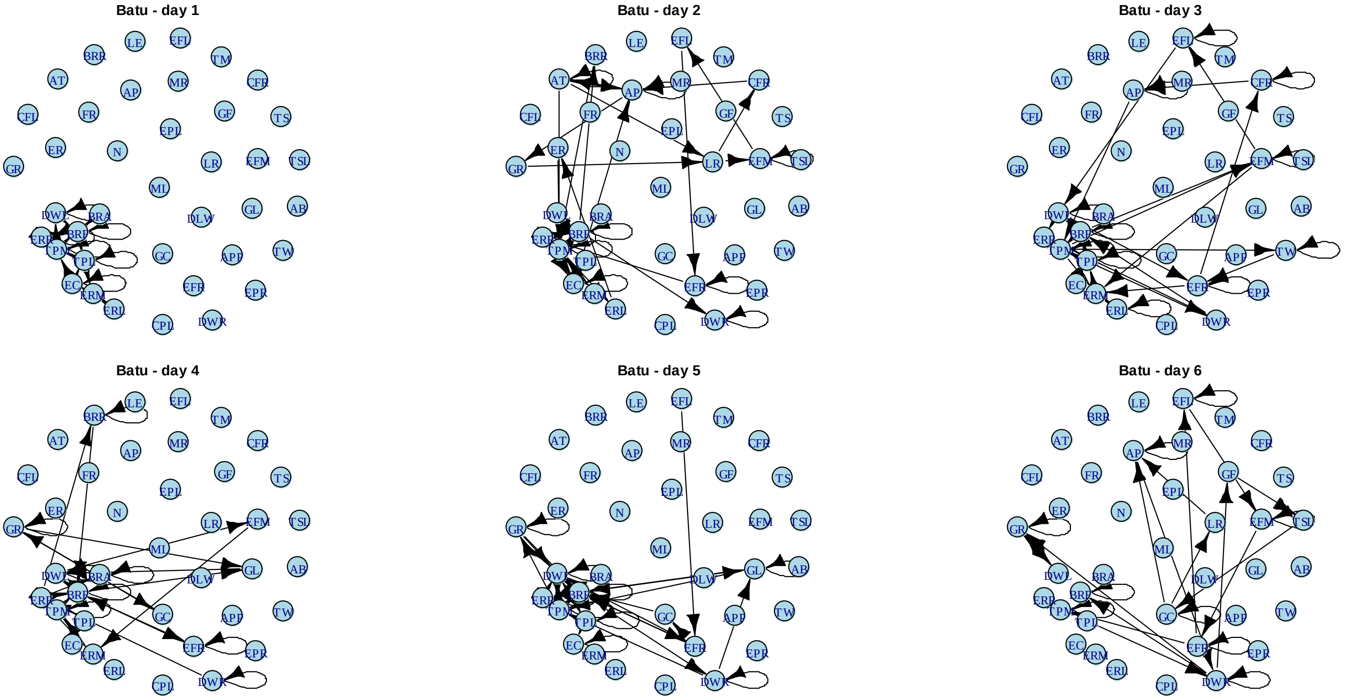


Figure 5.17 Daily space use networks for Batu. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between days, and do not relate to the spatial layout of the zones within the enclosure.

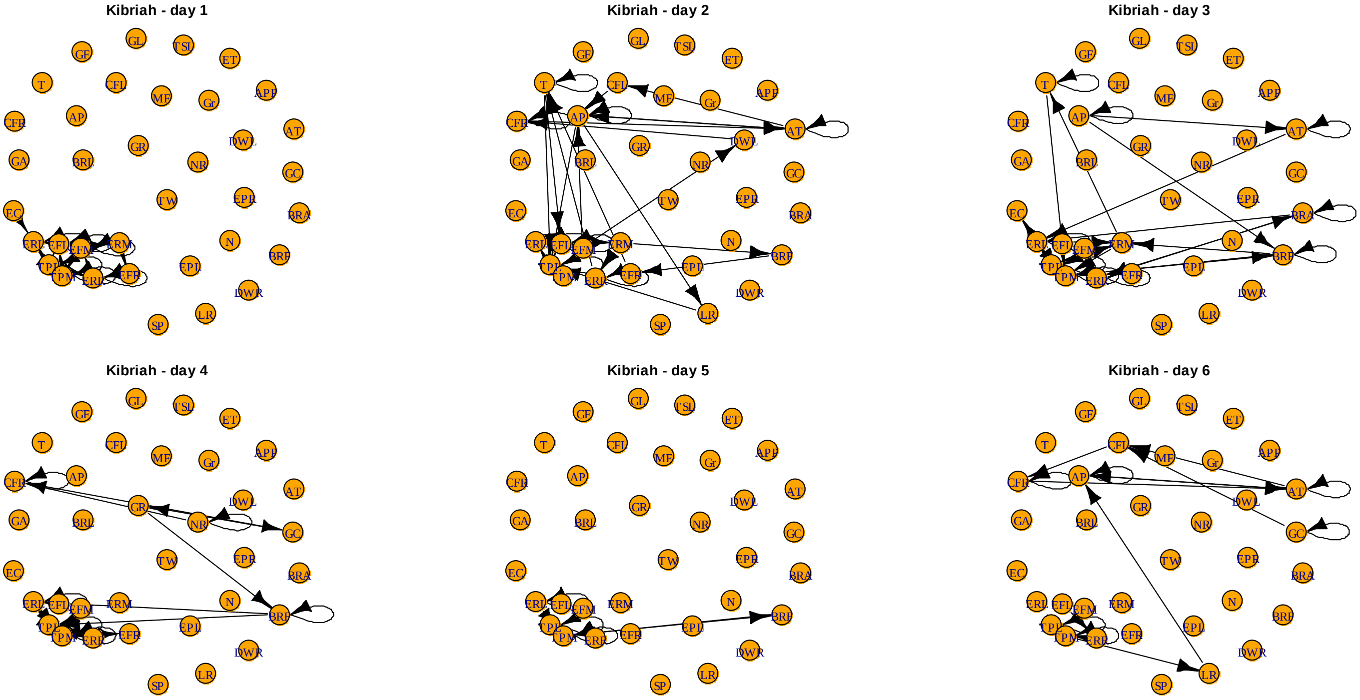


Figure 5.18 Daily space use networks for Kibriah. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices

were fixed in order to allow for easier comparison of networks between days, and do not relate to the spatial layout of the zones within the enclosure.

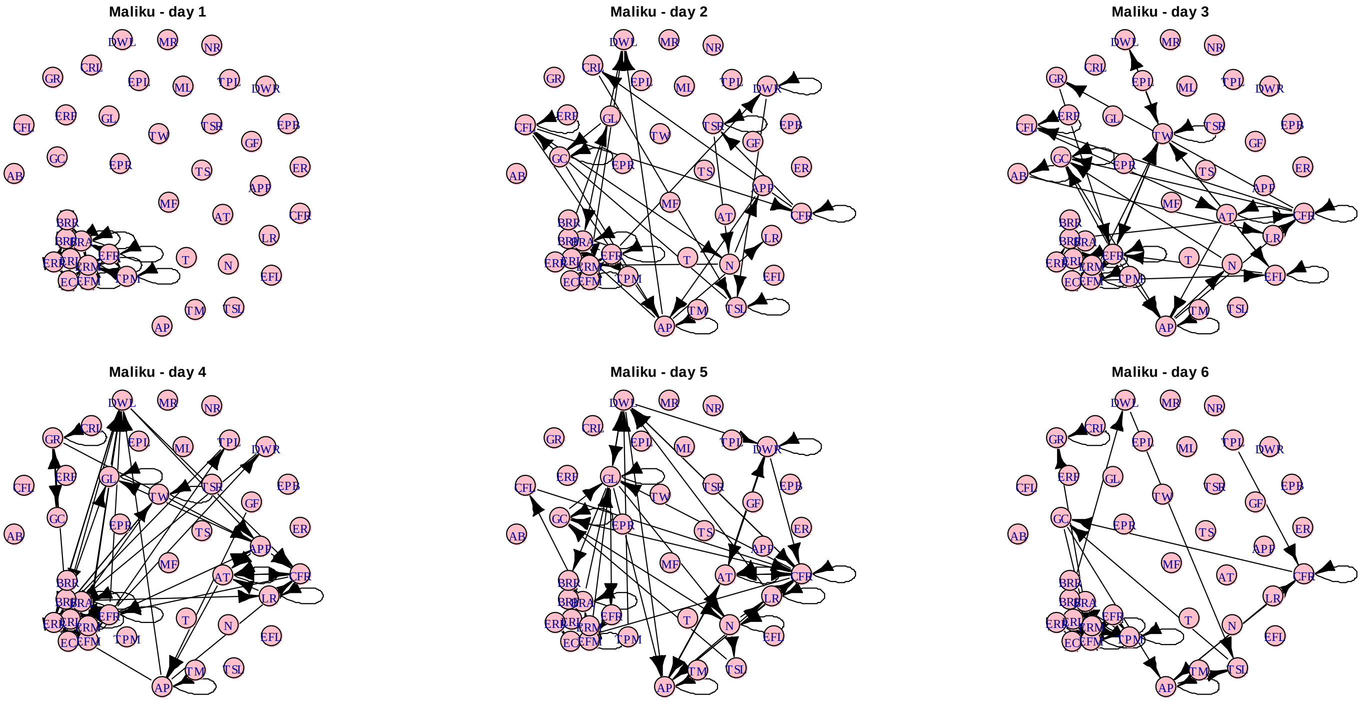


Figure 5.19 Daily space use networks for Maliku. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices **were fixed in order to allow for easier comparison of networks between days, and do not relate to the spatial layout of the zones within the enclosure.**

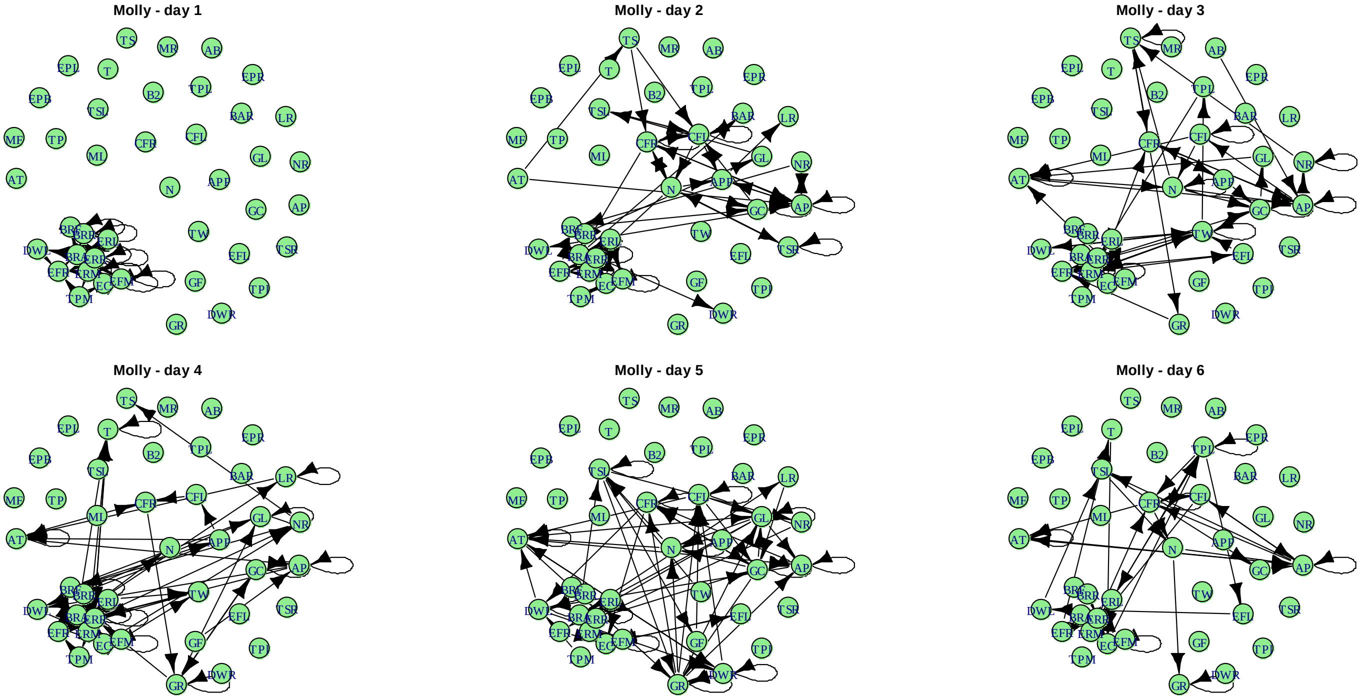


Figure 5.20 Daily space use networks for Molly. Only the behaviour networks from the first six days are shown for illustration. In order to increase clarity, vertices and edges are not weighted. In addition, the relative layout of the vertices were fixed in order to allow for easier comparison of networks between days, and do not relate to the spatial layout of the zones within the enclosure.

5.5 Discussion

The use of network analysis in a wide range of research areas (Erdös and Rényi, 1960; Rapoport & Horvath, 1961; Travers & Milgram, 1969) and its long mathematical history (Erdös and Rényi, 1960, Newman *et al.*, 2008) is testament to its power in facilitating understanding of complex systems. This has been demonstrated in the growing literature in the study of animal social networks (Fewell, 2003; Croft *et al.*, 2005; Croft *et al.*, 2008; Farine, 2017).

Part of the appeal of network analysis is the generality of the approach. Once information can be converted to a network form, the extensive analytical tools available can be brought to bear on the system under study. However, despite this generality, in animal research the study of social networks dominates in terms of where network analysis is utilised. This chapter demonstrates that a wider range of data collected as part of many animal behaviour studies can relatively easily be converted to network forms, and this opens up the potential for using network analysis methods more widely in animal behaviour research.

Two further types of network were presented in this chapter: behavioural and space-use networks. The construction of both types of networks is conceptually very simple. However, it should be recognised that often quite extensive data manipulation is required in preparation for generating the networks. This is because components of the data need to be related to each other and this often requires the data to be restructured from the layout in which it was originally recorded. This is not a trivial task when dealing with observations of many individuals over extended time periods. In addition, there may be further complexities where specialised algorithms need to be implemented. For example, in this work an algorithm needed to be constructed in order to detect when the observer took a break in this study, and to then match up the relevant behaviour and space-use data, whilst taking this break period into account.

Data were collected as part of five weeks of observations of the captive orangutans at Twycross Zoo, and behavioural and space-use networks were constructed using these data as a demonstration of these methods. The visualisation of these networks is part of the utility of network analysis, with the visual representations acting as powerful and intuitive summaries of complex systems.

The flexibility in how extra information can be presented in these visualisations is also appealing. For example, the vertices can be scaled in size to represent the relative frequency of observation of a particular behaviour, and the frequency of transitions between behaviours can be represented by weighting the thickness of the representative edge. However, the ability of the visualisations to clearly show structure in the complex data varied with the size and density of the networks. The behaviour networks summarising patterns in behaviour over the length of the study were relatively clear and differences between the networks for the different individuals were easily spotted. However, the equivalent space-use networks were much more complex and structure was very difficult to visually detect. This was because there were many more vertices in these plots and the number of edges was also much larger. Although a number of different algorithms have been developed for optimally arranging a network visualisation (Kolaczyk & Csárdi, 2009), it becomes very difficult to maximise the amount of information being shown and balance this with maintaining clarity in the plot. Thus, as behaviour and space-use networks get larger, the potential use of the visualisations of these networks is likely to decline.

A suggested way of improving this situation is to utilise the methods developed by researchers studying microbial communities. Microbial communities can be extensive and association networks representing the community structure can be very difficult to follow. Some researchers have proposed the use of the 'core microbiome' (Shade & Handelsman, 2012; Sweet & Bulling, 2017). Essentially, this approach tries to identify the most important species in terms of their abundance or their connectivity to other species (Sweet & Bulling, 2017). Once these species have been identified the rest of the network is excluded. A similar approach could be taken with behavioural and space-use networks where 'core' behaviours and spatial zones are identified and the relationships between these are displayed, and the rest are ignored. There are many intricacies in deciding what qualifies a species to be 'core' (Sweet & Bulling, 2017) but, in principle, this could be a way of trying to focus visualisations of complex behaviour and space-use networks on the most important structures in the networks, and hence improving clarity in the visualisations. There would obviously be a cost to this as information is lost, but it may be worth pursuing with the visualisation of larger behavioural and space-use networks.

Zoo staff and researchers may want to examine data at different temporal resolutions. For example, examining general behaviour over the long term (e.g. several weeks), may be required for general monitoring of behaviour and space-use patterns. However, researchers may be interested in the effect on behaviour and space-use of an enrichment treatment applied over a period of a week. They may want to compare networks summarising patterns in behaviour during the week that the enrichment was applied with the patterns in the week before and the week after the application of the enrichment treatment. It is also possible that they may want to examine how behaviour changes on a day to day basis during the week of the enrichment to see if the effect declines over time. The network framework is flexible enough to cope with all of these situations. As the networks are summaries of data collected over time, the time period from which data can be used to construct these networks can be altered to match the time period required by the zoo staff. This was demonstrated in this chapter by producing weekly and daily behaviour and space-use networks in addition to the equivalent networks summarising the whole data set. Interestingly, the structure of the weekly networks seemed to be reasonably consistent, but the daily networks showed considerable variation in structure. These differences highlight the potential for generating networks at different temporal resolutions. Strong changes in network structure on a daily basis could be related to particular causes, whereas strong changes in network structure over longer time scales will indicate more profound longer-lasting changes. If causes linked to the changes at these different temporal resolutions can be found, this would start to generate an understanding of which events cause short-term, and which cause long-term changes in behaviour or space use patterns.

With specific reference to the implications for animal welfare, there is great potential in the interpretation of the networks. Being able to map out the orangutan enclosure and track the movement of individuals around the enclosure means that zoo staff can identify pathways used to access features and resources. Within an enclosure, animals may use some features (e.g. nest sites) for greater periods of time than others (e.g. connecting ropes). Therefore, simply calculating the length of time for which the animals use this feature, would identify the lesser used features as not important. However, these features might be used as transitional features and could be necessary to enable animals to access other areas of the enclosure. Identifying these transitional features and ensuring that animals are using them in a species appropriate way (an example of this would be the orangutans using ropes to brachiate between nesting platforms) means that enclosure design can focus on maximising the potential for the display of natural behaviours.

Behavioural activity budgets are a widely used tool in animal behaviour research, showing the frequency of the different behaviours displayed by individuals. However, they are a static summary of behaviour as they do not indicate patterns of transition between different behaviours. This is where behavioural networks can supplement the use of ethograms as they explicitly look at the dynamic aspects of animal behaviour and space-use. Thus they add to our understanding of the frequencies of behaviours by indicating the patterns of transitions between behaviours or spatial locations shown by individuals.

The use of behaviour and space-use networks could be very helpful in helping to manage captive animals. For example, if an individual is exhibiting a problematic behaviour, there may be a particular sequence of behaviours that lead up to this behaviour being displayed. If this is the case then the pathways within the behaviour network will be weighted more heavily. This would enable the zoo to then try and identify ways to divert the individual from this temporal path leading to the problematic behaviour.

This chapter has demonstrated the construction and potential utility of behavioural and space-use networks in visualising patterns underlying animal behaviour studies. However, although informative and suggestive, the visualisations do not allow for a rigorous analytical approach. In the next chapter the quantification of behavioural and space-use network properties is explored, along with their interpretation from an animal behaviour perspective. Therefore, the next chapter builds on the work in this chapter by showing how the structure in behavioural and space-use networks can be quantified, leading to utilising these metrics within a statistical analytical framework.

5.6 Conclusion

This chapter demonstrated the potential for expanding the use of network analysis beyond the applications to animal social networks, and illustrated the mechanisms by which this could be done by producing behavioural and space-use networks using data based on observations over a five week period of the captive orangutans at Twycross Zoo. The utility of the visualisations of these networks was highlighted along with the potential difficulties as network size increases. Although a powerful visualisation tool, the utility of networks for behavioural studies extends further. However, this requires methods for quantifying network structure and this aspect is developed and illustrated in the next chapter.

Chapter SIX: Quantification and Interpretation of Behaviour and Space Use Network Characteristics

6.1 Chapter overview

The previous chapter demonstrated that there was substantial potential for extending the use of networks beyond the social network framework to produce behavioural and space use networks. The derivation of these networks was described and examples were generated based on data from a five week study of individuals in the orangutan enclosure at Twycross Zoo. The ability of networks to visually summarise complex behavioural and space use data was critically explored. This chapter expands this work by focussing on the use of quantitative metrics of network structure. These metrics are derived from the networks generated in the previous chapter, and their interpretation with reference to the behaviour of the orangutans and their patterns of use of the space within their enclosure is explored. This chapter demonstrates how the use of networks for the study of animal behaviour can go beyond the utility of visualisation of behaviour and space use patterns. It explains how enabling the quantification of these patterns in ways that are meaningful for gaining a deeper understanding of animal behaviour and provide powerful and insightful tools for managing captive animal enclosures. The chapter ends with a critical discussion of the potential use of network analysis with a wider range of behavioural data than social networks, and outlines current challenges and suggested directions for future research.

6.2 Introduction

Part of the utility of converting data to network form is the ability to visualise network structure and to gain a relatively intuitive overview of the structure of complex data (Croft *et al.*, 2008; Chapter five). However, the development of graph theory and the wide use of social networks has led to the development of an extensive range of quantitative metrics to describe network characteristics (Wilson & Watkins, 1990; Newman *et al.*, 2006; Croft *et al.*, 2008; Kolaczyk & Csárdi, 2009). Although the interpretation of such metrics is well developed for social networks (Wey *et al.*, 2007; Croft *et al.*, 2008), it has not been explored for the newly developed behavioural and space use networks (chapter five).

The previous chapter detailed the construction of behavioural and space use networks, and then visualised these networks over different time frames: daily, weekly and over the full five weeks of the study. This chapter complements the previous chapter by exploring the utility of network metrics of the behavioural and space use networks in animal behaviour studies. It firstly describes network metrics that are commonly used in studies of social networks, and then describes how these metrics can be interpreted within the behavioural and space use network frameworks. It then proceeds to calculate these metrics for the networks generated in the previous chapter, and critically assesses their utility as tools for understanding animal behaviour and space use.

6.3 Frequently used network metrics

In this section, commonly used network metrics are described. The metrics included in this section are based on those given in the review paper of Wey *et al.* (2008), one of the most frequently cited text books on animal social network analysis (Croft *et al.*, 2008), and a recent guide to animal social network analysis (Farine & Whitehead, 2015). These texts are often used as references in studies of social networks in animals and therefore the metrics they cover represent a central repository for the majority of animal behaviour studies incorporating network analysis.

Network metrics tend to fall into three main categories (Wey *et al.*, 2008), depending on their relative focus. 'Individual measures' focus on the characteristics of individual vertices or edges, 'group measures' focus on overall network characteristics, whilst 'intermediate measures' focus on characteristics of subnetworks within the whole network. Below, the metrics used in the majority of research in social network analysis are described, broken down into individual, group and intermediate measurement categories.

6.3.1 Individual measures

6.3.1.1 Degree

Degree is a property of individual vertices and is simply the number of edges connected to the focal vertex (Wey *et al.*, 2008).

This measurement can be refined further when networks are directed or weighted. In weighted networks, rather than counting the number of connections, the total weight of the connections can be summed (Barthélemy *et al.*, 2005), and this is sometimes referred to as 'vertex strength' (Farine & Whitehead, 2015). It should be noted that these metrics give related, but different information about the characteristics of the vertex. For example a vertex could have a low degree but have a high weighted degree (although the vertex has a low number of linked edges, those edges are heavily weighted). In directed networks, the 'in-degree' and the 'out-degree' can be calculated for each vertex. The former refers to the number of edges directed into the vertex, whilst the latter refers to the number of directed edges leading out from the vertex (Wey *et al.*, 2008). Both in-degree and out-degree metrics can also be calculated for weighted networks in the same way as for the basic degree measurement.

It is also possible to calculate the difference between in-degree and out-degree values for individual vertices (Wey *et al.*, 2008).

6.3.1.2 Centrality

Centrality is an attempt to quantify the structural importance of an individual vertex within the network (Friedkin, 1991). There are several ways of doing this. Degree (see previous section) is a measure of centrality. A second measurement is 'Closeness centrality'. This requires an understanding of 'shortest path lengths'. The 'path length' between two vertices is the lowest number of edges that need to be traversed in order to travel from one vertex to the other (Croft *et al.*, 2008). Closeness centrality is the sum of the inverse of the path lengths associated with the focal vertex. 'Betweenness centrality' is the number of shortest paths in the network as a whole, which pass through the focal vertex (Farine and Whitehead, 2015).

Importantly, closeness centrality and betweenness centrality can both also be calculated for a focal edge as well as for a focal vertex (Croft *et al.*, 2008), allowing centrality metrics to be focussed on particular connections in the network rather than objects (vertices).

6.3.2 Group (overall network) measures

6.3.2.1 Density

Density is a measure of overall connectedness of the network. It is calculated by dividing the total number of edges by the number of possible edges (Croft *et al.*, 2008). It has an equivalent in weighted networks, which is calculated by dividing the sum of all edge weights by the number of possible edges (Farine and Whitehead, 2015).

6.3.2.2 Average Path Length

This is simply the mean path length (see section on centrality above) when considering all path lengths in the network (Wey *et al.*, 2008).

6.3.2.3 Diameter

The diameter is the longest path length (see section on centrality above) when considering all path lengths in the network (Wey *et al.*, 2008).

6.3.3 Intermediate measures

6.3.3.1 Clustering coefficient (also known as transitivity)

This is a measurement of the level of clustering (relative density) in the subnetwork of a focal vertex (Wey *et al.*, 2008). However, there is variation in the literature in how the clustering coefficient is calculated (Kolaczyk & Csárdi, 2014). It is most frequently calculated as:

C = (3 x number of triangles) / (number of connected triples of vertices)

(Kolaczyk & Csárdi, 2014). Triangles are formed by three edges connecting three vertices in the form of a triangle, whereas triples are formed by three vertices connected by two edges. It is important to note that although the clustering coefficient is regarded as an intermediate measure, it is actually an individual measure as it is calculated for a focal vertex (behaviour). However, it is regarded as an intermediate measure as it is a measure of clustering (the frequency with which triples close to form triangles; Kolaczyk & Csárdi, 2014), which examines an extended subsection of the network around the focus vertex, and therefore can be regarded as an intermediate property of networks.

6.4 Interpretation of network metrics

There is an extensive range of network metrics which have been developed over the last 50 years (Wilson & Watkins, 1990; Newman *et al.*, 2006; Kolaczyk & Csárdi, 2014). Interest in social network analysis in behaviour studies has led to a favoured selection of metrics within this area, which are described in the previous section. Although these metrics are frequently used in studies of human and animal social networks, considerable care needs to be taken in interpreting the meaning of the metrics in terms of real world phenomena and behaviour (Farine & Whitehead, 2015). Some of the complexities of interpretation can result from calculating the same metric, but on different types of networks. A simple example is calculating the mean degree for an unweighted network and doing the same for a weighted network. The former reflects information about only the mean number of edges linked to the vertices, whereas the latter reflects this same characteristic but is moderated by the distribution of weights among the edges within the network. The conclusions reached based on the two measurements could be very different, and there is substantial scope for misinterpretation.

In the previous chapter, the use of network analysis for studying animal behaviour beyond that of social networks was advocated and the development of behaviour and space use networks was demonstrated. Given the care which is needed in interpreting metrics of social networks, noted in the previous paragraph, it is critically important that the interpretation of metrics applied to the newly developed behaviour and space use networks be carefully considered and outlined. This section considers each of the metrics described in the previous section in terms of their interpretation within the context of patterns of behaviour and patterns of space use.

6.4.1 Individual measures

In this section, each of the network metrics described above are examined in terms of how they should be interpreted with respect to what they indicate and how they relate to animal behaviour and space use. For clarity, the individual sections focus on behaviour networks, however the interpretation will be the same for spatial networks, behaviour type just being substituted for spatial zone. However, how these measures can be used and their utility does vary between behavioural and spatial networks and this is discussed in the final subsection of this section.

6.4.1.1 Degree

Degree is an unweighted measure of centrality or connectedness, with higher values indicating that the vertex is linked with higher numbers of other vertices.

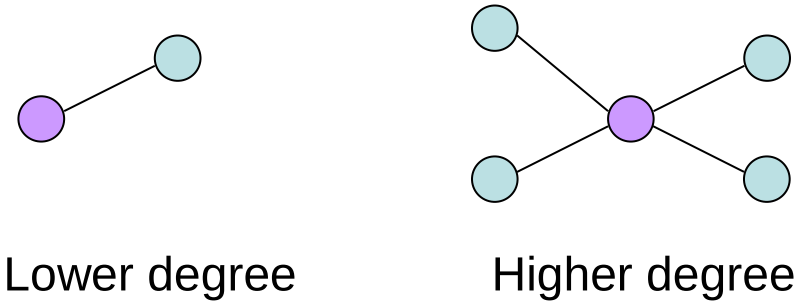


Figure 6.1 An illustration of a vertex (behaviour) with a relatively low degree and one with a higher degree. The degree measurement is for the focus vertex indicated by the purple purple colouring

With a behaviour network, a vertex with a low degree represents a behaviour that the animal transitions to or from a limited set of other behaviours. A vertex with a high degree represents a behaviour that the animal transitions to or from a large set of behaviours, or both. There is therefore an asymmetry in the information provided by this metric, depending on the degree of the vertex. With a low degree we know that the animal transitions to and from the behaviour from a limited number of other behaviours, whereas with a high degree it may transition to the behaviour from a large number of behaviours but transition from that behaviour to a low number of behaviours, or vice versa, or somewhere in between. However, as behavioural and spatial networks are directed, the extra resolution of the in-degree and out-degree can be extremely informative and enables us to establish if there is asymmetry and its form.

6.4.1.2 In-degree

A vertex with a high in-degree represents a behaviour that the animal transitions to from a high number of other behaviours.

6.4.1.3 *Out-degree*

A vertex with a high out-degree represents a behaviour that the animal transitions from to a high number of other behaviours.

6.4.1.4 *In-degree - out-degree*

The in- and out-degree metrics indicate if the animal is transitioning from or to a low or high number of other behaviours, respectively. However, on their own they do not provide an indication of the balance between the degrees of transitioning to or from the behaviour. This balance is given by subtracting the out-degree from the in-degree for the vertex representing the focus behaviour.

It is important to recognise that this difference measure needs to be interpreted within the context of the overall degree in order to get a full understanding of the balance in the behaviour transitioning. This is because the range of possible values for this metric is determined by the degree. An illustration of this is given in **Figure 6.2** where the degree of the vertex (behaviour) in the top and bottom rows is 2 and 4, respectively. This means that the possible range of the in-degree - out-degree metric in the top row is from -2 to 2, whereas the range in the bottom row is from -4 to 4. Therefore, not considering the degree at the same time gives no indication of the extent of the metric in relation to the possible range of values.

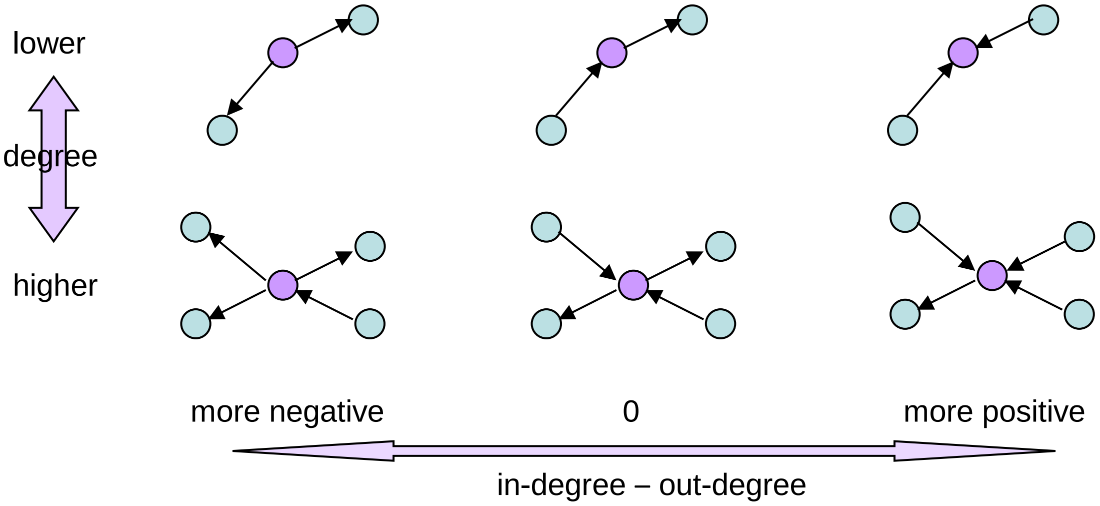


Figure 6.2 Figure illustrating how the network metric in-degree - out-degree generally indicates the level of asymmetry between the number of behaviours that transition into the behaviour (vertex) of interest and the number of behaviours that are transitioned to from the focus behaviour. However, the extent of this

metric must be taken within the context of the overall degree of the focus behaviour, which is lower in the top row and higher in the bottom row. The focus vertex is the one coloured purple.

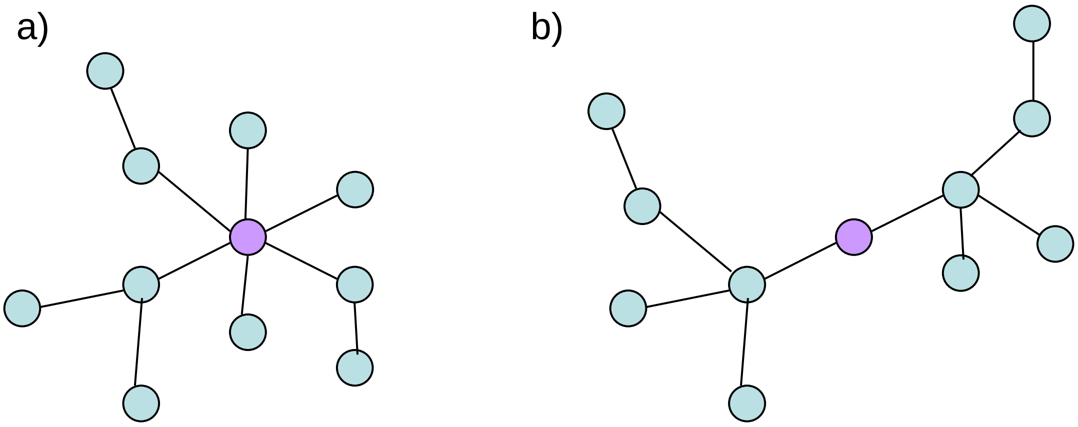
6.4.1.5 Closeness centrality

Closeness centrality is a measure of how few behaviours, on average, an individual transitions through to get from the focus behaviour to another behaviour. Thus, a high closeness centrality measure indicates that the animal tends to transition from the focus behaviour to other behaviours via few (if any) intermediate behaviours. This will tend to indicate that it is relatively difficult to predict what behaviour the individual will move to next when currently demonstrating the focus behaviour.

6.4.1.6 Betweenness centrality

Betweenness centrality is a metric that tries to measure the importance of a vertex (behaviour) as a link in the overall network. A vertex with a high betweenness centrality will frequently need to be passed through when moving between two other behaviours. It therefore indicates that the associated behaviour is a central behaviour in the behaviour network.

Closeness and Betweenness centrality are both measures of how well linked the behaviour is in the overall network structure. However, it is important to note that although they overlap in what they measure, differences in their relative values can indicate more subtle characteristics of the behaviour network. This is illustrated in **Figure 6.3** where two networks with equal numbers of vertices and edges are shown. However, the relative values of the two measures of centrality for the focus vertex (purple) will be different in the two situations. In the network on the left the vertex will have high values for both closeness and betweenness centrality metrics. However, in the network on the right it will have a high value for the betweenness centrality measure, but a relatively lower value for the closeness centrality measure. This difference is due to the level of overall clustering of the two networks being different. In the network on the right the focus vertex is forming a crucial link between two sub-networks, whereas the network on the left shows much less sub-clustering. Thus using the relative values of these two measures of centrality can highlight important structural characteristics of the overall behavioural network.



High betweenness centrality
High closeness centrality

High betweenness centrality
Lower closeness centrality

Figure 6.3 Two behavioural networks with the same number of vertices (behaviours) and edges (transitions between behaviours) but with different relative values in the two measures of centrality (betweenness and closeness) for the focus behaviour (coloured purple).

6.4.2 Group (overall) network measures

6.4.2.1 Density

Being the proportion of edges in a network relative to the number of possible edges, density is an overall measure of the connectedness of the behavioural network. For a given number of behaviours, a behaviour network with a greater density will have a larger proportion of pairwise behaviour transitions.

6.4.2.2 Average Path Length

Average path length is a complementary measure to density. Density gives a measure of how connected the average vertex is (i.e. the number of other behaviours that an animal transitions through to, and from, a particular behaviour). The average path length attempts to measure the overall centralisation network (i.e. does the individual tend to transition from one behaviour to another through a minimum number of other behaviours, or are their extended transition pathways between two behaviours?).

6.4.2.3 Diameter

The diameter is the longest path length. This is again a measure of overall centrality of the behaviour network. A smaller diameter indicates a more compact network where transitions between one behaviour and another are done via a small number of other behaviours. However, diameter refers only to the longest path length. Thus it is possible that the network is generally centralised, but there is a large diameter due to a small extended component of the network. Thus diameter and average path length should be used in conjunction, diameter giving a measure of the extreme of the network, average path length giving a measure of the general structure of the network.

6.4.3 Intermediate measures

6.4.3.1 Clustering Coefficient (transitivity)

In the previous section it was noted that although average path length and diameter are both measures of the general structure of the network, the former is a measure of general structure, whereas the latter is a measure of an extreme. The clustering coefficient complements these measures by being an intermediate level measure, being focussed on a particular behaviour, but including an extended sub-network around the focus behaviour. A vertex with a high clustering coefficient will be linked to vertices which are highly connected. Thus, within this sub-network, the animal will be transitioning quickly (i.e. through few intermediate behaviours) from one behaviour to the next.

6.5 Example application to orangutan behaviour networks

The network metrics outlined in the above sections were calculated for the behavioural networks derived in the previous chapter from the observational data from the orangutan enclosure at Twycross Zoo. This section presents some of the key results from these calculations. It forms a link between the details of the metrics and their interpretation in the previous sections, to an assessment of their utility and potential for behavioural studies of captive animals presented in the discussion section of this chapter. It should be noted that in this section, unless explicitly stated, measurements are based on unweighted versions of the behavioural networks. This has been done in an effort to increase clarity. Assessment and interpretation of network structure and the measurements of network structure are complex, and therefore adding in a further level of complexity (weighted and non-weighted networks) is likely to lead to a decrease in clarity with reference to the main topics addressed in this chapter. However, implications of the use of weighted networks and metrics associated with these networks, and a discussion of their utility and constraints are addressed in the discussion section of this chapter, building on the understanding gained from the unweighted networks.

6.5.1 Degrees

The distributions of the degrees for each behaviour (vertex) in the behaviour networks derived over all five weeks of the study for each individual are shown in **Figure 6.4**.

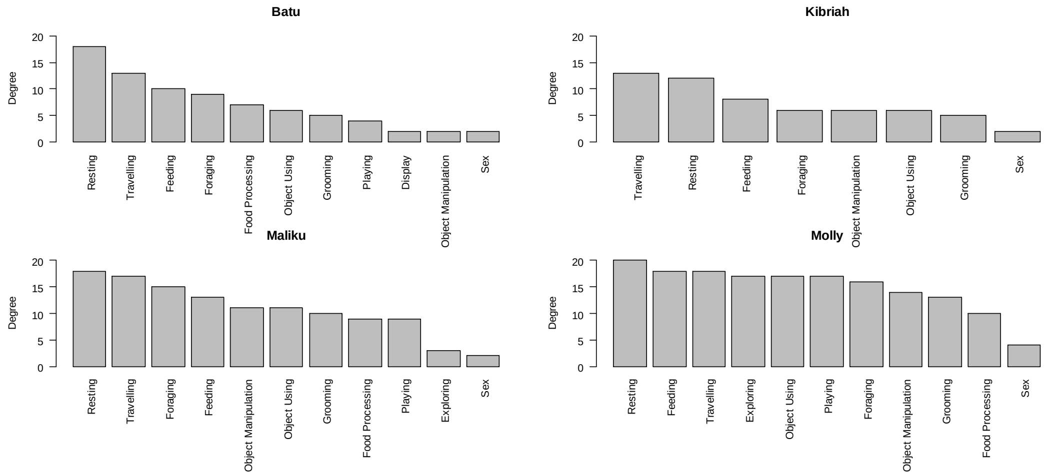


Figure 6.4 Barplots of degrees associated with each behaviour in the behaviour networks (directed) of the four orangutans derived from the data collected over the full five weeks of the study.

The associated balances between the in- and out-degrees for each behaviour are shown in **Figure 6.5**.

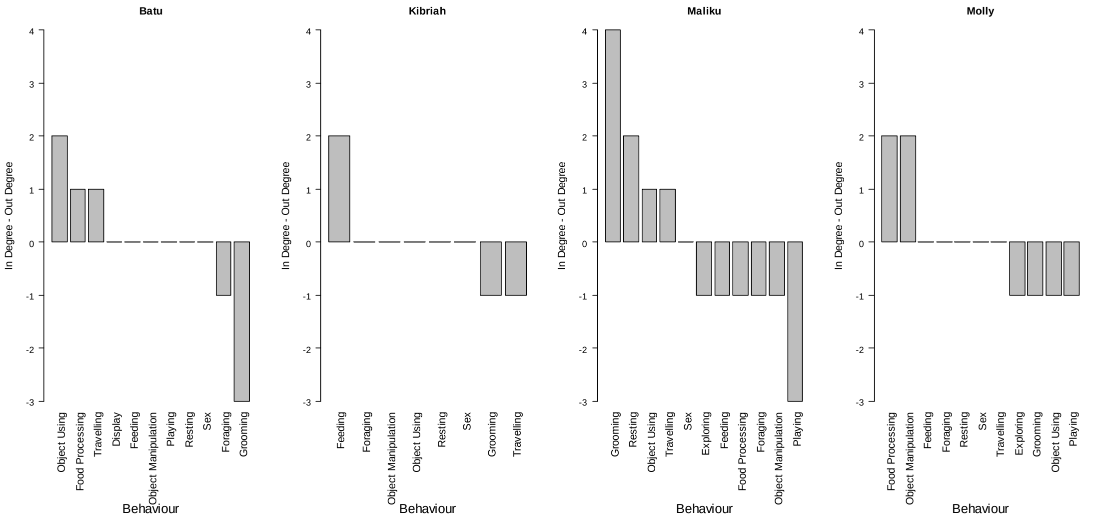


Figure 6.5 Barplots of in-degree - out-degree associated with each behaviour in the behaviour networks (directed) of the four orangutans derived from the data collected over the full five weeks of the study.

6.5.2 Betweenness centrality

Barplots of the vertex betweenness values calculated for each behaviour based on the behavioural network of each individual utilising the data from the full five weeks are shown in **Figure 6.6**.

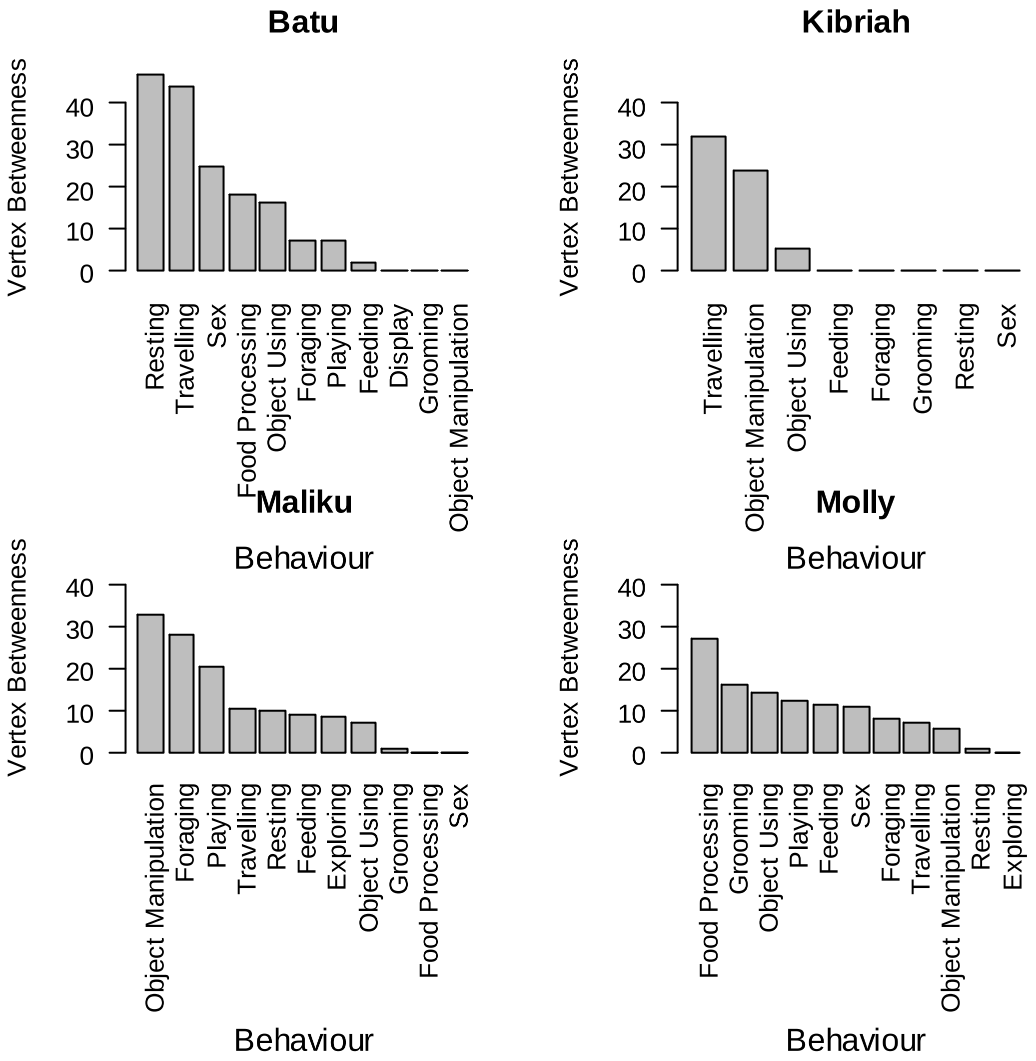


Figure 6.6 Barplots of vertex betweenness values calculated from the network for each individual based on the data collected over the full five weeks of the study.

6.5.3 *Density*

Density values for the behavioural networks based on the full five weeks of data collected for each individual were as follows: Batu 0.44; Kibriah 0.57; Maliku 0.66; Molly 0.80.

6.5.4 *Diameter*

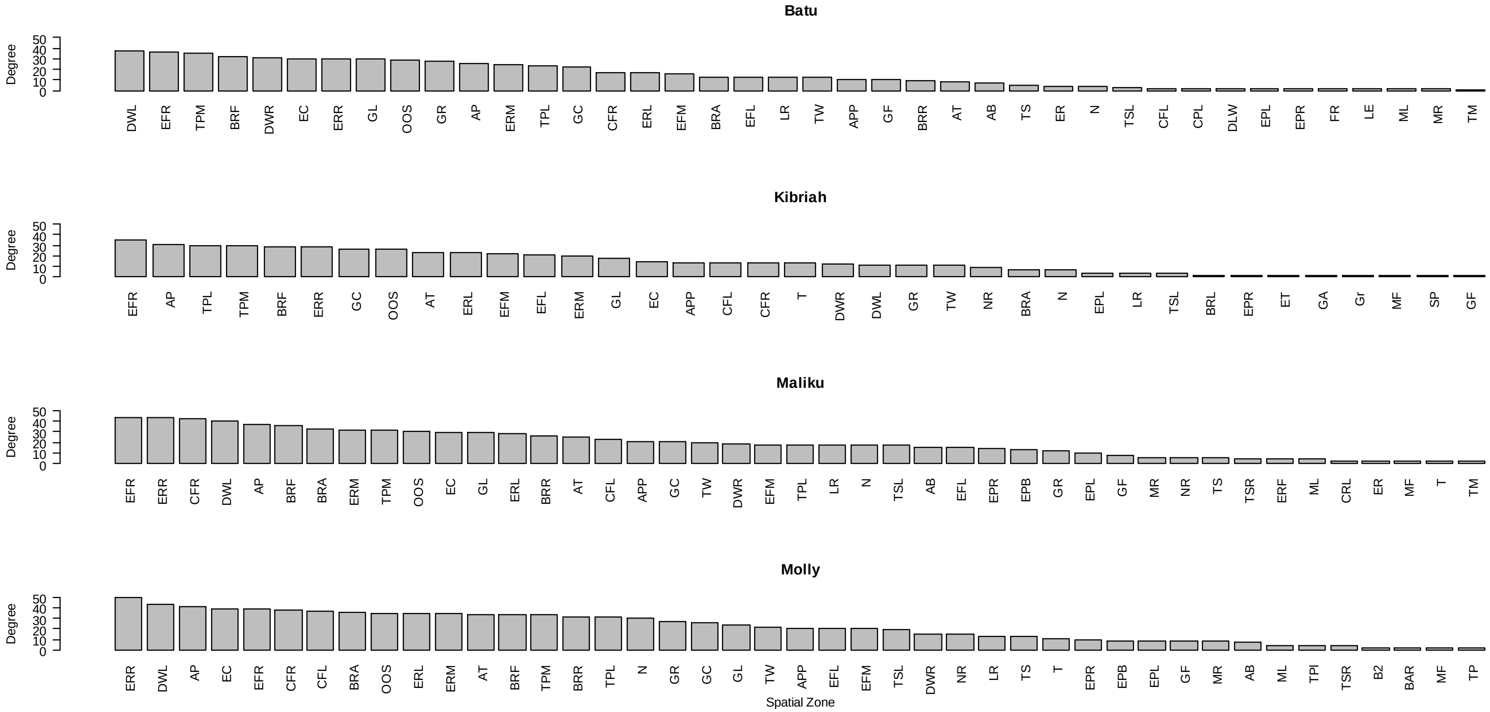
Diameters of the behavioural networks based on the full five weeks of data collected for each individual were as follows: Batu 6; Kibriah 4; Maliku 4; Molly 4.

6.5.5 *Transitivity*

Transitivity values for the behavioural networks based on the full five weeks of data collected for each individual were as follows: Batu 0.59; Kibriah 0.64; Maliku 0.76; Molly 0.87.

6.6 Example application to orangutan space use networks

Barplots of degree, in-degree minus out-degree and betweenness centrality for each vertex (spatial zone) of the space-use networks generated using the data from all five weeks of the study are shown (one for each individual) in **Figure 6.7**, **Figure *6.8*** Figure **6.9**, respectively.



Batu

Kibriah

Maliku

Molly

Figure 6.7 Barplots of ranked degree values for each vertex (spatial zone) in the overall space-use networks based on data collected over the full study period for each individual.

6.6.1 *In - out degree*

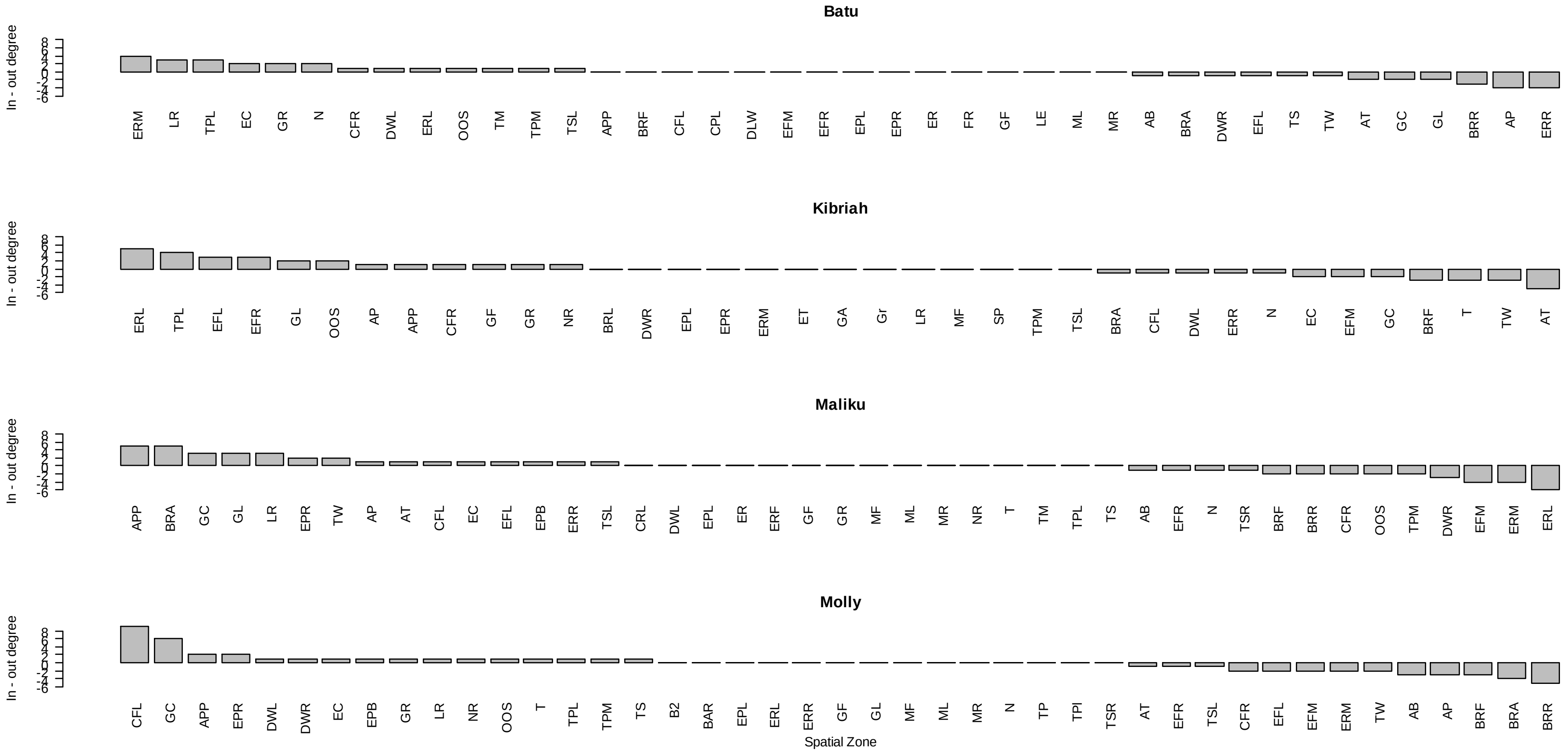


Figure 6.8 Barplots of ranked differences in degree values (in - out) for each vertex (spatial zone) in the overall space-use networks based on data collected over the full study period for each individual.

6.6.2 *Betweenness centrality*

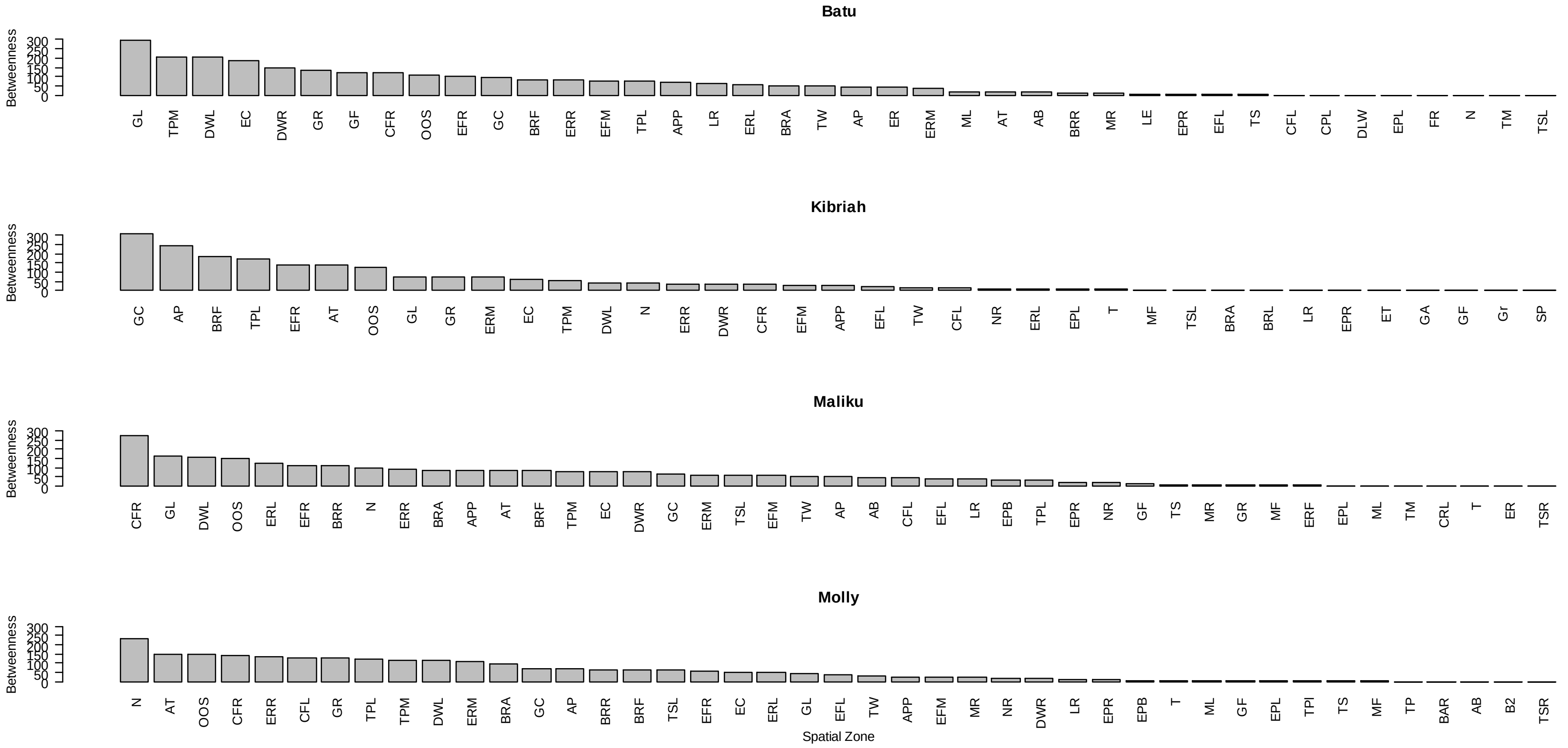


Figure 6.9 Barplots of ranked betweenness centrality for each vertex (spatial zone) in the overall space-use networks based on data collected over the full study period for each individual.

6.6.3 *Density*

Density values for the space use networks based on the full five weeks of data collected for each individual were as follows: Batu 0.19; Kibriah 0.20; Maliku 0.23; Molly 0.26.

6.6.4 *Diameter*

Diameter values for the space use networks based on the full five weeks of data collected for each individual were as follows: Batu 8; Kibriah 5; Maliku 6; Molly 6.

6.6.5 *Transitivity*

Diameter values for the space use networks based on the full five weeks of data collected for each individual were as follows: Batu 0.55; Kibriah 0.54; Maliku 0.53; Molly 0.57.

6.7 Variation in network metrics over time

Constructing networks based on data from an extended period of time gives a summary of behaviour or space use over the whole of that time. However, it does not indicate how consistent patterns of behaviour or space use are over time. One way to address this aspect is to calculate multiple networks which summarise behaviour or space use over sequential shorter periods of time, calculate the associated metrics and examine the level of variation in these networks. In the previous chapter, example behavioural and space use networks were constructed for data covering periods of one week and one day at a time. In this section examples of network metrics applied to networks calculated daily, thereby including variation in network metrics over time, are given.

The introduction of variation by calculating metrics daily rather than as a summary of all the data over the whole study period allows for the statistical analysis to assess for differences in network structure between individuals. As an example of the application of these methods to the network data, in this section linear regression is applied to a subset of the network metrics to test for differences in behaviour and space use network structures between individuals. Density and average path lengths were assessed in this way. Density was considered a good example metric as it is widely used in social network studies and is a measure of overall connectedness of a network. Average path length was also chosen as it serves as a useful comparison of applying these methods to networks of different sizes. The behaviour networks are likely to show little variation in this metric as there are few behaviours (vertices), whereas this is not the case with the space-use networks.

Linear regression was performed following Zuur *et al.* (2007), with the assumption of normality of residuals being assessed using Q-Q plots, homogeneity of variance being assessed using plots of standardised versus fitted residuals, and Cook's distances being used to check for overly influential data points. Where heterogeneity of variance was found, following Zuur *et al.*(2009) a generalized least squares (GLS) extension was applied (see chapter three for further details).

6.7.1 Network metrics for daily behaviour networks

6.7.1.1 Density

A boxplot of the density values for the daily behaviour networks for each of the orangutans is shown in **Figure 6.10**.

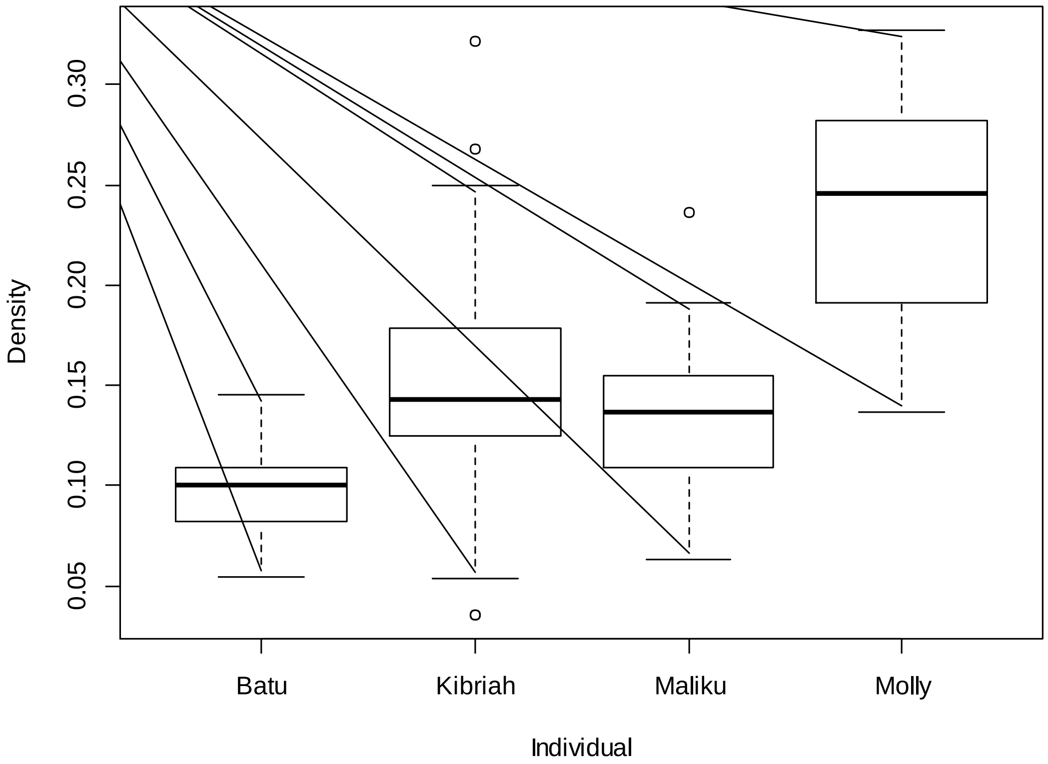


Figure 6.10 Density values calculated for daily behaviour networks for each of the individual orangutans.

Linear regression, with a GLS extension to model heterogeneity of variance, indicated significant differences between densities of the daily networks of the individual orangutans (*L*-ratio = 73.67, d.f. = 3, $p < 0.0001$). Pairwise comparisons between individuals indicated significant differences between all pairs of individuals apart from Kibriah and Maliku (*t*-value = -1.19, p =0.24). The corresponding statistics were Batu-Kibriah (*t*-value = -3.95, p = 0.0001), Batu-Maliku (*t*-value = 4.40, $p < 0.0001$), Batu-Molly (*t*-value = 12.17, $p < 0.0001$), Kibriah-Molly (*t*-value = 4.91, $p < 0.0001$) and Maliku-Molly (*t*-value = 7.89, $p < 0.0001$). Thus, the densities of Molly's networks were significantly greater than those for Kibriah and Maliku's, which in turn were significantly greater than those for Batu. It was also noticeable in the boxplot that the variation in densities in the networks for Batu and Maliku were less than for Kibriah and Molly, which was supported by the need for a GLS extension in the regression analysis.

6.7.1.2 Average Path Length

A boxplot of the average path length values for the daily behaviour networks for each of the orangutans is shown in **Figure 6.11**.

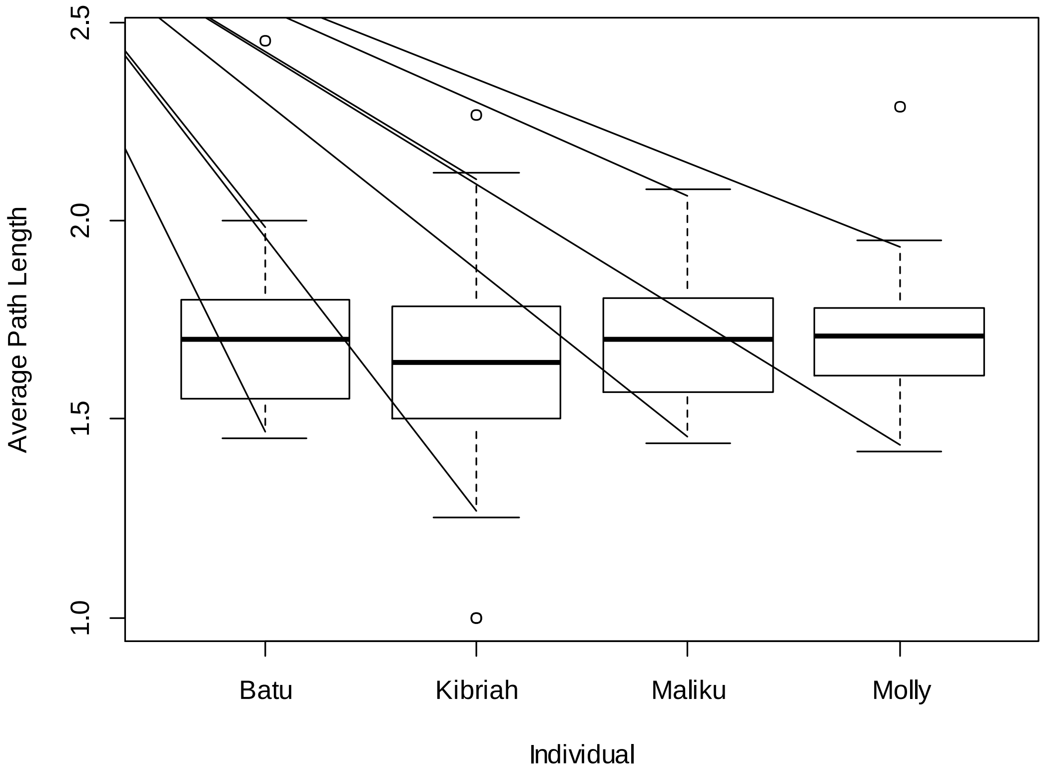


Figure 6.11 Average path length values calculated for daily behaviour networks for each of the individual orangutans.

Linear regression indicated no significant differences in the average path length value between individuals (F = 1.24, d.f. = 3, p = 0.30).

6.7.2 Network metrics for daily space-use networks

6.7.2.1 Density

A boxplot of the density values for the daily space-use networks for each of the orangutans is shown in **Figure 6.12**.

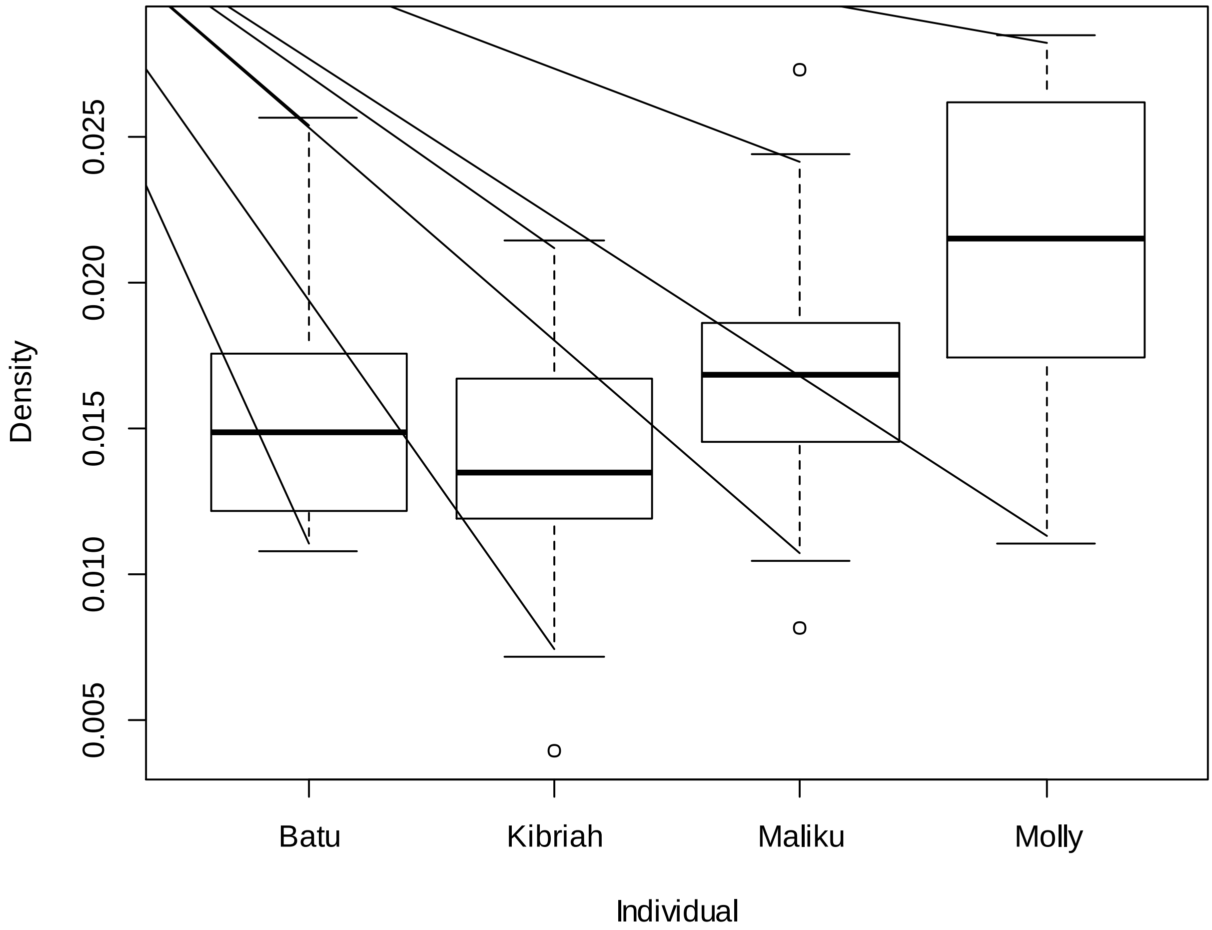


Figure 6.12 Average density values calculated for daily space-use networks for each of the individual orangutans.

A linear regression indicated significant differences in density values for the space-use networks between individuals (F = 13.54, d.f. = 3, p < 0.0001). Individual comparisons were Batu-Kibriah (*t*-value = -1.19, p = 0.24), Batu-Maliku (*t*-value = 1.16, p = 0.249), Batu-Molly (*t*-value = 4.83, p < 0.0001), Kibriah-Maliku (*t*-value = 2.35, p = 0.021), Kibriah-Molly (*t*-value = 0.02, p < 0.0001) and Maliku-Molly (*t*-value = 3.67, p < 0.001).

6.7.2.2 Average Path Length

A boxplot of the average path length values for the daily space-use networks for each of the orangutans is shown in **Figure 6.13**.

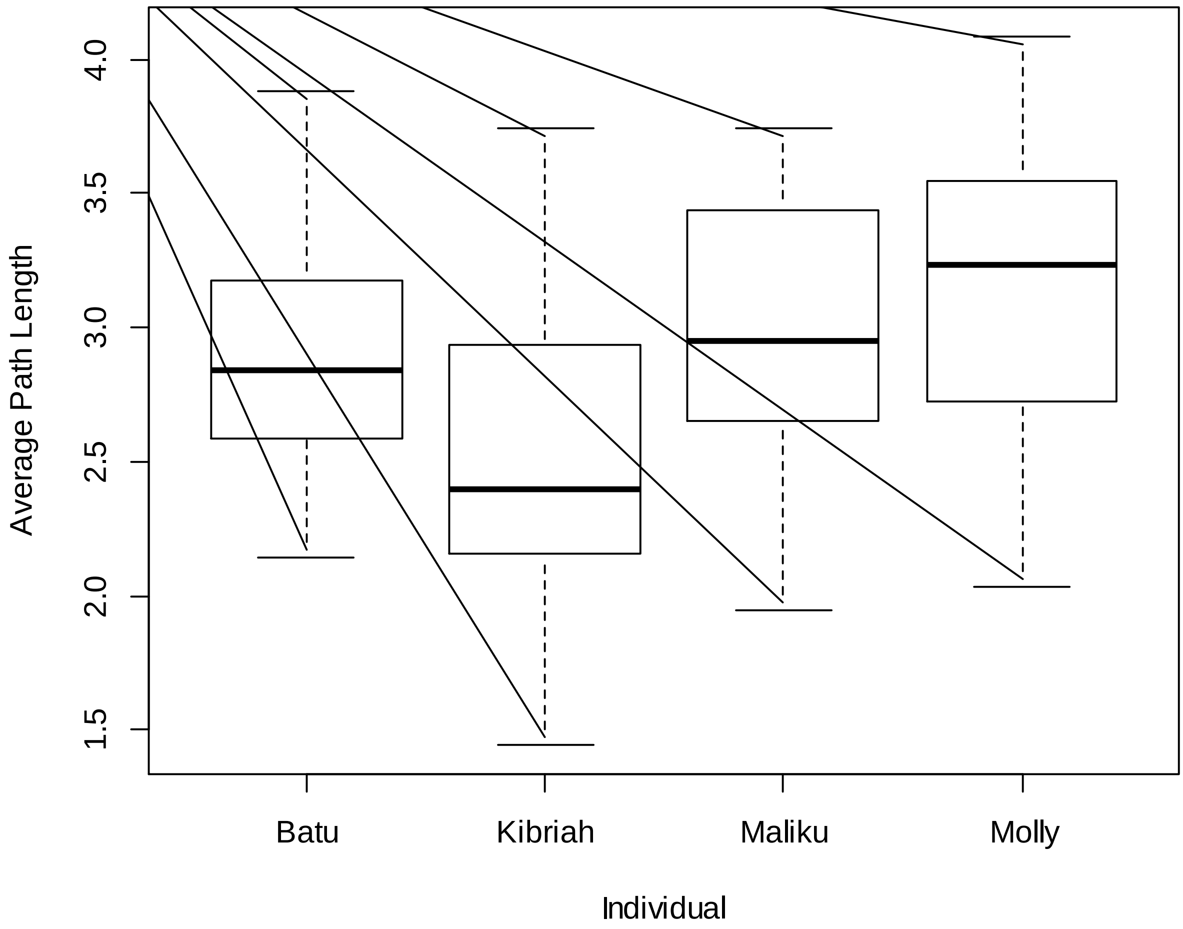


Figure 6.13 Boxplot of average path length values calculated for daily space-use networks for each of the individual orangutans.

Linear regression indicated significant differences in average path lengths between individuals (F = 5.29, d.f. = 3, p = 0.002). Individual comparisons indicated that Kibriah had significantly greater average path lengths than all other individuals, but that there were no differences between the other three individuals. The corresponding statistics were Batu-Kibriah (*t*-value = 2.17, p = 0.033), Batu-Maliku (*t*-value = -0.42, p = 0.67), Batu-Molly (*t*-value = 1.75, p = 0.084), Kibriah-Maliku (*t*-value = 2.56, p = 0.011), Kibriah-Molly (*t*-value = 3.92, p < 0.001), Maliku-Molly (*t*-value = 1.33, p = 0.19).

6.8 Discussion

The previous chapter detailed how the concepts behind social networks could be extended to produce behaviour and space-use networks, and illustrated these types of networks using data from observations of the orangutans at Twycross Zoo. The visual representation of these networks was informative but it was recognized that the wider use of behaviour and space-use networks in animal behaviour research would require quantification of network structure in order to facilitate formal tests within a statistical framework. This chapter described some of the most commonly used metrics for networks, indicated how these could be interpreted from an animal behaviour point of view, illustrated their use on the networks constructed in the previous chapter and then illustrated how these metrics could be used to conduct formal statistical tests.

Density is probably the most frequently calculated network metric as it gives an overview of how well connected a network is, and one of the primary use of networks is to describe patterns of connections. Within the examples given above, a high density behaviour network has been interpreted to mean that the individual displayed a high proportion of the available behavioural repertoire evenly across the respective period of study. A high space-use network density suggests that the individual utilised all enclosure zones equally. Both of these outcomes are often interpreted as indicators of positive welfare. Therefore, measuring density provides a direct indication of the welfare status of the focal individuals.

Looking at the behaviour networks summarising data over the full data collection period for each individual there were clear differences in density (Molly 0.80, Maliku 0.66, Kibriah 0.57, Batu 0.44). The two extremes match the interpretation of the visual plots of these networks, with the greater density Molly's behaviour network reflecting the much greater variety of paths that she followed in transitioning from one behaviour to another.

The pattern in density was matched by the transitivity measurements (Molly 0.87, Maliku 0.76, Kibriah 0.64, Batu 0.59). This suggests that in the overall behaviour networks, having multiple transition paths between behaviours is also leading to closure of triplets of behaviour, meaning that the path length between pairs of behaviours become even shorter. Thus Molly showed great diversity in the patterns of behaviour that she followed over time, whereas Kibriah and Batu were much more predictable in their transitions between behaviours.

Kibriah not having the lowest density in the overall behavioural networks is, at first, surprising given her low numbers of transitions in behaviour. However, caution needs to be exercised when comparing densities of behavioural networks containing different numbers of behaviours. The number of possible edges in an undirected network is given by n(n-1)/2, where n is the number of vertices. Thus the number of possible edges increases as a power relationship with n, i.e. faster than a linear relationship. Although the measurement of network density allows for this increase in rate as it is a proportional measure, it must be considered within the context that as the number of vertices increases the baseline of comparison increases at a faster rate. This is particularly important when comparing networks between individuals. The different individuals may show different numbers of behaviours, giving each a different baseline of comparison for the calculation of network density. This could potentially result in systematic bias in results if differences in numbers of behaviours are associated with groupings, e.g. demographic groupings. This also has implications for study design and the recording of behaviour patterns. For example, the ethogram derived for recording could include all behaviours exhibited in a previous study of the same species. However, there may be a behaviour which one demographic group do not exhibit e.g. aggression. Whether or not this particular behaviour is included in their networks (as it is a potential behaviour, even though in the current study it was not exhibited by the demographic group), or not will have a significant impact on the measurement of network density for individuals in this demographic group. Notably, this impact will increase as the number of behaviours in the ethogram increases.

Such difficulties are illustrated in comparing the density and transitivity measures between the overall behaviour networks and the overall space-use networks. The density measures for the behaviour networks range from 0.44 (Batu) to 0.80 (Molly), and matched the pattern in the transitivity measures ranging from 0.59 (Batu) to 0.87 (Molly). However, the density measures for the space-use networks were much lower and covered a much more restricted range of values, going from 0.19 (Batu) to 0.26 (Molly). Additionally, they did not match up with the order in the transitivity scores for the space-use networks, ranging from 0.53 (Maliku) to 0.57 (Molly). These contrasting behaviours in the metrics are likely to be due to the differences in the number of vertices in the behaviour and space-use networks. The latter have many more vertices and these require proportionately more edges in order to reach the same density level. This situation is even more pronounced for transitivity, as this requires the closure of triplets and this will only start to occur regularly once a high density of edges has been reached.

The above discussion suggests that the utility of particular metrics of networks in animal behaviour research is likely to vary according to the size of the networks. In the case of the orangutan data, both density and transitivity give pictures of patterns of behaviour that match up with those observed in the visual representations of the networks, and have a great enough sensitivity to clearly indicate differences in the structure of behaviour networks between individuals. The power of these metrics is much lower, and potentially redundant, when applied to the much larger (in terms of number of vertices) space-use networks. This in turn suggests that there may be a balance to be made in designing animal behaviour experiments. A larger number of vertices (categories) will provide greater resolution in the data, potentially increasing the level of understanding provided, but could lead to some metrics having very little power to inform. One strategy for dealing with these difficulties would be to start with high resolution data (large number of vertices) and if this leads to redundancy in metrics, then the high resolution data could be amalgamated in order to reduce the number of vertices.

It is interesting to note that many of the network metrics were originally designed to quantify very large networks such as the World Wide Web (Newman *et al*., 2006), but that their utility may be greater when describing smaller networks such as behaviour networks. However, this will not be the case for some metrics and this was illustrated by the results for the diameter metric for both the behaviour and space-use networks. The diameters of the behaviour networks ranged from 4 (Kibriah, Maliku and Molly) to 6 (Batu), and those for the space use networks ranged from 5 (Kibriah) to 8 (Batu). Although the networks for Batu had the greatest diameters, supporting that his networks were the least connected (as indicated by the density measures), the range and size of the diameter values limits the variation allowed to such an extent that diameter could not be used in formal statistical tests. The low values and range in diameter values is simply a reflection of the small size (number of vertices) of the behaviour and spatial networks.

So far this discussion has focussed on the single metrics which have been used to describe the structure of the overall networks generated using the data from the whole of the study. However, the calculation of metrics for the same networks, but focussing on a vertex level rather than the network level, provides a potentially deeper and more subtle level of understanding. Degree is a metric which is calculated very widely for a large number of network studies (Newman *et al*., 2006) and in studies of animal social networks (Croft *et al.*, 2008). In the behaviour networks, resting had the highest degree for all individuals apart from Kibriah, for whom resting had the second highest degree and travelling had the highest degree. Resting was the most frequent behaviour seen and its central location in the behaviour networks is likely to be a reflection of this. Again, because the number of vertices in the behaviour networks is low, the degrees of the behaviours will be restricted, potentially limiting the utility of this metric, even at a vertex level. This becomes even more pronounced for the differences between in and out degrees, which have values with such a small range of values that it becomes almost impossible to robustly conclude any insight into behaviour patterns.

Although the degree values for individual behaviours may provide limited information, the distributions of these degree values across the behaviours may be more informative. **Figure 6.4** shows the barplots of the degrees for each behaviour for each individual. They show a variety of structures ranging from a smooth and steep decline in degree values for Batu's network, but a fairly level and even distribution for Molly's network. These differences indicates that the vast majority of transitions between behaviours shown by Batu pass through the few behaviours with high degree, i.e. they are key transition behaviours. However, Molly is much more diverse in her transitions and the behaviours with the highest degrees are not so central to the overall patterns of her transitions between behaviours. This suggests that the shape of the distributions of vertex (and edge) network metrics are likely to provide a further level of information over and above the related summary mean or median values.

The space-use networks have larger numbers of vertices and therefore the potential for the vertex degree levels to provide useful information about patterns increases. As an example, the DWL (**Appendices 5.1 and 5.2**) zone has the highest degree in the space-use network for Batu. The DWL zone is the linking section between the indoor and outdoor parts of the enclosure. However, the high degree associated with this vertex could indicate two things; Batu moved through this zone to get to the inside and the outside areas and therefore the degree should be high because it represents a frequently used transition zone. However, by looking at the behaviour - space-use network for Batu (**Figure 7.14** in the next chapter) we see that Batu actually spent a lot of time resting in this zone. The degree values for the DWL vertex were also high for Molly and Maliku. However, examination of their behaviour - space-use networks indicates they spent very little time resting in this zone. This could indicate that Batu was using this key spatial transition zone to control and influence the other orangutans, as they would often have to pass by him in order to move between the inside and outside areas of the enclosure. This illustrates the potential for utilising networks to describe the relationships between two sets of behavioural data, and this is an aspect which is detailed and assessed in the next chapter.

The differences between the in-degree and out-degree values for vertices can also help to detail space-use patterns. As an example, the ERM zone had the highest value for this metric for Batu. This was a section that was high up within the indoor enclosure. In contrast, the ERR zone, which was just along from the ERM zone, had the lowest in - outdegree value (i.e. the most negative value). These marked differences indicate that Batu used multiple routes up to the ERM zone, but tended to use the same route down from this zone, via the ERR zone. In contrast, the in - outdegree values for these two zones are both reasonably high for Kibriah, suggesting that she had a wider range of routes down from the ERM zone. Thus, the in and out-degree values can start to highlight particular areas of an enclosure that are important in the patterns of space-use of individuals as well as providing more general information about how the individuals are using those zones to move to other zones. Those zones identified as being important can then be taken into account when making changes to the enclosure and when designing new enclosures. The zones known to support species typical behaviours can be carefully thought through and positioned in specific areas for maximum utilisation. The zones that are utilised the least may be altered to encourage utilisation (e.g. by adding enrichment), or may be removed from the enclosure to make way for more valuable features.

Linear regression methods were applied to the daily network metrics of density and average path length for both behaviour and space-use networks, in order to demonstrate how network metrics could be used within a statistical framework. As in much ecological data, there was often heterogeneity of variance, leading to the requirement for the application of a generalised least squares extension. This is not particularly problematic, but this extension does require greater amounts of information as it explicitly models the patterns of heterogeneity of variance. This necessitates a greater amount of data, otherwise the analysis becomes less robust and reliable. The data were available in this case due to the five week data collection period. However, in many zoo based studies, data collection occurs over shorter periods of time and this may reduce the ability of studies to use network metrics.

The patterns between individuals picked up by the analysis of density for the behaviour networks matched the patterns found in the density values calculated for the overall networks summarising the data from the whole of the study. Interestingly, although the patterns between individuals in density values based on the overall networks were much less clear in the space-use networks, significant patterns were detected in the linear regression analysis using density values based on daily networks. This suggests that using networks based on data at higher temporal resolutions may allow the detection of patterns that are hidden or partially hidden when using the overall network summarising the network for the complete data set. However, the lack of structure in the daily results for the average path lengths for the behaviour networks, suggests that limitations in the range of potential values due to the limited number of vertices in the network, will reach a point where even at a daily level there will not be enough variation for the metric to be useful.

6.9 Conclusion

This chapter has detailed commonly used network metrics that could be used for the analysis of behaviour and space-use networks. It has also suggested how these metrics could be interpreted in terms of animal behaviour and space-use. The networks presented here may aid zoo staff in making important decisions with regards to future enclosure developments and population dynamics within the group. Network metrics were calculated for the networks generated in the previous chapter (both summary networks and networks based on daily data). Patterns in these metrics were illustrated, and linear regression was applied to a subset of these data as an example of how the metrics could be used within a statistical framework. Finally, the use of these metrics was discussed in relation to understanding behaviour and space-use patterns, highlighting strengths and constraints of this approach. One of the key examples discussed indicated how the combination of different sets of data can provide deeper insights into aspects of animal behaviour and space-use. In addition, it was noted that future research is needed to understand how the range in values of the network metrics may change as the number of vertices changes. This will be important if different networks are going to be legitimately compared. The linking of different data sets using bipartite networks is explored in the next chapter, and the strengths of this approach as well as the potential limitations due to our lack of understanding of network metrics and the current limitations in using statistical analyses (due to the inherent lack of independence in network structure) are explored in more detail.

Chapter SEVEN: The Utility of Bipartite Networks in Extending the Analysis of Animal Behaviour and Space Use Data

7.1 Chapter overview

In the previous two chapters the use of networks for analysing animal behaviour and space use data has been developed and the strengths and weaknesses of the approach assessed. In recent years a particular form of network, bipartite networks, have been used within ecological research to examine patterns of relationships between two trophic or functional levels. These have proven to be extremely adept at both visualising and quantifying relationships between two complex communities. This has been particularly true in studies of pollinator species and wild flower communities. In this chapter it is argued that these bipartite networks can significantly extend the utility of network analysis of animal behaviour and space use data by providing a method of instantiating the relationships between two networks. For example, the patterns of an individual's behaviour over time can be related to its pattern of movement within an enclosure, allowing for the quantification of relationships between these patterns. This significantly extends the potential of network analysis in behaviour research as it provides a powerful framework for integrating pair-wise sets of behavioural data in a way that allows researchers to analyse how the patterns in one set may influence the patterns in another set. For example, bipartite networks could allow researchers to see how an individual's behaviour is influenced by its patterns of movement around the enclosure. This integration of datasets allows for a much more comprehensive understanding of behaviour by enabling multiple components of behaviour to be combined within a single framework.

This chapter begins by explaining how bipartite networks are constructed and briefly discusses how they have been used recently within ecological research. It then argues that the bipartite networks can be used with behavioural data and explains how this can be done. The data from the study of the orangutans at Twycross utilised in the previous two chapters are utilised to demonstrate the application of bipartite networks to behaviour and space use data. Example networks are shown and various quantification measures of network structures are calculated and discussed critically in the context of their potential use in studies of animal behaviour.

7.2 Introduction

Part of the power of representing data in the form of networks is that it helps to visualise and quantify complex patterns within the data. However, the majority of network studies have tended to focus on single levels of data. For example, in the social network literature, the network is comprised of relationships (the level) between individuals. Extra information such as the position of individuals within the dominance hierarchy can be added to these networks, but further levels of networks are not added. However, in the ecological literature, there has been considerable interest in examining the structuring of ecological communities through the interactions of species between trophic levels. These have included pollinator and flower interactions (Memmott *et al*., 2004; Greslin *et al.*, 2013), and predator-prey interactions (Tylianakis *et al*., 2007). Recently bipartite networks have been used to quantify the topological structure of these bi-trophic interactions (Dormann *et al.*, 2008; Greslin *et al.*, 2013).

Bipartite networks are constructed so that every member of one trophic level is only connected to members of the second trophic level. Thus, for pollinator-flower bipartite networks species in the pollinator level are connected to the species of flower which they pollinate, but there are no pollinator-pollinator edges or flower species-flower species edges. Therefore the networks focus on the patterns of interactions between trophic levels rather than interaction patterns within trophic levels. Bipartite networks can therefore be regarded as specialised directed networks as there is only one possible direction in the edges, between one trophic level and the second. They can, however, be weighted, with the edges being weighted according to the relative frequency with which the particular interaction occurred. Thus bipartite networks form a distinct subgroup of networks.

Although bipartite networks have been developed to study the structure of interactions between two trophic levels, conceptually they can be regarded as representing patterns of linkages between two sets. Recognising this generalisation means that there is greater potential for utilising bipartite networks on a wider range of data. The data simply need to consist of two distinct sets of units which interact with each other. In the pollinator networks these sets consist of pollinator and flowers, each containing unique sets of species (units), and the edges represent the occurrence of pollination between individual pollinator species and individual flower species. However, the trophic levels could be substituted for other sets of data. For example, one level could consist of behaviours exhibited by an individual and the second level could consist of locations. The links between these layers indicate the locations where the individual performed particular behaviours. Thus, in behaviour studies, bipartite networks open up the possibility of visualising and quantifying patterns of interactions between different components of behaviour.

This chapter gives an overview of how bipartite networks can be constructed with behavioural data detailing how specific combinations of types of behavioural data could be productively utilised by animal behaviour researchers. It then utilises the same data as the previous two chapters to show how the sets of data used to produce two individual networks can be used to construct bipartite networks representing patterns of interactions between the two networks. Examples of these networks are shown, highlighting their utility in providing more information about patterns of behaviour within a broader context, which is complementary to the results from the behaviour and spatial networks constructed in the previous two chapters. The chapter ends with a critical discussion of the strengths and weaknesses of the use of bipartite networks for animal behaviour studies, and makes suggestions for extending the utility of these networks in behaviour studies.

7.3 Constructing bipartite networks

For clarity, this section describes the construction of a particular kind of bipartite network, a behaviour-space use bipartite network. This details the core construction process. However, a wider range of bipartite networks can be constructed from behavioural data and these are detailed in the next section.

To construct a behaviour-space use bipartite network, records are needed for both what behaviours were performed by an individual and the spatial location where these behaviours were performed. A matrix can then be constructed, with rows (i) representing individual behaviours and columns (j) representing individual locations. The frequency with which the individual performed behaviour i in location j is then recorded at location (i,j). This matrix then represents all the information required to construct the bipartite network. The sum of each row i, represents the total frequency with which the corresponding behaviour was observed, whilst the sum of each column represents the total frequency of (all) behaviours being observed in the corresponding location. The frequency recorded at matrix location (i, j) is then represented in the bipartite network as the weighting (thickness) of the edge connecting behaviour i with location j.

7.4 Utilising bipartite networks for behaviour studies

Conceptually bipartite networks are simple, relating the strengths of connections between two sets of data. However, this simplicity underlies part of the extensive potential utility of these networks for behaviour studies in that it allows a wide range of data layers to be related to each other. In the previous two chapters networks were constructed based on behaviour and space use data collected using standard experimental protocols. These networks provided useful visualisations and metrics for understanding the behaviour and space use patterns of the individual orangutans. Bipartite networks allow this to be extended by relating different sets of behavioural data to each other. Behaviour data from an individual can be related to the space use data from that individual collected at the same time, allowing for the structure in the patterns of behaviour to be understood in the context of the location of the individual within the enclosure. Behaviour data from one individual can be mapped to the behaviour data of another individual, the data being collected at the same time, allowing the behaviour of the first individual to be related to the behaviour of the second individual. The same approach can be taken with the space use data from two individuals, allowing an assessment of how the space use of one individual may be affecting the space use patterns of a second individual.

In this chapter the utility of bipartite networks for animal behaviour studies is assessed. The behaviour and space use data for the captive orangutans at Twycross Zoo utilised in the previous two chapters are used here. Bipartite networks are constructed in the following ways; individual behaviour and space use networks (behaviour - space use bipartite networks, BSB networks), individual behaviour related to the behaviour of a second individual (behaviour - behaviour bipartite networks, BBB networks), individual space use related to the space use of a second individual (space use - space use bipartite networks, SSB networks).

7.5 Bipartite network metrics

There are at least 46 basic metrics which can be calculated for a bipartite network using the bipartite package (Dormann *et al.*, 2008) in the R statistical software (R Core Team, 2016). However, the understanding and interpretation of these metrics in ecological research is at an early stage, and almost non-existent for behaviour and space use studies, making it necessary to be cautious in utilising and interpreting these metrics (see the discussion in this chapter). Therefore a small subset of metrics were calculated for the behaviour - behaviour and the behaviour - space use bipartite networks generated from the data collecting from observing the orangutans at Twycross Zoo. These are used to illustrate the potential utility of bipartite metrics in understanding animal behaviour as well as some of the potential difficulties associated with such an approach. These metrics were not applied to the space use - space use bipartite networks as the level of connectivity and complexity of these networks with large numbers of units in each level reduced the robustness of these measures and it was felt made their use unreliable without more extensive testing, which was not the aim of this chapter.

The metrics which were calculated were selected based on their being a summary of the broad structure of the bipartite network (rather than focussing on a small sub-component), having reasonable provenance in the literature, thought to have an intuitive and relatively straight forward interpretation in terms of behaviour and space use, and thought to have potential utility in providing greater understanding of animal behaviour and space use. These metrics are outlined in the following subsections.

7.5.1 Alatalo Interaction Evenness

This is a measure of the evenness of the proportions of the links between the two layers of the bipartite network. It was originally suggested by Alatalo (1981) and was utilized by Müller *et al*. (1999), and relates to evenness as calculated in many biodiversity studies where p_k is the proportion of the k^{th} 'species' (link). This measure has the advantage of being robust to extremes in proportions (Müller *et al*., 1999).

$$E = \frac{(\Sigma p_k^2)^{-1} - 1}{\Pi p_k^{p_k} - 1}$$

Equation 1

7.5.2 Interaction Evenness

A second measure of the evenness of the proportions of the links, and is the equivalent of Shannon's evenness, being calculated as the diversity of 'species' (links) divided by the maximum possible diversity, given the number of links. This measure of evenness has a long and extensive history in the ecological literature (Magurran & McGill, 2011).

7.5.3 Linkage Density

This measures the total diversity of interactions across all 'species' (behaviours or spatial locations) in both levels of the bipartite plot, weighted according to the frequency (proportion) of each 'species' (Dormann *et al.*, 2008). As a diversity measure, it differs from the two evenness indices introduced above, in that its value is a reflection of both the number of 'species' in each level and the evenness with which the number of links are distributed, rather than focussing just on the evenness.

7.5.4 Connectance

This is the number of links in the bipartite networks as a proportion of the number of possible links as used by Dunne *et al*. (2002). It is the bipartite version of the density metric used for measuring the proportion of realised links compared to the maximum possible in normal networks.

7.5.5 Weighted Connectance

The linkage density (see above) divided by the number of 'species' (behaviour / spatial location) in the network, as used by Bersier *et al*. (2002).

7.5.6 Links per species

This is the mean number of links per 'species' (behaviour / spatial location), i.e. the total number of links divided by the number of 'species' in both levels (Dormann *et al.*, 2008).

As an illustration of their utility, these metrics were calculated for the overall BBB networks constructed using all the data collected across the five week study period, and for the BSB networks generated using the daily data. As all of the network metrics listed above are related in some way in that they are quantifying some aspect of the level of connectivity of the bipartite networks, patterns of consistency between the metrics were assessed using Pearson's correlation coefficients.

The aim of this work is to demonstrate the utility and potential in using bipartite networks for animal behaviour studies. So far the generation of bipartite networks using behavioural and spatial use data has been described as well as some potentially useful metrics. Although the visualisation and quantification of bipartite network structure are useful in themselves, if bipartite networks are going to be used widely in future studies of behaviour statistical analyses will need to be utilised in order to make comparisons, e.g. between bipartite networks constructed for individuals. To demonstrate this process linear regression was used to test for differences in the connectance of daily BBB networks. Assumptions of normality were assessed using residual plots (Zuur *et al*., 2007), and backward stepwise selection was used to assess the significance of the individual-individual pairing (Quinn & Keough, 2002).

The visualisation and generation of metrics for the bipartite networks was conducted using the bipartite package (Dormann *et al.*, 2008) within the statistical software R (R Core Team, 2016). All network metrics were calculated using the **networklevel** function in the bipartite package (Dormann *et al.*, 2008).

Below, example BSB, BBB and SSB networks are shown, both for networks summarising data from the full five weeks of the study, and networks generated using behaviour and space use data at a daily resolution.

7.6 Results

7.6.1 Behaviour-Behaviour Bipartite (BBB) networks

7.6.1.1 BBB networks summarising results across the five week study period

Three example BBB networks summarising pair-wise patterns of behaviour between pairs of individuals over the full five weeks of the study period are shown in **Figure 7.1**, **Figure 7.2** and **Figure 7.3**. Edges between behaviours illustrate behaviours that occurred simultaneously. For example, in **Figure 7.1** a heavily weighted edge exists between resting for both individuals. This illustrates that both individuals rested at the same time for a large proportion of the total time of the observations. However, when Batu was OOS (out of sight) Kibriah was mostly resting and only occasionally also OOS.

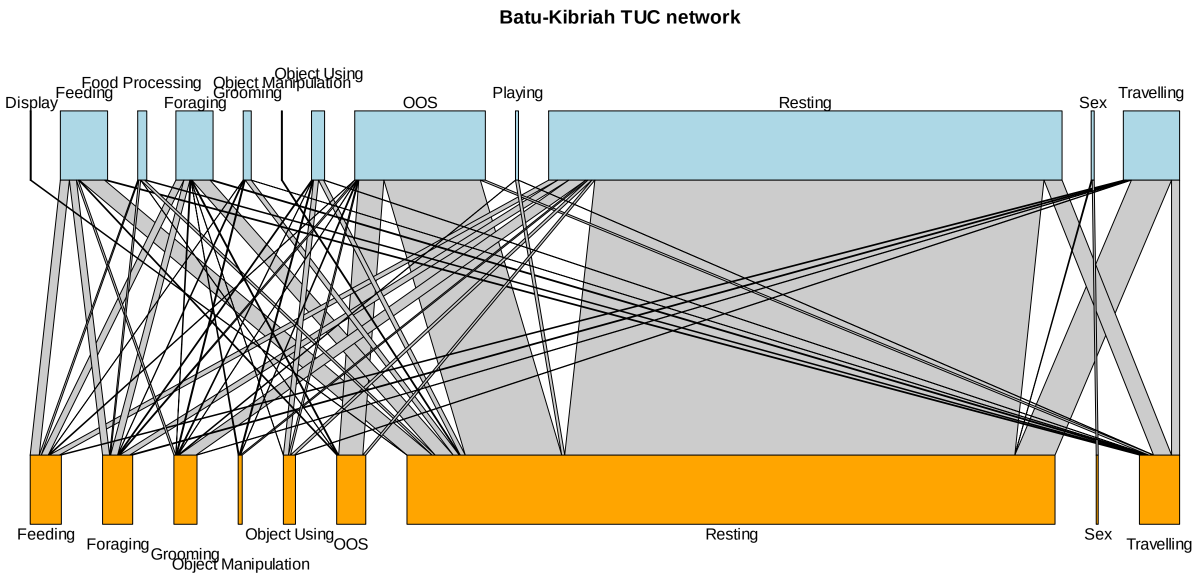


Figure 7.1 The behaviour-behaviour bipartite network summarising pair-wise patterns of behaviour for Batu (top and blue) and Kibriah (bottom and orange) over the five week study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the respective behaviour. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was performing the behaviour at the same time as the 'bottom' individual was performing the respective behaviour.

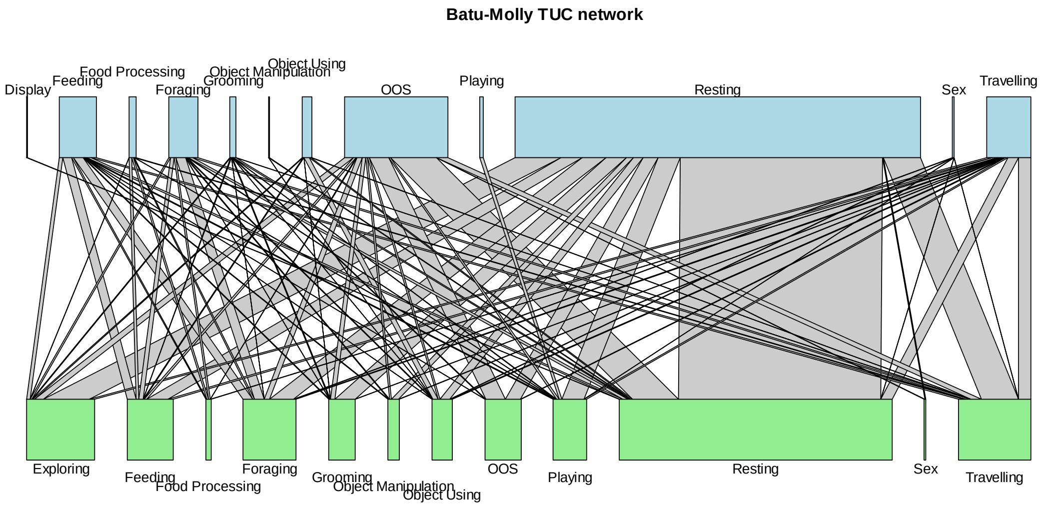


Figure 7.2 The behaviour-behaviour bipartite network summarising pair-wise patterns of behaviour for Batu (top and blue) and Molly (bottom and green) over the five week study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the respective behaviour. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was performing the behaviour at the same time as the 'bottom' individual was performing the respective behaviour.

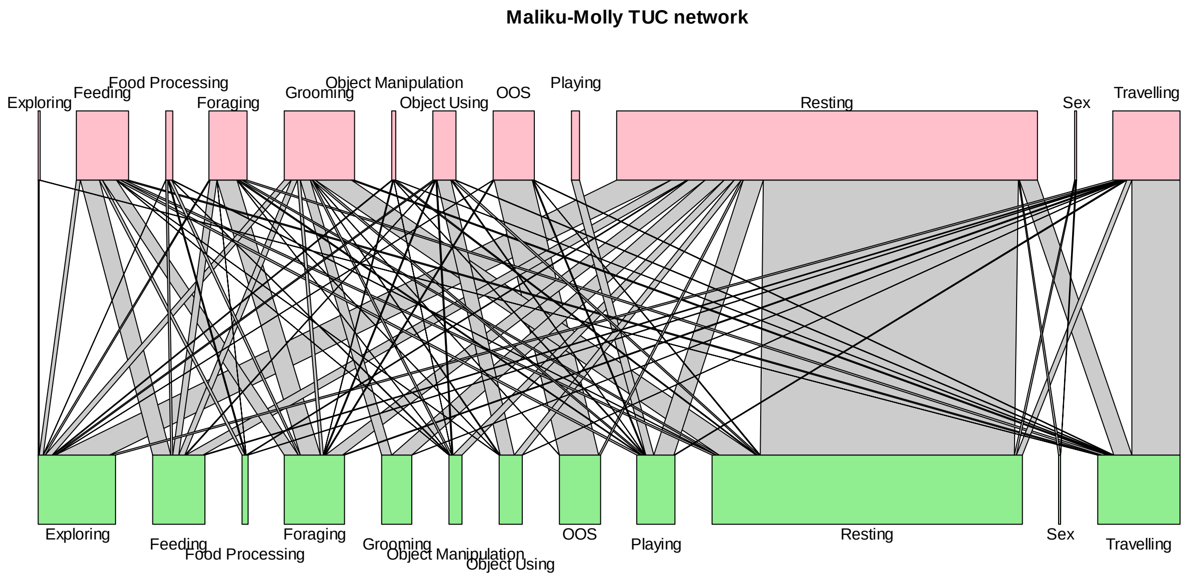


Figure 7.3 The behaviour-behaviour bipartite network summarising pair-wise patterns of behaviour for Maliku (top and pink) and Molly (bottom and green) over the five week study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the respective behaviour. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was performing the behaviour at the same time as the 'bottom' individual was performing the respective behaviour.

Figure 7.4 shows the correlation levels between five measurements of the extent of links between the two levels across each of the individual-individual BBB networks generated using data from all five weeks of the study.

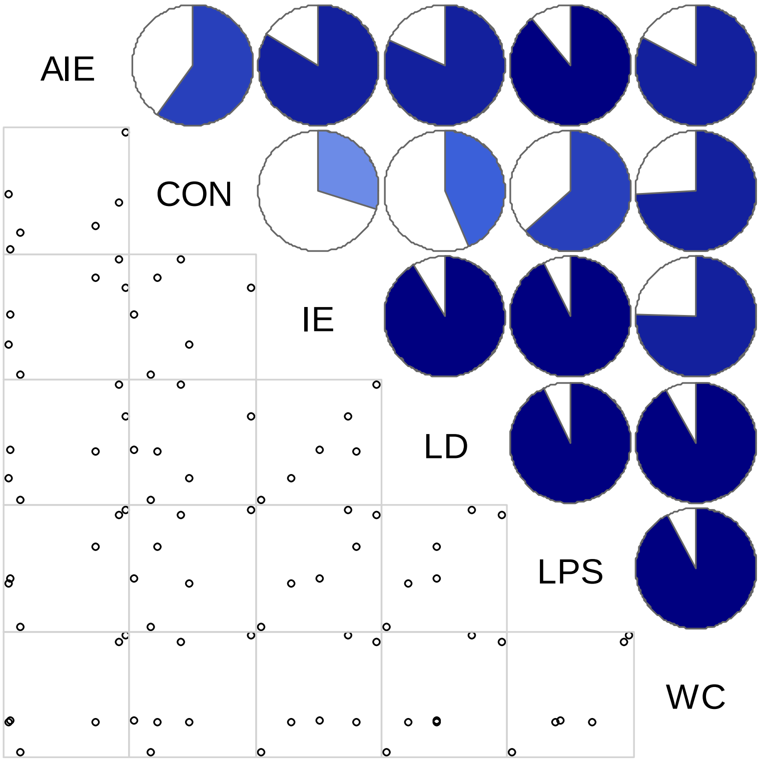


Figure 7.4 Correlation (Pearson's) coefficients of the five metrics calculated (AIE, Alatalo Interaction Evenness; CON, Connectance; IE, Interaction Evenness; LD, Linkage Density; LPS, Links Per Species; WC, Weighted Connectance) on the six behaviour-behaviour pair-wise bipartite networks summarising behaviour patterns across all five weeks of the study. The lower triangular section of the matrix shows pair-wise scatterplots of the respective measures, one point for each individual-individual based network. The upper triangular section of the matrix indicates the levels of correlation, the more extensive the blue coverage the higher the level of positive correlation between the two respective metrics (all dark blue indicates a correlation coefficient of 0.45-1, all pale blue indicated a correlation coefficient of 0.25-0.45, all light blue indicates a correlation coefficient of 0-0.15).

7.6.1.2 BBB networks summarising daily patterns of behaviour

Two sets of example BBB networks summarising daily patterns of behaviour are shown in **Figure 7.5** and **Figure 7.6**.

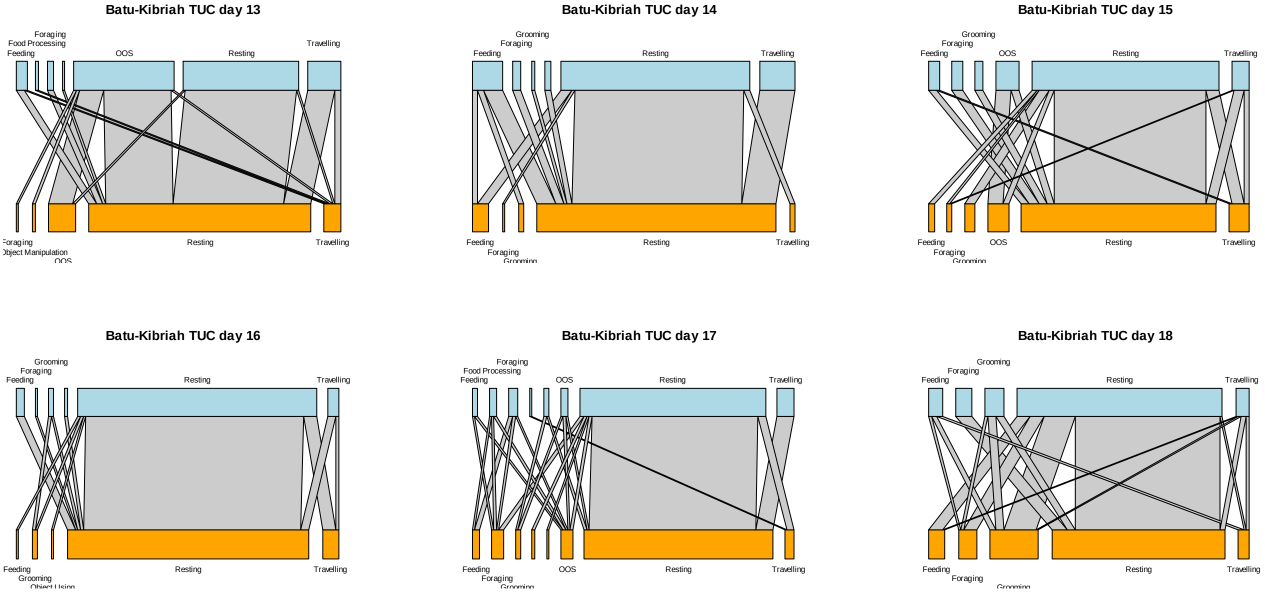


Figure 7.5 The behaviour-behaviour bipartite network summarising daily pair-wise patterns of behaviour for Batu (top and blue) and Kibriah (bottom and orange) for days 13 to 18 in the study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the respective behaviour. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was performing the behaviour at the same time as the 'bottom' individual was performing the respective behaviour.

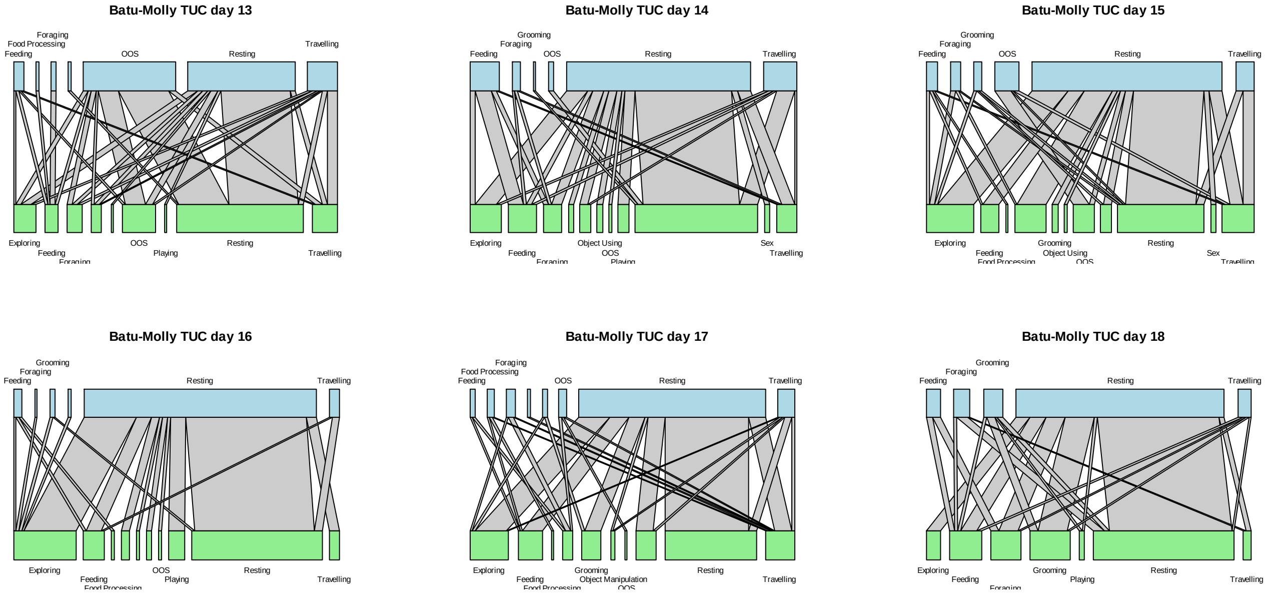


Figure 7.6 The behaviour-behaviour bipartite network summarising daily pair-wise patterns of behaviour for Batu (top and blue) and Molly (bottom and green) for days 13 to 18 in the study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the respective behaviour. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was performing the behaviour at the same time as the 'bottom' individual was performing the respective behaviour.

As the correlation of connectance with the other metrics measured was the lowest, it was thought that this measure may be highlighting different, potentially more variable, aspects of the bipartite network structures. Thus, this metric was explored in more detail as an example of how bipartite metrics can be used in animal behaviour studies. A boxplot showing the variation in values of the connectance metric calculated on each of the daily BBB networks for each of the pair-wise individual combinations is shown in **Figure 7.7**.

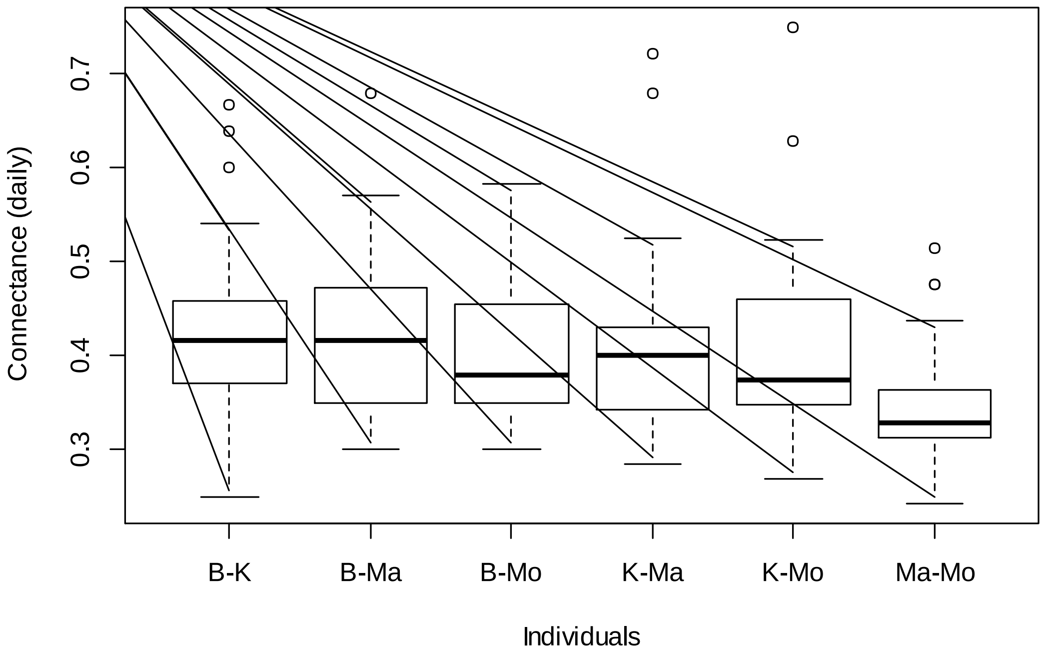


Figure 7.7 A boxplot of the connectance metric calculated on each of the daily BBB networks for each of the pair-wise individual combinations (B = Batu, K = Kibriah, Ma = Maliku, Mo = Molly).

A linear regression was conducted with daily bipartite network connectance as the dependent variable and the individual-individual combination as the independent variable. The connectance was found to be significantly affected by the individual-individual combination (F = 2.66, d.f. = 5, p = 0.025). However, this was due only to the Maliku-Molly combination having significantly lower connectance than any of the other combinations.

As an aid to searching for variation and consistency in the patterns of connectance in the pair-wise BBB networks, the connectance for each pair-wise combination was plotted over time at a daily resolution (**Figure 7.8**).

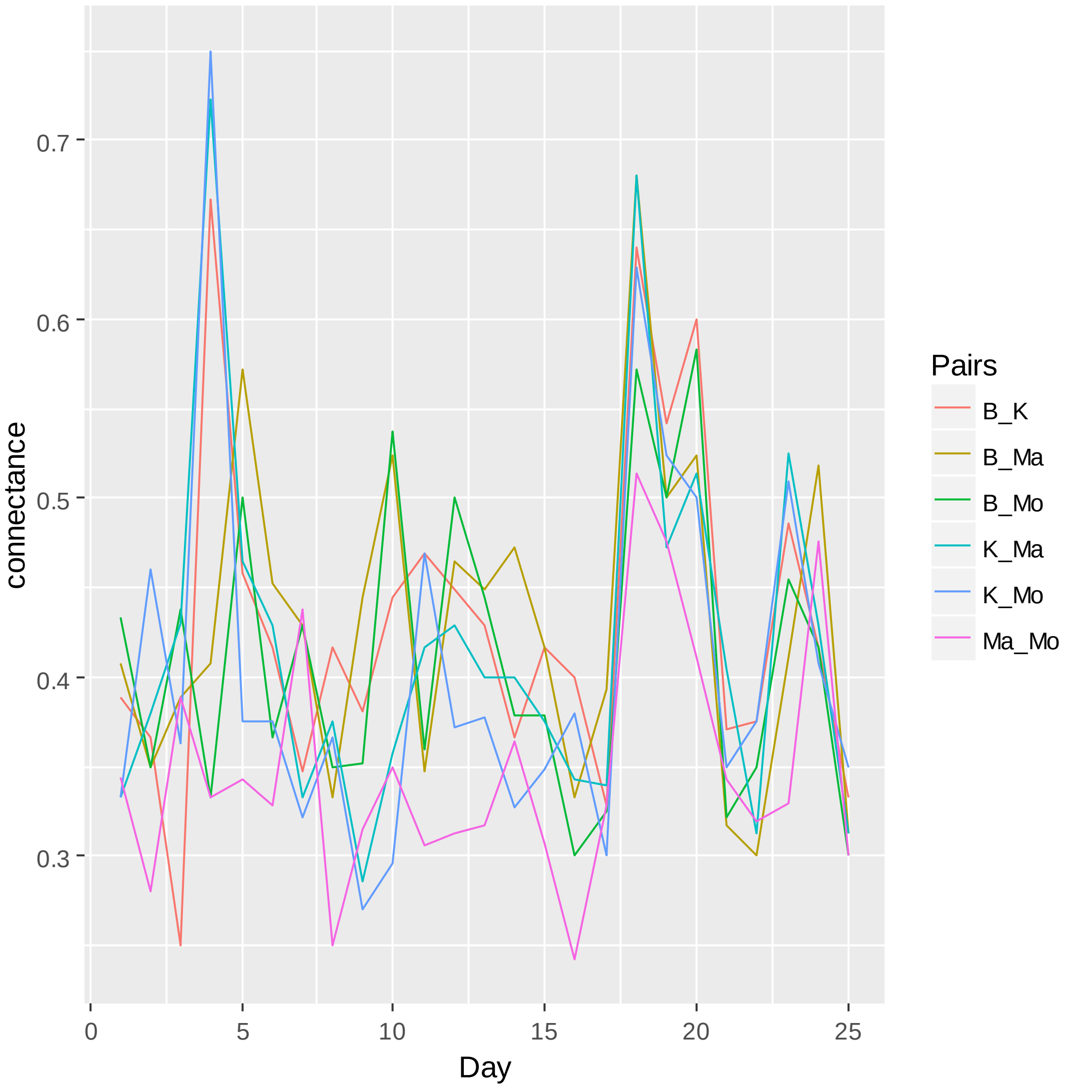


Figure 7.8 Connectance metric calculated on each of the daily BBB networks for each of the pair-wise individual combinations (B = Batu, K = Kibriah, Ma = Maliku, Mo = Molly) through time, over the period of study. For ease of visual comparison between pair-wise combinations, the time has been treated as continuous, but it should be noted that every 5 days there was actually a two day break (the weekend) when no observations were made.

As the variation in the connectance metric was found to be the largest in the metrics used to describe the bipartite networks summarising results across the whole of the study, it was regarded as potentially the most sensitive metric to changes in network structure. In order to investigate this further at a higher temporal resolution, correlation (Pearson's) levels in the values of network connectance of the daily BBB networks were calculated for each individual-individual pair. The patterns in these correlations are shown in **Figure 7.9**.

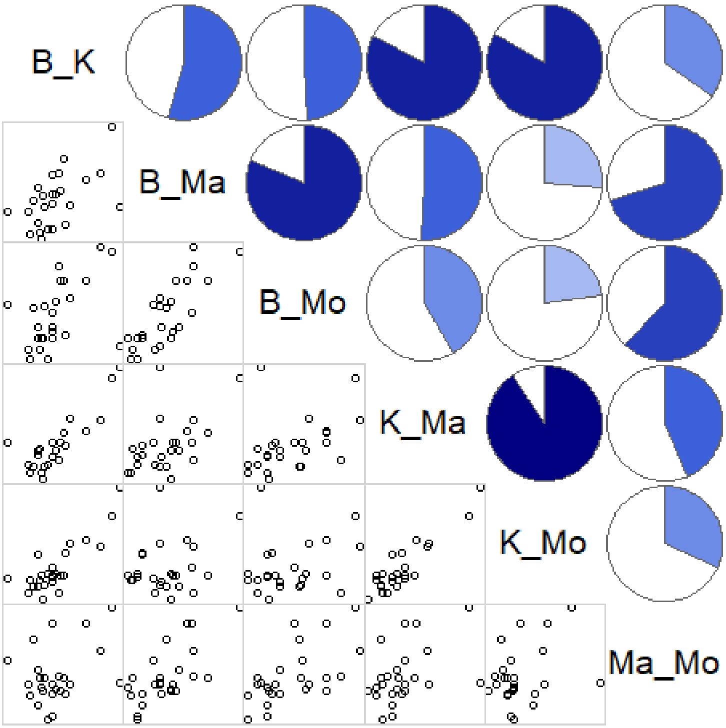


Figure 7.9 Correlation (Pearson's) coefficients for the connectance metric calculated for the behaviour-behaviour pair-wise bipartite networks summarising behaviour at a daily temporal resolution. The lower triangular section of the matrix shows pair-wise scatterplots of connectance, one point for each daily connectance value calculated for each of the two individual-individual based networks in the pair-wise comparison (B = Batu, K = Kibriah, Ma = Maliku, Mo = Molly). The upper triangular section of the matrix indicates the levels of correlation, the more extensive the blue coverage the higher the level of positive correlation between the two respective metrics (all dark blue indicates a correlation coefficient of 0.45-1, all pale blue indicates a correlation coefficient of 0.25-0.45, all light blue indicates a correlation coefficient of 0-0.15).

7.6.1.3 SSB networks summarising results across the five week study period

Two example SSB networks summarising pair-wise patterns of behaviour between the space use of two individuals over the full five weeks of the study period are shown in **Figure 7.10** and **Figure 7.11**. Edges between individuals identify zones that were occupied by the respective individuals at the same time. For example on **Figure 7.10** a heavily weighted edge exists between AP for Batu and AT for Kibriah. This illustrates that for this proportion of observations, when Batu was located at AP, Kibriah was located at AT.

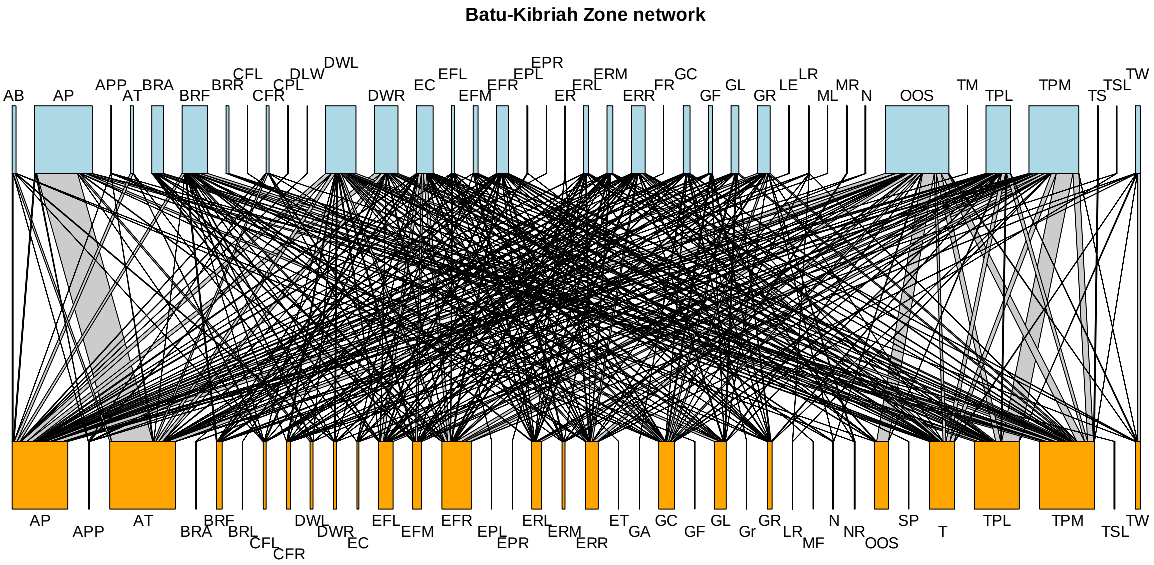


Figure 7.10 The space use - space use bipartite network summarising pair-wise patterns of behaviour for Batu (top and blue) and Kibriah (bottom and orange) over the five week study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed within the respective spatial zone. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was in the particular spatial zone at the same time as the 'bottom' individual was within the respective spatial zone.

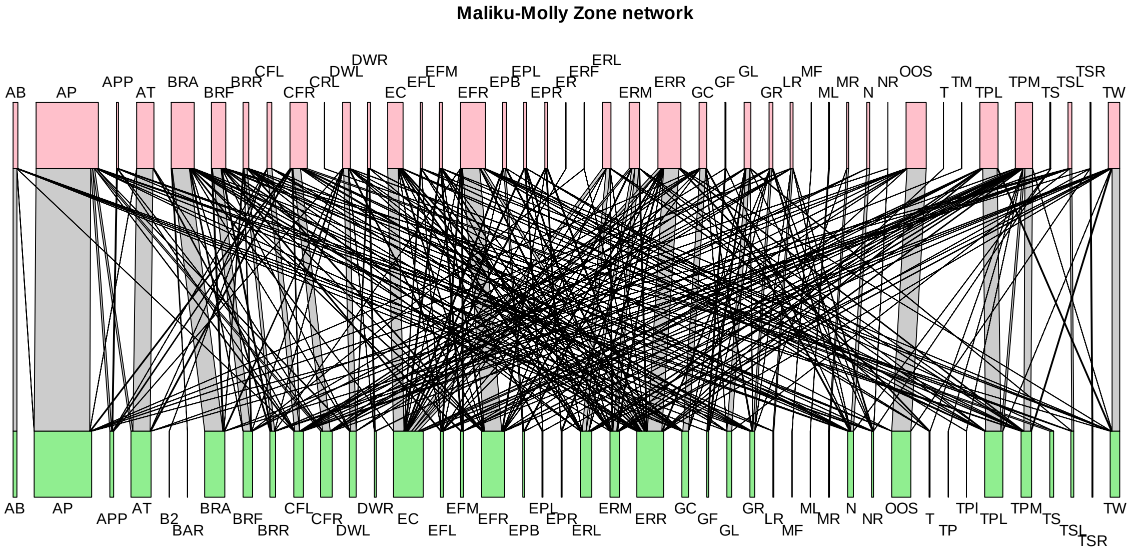


Figure 7.11 The space use - space use bipartite network summarising pair-wise patterns of behaviour for Maliku (top and pink) and Molly (bottom and green) over the five week study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed within the respective spatial zone. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was in the particular spatial zone at the same time as the 'bottom' individual was within the respective spatial zone.

7.6.1.4 SSB networks summarising daily patterns of behaviour

Two sets of example SSB networks summarising daily patterns of space use are shown in **Figure 7.12Figure 7.13**.

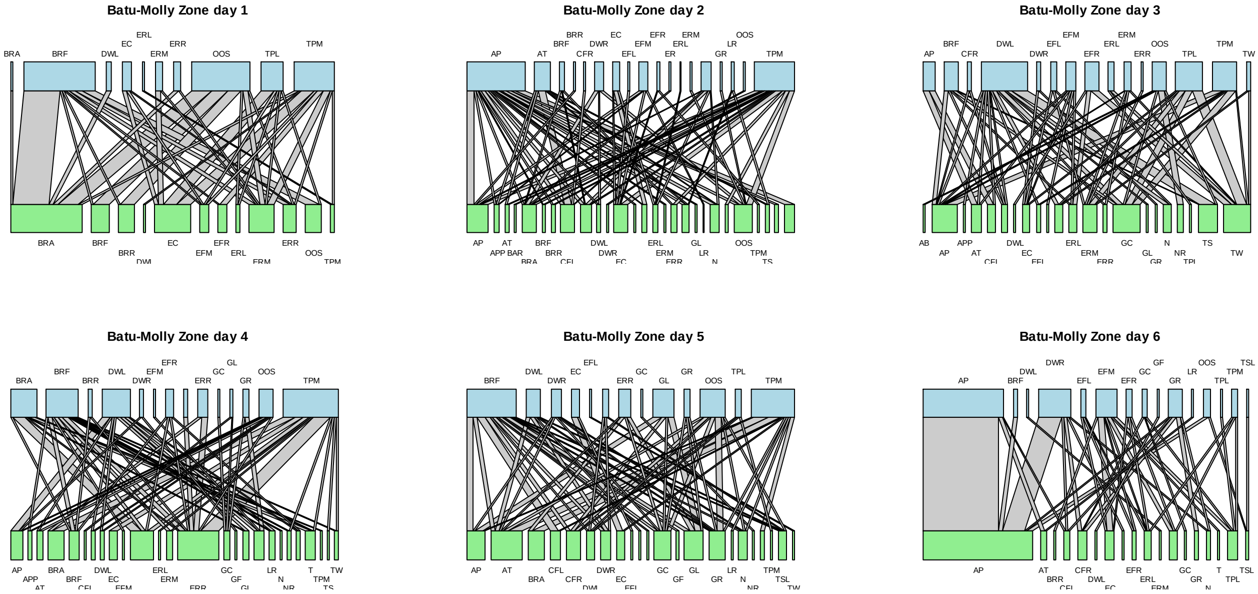


Figure 7.12 The daily space use - space use bipartite network summarising pair-wise patterns of behaviour for Batu (top and blue) and Molly (bottom and green) for days 1 to 6. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed within the respective spatial zone. The thickness of the grey links between the levels indicates the relative frequency with which the 'top' individual was in the particular spatial zone at the same time as the 'bottom' individual was within the respective spatial zone.

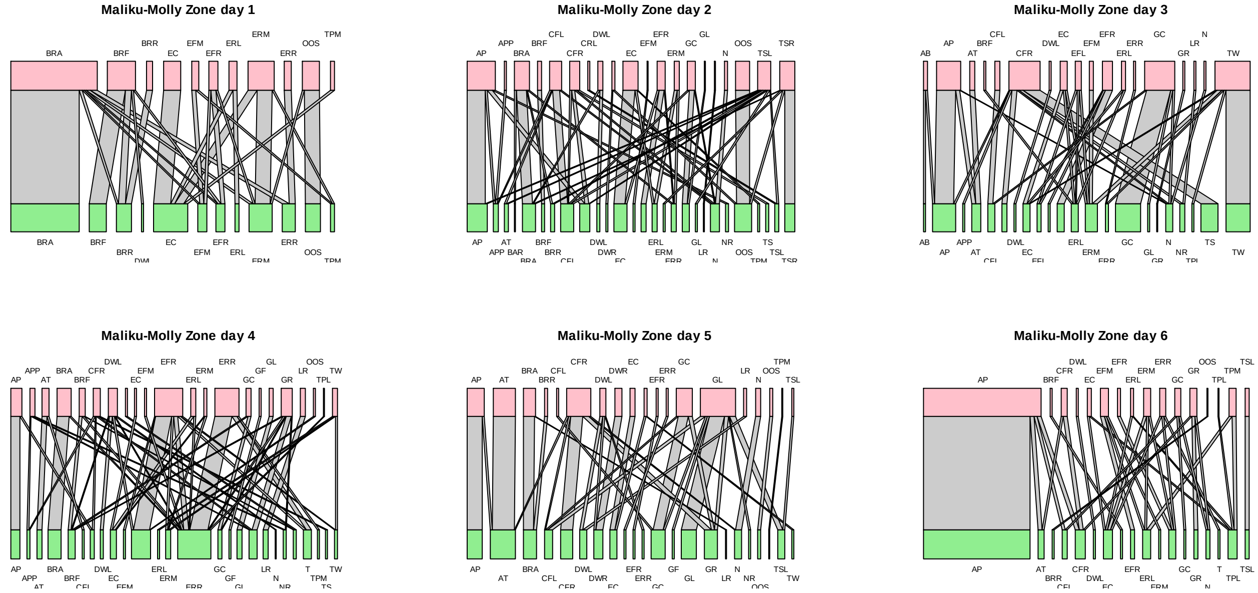


Figure 7.13 The daily space use - space use bipartite network summarising pair-wise patterns of behaviour for Maliku (top and pink) and Molly (bottom and green) for days 1 to 6. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed within the respective spatial zone. The thickness

of the grey links between the levels indicates the relative frequency with which the 'top' individual was in the particular spatial zone at the same time as the 'bottom' individual was within the respective spatial zone.

7.6.1.5 BSB networks summarising results across the five week study period

Two example BSB networks summarising patterns of behaviour in relation to space use for two individuals (Batu and Molly) over the full five weeks of the study period are shown in **Figure 7.14** and **Figure 7.15.** Edges between behaviours and zones indicate the proportion of observations during which the individual was observed displaying the behaviour in that zone. For example **Figure 7.14** illustrates that Batu rested for a large proportion of observations at AP, DWL and TPM.

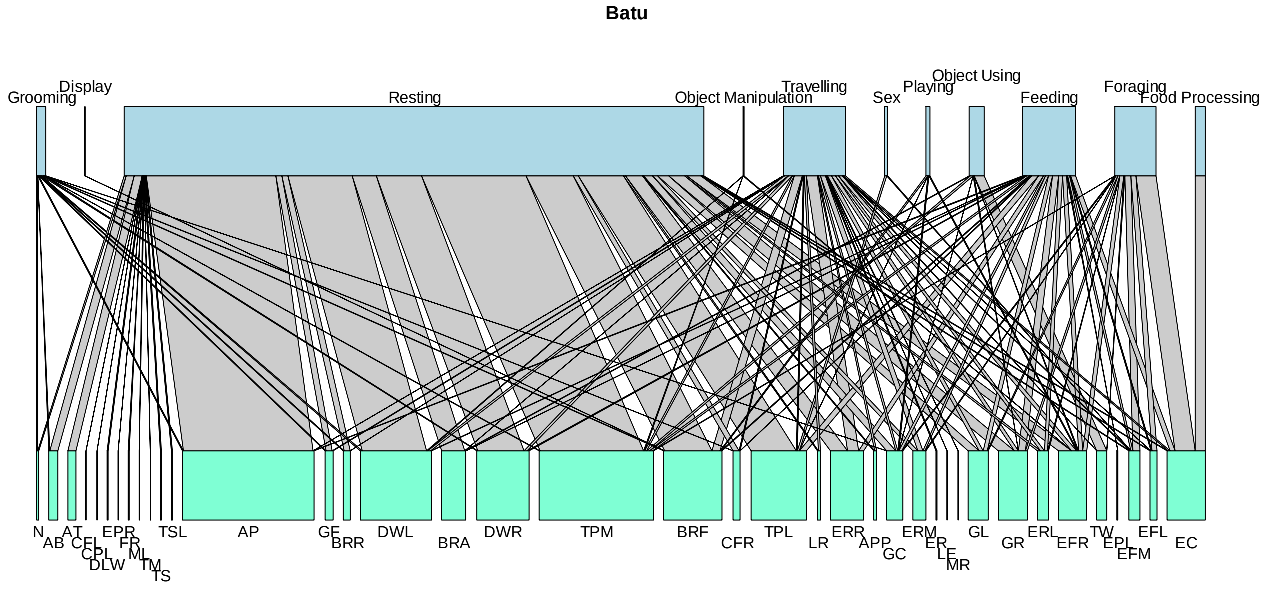


Figure 7.14 The overall behaviour - space use bipartite network summarising patterns of behaviour (top level) in relation to spatial location (bottom level) for Batu based on data from the whole of the five week study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the behaviour (top level) or located in the particular spatial zone (bottom level). The thickness of the grey links between the levels indicates the relative frequency with which the individual was exhibiting the behaviour in the particular spatial zone.

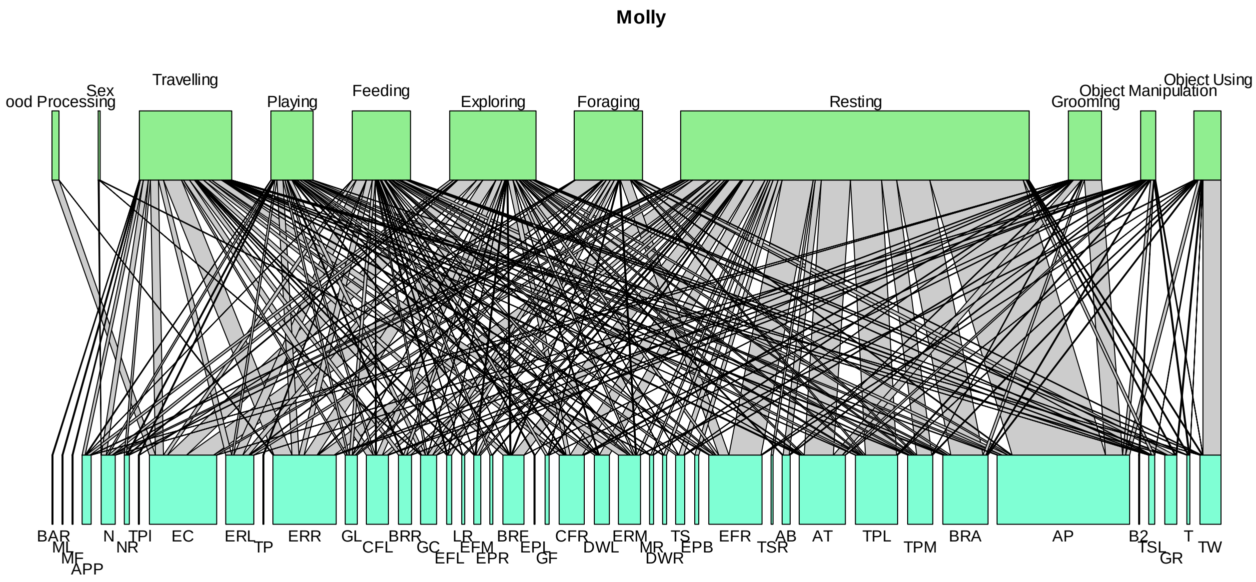


Figure 7.15 The overall behaviour - space use bipartite network summarising patterns of behaviour (top level) in relation to spatial location (bottom level) for Molly based on data from the whole of the five week study period. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the behaviour (top level) or located in the particular spatial zone (bottom level). The thickness of the grey links between the levels indicates the relative frequency with which the individual was exhibiting the behaviour in the particular spatial zone.

In order to illustrate the potential for use of bipartite network metrics, the five metrics examining patterns of links between the two levels in the networks were calculated for the BSB networks generated for each orangutan individual. The patterns of correlations (Pearson's) between the metric values calculated for these four bipartite networks are shown in **Figure 7.16**.

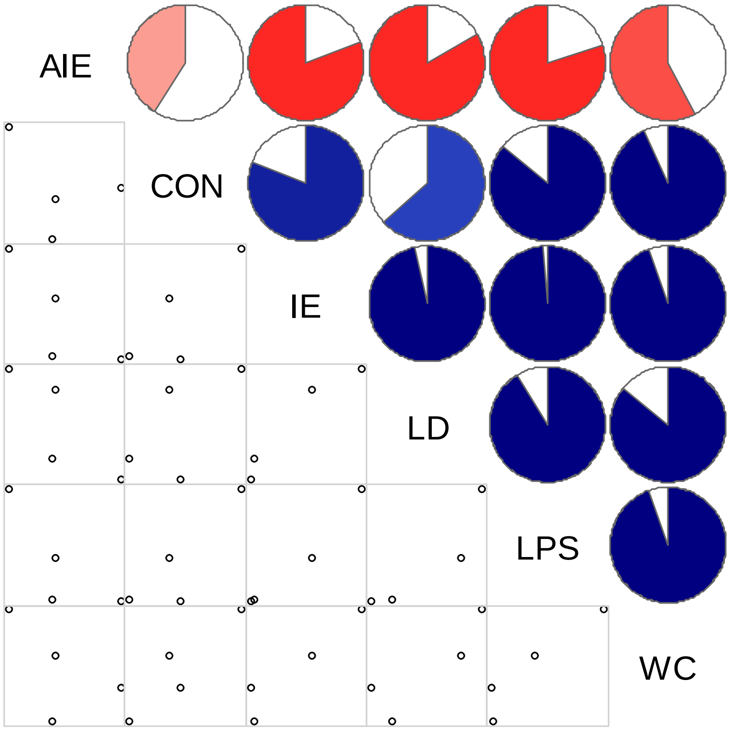


Figure 7.16 Correlation (Pearson's) coefficients of the five metrics calculated (AIE, Alatalo Interaction Evenness; CON, Connectance; IE, Interaction Evenness; LD, Linkage Density; LPS, Links Per Species; WC, Weighted Connectance) on the four behaviour - space-use bipartite networks summarising behaviour patterns across all five weeks of the study for each of the four individuals. The lower triangular section of the matrix shows pair-wise scatterplots of the respective measures, one point for the network of each individual. The upper triangular section of the matrix indicates the levels of correlation, the more extensive the blue coverage the higher the level of positive correlation between the two respective metrics (all dark blue indicates a correlation coefficient of 0.45-1, all pale blue indicates a correlation coefficient of 0.25-0.45, all light blue indicates a correlation coefficient of 0-0.15). Red indicates negative correlation (all dark red indicates a correlation coefficient of $^-$0.45 -$^-$1, all pale red indicated a correlation coefficient of $^-$0.25-$^-$0.45. all light red indicates a correlation coefficient of 0-$^-$0.15).

7.6.1.6 BSB networks summarising results on a daily basis

Two example sets of BSB networks summarising patterns of behaviour in relation to space use for two individuals (Batu and Molly) on a daily basis are shown in **Figure 7.17** and **Figure *7.18***.

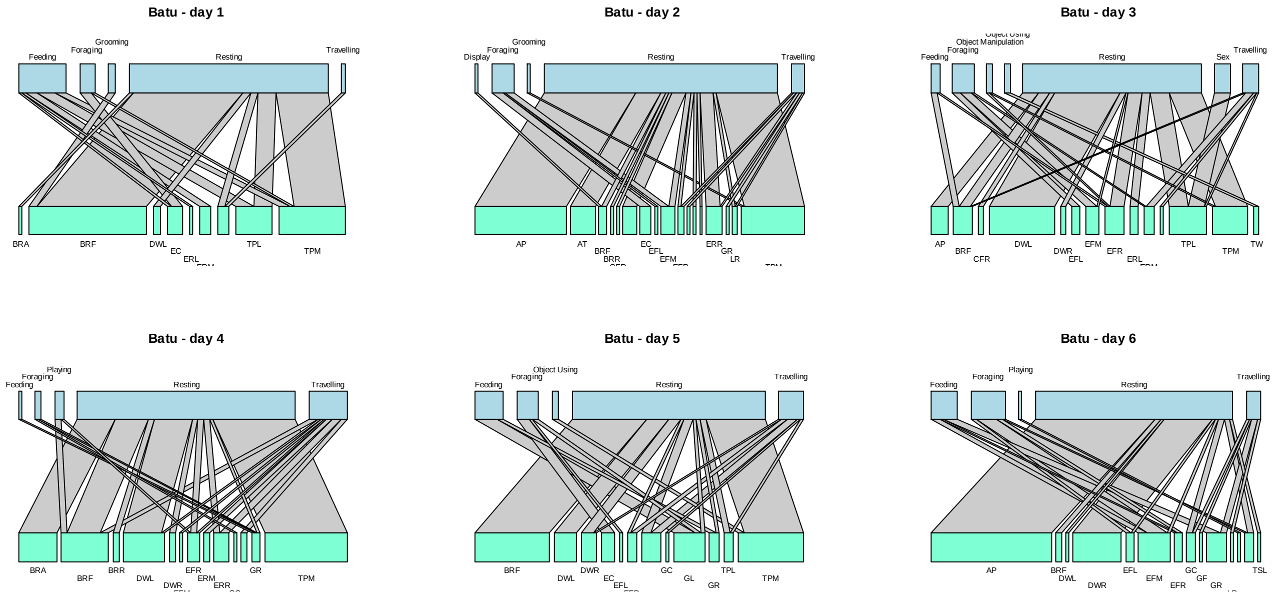


Figure 7.17 The daily (day 1 to 6) behaviour - space use bipartite network summarising patterns of behaviour (top level) in relation to spatial location (bottom level) for Batu based on data at a daily temporal resolution. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the behaviour (top level) or located in the particular spatial zone (bottom level). The thickness of the grey links between the levels indicates the relative frequency with which the individual was exhibiting the behaviour in the particular spatial zone.

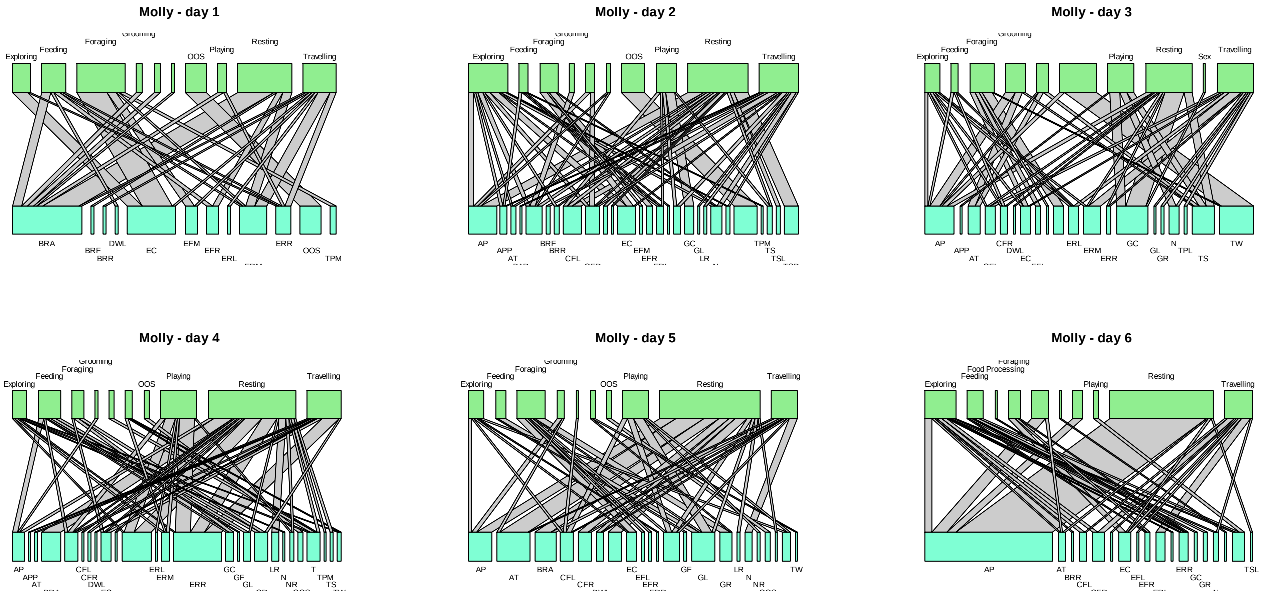


Figure 7.18 The daily (day 1 to 6) behaviour - space use bipartite network summarising patterns of behaviour (top level) in relation to spatial location (bottom level) for Molly based on data at a daily temporal resolution. The horizontal extent of the boxes on each level indicates the relative frequency with which the individual was observed exhibiting the behaviour (top level) or located in the particular spatial zone (bottom level). The thickness of the grey links between the levels indicates the relative frequency with which the individual was exhibiting the behaviour in the particular spatial zone.

As an aid to searching for variation and consistency in the patterns of connectance in the pair-wise BSB networks and as a comparison of these patterns to those found within the BBB networks (**Figure 7.18**), the daily connectance in the BSB network for each individual was plotted over time (**Figure 7.19**).

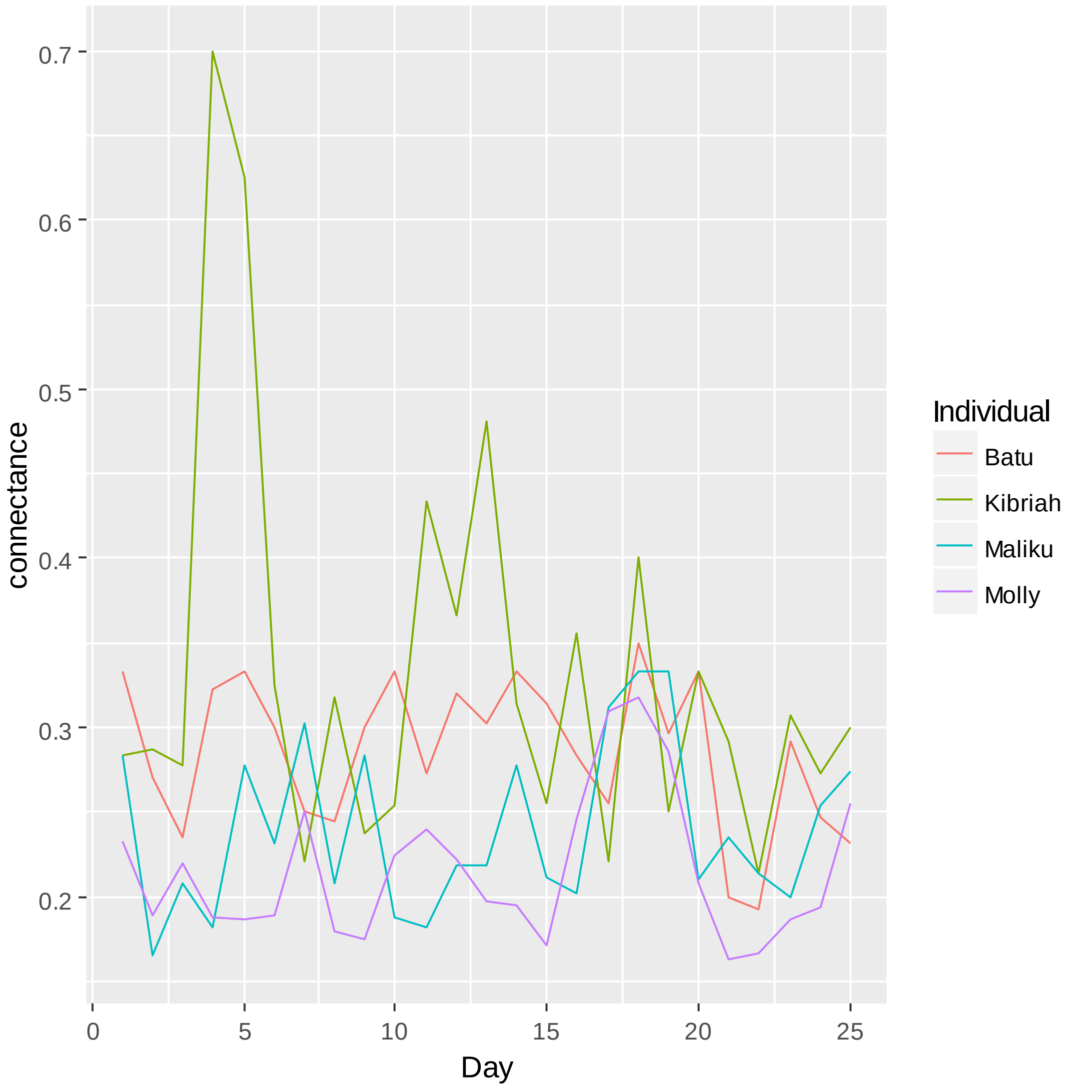


Figure 7.19 A plot of the connectance metric calculated on each of the daily BSB networks for each of the individual orangutans (B = Batu, K = Kibriah, Ma = Maliku, Mo = Molly) through time, over the period of study. For ease of visual comparison between individuals, the time has been treated as continuous, but it should be noted that every 5 days there was actually a two day break (the weekend) when no observations were made.

To assess the level of similarity in temporal patterns of connectance over time between individuals, Pearson's correlation coefficients were calculated for each individual-individual comparison. Results are shown in **Figure 7.20**.

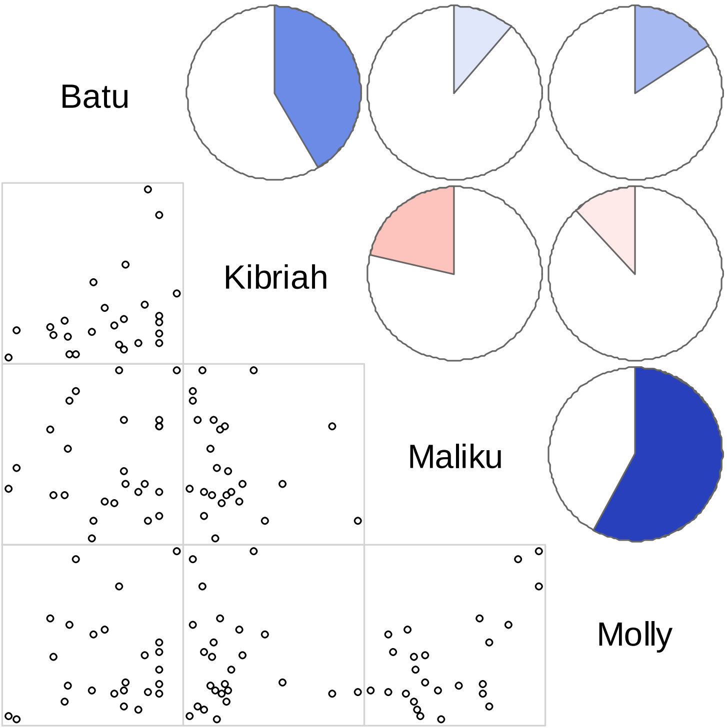


Figure 7.20 Correlation (Pearson's) between the daily connectance of the BSB networks of each of the individual - individual pair-wise comparisons . The lower triangular section of the matrix shows pair-wise scatterplots of the respective measures, one point for the daily network of each individual. The upper triangular section of the matrix indicates the levels of correlation, the more extensive the blue coverage the higher the level of positive correlation between the two respective metrics (all dark blue indicates a correlation coefficient of 0.45-1, all pale blue indicates a correlation coefficient of 0.25-0.45, all light blue indicates a correlation coefficient of 0.1-0.15, all pastel blue indicates a correlation coefficient of 0-0.1). Red indicates negative correlation (all dark red indicates a correlation coefficient of $^-$0.45 -$^-$1, all pale red indicated a correlation coefficient of $^-$0.25-$^-$0.45. all light red indicates a correlation coefficient of $^-$0.1-$^-$0.15, all pastel red indicates a correlation coefficient of 0-$^-$0.1).

7.7 Discussion

In the previous two chapters the use of network analysis for studying animal behaviour in a wider context than social networks was demonstrated using behaviour and space use data from observations of the captive orangutans in Twycross Zoo. The utility of such approaches, both in visual and quantitative form, was demonstrated pointing to substantial potential for the use of such methods in future behaviour studies. Much of what these network approaches can add to current behaviour study methods lies in the way that they allow the dynamics of patterns of behaviour to be studied in addition to overall summaries of frequencies of behaviour. In this chapter, the use of networks was extended through the use of bipartite networks. These supplement the previous work by explicitly examining the relationships between two sets of information. Rather than examining patterns within a set of data, they allow the examination of patterns between sets of data, e.g. behaviour of an individual and its spatial location. This framework has been utilised in biodiversity and ecological community studies to great effect (Dormann *et al.*, 2008; Greslin *et al.*, 2013), and the work in this chapter argues that this could also be true for future studies of animal behaviour. Thus, bipartite networks allow for the visualisation and quantification of patterns of the relationships between two sets of behaviour data, extending the potential depth and complexity of behaviour data analysis. Below, the strengths and limitations of the use of bipartite networks in the analysis of behaviour data are discussed in light of the results obtained from their use in understanding the behaviour and space use data collected from observations of the orangutans at Twycross Zoo.

7.7.1 An overview of the application of bipartite networks to the orangutan behaviour and space use data, and implications for other studies

One of the powerful advantages of bipartite networks is their ability to visually represent in an intuitive way, the complexities of how two sets of data relate to each other. Effectively, in a BBB network the boxes in the two levels provide the proportion of the behaviour of the two individuals (e.g. **Figure 7.1**), with the links providing a further depth to this representation by showing how the two sets of behaviour are related to each other. Therefore, they provide a very powerful tool for visually representing behaviour data. This is extended by the flexibility of being able to combine different sets of behaviour data, space use data, and combinations of the two. Thus, in **Figure 7.1** it was easy to see that Batu and Kibriah both spent a large proportion of their time resting and that they tend to do this at the same time. The bipartite plot in **Figure 7.1** also demonstrates an asymmetry between the individuals in that each tended to exhibit travelling behaviour when the other was resting, with a small proportion of time when they were both travelling at the same time. Similarly in **Figure 7.2**, the bipartite network showed that Molly did the majority of her resting when Batu was resting, but she also displayed a large range of behaviours when Batu was resting. Noticeably she also tended to not rest when Batu was travelling. These examples illustrate the ability of bipartite networks to highlight how patterns of behaviour in two individuals are related. Thus they can be used by zoo staff to explore how the behaviour of one individual starts to impact on the behaviour of other individuals. This is illustrated very clearly in **Figure 7.3** in the BBB network for Maliku and Molly. The structure in this network is very different to that of the previous two in that there are clear 'parallel' behaviours being exhibited, in that Maliku and Molly were often performing the same behaviour at the same time. In this case it reflects that Maliku is Molly's mother and that Molly is not yet independent of her mother. However, the different structures in the networks shown here could also be used to indicate other aspects of behaviour. For example in a study involving larger groups, similarity in the timing of behaviours between individuals could help to identify subgroups of individuals, and extensive asymmetry in these networks could indicate avoidance (or response to) of other individuals. This information along with the biological interpretation may be an invaluable tool for zoo staff when making decisions around group housing. Understanding the effect individuals have on one another will help determine which animals should or should not be housed together, the length of time mothers and infants should remain housed together and can be shared with other institutions to instruct them on similar grouping decisions.

The ability to produce bipartite networks over different time periods (i.e. different temporal resolutions) has the same advantages as for the behavioural and space use networks discussed in the previous two chapters. For example, comparisons of daily networks can show how consistent the patterns in the networks based on the complete data set are. For example, in **Figure 7.5** the BBB network shows that both Batu and Kibriah spent extensive amounts of time resting. However, there were then days (e.g. day 18 in **Figure 7.5**) when Kibriah spent significantly more time displaying other behaviours whilst Batu remained resting. Similarly, in **Figure 7.6**, it is clear that the overall picture of Molly displaying many behaviours whilst Batu generally spent time resting (**Figure 7.2**), is a good overall representation, but there is extensive variation in distributions of the sets of behaviours exhibited by Molly on a day to day basis. The ability to detect departures from 'normal' behaviours using networks at greater temporal resolution can potentially help to pinpoint causal factors for these changes. In addition, the BBB bipartite networks would allow zoo staff to quantify the form of these changes in behaviour and potentially assess the levels to which different individuals were affected. This could be a powerful analytical framework for studies of enrichment, as not only could changes in individual behaviours be assessed, changes in patterns of behaviours between individuals could also be quantified. This could of course be extended to the SSB and the BSB networks as well.

Caution must be used when interpreting bipartite network metrics as is discussed in the section that follows. However, as an example of utilising such metrics the connectance values for the different BBB networks were calculated on a daily basis (**Figure 7.6**) and plotted through time (**Figure 7.7**). Both these figures highlight the extent of variation in network connectance at a daily resolution. Importantly, however, **Figure 7.7** shows that the changes through time for the different individual-individual pairings had periods where there was little correlation between the pairings, and periods when the correlation was strong, suggesting that the group dynamics change over periods of a few days. The wide range of values calculated for connectance suggests that such metrics are sensitive enough to be able to detect differences in network structure. However, as is discussed in the following section, understanding how the metrics change with network structure is at an early stage and much work needs to be done before these metrics can be reliably used and interpreted within the context of welfare and behaviour studies.

Similar advantages gained in the use of BBB networks were gained using BSB and SSB networks for the orangutan behaviour and space use data. For example, in **Figure 7.10**, it was clear that Batu and Kibriah had little overlap in their spatial use of the enclosure, and there were many links in the SSB network. However, it was noticeable that Batu spent some time in section AP, and when he did Kibriah tended to be in section AT. To a lesser extent, this pattern was also found when Batu was in zone TPM, Kibriah tended to be in zone TPL. The SSB networks can also be used to detect patterns in spatial associations between individuals. Batu and Kibriah did not tend to be in the same location together, but looking at the SSB network for Maliku and Molly (**Figure 7.11**), it is clear that they spent extensive periods of time in the same zone. In addition, looking at the SSB networks at a daily temporal resolution indicates both general consistencies in space use patterns, but also that there is clear variation from day to day. For example the SSBs for Batu and Molly over a six day period (**Figure 7.12**) showed little general overlap in space use between the two. However, on day 6 they spent substantial proportions of time together in zone AP. Identifying areas within an enclosure where individuals are comfortable being together and those where they are not could allow zoo staff to identify characteristics of the spatial locations leading to these differences. This leads to the potential for using these networks to identify areas within an enclosure which could be altered to allow for individuals to more readily mix and potentially to reduce levels of tension between individuals. The BSB networks would support this work as they would identify zones where behaviours linked to low and high welfare are displayed. As an example, the BSB of Batu

(**Figure 7.14**) showed that although Batu spent a significant amount of time resting, he did this in a large number of zones, suggesting that he was comfortable in many locations within the enclosure, in turn suggesting high welfare levels. Molly's BSB (**Figure 7.15**) shows a more even spread in time spent in different behaviours, and each of these behaviours were displayed in a wide range of locations. Again, as none of these behaviours demonstrated high stress or poor welfare, it suggests that Molly was comfortable in a wide number of zones within the enclosure. The daily BSB plots are also informative (e.g. **Figure 7.17** and **Figure 7.18**) showing smaller subsets of behaviour being exhibited within a day than over the study period, but still showing a marked difference between Batu and Molly, with Molly showing more behaviours and greater use of the space within the enclosure on a daily basis. Although no abnormal or stereotypic behaviours were observed during this study, should a captive group raise concerns, the bipartite networks may be implemented to analyse the specific areas of enclosure in which abnormal or stereotypic behaviours take place. It may be that peripheral areas, close to viewing crowds are heavily associated with pacing behaviour, for example. Identifying and being able to quantify this relationship over time, means that zoo managers cannot only implement strategies to reduce such behaviours but also monitor the effects of the intervention on both behavioural and space use patterns in the short and long term.

Thus the example application of bipartite networks to the behaviour and space use data for the orangutans collected over the five week period has highlighted many of the potential uses of these networks for animal behaviour and welfare studies. Below the use of network metrics is discussed and then the current limitations of the use of bipartite plots in behaviour studies are addressed.

7.7.2 Bipartite network metrics

The use of bipartite networks in biological research is at an early stage and increasing, but has primarily been utilised in studies of plant-pollinator networks (e.g. Dormann, 2011; Greslin *et al.*, 2013). Despite the early stage of this research, the ability to visualise and calculate quantitative measures of bipartite network structure is substantial through the use of the bipartite package (Dormann *et al.*, 2008) for the R statistical software (R Core Team, 2016). Within the bipartite package, there are at least 46 basic metrics which can be calculated for a bipartite network (Dormann *et al.*, 2008). However, as is acknowledged in the documentation for this package (Dormann *et al.*, 2008) and elsewhere (Dormann, 2011), the interpretation of these metrics is little understood, and indeed some of the algorithms for calculating the metrics vary between implementations (Dormann *et al.*, 2008). Although the assessment of network metrics for use in ecological research is beginning to gain momentum (Dormann, 2011; Borrett & Lau, 2014; Leger *et al.*, 2015; Strona & Veech, 2015), such assessment has been heavily biased towards research in social networks and is at an early stage in other ecological areas, particularly in relation to bipartite networks. An example which highlights the need for such work is the use of the metric 'interaction strength asymmetry'. This was proposed by Bascompte *et al.* (2006) as a measure of the asymmetry in interaction strength between species of pollinators and plants, and proved instrumental in developing theory to understand mechanisms of biodiversity maintenance. However, more recently this measure has been critically reviewed by Blüthgen (2010) and modifications have been suggested. Such potential pitfalls in robustly interpretation network metrics are likely to be more frequent when utilising networks in new areas of biology and calculating metrics which have been developed in other areas of biological research. Thus, although in this chapter the use of bipartite networks in animal behaviour studies indicates significant potential in supporting a more extensive understanding of animal behaviour and space use patterns, caution should be applied in the use of network metrics before extensive testing and assessment of these metrics has been carried out.

As an example of utilising network metrics within a statistical framework, linear regression analysis was implemented using the daily connectance values for the BBB networks. However, such utilisation of network metrics needs to be undertaken carefully due to the inherent lack of independence in network data (Croft *et al*., 2008), which is a basic assumption of many statistical frameworks including general linear models (Zuur *et al.*, 2007). By their very nature networks involve information which is connected and therefore not independent. Bipartite networks present an added complication in the form of additional lack of independence in that they include information on the links between two sets of data. Finally, there is also a potential lack of independence if metrics are calculated on networks developing over time, as the structure of a network on t+1 is likely to be dependent on the structure of the network at time t. To address the lack of independence within a network, randomisation methods have been developed for social networks (Croft *et al*., 2008; Croft *et al.*, 2011; Farine, 2017). However, these are not easy methods to implement and usually require bespoke programming as they need to be tailored to the structure of the data collected and the specific questions being asked (Farine, 2017). In addition these randomisation methods are designed to assess whether single networks support or reject the null hypothesis and work on the basis of randomising particular components of the single network. However, most of the interest in the use of bipartite networks will be in the comparison of two or more networks, making it difficult to see how these randomisation methods can be extended to use with bipartite networks. The Mantel test (Mantel, 1967) has been used to compare networks (Croft *et al.*, 2008). However, recently its reliability has been questioned (Guillot *et al.*, 2013; Legendre *et al.*, 2015). In addition, the Mantel test requires the two matrices representing the two networks to be of equal size. With bipartite networks this is often not the case due to different individuals exhibiting different ranges of behaviour or space use. There are therefore strong challenges ahead in developing robust statistical frameworks to compare bipartite networks. However, recent work on the analysis of temporal networks (Blonder *et al.*, 2012) holds much promise as temporal networks present similar problems in analysis to bipartite networks.

7.7.3 Limitations of the use of bipartite networks in animal behaviour studies

A key strength of the behaviour and space use networks introduced in the previous two chapters is the ability to extend the examination of patterns of behaviour beyond analysis of frequencies of behaviour to include the patterns in the dynamics of behaviour e.g. the patterns in sequences of behaviour exhibited by individuals. One of the key strengths of the bipartite networks is their ability to examine the patterns of links between two sets of behaviour data. However, bipartite networks do not currently allow the advantages of these two types of behaviour to be combined, i.e. incorporating both the dynamics within the two layers of behaviour data as well as the patterns in the relationships between the two layers. Thus a thick link in a BSB network indicates that the individual was often observed performing the particular behaviour in the associated spatial location. However, it gives no information about whether the individual spent long periods performing the behaviour, or performed the behaviour for relatively short periods but very frequently. Currently, in order to examine dynamic patterns in behaviour through time and the relationships between two layers of behaviour data, the behaviour and space use networks (previous two chapters) would have to be used in conjunction with each other.

As bipartite networks examine the relationship between two sets of data, the amount of data required to construct them will tend to be larger; two datasets rather than one need to be collected. In addition, this ability to link patterns between layers of data, should require us to provide greater amounts of data for each layer than in normal behaviour studies. The reason for this is a statistical one. As the outcome is quantification of relationships between two sets of data, this is combining uncertainty in two sets of data, requiring a larger amount of data to counteract this increase decrease in confidence. This requirement for larger datasets will be more pronounced when working with large groups of individuals, or individuals who are very active and dynamic, or where individuals are often out of sight. This is because all of these circumstances will increase the uncertainty in the relationship between the sampled behaviour patterns and the true patterns.

For the bipartite networks developed in this chapter, the two sets of data need to be collected simultaneously as units in both layers (e.g. behaviour i and location j) are matched by occurring at the same time point. One of the reasons that the orangutans at Twycross were used to provide test data for this chapter was that there were only four individuals, they tend not to move rapidly and the enclosure provides extensive observer visibility. Thus it was relatively easy to record the necessary data. However, in studies where these conditions do not hold the collection of data for each level (e.g. behaviour and space use) will be more difficult in itself, but the simultaneous collection will be even more difficult. Within a captive environment, the use of multiple remote cameras could provide an answer to these difficulties. The quality of images are constantly improving and the cost of wireless cameras is consistently decreasing, with the main limitation currently being the battery life. These problems of data collection for bipartite networks will be exacerbated when collecting data in the field, and although the use of remote cameras is likely to help, it is unlikely to help to the extent as when studying captive animals. To the best of my knowledge, the potential impact on the generation and interpretation of bipartite networks of reduced amounts of data has not been investigated, but it will need to be addressed if bipartite networks are going to be more widely used in the study of animal behaviour.

The majority of the current key metrics of bipartite networks focus on the links between the two layers (Dormann *et al.*, 2008). The patterns and strengths of these links will, in part, be determined by the resolution (number of units) in the two layers. As the resolution in the two layers increases the number of possible links increases, as the number of possible links is simply the number of units in the top layer multiplied by the number of units in the bottom layer. Thus the chosen resolutions of these layers will directly affect many of the metrics which can be calculated for bipartite networks (e.g. linkage density and links per species) as well as indirectly affecting others (e.g. connectance) which utilise the number of possible links in their calculations. This makes comparisons of metrics between studies which have observed different species or different environments difficult as the different studies are likely to have varying numbers of behaviours and spatial locations.

These difficulties also extend to the comparison of metrics between individuals within the same study. For example, in the current study, Kibriah demonstrated a smaller set of behaviours than the other individuals, particularly Molly. This directly reduces the total number of possible links in bipartite networks involving Kibriah, but potentially increases the density of links as there are fewer degrees of freedom in terms of where the links can occur. It can be argued that these differences and the resulting constraints on the structure of the bipartite network are a reflection of reality; if an individual performs few behaviours this does limit the number of possible links in a BBB network, and this should be reflected by altering the network metrics. This is a reasonable argument, but currently there is a lack of understanding of how the differences in numbers of behaviours between individuals maps through to the calculation of the various bipartite network metrics, e.g. linearly compared with non-linearly. These difficulties emphasise the need for careful thought at the experimental design stage about how behaviours and space use should be categorised. There is potential to conduct a form of sensitivity analysis in this respect by reducing the resolution of data recording (i.e. amalgamating behaviour or space use categories), and recalculating the network metrics to determine the impact of data resolution. These problems are also exacerbated by the fact that detailed understanding of the bipartite network metrics are at an early stage and there has already been marked misunderstandings of some of their behaviours (Dormann, 2008; Blüthgen, 2010).

Although there are substantial difficulties ahead in understanding and interpreting the values of the various bipartite network metrics, it should be noted that there is rapidly increasing interest in these networks and this includes both the development of new metrics and research into the behaviour of the current metrics (Blüthgen, 2010; Borrett & Lau, 2014; Podani *et al.*, 2014). Whilst sophisticated statistical analysis is not a skill that is usually desired of zoo staff, it is known that many zoos recruit degree students to conduct research and answer questions regarding their specimens. Therefore, as the understanding of network analysis and the sophistication of data analysis develops, students at post- or undergraduate level may be able to assist in processing the data and producing networks like those presented here.

7.8 Conclusions

This chapter has demonstrated how the use of networks in animal behaviour studies can be greatly extended by using bipartite networks to visualise and quantify patterns between two sets of data. Three forms of bipartite network were constructed: individual behaviour - individual behaviour (BBB), individual behaviour - individual space-use (BSB), and individual space-use - individual space-use (SSB) networks. Examples of networks were illustrated and the utility and limitations of calculating network metrics and using them in statistical analyses were explored and discussed. In conclusion, bipartite networks offer substantial potential for gaining deeper insights into animal behaviour, particularly in captive environments. The visual outputs may be interpreted with little prior knowledge of their construction, allowing zoo staff to identify inter-individual interactions as well as behaviour-space-use relationships. Ultimately these can aid planning decisions around animal management and enclosure design. Currently their utility is biased towards their use as a visual tool rather than as a statistical tool. This is because although there is an extensive range of metrics for quantifying the structure of bipartite networks, the behaviour of these metrics has not been widely explored. In addition there are several difficulties in developing robust statistical frameworks for comparing these metrics due to the inherent lack of independence in the networks, and research into this is currently at a very early stage.

Chapter EIGHT: General Discussion of Thesis Findings and Future Directions

8.1 Introduction

Captive housing poses restrictions on all aspects of an animal’s life and these restrictions have the potential to cause welfare problems including abnormal behaviours and immunosuppression. The problems associated with poor welfare pose a significant risk to the value of animals as representatives of their species in conservation and research (Shepherdson, 1994; Swaisgood, 2007). It is thought that animals with higher cognitive functioning are most vulnerable to suffering welfare problems as a result of the restrictions placed on them by captivity (McGrew, 1981). Therefore, it follows that apes should be on the list of priority species to be included in welfare research and in the provision of enrichment, considered the most useful tool in combating welfare problems (Carlstead and Shepherdson, 2000).

As one of the apes most at risk of extinction in the wild (IUCN, 2016a, 2016b), orangutans present a valuable case for welfare research in captivity. Captive orangutan populations are a unique case in that their semi-solitary social structure is not catered for in many captive settings, leaving them particularly vulnerable to welfare problems (Weingrill *et al.*, 2011). Resources in zoos, in terms of time and financial budgets are limited and so, when assessing animal welfare, there must be a strategic and robust approach which yields useful information. The animal welfare literature points to two avenues for assessing animal welfare: behavioural observations and monitoring of the physiological stress response (Moberg, 1985; Maki and Bloomsmith, 1989; Carrasco *et al.*, 2009; Koknaroglu and Akunal, 2013; Mason, 2010). Using a combination of these methods a, comprehensive understanding of animal welfare can be established (Maple and Perdue, 2013). However, given the costs associated with such investigations and the recognised budget constraints of zoos, it would be advantageous to determine the relative strengths and weaknesses of different methods of welfare assessment, and how they can potentially complement one another. Being able to identify an efficient and robust method of assessing welfare has the potential to significantly reduce the amount of time and money spent on other methods commonly utilised.

Recognising the importance of assessing and quantifying animal welfare, as well as the restrictions faced when implementing the available methods, this thesis aimed to compare the methods of quantifying and assessing animal welfare and in doing so provide recommendations for the future assessment of animal welfare, using Bornean orangutans as a model species. To achieve these aims the thesis sought to address a number of objectives. Primarily the thesis needed to implement the existing methods of assessing animal welfare and provide a narrative of data collection, analysis and interpretation of results. There then needed to be a comparison of these methods in terms of implementation requirements and in terms of information gained. The study of animal behaviour and welfare is a developing field and new technology such as field cameras as well as new methods of analysis are currently being developed. Network analysis has been particularly useful in the study of animal social networks over the last decade

(Croft *et al.*, 2008, 2011; Farine, 2017). However, the analytical framework has the potential to be expanded and used with a wider range of behavioural data and enhance many animal behaviour studies. These methods were demonstrated by applying them to behaviour and space-use data derived from observations of the captive orangutans studied in earlier parts of the thesis. The utility, implementation and constraints of the methods, and how they might complement more traditional forms of analysis of behaviour data were assessed.

This final chapter briefly summarises the key findings of the thesis. The strengths and weaknesses of the methods of study used are identified, and potential future directions in animal behaviour research are considered in the light of the findings of the thesis.

8.2 Major findings

8.2.1 Systematic review

Investigation of the trends in research of the effects of enrichment in the great apes showed an overall increase in publication rate between 1982 and 2014, in agreement with the prediction of Bloomsmith *et al*. (1991), but did not support the exponential growth identified for enrichment research across all captive animals (de Azevedo *et al*., 2007). Chimpanzees were the focus of the vast majority of these studies, mainly due to their wide use in the biomedical research. Recent changes in legislation have led to a decline in the use of chimpanzees in medical research (Conlee, 2007), suggesting that research investigating the use of enrichment to improve the welfare of captive chimpanzees will decrease. This species bias in the research literature does not match up with the declines in wild populations found in all species of ape. Captive populations represent increasingly valuable sources of information about these species and routes for a greater understanding of their ecology, physiology and behaviour. The use of enrichment is instrumental in preserving the value of captive animals (Shepherdson, 1994) and the availability of empirical knowledge pertaining to the implementation and outcomes of enrichment governs their use by animal carers (Hoy *et al*., 2010). A call for greater investigation of enrichment methods for apes (Nelson and Mandrell, 2005) has generally not been answered. Inconsistencies in the temporal length of enrichment research, the types of enrichment used and the numbers of subjects included in these studies leads to a need to develop a framework for structuring future research.

8.2.2 Behavioural observations

Previous attempts at using enrichment to increase the welfare of captive orangutans yielded varied results (Tripp, 1985; Wright, 1995; Perdue *et al.*, 2012; Mallavarapu *et al.*, 2013; Ritvo and MacDonald, 2016). The aims of this first experimental chapter were to make a contribution to the ape enrichment literature by utilising multiple enrichment devices over an extended period of time. The study aimed to determine the effect of using frozen fruit cups and an artificial termite mound on the welfare of the orangutans. In addition, the study aimed to assess the use of behavioural observations as a method for quantifying and assessing animal welfare.

The frozen food enrichment programme fulfilled the role of promoting behaviours displayed in the wild (Chamove, 1989). This was probably due to the provision feeding mimicking the fruit masting events experienced in Bornean and Sumatran rainforests (Ashton *et al.*, 1988; Curran and Leighton, 2000; Harrison *et al.*, 2010), encouraging an increase in feeding behaviour during the provision of enrichment. The artificial termite mount was associated with an increase in behaviours associated with the manufacture and use of tools. Individual differences in the behavioural response to enrichment were evident. The adult male ceased to use the termite mound after the first day of exposure, one of the adult females maintained the use of the mound over a two week period. These findings support the use of artificial termite mounds as enrichment devices, and reinforce conclusions from previous studies involving both chimpanzees and orangutans (Nakamichi, 2004; Nash, 1982). Importantly, it also showed that responses to enrichment can vary considerably between individuals. A significant reduction in natural behaviours coincided with the removal of all enrichment and highlighted the potential adverse effects of withdrawing enrichment devices.

Behavioural observations built a detailed account of the behavioural repertoire of the individuals and the data allowed for a comprehensive exploration of individual behaviour in relation to the enrichment programme. However, the traditional forms of analysing these data focus on relative frequencies of behaviours and do not examine greater complexities such as temporal patterns in behaviour, or links between behaviour and locations within the enclosure, potentially limiting the information gained from such behavioural studies.

8.2.3 Hormone analysis

The characteristics associated with captive housing have been identified as potential stressors for the captive animals (Hosey, 2005), and the activity of the adrenal cortex as part of the physiological stress response has become an established method for quantifying the condition of captive animals (Möstl and Palme, 2002). The fourth chapter of the thesis applied hormone analysis as a method of assessing physiological stress in relation to the enrichment programme implemented as part of chapter three. Recognising the potential for animal behaviour to be correlated with adrenal cortex activity, the study aimed to establish: 1) if the enrichment programme presented a stressful stimulus to the orangutan group and 2) if fGCM concentrations can act as predictors of animal behaviour patterns

High levels of variation both within and between individuals were identified but did not exceed the limits that would classify as a stress response as recognised by Weingrill *et al*. (2011). This confirmed the absence of a significant stress response in reaction to the environmental enrichment and thus provided further support for the use of enrichment with captive orangutans. The fGCM concentrations produced by the individuals were attributed to their age and sex. The adult male maintained the most elevated levels consistently over the 12 weeks as would be expected from high ranking males of other primate species (Gesquiere *et al*., 2011). The lower levels produced by the youngest group member were indicative of the social-buffering phenomenon (Kikusui *et al*., 2006). However, further investigation into fGCM production in a greater number of orangutans would be necessary to validate these conclusions and it was noted that there were very few studies with which to compare these results.

Both play and resting behaviour were negatively correlated with fGCM levels but this was only true for the two adult females. Previously, abnormal behaviour (scratching) had been shown to indicate physiological stress (Amrein *et al*., 2014). However, behavioural observations did not capture any behaviours in the group indicating stress or poor welfare, and so the effect of enrichment in terms of reducing behaviours associated with these conditions could not be assessed. However, behaviours associated with good welfare (natural behaviours) were able to predict reduced fGCM production. Adrenal cortex activity is associated with physiological stress and not events promoting pleasure. Therefore further exploration of the physiological indicators of pleasure would be needed to determine the potential of hormonal analysis for the identification of good welfare. The need to focus on indicators of good welfare as well as poor welfare has been highlighted in the research (Melfi, 2009).

8.2.4 Network analysis

Over the last decade there have been dramatic developments in analytical methods used in ecology (Kéry & Royle, 2016), allowing for marked increase in the amount of information gained from ecological data. Part of these developments has been the increasing use of network analysis in ecology (Tylianakis *et al*., 2007; Greslin *et al.*, 2013). Within the animal behaviour research community networks have been extensively used to study social structures in the form of social network analysis (Farine, 2017). This thesis proposes that these methods can be more widely used for the study of animal behaviour. The proposed extensions were demonstrated by constructing behavioural and space-use networks based on data collected from observing the orangutans at Twycross Zoo over a five week study. This was then built upon by illustrating how network structures could be quantified and related to characteristics of behaviour and space-use patterns. Finally, bipartite networks, previously used in analysing community interactions between species on two trophic levels (Dormann *et al.*, 2008; Greslin *et al.*, 2013), were utilised to relate two sets of behavioural data together in novel ways. This allowed a much greater level of detail to be gained from traditionally collected behavioural observation data.

8.2.5 Strengths and weaknesses

Given the limited time and financial budgets available to zoos and animal carers, careful consideration of the potential benefits resulting from the implementation of a behaviour study must be balanced against the costs required. The work conducted for this thesis has utilised a wide array of behaviour study techniques. In addition these have been instantiated for the same group of captive orangutans at the same location allowing for a direct comparison of these different methods. Strengths and weaknesses of these methods have been summarised in **Table 8.1**.

Table 8.1 The strengths and weaknesses of the methods of study and analysis utilised within the thesis, based on their implementation as part of this research.

Strengths	Weaknesses
Traditional behavioural observations	
• Made possible by small group and enclosure • Provided a detailed account of orangutan activity • Required little technical knowledge • Ethogram allowed for simple identification of behaviours • Allowed for analysis of the behaviour of each orangutan as an individual • Impact of enrichment on behaviours was easily identified via linear regression analysis • Analysis produced a range of visual and numerical outputs	• Single observer meant establishing behaviours of all individuals was not always possible • Transforming data into a digital spreadsheet was particularly time consuming • Allowed for observation of only one group over the 12 week period
Hormone analysis	
• Uninvasive • Required very little alteration to the husbandry routine • Samples collected alongside behavioural observations • EIA validated for use on orangutan faecal samples • Allowed for analysis of adrenal cortex activity of each individual • When coupled with behaviour data, established the extent of behaviour as an indicator of fGCM production	• Limited in its application as a positive welfare indicator • Relatively expensive • Requires expertise for processing samples • Requires expertise for interpreting chemical output • Uncertainty over the effect of metabolism on hormone levels • Uncertainty over the effect of storage on hormone levels
Network analysis	

• Data collection follows traditional methods • Free software available for implementing analyses • Visual output is intuitive to understand • Many metrics available • Bipartite networks allow for relating one component of behaviour to another • Add a deeper level of analysis	• Resolution of data collection can affect results and currently there are no methods for optimising the resolution • Data preparation can be complex • The behaviour of many of the network metrics is currently uncertain • Clarity of visual output is reduced as the size of the networks grow • Significant problems with applying statistical techniques due to the inherent lack of independence within the networks (particularly problematic in the bipartite networks) - although there is substantial promise with the development of randomisation methods • Requirement for greater amounts of data • Difficult to implement in the wild

8.2.6 Conclusions

The proportion of animal research which focuses on the use of enrichment with captive apes is surprisingly low, considering the global threat of extinction faced by all great ape species. The need to establish robust and repeatable methods of welfare assessments has never been greater, yet consistency and comprehensiveness within great ape enrichment research is still lacking. Behavioural observations were utilised in all enrichment studies analysed, yet, the time taken to collect the data, the potential for misinterpretation and the absence of context to observed behaviours means that a uni-dimensional picture of animal welfare is formed when using behavioural observation alone.

A detailed understanding of the physiological facet of animal welfare can be gained by monitoring stress hormone production over time. However the great variation in daily concentrations, the need to acquire a large quantity of samples over an extended period of time and the lack of existing data with which to compare results, means that hormone analysis, whilst easily quantifiable is not likely to be the most accessible method of animal welfare assessment for captive animal managers. Hormone concentrations also do not appear to alter significantly on a day-to-day basis and therefore may only be useful during and after particularly stress inducing events. However, the potential relationship between behavioural indicators of good welfare and low hormone concentrations warrants further investigation.

To address the problems faced by methods currently used to assess animal welfare, a novel method has been developed. Using behavioural observations already gathered routinely, and simply adding space use as an observation, the data may be transformed into visual networks. Networks can be used as simple graphs to illustrate an extensive range of paired data and also be quantified to track changes over time. The results may inform carers of the extent of enclosure use, the appropriateness with which animals are using enclosure features and the effect of any enclosure or group member changes. With current developments in software, the complex data processing involved will soon be accessible to the undergraduate students recruited by zoos, who will be able to produce elegant graphs that require only basic understanding of animal behaviour to interpret. Ultimately, zoo staff and researchers are now able to evaluate their resources, restrictions and rationale and, using the comprehensive evaluation presented here, determine the most appropriate method for assessing animal welfare in a given set of circumstances.

8.2.7 Future directions

With current rates of species extinctions, habitat destruction and climate change there has never been a greater need for effective and informative studies of animal behaviour. However, there is great potential for dramatically improving the extent, quality and information gained from animal behaviour studies. This results from the coalescing of a number of factors. Firstly, the rate of development in technology used in monitoring animals in captivity and the wild is increasing dramatically. Thus, behaviour can be captured on cameras which, prodeuce imagies with very high resolution (both during the day and night), have increasing battery life and are robust to being outside in rough weather for extended periods. Remote sensing technology in the form of data logging collars, proximity collars, drones and GPS trackers is becoming affordable and widely used. Secondly, the sophistication and power of analytical methods is developing at an equally fast rate. This means that there will be increasing amounts of high quality, high resolution robust data available, which can in turn be analysed using cutting edge analytical methods. There is therefore substantial potential for future animal behaviour studies.

However, technology and sophisticated analyses will not necessarily lead to greater understanding of animal behaviour. As highlighted by the development of the network analyses in this thesis, a thorough understanding of the complications and implementation of these methods will be required if misinterpretations of data and misunderstanding are not going to be widespread. This means that there will need to be a mixture of technical, analytical and behavioural experts working together if the potential of using the new technology and analyses is going to be realised. A number of different methods of study were applied in this thesis, all having their strengths and weakness (see **Table 8.1**), but they all gave different insights into the behaviour patterns of the orangutans. The ideal will be animal behaviour studies using an integrated methods approach allowing the linking of the different insights into a more comprehensive understanding of behaviour. In reality, the probability of this occurring will decrease due to the cost and logistic implications. However, improvements in technology and decreasing prices, may go some way to counteract this negative impact. Until the idealised integrated methods approach becomes widely available, researchers will have to continue to try and utilise the methods which will address their question most readily within the logistic and cost constraints imposed on them. It is hoped that the work in this thesis will firstly demonstrate the potential of the integrated methods approach and, secondly, help animal behaviour researchers to balance the costs and benefits of the different methods used.

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Appendices

3.1 - Twycross Zoo Application Form Undergraduate and Postgraduate projects

RESEARCH POLICY

1. Research will be encouraged, particularly which links with, or is relevant to, Twycross Zoo's research strategy, its conservation or education programmes.
2. All research undertaken must be for the net benefit of the individual subject(s) and / or species that are the subject(s) of the research, and / or the ecosystem that the species are part of, and / or for the benefit of the organisation. **You must demonstrate that you have clear research outcomes for your application** to have any chance of approval being given.
3. Research may be undertaken in association with Twycross Zoo through the study of the living collections, examination of other aspects of work within the zoo or through the involvement of Twycross Zoo personnel. Please note: Twycross Zoo current collection list can be viewed at: http://www.twycrosszoo.org/Modules/Animal/Listing.aspx?contentID=853
4. **All research to be undertaken in association with Twycross Zoo must be assessed and approved prior to commencement** to ensure scientific and ethical validity, that animal welfare is not compromised and that the research complies with relevant UK and EU legal requirements.
5. **All research carried out must be reported in full**, with a copy of that report being presented to the zoo at the appropriate time within the duration of the research or course. **The zoo should be advised in advance of publication plans when aspects of the Zoo's work, personnel or collection are involved**.
6. **Copies of any publication resulting from research undertaken at or through Twycross Zoo are to be provided to the Zoo.**
7. **All reports and publications resulting from research at or through Twycross Zoo (including samples) should acknowledge fully Twycross Zoo and the East Midlands Zoological Society, with copies lodged at Twycross Zoo**. It is expected that individual zoo staff contributing to or supervising research projects must be acknowledged through co-authorship of any publications as well as by name in the relevant section of reports.
8. A **copy of all raw data relating to Twycross Zoo**, appropriately annotated to allow independent interpretation, **must be lodged with the Zoo on completion of a study**. Twycross Zoo reserves the right to use all data collected at the zoo for its own publications if they have not been published (or submitted for publication) elsewhere within one year of the termination of the research project. In such cases, due acknowledgement of contribution and intellectual property will be given.
9. Registration Fees: to access the zoo for the duration of the project, all researchers must obtain a researchers' pass. The basic research fee for 2012 is £50.00, to be paid before commencement of any research (see p 9 for details). There is fee of £100.00 for more involved projects or those requiring supervision by the Zoo.

   Please NOTE: Fees may be waived in cases where researchers are affiliated with specific partner institutes or where research has been commissioned by Twycross Zoo.

In these cases, such a waiver (with brief reason) should be included within the application document.

Please complete the following form so it is easily legible. All boxes **must be completed** for your application to be considered. **Please note: Twycross Zoo – East Midland Zoological Society Limited are now charging *successful* applications for using the zoo + facilities to carry out research (see agreement section for details).**

For Office use only:

Project Ref:

1) General information

First names

Ruth

Surname (Family name)

Bentley

Title

Miss

E-mail address

R.Bentley@Derby.ac.uk

Address

6 Warwick Road
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Home phone

0116 2881369

Mobile phone

07540397119

Researcher details

A) Title of course or job title
B) Name and address of institution

PhD
University of Derby
Kedleston Road
Derby
DE22 1GB

Supervisor details (if applicable)

A) Title, name and contact details of supervisor (if applicable)
B) Position (and qualifications if not apparent from title)

A) Prof. Karim Vahed (Second Supervisor)
Dept of Biological and Forensic Sciences
University of Derby
Kedleston Road
Derby
DE22 1GB

B) Reader in Behavioural Ecology

Charge level (please tick) : Basic ☐ Supervisional ☑ See page 8 for details

Level of study

Degree (Undergraduate Degree / MSC / PHD) / POST DOCTORAL /OTHER

PhD

2) Project information

Please give details of your **research questions and how you intend** to meet these with research at the zoo. In particular, we need to know what you need from and expect from the zoo. You can append further information if you think it would help us to assess the project. If you project involves any intervention in the animal enclosures or with the zoo routine then you must provide FULL details. **If the research includes a questionnaire then it must be included with this form.**

Start date at the zoo	**Finish date**
30th June 2014	19th September 2014

Project title

The impact on welfare of utilising food-based and tool-use enrichment on a group of captive Bornean orangutans (*Pongo pygmaeus*)

Research Animals (species / group)

Bornean orangutans	For Office use: Sections involved:-

Project aims and testable hypotheses

AIMS:	OBJECTIVES:
To assess the role of enrichment in improving the welfare of primates.	To determine the impact of two enrichment devices on the behaviours of captive orangutans such as; feeding, foraging, travelling and tool-use. To understand the effect on social interactions of implementing an enrichment program with captive orangutans. To establish baseline faecal cortisol levels in captive orangutans and monitor changes in cortisol levels throughout an enrichment program.

SUMMARY OF PROJECT (max 120 words):

In order to assess the impact that enrichment has on captive orangutan welfare, behavioural observations will be carried out during the treatments of pre, during and post enrichment. Food-based enrichment will be utilised to promote natural feeding and foraging behaviour whereas tool-use enrichment will target the further tool use within the enclosure. As wild orangutans spend considerable time foraging and building nests, it is hoped that the enrichment items will increase these natural behaviours, indicating a positive effect on their welfare.

Current state of the research: (e.g. not started/pilot/second year etc.)

First year of PhD project

Methodology (give full information about what you are hoping to do, how you intend doing it and what you require from the zoo)

ON-SITE METHODOLOGY & REQUIREMENTS FROM THE ZOO
(if samples – please list on page 5)

The study will run for a total of 12 weeks. The initial three weeks of study will comprise of baseline observations only. The orangutans will be observed each day for the total time that they are on view to the public. Observations last for 1 hour with 20 minute intervals and will follow the method of Time Use Categories (TUCs) and Social Contexts (SCs) used by Wood (1998). This has been adapted for orangutan behaviours following a previous study by Bentley and Bulling (in prep). This baseline data will establish levels of variation in individual and group behaviour.

The study will then progress through three enrichment blocs, each comprising three weeks of observations. Each bloc will adhere to an ABA experimental design, one week of no enrichment will be followed by one week of exposure to enrichment before another week without enrichment. This will allow researchers to determine any effects of removing the enrichment on orangutan behaviour.

The middle week of each bloc will involve a different enrichment treatment. The study will require enrichment to be added to the outdoor orangutan enclosure each morning during the keeper's normal husbandry routine.

1) Feeding enrichment – plastic bottles filled with grapes, sultanas and fruit flavoured cordial will be frozen to provide an alternative presentation of the orangutan's daily food intake. This enrichment was successfully used in Bentley and Bulling (in prep), yielding positive increases in the welfare of individuals.
2) Tool-use enrichment – Students at the University of Derby along with the researcher will be carrying out extensive research into effective enrichment devices that require great apes to use tools. An enrichment device will be designed and manufactured to provide a cognitive challenge to the orangutans whilst also being easily installed and maintained. It is hoped that this enrichment will appeal to the intelligence of the orangutans, exercising their cognitive ability as well as providing an alternative use of their time budget. This enrichment treatment is currently under development. However, it will be finalised under the advice of the zoo keepers and staff at Twycross Zoo.
3) The final enrichment treatment will combine the feeding enrichment and the tool-use enrichment in order to investigate the combined effect on the welfare of the Bornean orangutans at Twycross Zoo. This will provide further behavioural choices to the orangutans with the flexibility to carry out more species specific behaviours.

At each stage of this study, the researcher will be accompanied by two students from the University of Derby, these students will be recording behaviour at the same time, but completely independently. This parallel data can then be used to assess levels of variation between observers and inter-observer reliability.

A linear regression on individual behavioural data will be used to assess changes in behaviours that across each section of each bloc (pre, during and post enrichment). A hierachical regression framework will be used to examine differences in changes across sections, nested within the three treatments listed above. For example, significant increases in TUCs such as feeding, foraging and travelling will suggest a positive impact on welfare. The hierachical regression will be carried out separately for each individual. In order to describe and assess changes in the suite of behaviours as a whole within individuals, multivariate similarity indices will be calculated and hierachical clustering algorithms applied. In addition, methods such as non-metric multi-dimensional scaling (NMDS) will be used to cluster patterns of similar behaviour.

OFF-SITE METHODOLOGY (Summary: please add an extra sheet if you need to)

Prior to the implementation of enrichment, the researcher and students of the University of Derby will carry out research into previously tested enrichment devices and design a tool-use enrichment device. The device will require the orangutans to select the most appropriate tool to complete a task to access a food reward. The device will be designed to be safe and appropriate for each of the orangutans to use successfully, easily fixed to the enclosure and easily refilled as part of the keeper's morning routine. Staff from Twycross zoo will be fully consulted in the design and implementation of the device.

After the data collection period of the study, all data will be gathered and analysed using the methods outlined in the previous section, to look for significant changes in behaviours displayed across and within each three week treatment period for each individual.

Hormone assays will be analysed in a similar way to the behaviour data, although the reduced number of data points will reduce the power and resolution of such analyses. However, this study will provide a database of considerable length, against which conclusions from behavioural observations can be compared, particularly for consistent patterns..

Samples. If your project includes a request for animal samples then please give full protocols for collection, storage and transport of the samples as appropriate (continue onto further sheets if necessary.) Otherwise, please put '*n/a*'. Please note that we will need *you to supply containers etc. and pay for any shipment prior to samples being collected or sent*. We have very limited freezer capacity on site and cannot store samples for long periods.

If your research proposal is successful, we will ask your supervisor to sign a short samples agreement form specific to the project.

Animals: Bornean Orangutans
Samples: Faeces
Protocols: Samples will need to be collected and stored in container provided and stored at -10°C or below. The project will enlist the assistance of The German Primate Centre. The centre provide an independent endocrine service in the measurement of steroid hormones such as cortisol. Researchers will be working closely with the The German Primate Centre to identify the quantities required for hormone analysis and the storage and transport of samples. Once all samples have been collected, they will be shipped to The German Primate Centre for extraction, hormone quantification, calculation of results and interpretation. Please note; a more detailed protocol will be provided upon further communications with The German Primate Centre before the commencement of the project.

Zoo areas you wish to use in your project research (important if you haven't specified an animal, or are specifying a particular group)

The visitor viewing areas of the orangutan enclosure

How will this research be of benefit to animal management and/or to Twycross Zoo?

Zoos aim to minimise abnormal behaviour in their animals and promote healthy environments, an aim that is highlighted as a priority in the stated visions of Twycross Zoo. Enrichment devices offer a method of helping to accomplish these targets and are increasingly utilised. As well as being effective, this methodology can also be relatively cheap and easily implemented, characteristics that are important with the time and budget constraints of modern zoos.

We hope that, as well as being effective in enhancing welfare, the enrichment tools used in the research will be easily implemented by zoo staff, requiring little of their time, as well as being inexpensive. The study aims to test all of these objectives and hopefully provide the zoo with a scientific appraisal of the resulting welfare benefits, as well as documenting any lag effects, post treatment, and how combining treatments could be used effectively.

Visitor interest may be stimulated by observing the implementation of the enrichment devices. As such, there is a possibility for supporting this interest in the form of outreach, perhaps with a poster, explaining the research being carried out.

How will this research be of benefit to conservation of the species?

Orangutans of both species are classified as endangered on the International Union for the Conservation of Nature's (IUCN) Red List, their desperately low numbers in the wild can be attributed to the growing palm oil trade across Indonesia as well as the illegal pet trade. There have been outstanding efforts by various charities to rehabilitate orphaned and ex-captive orangutans into the wild or into other captive settings such as zoos and sanctuaries aimed at promoting their welfare. If the enrichment treatments proposed are successful in promoting natural behaviours in captive orangutans, there is strong hope that it may also be implemented in the rehabilitation and reintroduction of captive orangutans.

One of the issues facing reintroduced orangutans is their familiarity with humans. They often seek human interaction in the wild and are often maimed or killed as a result. Enrichment that promotes natural behaviours such as foraging and nest building without human interaction could avoid these fatal interactions.

For those orangutans remaining in captivity, their reproduction and longevity is a priority to care staff. The hormonal responses to chronic stress inhibit the reproductive system and the immunoresponse of vertebrates. Enrichment that improves the welfare of captive orangutans has the potential to improve reproductive potential and extend the life-span of captive individuals, making great contributions to captive breeding programs and to the conservation for the species.

There is no exact definition for animal welfare as yet, but the literature supports the statement that animal welfare is; the provision of an environment in which animals can freely display their natural behaviours (Koknarolgu & Akunal, 2013). Most researchers support the method of measuring animal behaviour to assess welfare through observational studies (Yeates, 2008). Recently however, there has been an increase in taking physiological measures to assess welfare. The recent development of non-invasive hormonal sampling protocols has led to an increase in the use of cortisol concentrations as welfare indicators (Witham & Weilebnowski, 2013). Cortisol is a substance released from the adrenal gland in response to stressful stimuli and helps prepare the body to cope with the stress (Koknarolgu & Akunal, 2013). Baseline concentrations can indicate the effects on the animals physiology of its energy allocation and habitat quality (Romero, 2004 cited in Weingrill *et al*, 2011). Chronic stress can cause the production of such high cortisol concentrations that internal systems such as the reproductive system and immune response are inhibited (Shepherdson, 1993).

Environmental enrichment is widely used to address welfare problems in captive populations and to reduce stereotypic behaviours caused by chronic stress. Primates receive most attention in published enrichment studies (de Azevedo *et* al, 2007). Their high level of intelligence and problem solving ability means they are easily studied and are more likely to succumb to stress induced abnormal behaviours. A review of the literature indicates a strong bias towards chimpanzees and gorillas within published enrichment research. Orangutans appear to be neglected in this field of study, yet their 'critically endangered' status on the IUCN red list should ensure they are given more attention than is currently afforded to them.

de Azevedo, C.S., Cipreste, C.F. & Young, R.J. (2007) Environmental Enrichment: A GAP analysis, *Applied Animal Behaviour Science*, 102, pp. 329-343

Koknaroglu, H. & Akunal, T. (2013) Animal welfare: An Animal Science Approach, *Meat Science*, 95, pp.821-827.

Shepherdson, D. (1003) The role of environmental enrichment in the captive breeding and reintroduction of endangered species, In: Olney, P.J.S, Mace, G.M. & Felstner, A.T.C. (eds) Creative Conservation: Interactive management of wild and captive animals. Chapman & Hall: London.

Weingrill, T., Willems, E.P., Zimmerman, N., Steinmetz, H. & Heistermann, M. (2011) Species-specific patterns in faecal glucocorticoid and androgen levels in zoo-living orangutans (*Pongo* spp.), *General and Comparative Endocrinology*, 72, pp. 446-457.

Where could this work be published or presented:

We would expect this work to be published and/or presented and we would expect the zoo to be fully credited or acknowledged. Include student presentations and student-oriented publications (e.g. Biohorizons, OUP: BIAZA conferences, JZAR, etc.).

Submission to a particular journal is obviously contingent on the structure and form of experimental results and conclusions. However, we would aim to publish in at least one of the following journals:

1) Animal Behaviour (Academic Press)

2) Applied Animal Behaviour Science (Elsevier Science)

3) Zoo Biology (Wiley-Liss)

4) Biohorizons

3) Research guidelines

1. **We cannot accept studies that would compromise animal welfare or adversely affect animal management**. Our keepers are very helpful but you must co-operate with them and respect their constraints and requirements.
2. Any equipment to be used or questionnaires must be seen and agreed to by the zoo in advance.
3. There must be no harm or distress caused to any of our animals during your study.
4. You must appreciate that animals may be moved or not available for study at short notice if there are emergencies, such as veterinary problems. We will do our best to sort out any problems that affect your study and keep you informed of any necessary changes.
5. You may find it helpful to arrange a preliminary visit to the zoo before commencing your research to see the zoo and be sure that your proposal will work before any research commences.
6. You need to arrange accommodation before arrival at the zoo and you should arrange your daily travel to the zoo as the zoo is quite a distance from nearby towns.
7. Please be aware that if your application is successful, **Twycross Zoo East Midlands Zoological Society Limited will invoice you or your supervisor for the required rate (£50.00 basic, £100.00 supervision)**. Exact details of what this entails is shown on page 8.
8. **You will need to provide a short summary of the research that you have carried out and a copy of the finished research project when you complete it. You will also need to provide us with a copy of your raw data**.
    - In the event that this agreement is terminated, and / or the student fails, for any reason, to write up the results within three (3) months of the conclusion of the scholarship, the zoo shall retain the right to write up and publish the results
9. **Twycross zoo must be credited in the main body of your report and any subsequent publications**. If personnel at Twycross have made a significant contribution to the research, co-authorship on resulting publications will be expected.
10. Your details will be held in various databases for our use only and will not be passed to third parties without your consent.
11. Twycross Zoo will not be held responsible for any problems arising from research carried out at the zoo.

Please tick the relevant box and return payment with this form to the Research Department:-

	I have included / will include payment (prior to starting) for the basic research rate (£50.00)
✓	**I have included / will include payment (prior to starting) for the supervision research rate (£100.00)**

Cheques should be made payable to Twycross Zoo, payment by bank transfer will require further communication. Payment will be returned if the proposal is not approved and is non-refundable once the researchers' pass has been issued.

I have read and understood the above guidelines and agree to abide by them. I will submit a copy of my research to the zoo by my institutional deadline.

Applicant signature ………………………………………………………….……………..

Date ……….15/04/2014……………………..

Twycross Zoo Charging

There are currently two costing streams within the research strategy for Twycross Zoo – East Midlands Zoological Society Limited. These apply for the period 2010 – 2012, although TWZEMZS Limited reserves the right to review and alter the charging regime in September 2012. There is a free initial consultation period for all applications, should this be required by your supervisor, but this cannot include any actual research.

Basic research rate : £50.00
This provides you with –

- Free access into the zoo to do your research (through supply of a badge, which you will receive on your first day, and will need to return at the end of your on-site research period)
- Free access to the zoo library and records system when on-site
- Free access to the visitor viewing areas, and consultations with the keepers and other staff, as outlined in the induction. *You will not be allowed access to the animal enclosures*.
- The chance to provide simple enrichment objects only. *These enrichment devices must be supplied to the keepers for them to include in the enclosures during their normal daily routines*.
- Access to the staff cafe.

Supervision research rate : £100.00
This provides you with all the above, and additional (ongoing) use of staff time, as well as more involved projects such as more complex enrichment devices, nutritional studies, etc. As above, *you will not be allowed access to the animal enclosures except under specific agreements*, while *all enrichment devices must be supplied to the keepers for them to include in the enclosures during their normal daily routines.*

- Any other hands-on applications will be looked at on a case-by-case review, so it is best to make consultations before applying to do research at the zoo.

- If during the course of a ‘basic research rate’ project it becomes apparent that you are taking up staff time, then your project will be assigned the ‘supervision research rate’ pricing requirement.

Twycross Zoo Timelines for Applications

Please note that you can submit applications at any time of the year. However, all applications take about 6-7 weeks for us to process, so you will need to plan accordingly. For guidance, we have included some examples of deadlines below to allow you to estimate your submission timeline:-

Submit application : **Mid November** :: *Start research* : **Early January**
Submit application : **Late February** :: *Start research* : **Early April**
Submit application : **Mid April** :: *Start research* : **Early June**
Submit application : **Early August** :: *Start research* : **Early October**

For completion by your supervisor (if appropriate):

We would welcome any comments on the proposed project. It is a condition of the zoo that the student provides a copy of their finished report at the end. The zoo is not prepared to sign off the work as complete until the project report is received and we hope that you will co-operate with us on this. In this way the work can be of real benefit to the zoo as well as to the student.

Please note that as of September 2010, Twycross Zoo – East Midland Zoological Society has put in place research charges for use of its facilities. Please see page 8 for what the two charging rates incorporate. Please note that we expect charges to be paid before any research can commence.

Charge level (please tick) :	Basic (£50.00)	☐	Supervisional (£100.00)	✓	(see page 8 for details)

Supervisor' signature……………………………………………………………………………

Supervisor's comments:

1) In 2011, Ruth conducted a similar research project, albeit on a smaller scale, for her third year undergraduate project. We were so impressed with this project and the enthusiasm that Ruth showed that we put in a grant proposal to continue the work. The proposal was successful, and although funding was initially delay, this work will be the first stage in a PhD study carried out by Ruth.

2) We recognise that there are currently discussions between Twycross Zoo and the University of Derby over closer collaboration and support, and we hope that this project would represent a considerable step forward in this process.

3) We recognise that the methodology outlined above may have to be altered in light of

Please return your completed application form to kevin.caley@twycrosszoo.org.

3.2 – Results of linear regressions performed on all individuals and behaviours

Table 9.1 Results of the linear models and those with which the GLS extension was applied for the data representing the baseline observations. None of the models produced a significant p-value

Baseline					
Orangutan	Method of Analysis	P-Value	L-Ratio	F-Statistic	DF
Batu					
Feeding	LM	0.067		3.410	12
Foraging	LM	0.937		0.065	12
Object Using	LM	0.396		1.000	12
Travelling	GLS	0.610	0.990		2
Object Manipulation	GLS	0.446	1.610		2
Resting	GLS	0.501	1.383		2
Kibriah					
Feeding	GLS	0.400	1.833		2
Foraging	LM	0.969		0.031	12
Object Using	LM	0.402		0.986	12
Travelling	GLS	0.818	0.402		2
Object Manipulation	LM	0.467		0.812	12
Resting	GLS	0.655	0.845		2
Maliku					
Feeding	LM	0.309		1.298	12
Foraging	LM	0.947		0.055	12
Object Using	GLS	0.539	1.236		2
Travelling	LM	0.263		1.496	12
Object Manipulation	GLS	0.229	2.945		2
Resting	LM	0.606		0.522	12
Molly					
Feeding	GLS	0.080	5.043		2
Foraging	GLS	0.544	1.220		2
Object Using	GLS	0.070	5.322		2
Travelling	GLS	0.431	1.685		2
Object Manipulation	GLS	0.396	1.853		2
Resting	LM	0.763		0.277	12

Table 9.2 Results of the linear models and those with which the GLS extension was applied for the data gathered during the food enrichment treatment. Significant p-values are highlighted in bold.

Feeding Enrichment					
Orangutan	Method of Analysis	P-Value	L-Ratio	F-Statistic	DF
Batu					
Feeding	GLS	0.106	4.497		2
Foraging	GLS	0.564	1.145		2
Object Using	LM	0.415		0.971	9
Travelling	GLS	0.556	1.174		2
Object Manipulation	LM	0.542		0.656	9
Resting	GLS	0.672	0.796		2
Kibriah					
Feeding	GLS	0.378	1.948		2
Foraging	GLS	0.357	2.060		2
Object Using	GLS	0.661	0.828		2
Travelling	GLS	0.624	0.943		2
Object Manipulation	LM	0.239		1.687	9
Resting	GLS	0.359	2.051		2
Maliku					
Feeding	GLS	**0.046**	6.141		2
Foraging	GLS	0.579	1.092		2
Object Using	GLS	0.172	3.521		2
Travelling	LM	0.922		0.082	9
Object Manipulation	GLS	0.126	4.143		2
Resting	LM	0.315		1.316	9
Molly					
Feeding	GLS	**0.048**	6.100		2
Foraging	GLS	0.143	3.890		2
Object Using	GLS	0.443	1.629		2
Travelling	GLS	0.664	0.819		2
Object Manipulation	LM	**0.002**		13.360	9
Resting	LM	0.589		0.562	9

Table 9.3 Results of the linear models and those with which the GLS extension was applied for the data gathered during the second period of baseline observations. Significant p-values are highlighted in bold.

Baseline 2					
Orangutan	Method of Analysis	P-Value	L-Ratio	F-Statistic	DF
Batu					
Feeding	LM	0.235		1.657	11
Foraging	GLS	0.878	0.260		2
Object Using	LM	0.587		0.560	11
Travelling	GLS	0.431	1.682		2
Object Manipulation	NA	NA	NA	NA	NA
Resting	GLS	0.816	0.408		0
Kibriah					
Feeding	GLS	0.382	1.927		2
Foraging	LM	0.421		0.938	11
Object Using	LM	0.142		2.339	11
Travelling	GLS	0.406	1.802		2
Object Manipulation	GLS	0.389	1.889		2
Resting	GLS	0.609	0.993		2
Maliku					
Feeding	LM	0.335		1.212	11
Foraging	LM	0.752		0.232	11
Object Using	GLS	0.135	4.011		2
Travelling	GLS	**0.040**	6.428		2
Object Manipulation	GLS	0.399	1.838		2
Resting	LM	0.329		1.234	11
Molly					
Feeding	GLS	0.280	2.543		2
Foraging	LM	0.876		0.134	11
Object Using	GLS	0.160	3.663		2
Travelling	LM	0.233		1.669	11
Object Manipulation	LM	0.462		0.829	11
Resting	GLS	0.605	1.005		2

Table 9.4 Results of the linear models and those with which the GLS extension was applied for the data gathered during the too use enrichment treatment. Significant p-values are highlighted in bold.

Tool use enrichment					
Orangutan	Method of Analysis	P-Value	L-Ratio	F-Statistic	DF
Batu					
Feeding	GLS	**0.033**	6.802		2
Foraging	LM	0.973		0.028	11
Object Using	LM	0.251		1.570	11
Travelling	GLS	0.180	3.430		2
Object Manipulation	LM	**0.016**		6.231	11
Resting	LM	0.897		0.110	11
Kibriah					
Feeding	GLS	**0.033**	6.847		2
Foraging	LM	0.524		0.685	11
Object Using	LM	0.150		2.264	11
Travelling	GLS	**0.046**	6.144		2
Object Manipulation	LM	0.309		1.310	11
Resting	GLS	**0.002**	12.702		2
Maliku					
Feeding	LM	**0.014**		6.525	11
Foraging	LM	0.110		2.708	11
Object Using	GLS	**0.015**	8.444		2
Travelling	GLS	0.336	2.182		2
Object Manipulation	GLS	0.316	2.307		2
Resting	GLS	0.184	3.385		2
Molly					
Feeding	LM	0.113		2.734	10
Foraging	LM	0.120		1.902	10
Object Using	LM	0.322		1.272	20
Travelling	LM	0.241		1.646	10
Object Manipulation	LM	0.431		0.916	10
Resting	LM	0.706		0.360	10

4.1 – Sample Collection Protocol

Protocol for collection, storage and shipment of urine and faecal samples from captive-housed animals

Urine Samples

- Collect sample as fresh as possible, e.g. observe animal for urination or separate it from group to facilitate sample collection.
- Ideally collect first morning urine or, if not possible, at roughly the same time of day in order to minimize the potential influence of diurnal variation on hormone levels (important mainly in the case of testosterone and cortisol analysis).
- Collect only samples that are not contaminated with faeces. A volume of 0.5-2.0 ml (depending on species) is sufficient.
- Also avoid contamination of the sample with water (e.g. from cage cleaning) as this may render the sample too dilute.
- Aspirate sample (using a pipette or syringe) from the surface and place it into a collection tube (e.g. 2 ml plastic cup). (If necessary, sample containers can be ordered at us).
- Label the tube properly with animal ID and date and daytime of collection using a waterproof black marker pen or, better, a freeze-resistant sticky label which should be additionally protected with adhesive tape.
- Freeze sample at -18°C as soon as possible after collection (latest within 2 hours).
- Prepare a sample collection list (i.e. excel file) for cross-checking purposes and recording of relevant information and notes.

Faecal Samples

- Collect sample as fresh as possible, e.g. observe animal for defecation or separate it from group to facilitate sample collection.
- Collect sample at roughly the same time of day in order to minimize the potential influence of diurnal variation on hormone levels (important mainly in the case of testosterone and cortisol analysis).
- Collect only samples that are not contaminated with urine or water.
- In case of big sample volumes, homogenize whole sample (e.g. with spatula, plastic coffee spoon or gloved hands before collecting a 3-10 g portion (thumb-nail size) into a small plastic container with wide opening (e.g. 30 mm, see http://www.carlroth.com/website/de-

de/carl-roth_index.jsp; search for Art. No. EA72.2); sample containers can be ordered at us if necessary). **Do not completely fill the container since this makes drying of the sample for hormone analysis very timeconsuming.**

In case of elephants and rhinos, it is recommended to take the sample aliquot from the middle of a bolus. Since elephant and rhino faeces is strawy, the sample container should have a preferable size of ca. 40 mm wide and 40 mm high, e.g. see http://www.carlroth.com/website/de-de/carl-roth_index.jsp; search for Art. No. EA73.2). The tube should be completely filled with faecal material, but please do not compact faeces too much. **Do not collect faeces into plastic bags**.

- In case of a very small sample, the entire sample should be collected, even if it is less than the specified amount.
- Label the tube properly with animal ID and date and daytime of collection using a waterproof black marker pen or, better, a freeze-resistant sticky label which should be additionally protected with adhesive tape.
- Freeze sample in a regular freezer (-18°C) as soon as possible after collection (latest within 2 hours).
- Prepare a sample collection list (i.e. excel file) for cross-checking purposes and recording of relevant information and notes.

Shipping

- Please contact us first, prior to shipment; to be sure someone will be on hand to receive the samples.
- Please ship only Monday-Wednesday so samples will definitely arrive on a weekday (latest by Friday 2 p.m.).
- All samples should be shipped frozen using dry ice (or frozen cold packs, if dry ice is not available).
- Make sure that shipment will be done within 24 hours (when only frozen cold packs are used) or 48 hours (in case dry ice is used). Using overnight courier is highly recommended

For any further questions on sample handling and shipment contact Dr. Michael Heistermann (Head of the Endocrinology Lab) at ++49-(0)551-3851290, or at mheistermann@dpz.eu

Shipping Address:
Dr. Michael Heistermann
German Primate Centre
Endocrinology Laboratory
Kellnerweg 4, 37077 Goettingen, Germany

4.2 – Results of Enzyme Immunoassay from the German Primate Centre

Sample ID number	**Name of Orangutan** (if known)	**Time of collection**	**Date**	Faecal weight (g)	11ß-hydroxy… dilution	11ß-hydroxy… (pg/50µl)	11ß-hydroxy… (ng/g faeces)
002	Batu	8.20	30.06.14	0.0511	60	24.1	1697.8
008	Batu	10.00	02.07.14	0.052	60	17.6	1218.5
012	Batu	8.30	04.07.14	0.061	60	20.7	1221.6
015	Batu	10.00	05.07.14	0.0545	60	21.2	1400.4
019	Batu	10.30	06.07.14	0.0596	60	32.5	1963.1
026	Batu	10.30	10.07.14	0.0663	60	19.5	1058.8
033	Batu	10.30	13.07.14	0.0567	60	31.3	1987.3
036	Batu	10.45	15.07.14	0.0663	60	30.5	1656.1
039	Batu	10.45	16.07.14	0.0641	60	16	898.6
043	Batu	9.30	18.07.14	0.0543	60	15.1	1001.1
046	Batu	10.30	19.07.14	0.0687	60	13.4	702.2
048	Batu	10.20	20.07.14	0.0601	60	24.3	1455.6
049	Batu	9.00	21.07.14	0.0508	60	15.7	1112.6
052	Batu	11.30	24.07.14	0.062	60	18.7	1085.8
054	Batu		25.07.14	0.0639	60	23.6	1329.6
062	Batu	11.45	28.07.14	0.0517	60	14.3	995.7
063	Batu	10.00	29.07.14	0.0537	60	13.7	918.4
067	Batu	10.45	30.07.14	0.0604	60	29.7	1770.2
073	Batu	10.30	02.08.14	0.0565	60	20.7	1318.9
076	Batu	10.00	03.08.14	0.0526	60	16.4	1122.4
078	Batu	10.00	04.08.14	0.0524	60	17.1	1174.8
081	Batu	10.30	05.08.14	0.063	60	25.5	1457.1
088	Batu	15.15	07.08.14	0.0582	60	4.5	278.4
089	Batu	10.00	08.08.14	0.0644	60	17.2	961.5
093	Batu	9.00	10.08.14	0.0596	60	21.7	1310.7
096	Batu	9.30	11.08.14	0.0535	60	13.4	901.7
104	Batu	10.30	13.08.14	0.0498	60	13.8	997.6
107	Batu	10.45	14.08.14	0.0564	60	8.9	568.1
112	Batu	11.45	16.08.14	0.0506	60	12.3	875.1
115	Batu	10.00	17.08.14	0.0564	60	18.3	1168.1
117	Batu	10.50	19.08.14	0.0554	60	14.6	948.7
121	Batu	10.00	22.08.14	0.0508	60	17.2	1218.9
125	Batu	10.00	24.08.14	0.0532	60	26.4	1786.5
127	Batu	10.30	26.08.14	0.0511	60	11.3	796.1
131	Batu	10.30	27.08.14	0.055	60	13.6	890.2

134	Batu	10.00	28.08.14	0.0596	60	16.6	1002.7
138	Batu	10.30	29.08.14	0.0656	60	13.5	740.9
140	Batu	10.00	31.08.14	0.0522	60	8.6	593.1
142	Batu	12.00	02.09.14	0.0587	60	24.6	1508.7
150	Batu	9.30	04.09.14	0.057	60	19.1	1206.3
154	Batu	10.00	06.09.14	0.0626	240	10.8	2484.3
155	Batu	8.30	07.09.14	0.0523	60	19.2	1321.6
158	Batu	9.00	08.09.14	0.05	60	9.8	705.6
160	Batu	9.30	09.09.14	0.0584	60	27.5	1695.2
163	Batu	10.00	10.09.14	0.0545	60	16.7	1103.1
167	Batu	9.00	12.09.14	0.0563	60	16.8	1074.2
170	Batu	16.00	13.09.14	0.0498	60	25.1	1814.5
171	Batu	9.30	14.09.14	0.0507	60	16.9	1200.0
176	Batu	9.46	16.09.14	0.053	60	21.2	1440.0
179	Batu	8.55	17.09.14	0.0596	60	26.2	1582.6
181	Batu	9.00	18.09.14	0.0511	60	17.2	1211.7
001	Kibriah	8.05	30.06.14	0.0501	60	14.4	1034.7
005	Kibriah	10.00	02.07.14	0.0529	60	14.7	1000.4
017	Kibriah	10.20	05.07.14	0.0508	60	2.1	148.8
023	Kibriah	9.00	07.07.14	0.05	60	14.1	1015.2
025	Kibriah	10.30	10.07.14	0.0535	60	16	1076.6
032	Kibriah	10.30	13.07.14	0.0547	60	14.9	980.6
035	Kibriah	10.45	16.07.14	0.054	60	15.8	1053.3
066	Kibriah	10.45	30.07.14	0.0593	60	11.8	716.4
074	Kibriah	10.30	02.08.14	0.0519	60	8.9	617.3
075	Kibriah	10.10	03.08.14	0.0566	60	9.6	610.6
077	Kibriah	10.00	04.08.14	0.0589	60	12.8	782.3
091	Kibriah	10.20	08.08.14	0.0531	60	9.5	644.1
092	Kibriah	9.00	10.08.14	0.0565	60	11.3	720.0
099	Kibriah	10.00	11.08.14	0.0514	60	21.3	1491.8
116	Kibriah	10.50	19.08.14	0.0595	60	11.6	701.8
124	Kibriah	10.20	22.08.14	0.0586	60	1.9	116.7
126	Kibriah	10.30	26.08.14	0.0518	60	14.4	1000.8
135	Kibriah	10.30	29.08.14	0.0574	60	9.8	614.6
146	Kibriah	9.15	03.09.14	0.0552	60	14.2	926.1
147	Kibriah	9.15	04.09.14	0.0553	60	12.1	787.7
157	Kibriah	8.30	08.09.14	0.0561	60	11.7	750.8
162	Kibriah	10.00	10.09.14	0.0582	60	8.8	544.3
175	Kibriah	9.42	16.09.14	0.05	60	14	1008.0
180	Kibriah	9.07	17.09.14	0.0588	60	12.9	789.8
003	Maliku	8.20	30.06.14	0.0599	60	27.3	1640.7

007	Maliku	10.00	02.07.14	0.0527	60	3.9	266.4
014	Maliku	10.00	04.07.14	0.0535	60	5.2	349.9
016	Maliku	10.00	05.07.14	0.0506	60	11.7	832.4
020	Maliku	10.30	06.07.14	0.0581	60	16.8	1041.0
024	Maliku	10.00	09.07.14	0.0571	60	9.6	605.3
027	Maliku	10.30	10.07.14	0.0577	60	18.2	1135.5
029	Maliku	10.45	11.07.14	0.0582	60	20.4	1261.9
031	Maliku	10.30	12.07.14	0.059	60	8.1	494.2
034	Maliku	10.30	13.07.14	0.0582	60	12.3	760.8
037	Maliku	10.45	15.07.14	0.055	60	15.2	994.9
040	Maliku	10.45	16.07.14	0.0551	60	17.4	1136.8
042	Maliku	9.30	18.07.14	0.0501	60	12.6	905.4
044	Maliku	10.30	20.07.14	0.0583	60	28.7	1772.2
047	Maliku	10.20		0.0573	60	16.3	1024.1
050	Maliku	9.00	21.07.14	0.0554	60	6.7	435.4
053	Maliku	11.30	24.07.14	0.0542	60	5.5	365.3
055	Maliku		24.07.14	0.0521	60	6.7	463.0
061	Maliku	11.45	28.07.14	0.0527	60	7.6	519.2
065	Maliku	10.00	29.07.14	0.0582	60	10.2	630.9
068	Maliku	10.45	30.07.14	0.0522	60	15.7	1082.8
070	Maliku	9.50	01.08.14	0.0524	60	15.8	1085.5
072	Maliku	10.30	02.08.14	0.0533	60	16.2	1094.2
079	Maliku	10.00	04.08.14	0.0551	60	7.3	477.0
082	Maliku	10.30	05.08.14	0.0576	60	8.1	506.3
085	Maliku	10.15	06.08.14	0.0558	60	9.2	593.5
086	Maliku	10.40	07.08.14	0.0517	60	27.6	1921.9
090	Maliku	10.00	08.08.14	0.0543	60	15.2	1007.7
094	Maliku	9.00	10.08.14	0.056	60	12.8	822.9
097	Maliku	9.30	11.08.14	0.0582	60	11.2	692.8
100	Maliku	12.00	12.08.14	0.0502	60	12	860.6
106	Maliku	10.30	13.08.14	0.0527	60	19.5	1332.1
108	Maliku	10.45	14.08.14	0.0551	60	14.5	947.4
110	Maliku	11.45	16.08.14	0.0567	60	11.9	755.6
114	Maliku	10.00	17.08.14	0.0509	60	11.3	799.2
118	Maliku	10.50	19.08.14	0.0511	60	8.2	577.7
123	Maliku	9.45	22.08.14	0.0554	60	12.2	792.8
128	Maliku	10.30	27.08.14	0.0568	60	16.1	1020.4
133	Maliku	10.00	28.08.14	0.0573	60	22.5	1413.6
137	Maliku	10.30	29.08.14	0.0567	60	14.7	933.3
143	Maliku	12.00	02.09.14	0.0521	60	10.6	732.4
149	Maliku	9.30	04.09.14	0.0592	60	19.6	1191.9

153	Maliku	9.00	06.09.14	0.0553	60	17.5	1139.2
156	Maliku	8.30	07.09.14	0.0501	60	22.7	1631.1
159	Maliku	8.50	09.09.14	0.0536	60	14.1	947.0
164	Maliku	10.00	10.09.14	0.055	60	17.8	1165.1
166	Maliku	9.00	12.09.14	0.0525	60	14.2	973.7
169	Maliku	10.00	13.09.14	0.0552	60	29.3	1910.9
172	Maliku	9.30	14.09.14	0.0523	60	14.9	1025.6
173	Maliku	10.30	15.09.14	0.0517	60	11.5	800.8
177	Maliku	9.46	16.09.14	0.0563	60	20.3	1298.0
182	Maliku	9.00	18.09.14	0.056	60	21.3	1369.3
184	Maliku	9.30	19.09.14	0.0578	60	20.1	1251.9
004	Molly	8.20	30.06.14	0.054	60	9.6	640.0
006	Molly	10.00	02.07.14	0.0594	60	11.3	684.8
013	Molly	10.00	04.07.14	0.0519	60	7.1	492.5
018	Molly	10.00	05.07.14	0.05	60	10.4	748.8
021	Molly	10.30	06.07.14	0.0586	60	10.5	645.1
028	Molly	10.30	10.07.14	0.0599	60	7.4	444.7
030	Molly	10.45	11.07.14	0.0544	60	8.4	555.9
038	Molly	10.45	15.07.14	0.0524	60	4.7	322.9
041	Molly	10.45	16.07.14	0.0524	60	6.2	426.0
051	Molly	11.30	24.07.14	0.0541	60	4.4	292.8
056	Molly		25.07.14	0.0513	60	4.9	343.9
064	Molly	10.00	29.07.14	0.0554	60	10.9	708.3
069	Molly	10.45	30.07.14	0.0563	60	15.5	991.1
071	Molly	10.30	02.08.14	0.0583	60	9.9	611.3
080	Molly	10.00	04.08.14	0.0574	60	6.2	388.9
083	Molly	10.30	05.08.14	0.0571	60	5.2	327.8
084	Molly	10.15	06.08.14	0.0516	60	15.7	1095.3
087	Molly	10.40	07.08.14	0.0533	60	6.3	425.5
095	Molly	9.00	10.08.14	0.0582	60	11.3	699.0
098	Molly	9.30	11.08.14	0.0539	60	13.8	921.7
101	Molly	12.00	12.08.14	0.055	60	9.6	628.4
105	Molly	10.30	13.08.14	0.0518	60	13.2	917.4
111	Molly	11.45	16.08.14	0.0524	60	5.6	384.7
113	Molly	10.00	17.08.14	0.0592	60	15.8	960.8
119	Molly	10.50	19.08.14	0.0573	60	6.1	383.2
120	Molly	10.00	20.08.14	0.0591	60	5.9	359.4
122	Molly	9.45	22.08.14	0.0548	60	8.9	584.7
129	Molly	10.30	27.08.14	0.0538	60	9	602.2
132	Molly	10.00	28.08.14	0.051	60	10.2	720.0
136	Molly	10.30	29.08.14	0.0581	60	4.8	297.4

139	Molly	10.00	30.08.14	0.0569	60	14.5	917.4
144	Molly	12.00	02.09.14	0.0571	60	15.1	952.0
145	Molly	9.00	03.09.14	0.0522	60	8.4	579.3
148	Molly	9.30	04.09.14	0.0515	60	6.2	433.4
161	Molly	9.30	09.09.14	0.0566	60	19.3	1227.6
165	Molly	10.00	10.09.14	0.0571	60	9.5	598.9
168	Molly	10.00	13.09.14	0.0599	60	18.5	1111.9
174	Molly	10.30	15.09.14	0.0507	60	10.9	774.0
178	Molly	9.46	16.09.14	0.0535	60	14.5	975.7
183	Molly	9.00	18.09.14	0.0521	60	7	483.7
185	Molly	9.30	19.09.14	0.054	60	9.5	633.3
009	Unknown	8.00	03.07.14	0.0526	60	16.4	1122.4
010	Unknown	8.00	03.07.14	0.0587	60	24.5	1502.6
011	Unknown	8.00	03.07.14	0.0513	60	10.5	736.8
057	Unknown	16.40	27.07.14	0.0579	60	25.8	1604.1
058	Unknown	16.40	27.07.14	0.0612	60	17.8	1047.1
059	Unknown	16.40	27.07.14	0.0635	60	3.1	175.7
060	Unknown	16.40	27.07.14	0.0561	60	38.4	2464.2
102	Unknown	16.20	12.08.14	0.0511	60	20.2	1423.1
103	Unknown	16.20	12.08.14	0.0517	60	16.2	1128.0
141	Unknown	10.30	01.09.14	0.0516	60	16.6	1158.1
151	Unknown	16.30	05.09.14	0.0607	60	14	830.3
152	Unknown	16.30	05.09.14	0.0557	60	12.3	795.0
022							
045							
109							
130							

5.1 – Partitioning of orangutan enclosure

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Figure 9.1 Aerial view of orangutan enclosure at Twycross Zoo with zones marked in red, blue and yellow.

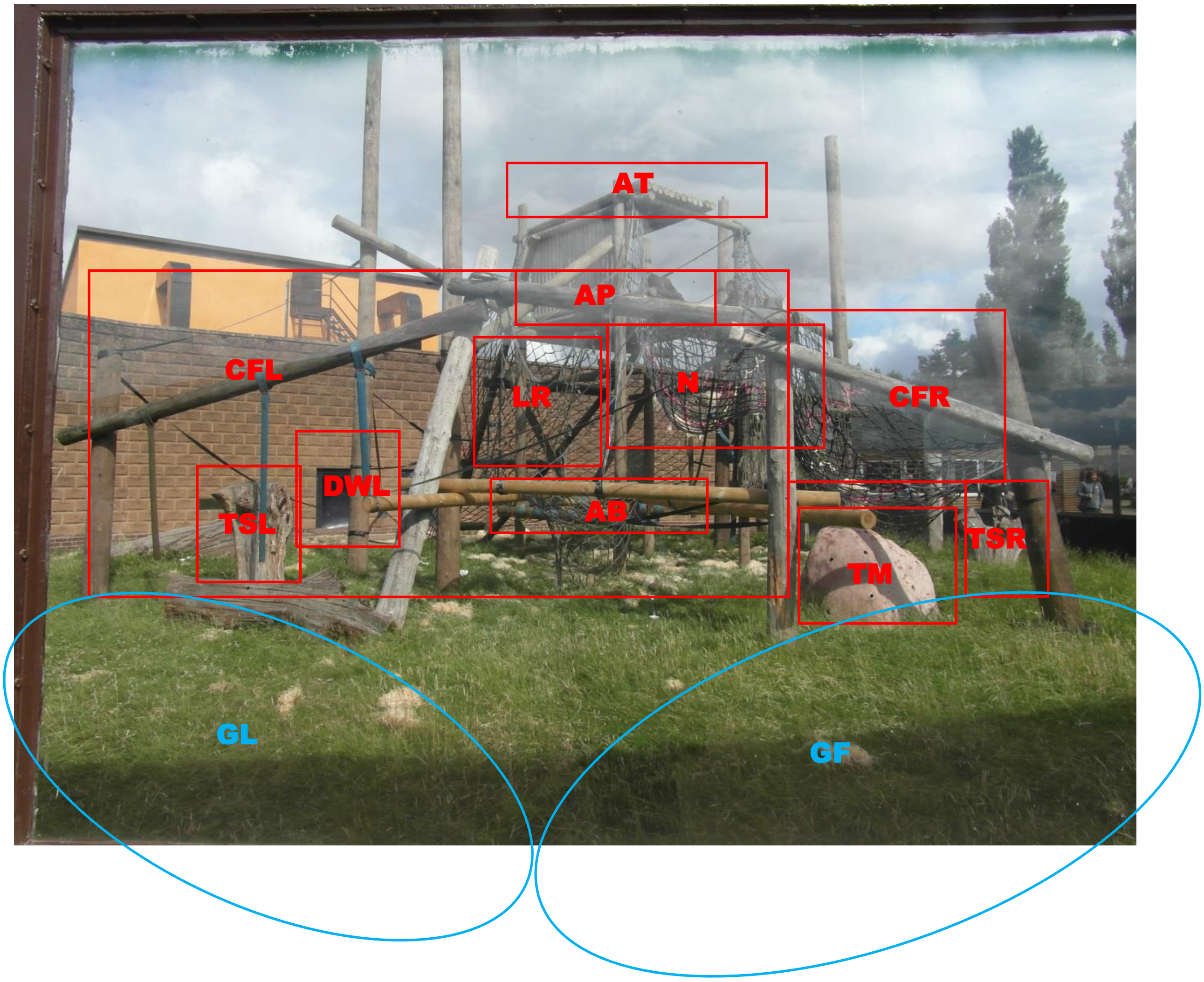

Figure 9.2 Left hand view of the outdoor area of the orangutan enclosure at Twycross Zoo with zones marked in red and blue.

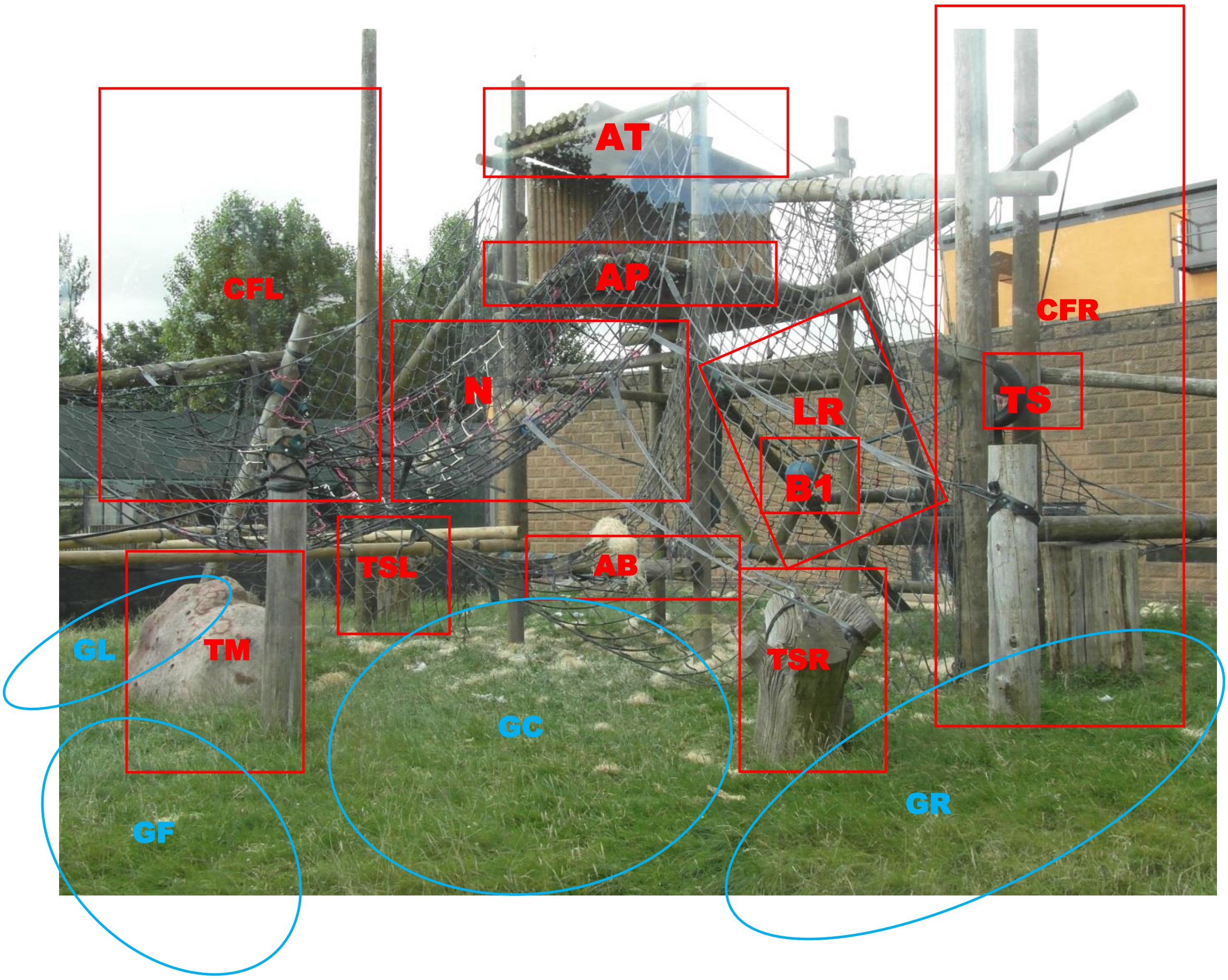

Figure 9.3 Right hand view of the outdoor area of the orangutan enclosure at Twycross Zoo with zones marked in red and blue.

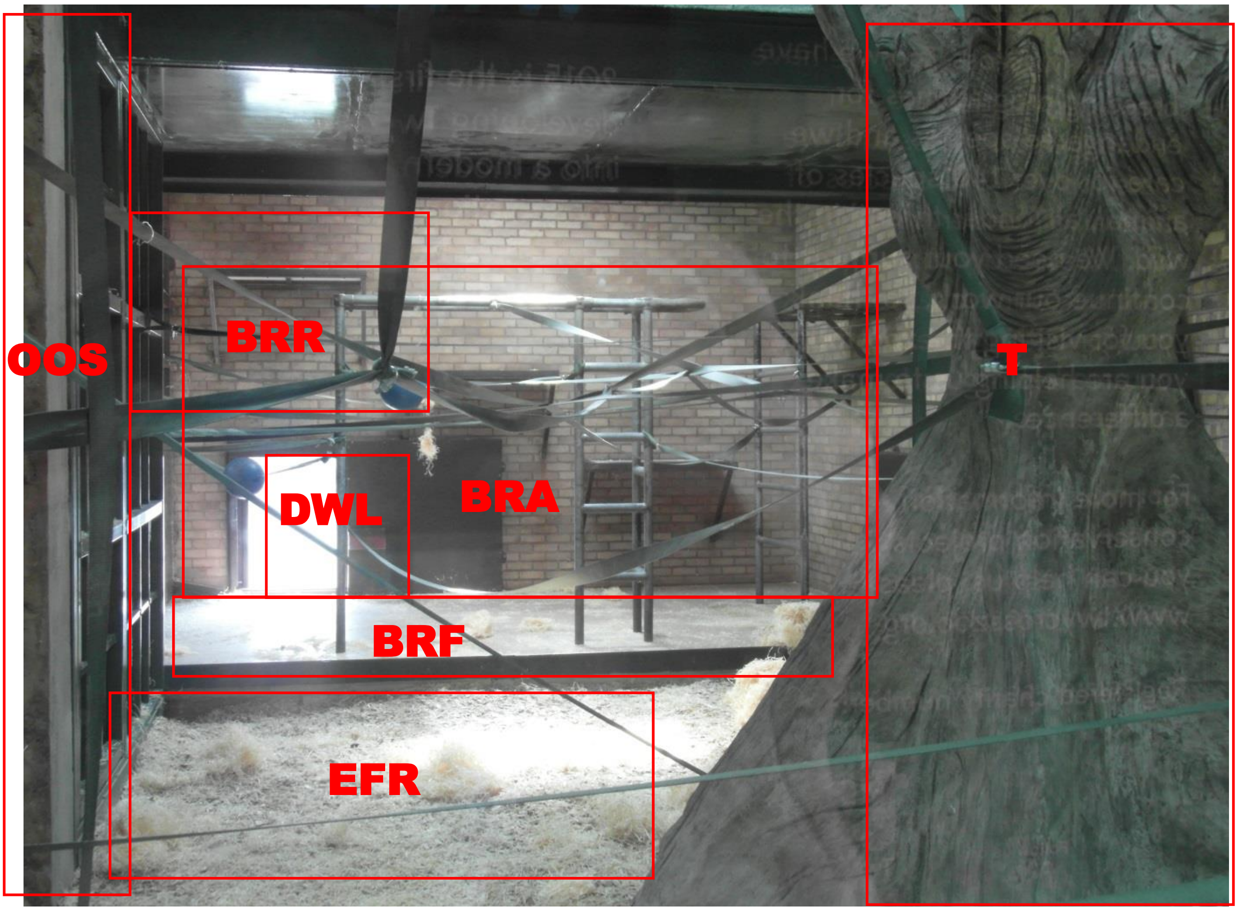

Figure 9.4 Right hand view of the indoor area of the orangutan enclosure at Twycross Zoo with zones marked in red.

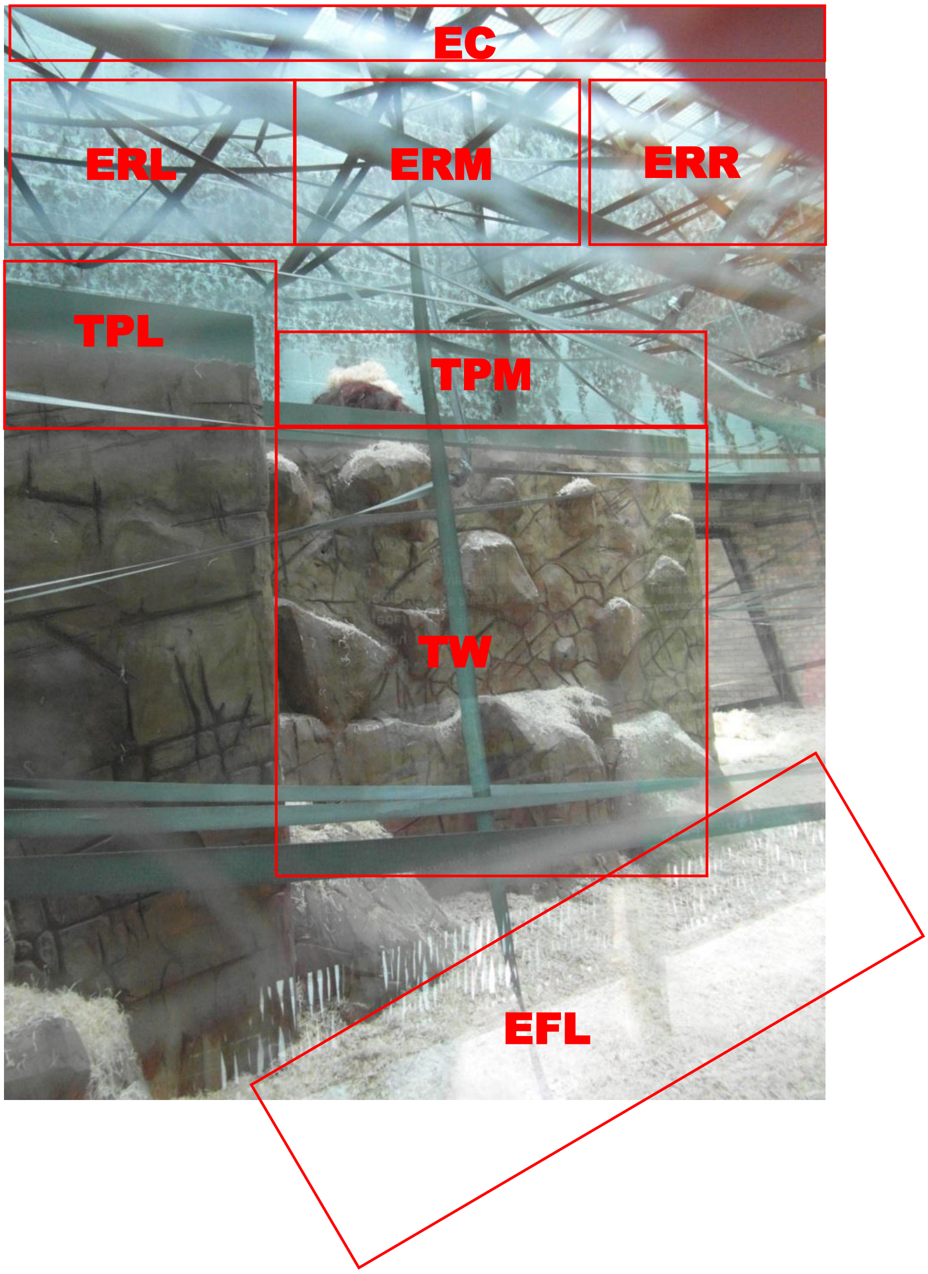

Figure 9.6 Left hand view of the indoor area of the orangutan enclosure at Twycross Zoo with zones marked in red.

5.2 – Zone key for partitioning of orangutan enclosure

Zone code	Description	Zone type
DWL	Doorway Left	Terrestrial
DWR	Doorway Right	Terrestrial
LR	Logs Rear	Terrestrial
CFR	Climbing Frame Right	Mid canopy
CFL	Climbing Frame Left	Mid canopy
TSR	Tree Stump Right	Mid canopy
TSL	Tree Stump Left	Mid canopy
TM	Termite Mound	Termite mound
N	Netting	Mid canopy
NR	Net Rear	Mid canopy
AT	Apparatus Top	Top canopy
AP	Apparatus Platform	Top canopy
APP	Apparatus	Vertical structure
AB	Apparatus Bottom	Mid canopy
GR	Grass Right	Terrestrial
GL	Grass Left	Terrestrial
GF	Grass Front	Terrestrial
GC	Grass Centre	Terrestrial
MR	Moat Right	Terrestrial
MF	Moat Front	Terrestrial
ML	Moat Left	Terrestrial
TS	Tyre Swing	Mid canopy
B1	Ball 1	Mid canopy
B2	Ball 2	Mid canopy
OOS	Out of Sight	Out of Sight
BRR	Back Room Ropes	Mid canopy
BRA	Back Room Apparatus	Vertical structure
BRF	Back Room Floor	Terrestrial
T	Tree	Top canopy
EFR	Extension Floor Right	Terrestrial
EFL	Extension Floor Left	Terrestrial
TW	Termite Wall	Termite mound
TPL	Termite Platform Left	Top canopy
TPM	Termite Platform Middle	Top canopy
ERL	Extension Ropes left	Mid canopy
ERM	Extension Ropes Middle	Mid canopy
ERR	Extension Ropes Right	Mid canopy
EC	Extension Ceiling	Top canopy
EPB	Extension Platform Back	Top canopy
EPR	Extension Platform Right	Top canopy
EPL	Extension Platform Left	Top canopy