Assessing the welfare of captive male giraffes in a bachelor herd using behavioural and hormonal analysis.

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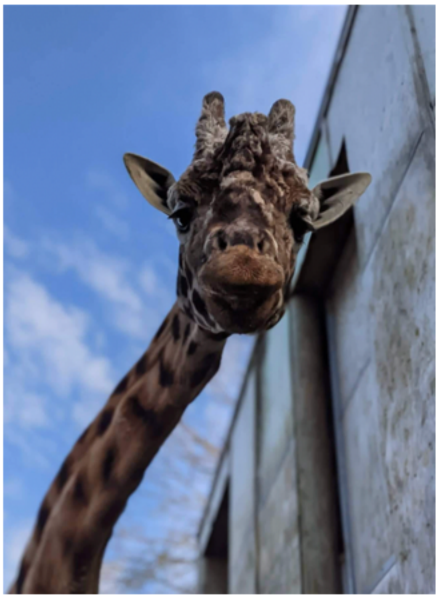

Masamba

Dume

Ndale

Ndoki

"Wildlife is something which man cannot construct. Once it is gone, it is gone forever.
Man can rebuild a pyramid, but he can't rebuild ecology, or a giraffe."

- Joy Adamson.

Abstract

As biodiversity continues to decline, zoos play an essential role in ex-situ species conservation. An animal's ability to feel pain and pleasure drives the need to ensure zoos maintain high animal welfare standards. This thesis aimed to assess the welfare of four male giraffes housed in a bachelor herd at Hamilton Zoo due to concerns about some individuals' social and stereotypical behaviour. This aim was achieved using non-invasive behavioural observations and faecal cortisol metabolite (FCM) analysis.

Behavioural observations and the collection of faecal samples occurred over 12 weeks. Behavioural analysis investigated the giraffes' social dynamic, stereotypical behaviour, and enrichment engagement. Faecal cortisol metabolite analysis investigated how each giraffe's behaviour correlated to their stress levels. Faecal cortisol metabolite analysis also included three giraffes housed in another bachelor herd at Gibbs Farm to identify if the giraffes at each institution experienced similar welfare concerns.

At Hamilton Zoo, the giraffes' social dynamic was influenced by a hierarchy. The hierarchy was similar to that displayed by wild males and likely reduced social conflict. The expression of same-sex sexual behaviour such as following and investigating also influenced the giraffes' social dynamic. All four giraffes expressed oral stereotypes with the underlying cause likely feeding motivation. One giraffe displayed pacing stereotypes more frequently than the others. Pacing occurred more regularly before movement to the day enclosure, and there was a positive correlation between pacing and being followed by another giraffe. Therefore, pacing likely occurred due to anticipation or social stress associated with being followed. Foraging enrichment had little impact on the giraffes' behaviour.

In the wild, subordinate males often experience higher FCM levels. However, the subordinate males at Hamilton Zoo did not experience the highest FCM levels. In comparison, the subordinate male at Gibbs Farm did exhibit the highest FCM levels. These variations may be due to the different herd and enclosure sizes at each institution. The expression of pacing stereotypes did not correlate with elevated FCM levels suggesting that stress may not be the underlying cause of that behaviour. Interestingly, the dominant male at Hamilton Zoo displayed the highest FCM levels. These FCM levels were likely due to pain associated with his hoof-related health issues. Foraging enrichment did reduce one giraffe's FCM levels, but additional data would be needed to draw more definite conclusions about this.

Behavioural observations and FCM analysis often provided contrasting results about the giraffes' welfare at Hamilton Zoo. Contrasting results are not uncommon and they often spark conflict over what defines optimal welfare and what assessment methods are valid. Perhaps the best welfare assessments will be achieved when scientists consider all views and use multiple assessment methods in conjunction. Hamilton Zoo can use the findings from this thesis to guide its husbandry routines to ensure they support each giraffe's welfare needs. High welfare standards will enable zoos to act as ethical arks that spark a passion for conservation in their community.

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Chapter 1

General introduction

1.1 Zoos

Biodiversity refers to the variation found amongst living species due to differences in genetics and functional traits. Research has identified that biodiversity increases ecosystem stability and productivity (Cardinale *et al.*, 2012). As anthropogenic and climate change pressures increase, the world is witnessing rapid declines in biodiversity (Leclère *et al.*, 2020). Alongside factors such as drought, ultraviolet radiation, and increased carbon dioxide, biodiversity loss could become a major factor reducing ecosystem productivity (Hooper *et al.*, 2012). In these conditions, zoos play an essential role in ex-situ species conservation (Lueders & Allen, 2020; Mooney *et al.*, 2020). Early zoos resembled museums with animals managed in small areas displayed to the public. These institutions mainly focused on human entertainment (Rutledge *et al.*, 2011). At the end of the twentieth century, more zoos began focusing on species conservation. The need to address increasing extinction rates and public demand for zoos to contribute to species conservation drove this change (Kaufman *et al.*, 2019). The ark paradigm emerged in the 1980s, suggesting that ex-situ populations act as genetic reserves for species whose in-situ populations are declining due to anthropogenic expansion and climate change (Brichieri-Colombi *et al.*, 2019). Modern-day zoos provide safe havens and breeding opportunities for a range of vulnerable and endangered species. Individuals in both in-situ and ex-situ populations can be considered in conservation programs as, despite their location, they all contribute to a species' genetic diversity (Lueders & Allen, 2020).

Modern-day zoos contribute to conservation through reintroduction programs, funding, education, and research. Ex-situ populations can contribute to in-situ conservation through reintroduction programs. For example, zoo-bred migrating northern bald ibises (*Geronticus eremita*) were reintroduced to European wintering sites where they had become extinct in the seventeenth century (Rutledge *et al.*, 2011). Zoo-bred Arabian oryx (*Oryx leucoryx*) were also introduced to locations in the Middle East after in-situ populations had decreased to 100- 200 individuals (Rahbek, 1993). In New Zealand, zoos have enhanced reintroduction programs by partnering with government groups and other non-profert organizations such as the Department of Conservation. Tuatara (*Sphenodon*), blue duck (*Hymenolaimus malacorhynchos*), kaka (*Nestor meridionalis*), and brown teal (*Anas chlorotis*) are examples of species that have been bred for release in New Zealand zoos (Armstrong *et al.*, 2015). However, reintroduction programs are not always successful as captive-breed animals may not exhibit appropriate behaviours for survival in the wild. For example, captive chimpanzees (*Pan troglodytes*) reintroduced to the wild often experience negative encounters with wild males. Challenges such as disease transmission must also be overcome when translocating animals from ex-situ to in-situ environments (Fa *et al.*, 2011; Brichieri-Colombi *et al.*, 2019). When reintroductions are not possible, advances in assisted reproductive technologies may provide another way for in-situ populations to provide genetic rescue to ex-situ ones (Herrick, 2019; Lueders & Allen, 2020).

Zoos can also contribute to in-situ conservation by raining awareness and funding. Through funding, zoos can make a considerable contribution to in-situ species conservation. Together zoos and aquariums around the world raise $350 million for in-situ conservation each year. Zoos raise these funds through visitor attendance fees and additional fundraising events. Individual zoos can strengthen their contribution to conservation by increasing their financial investments in in-situ conservation programs. Several institutions could also combine their funding efforts to increase their impact on in-situ conservation (Gusset & Dick, 2010; Mooney *et al.*, 2020).

A goal shared by many zoos is to provide education that sparks a passion for conservation amongst their visitors. People living in large cities often have little understanding of the natural world and frequently underestimate the devastating impacts of habitat loss, invasive species, and climate change. Zoos provide people with the opportunity to interact with and develop deeper connections with nature (Ballantyne & Packer, 2005; Mantyka-pringle *et al.*, 2012). After viewing and connecting with animals, people may feel inspired to learn more about the threats they face and how they can assist in their conservation. Information about species, such as their habitat, behaviour, and conservation status, is often displayed outside enclosures to prompt education. Events such as keeper talks and displays of animal training can also enhance education. People may be more interested in learning about a species after engaging with the animals and zookeepers (Pearson et al., 2014).

Zoos also provide many novel research opportunities. Research in zoos can expand our understanding of animal behaviour and a species role within an ecosystem. For example, captive populations have allowed us to describe and understand the mating behaviours of Suriname toads (*Pipa pipa*) and the feeding behaviours of snakes (*Serpentes*). Findings from these studies can help advance in-situ conservation efforts (Bashaw *et al.*, 2019). Reproductive physiology, including species ovarian mechanisms and ejaculation traits, have also been studied in captive animals, with findings used to develop assisted reproductive technologies for both captive and wild animals (Herrick, 2019; Lueders & Allen, 2020). Research on captive populations also helps advance captive animal husbandry and animal welfare standards. For example, studies on stereotypical behaviour have developed our understanding of an animal's ability to display appropriate behaviour in captive environments. Studies have also investigated behavioural changes across different enclosures to help identify environments that support optimal welfare (Razal *et al.*, 2017; Bashaw *et al.*, 2019). Research must continue to explore captive animal welfare and ways to improve it. When captive animals have enhanced welfare, zoos may have increased breeding success and increased visitation rates, enhancing their ability to contribute to conservation (Maple & Perdue, 2013).

1.2 Giraffes

Giraffes (*Giraffa*) are the tallest land mammals, occupying habitats across Africa. While debate surrounds their taxonomy, recent genetic analysis has suggested that four different giraffe species exist. The four species are- reticulated giraffe (*Giraffa reticulata*), northern giraffe (*Giraffa camelopardalis*), southern giraffe (*Giraffa giraffa*), and Masai giraffe (*Giraffa tippelskirchi*). The species have adapted to habitats ranging from arid deserts to woodlands and savannas (Fennessy *et al.*, 2016; O'Connor *et al.*, 2019). Northern giraffe populations are fragmented and occupy habitats across nine countries, including Niger, Central African Republic, and Uganda. Reticulated giraffe populations seem to be connected, and the majority are found throughout northern Kenya, Somalia, and southern Ethiopia. Masai giraffes also occupy habitats in Kenya as well as Tanzania. The southern giraffe inhabits nine countries, including Namibia, Botswana, and South

Africa (Figure 1.1). In all these locations, giraffes provide many ecosystem functions, including seed dispersal, facilitation of plant growth, and the opening of habitat spaces (O'Connor *et al.*, 2019).

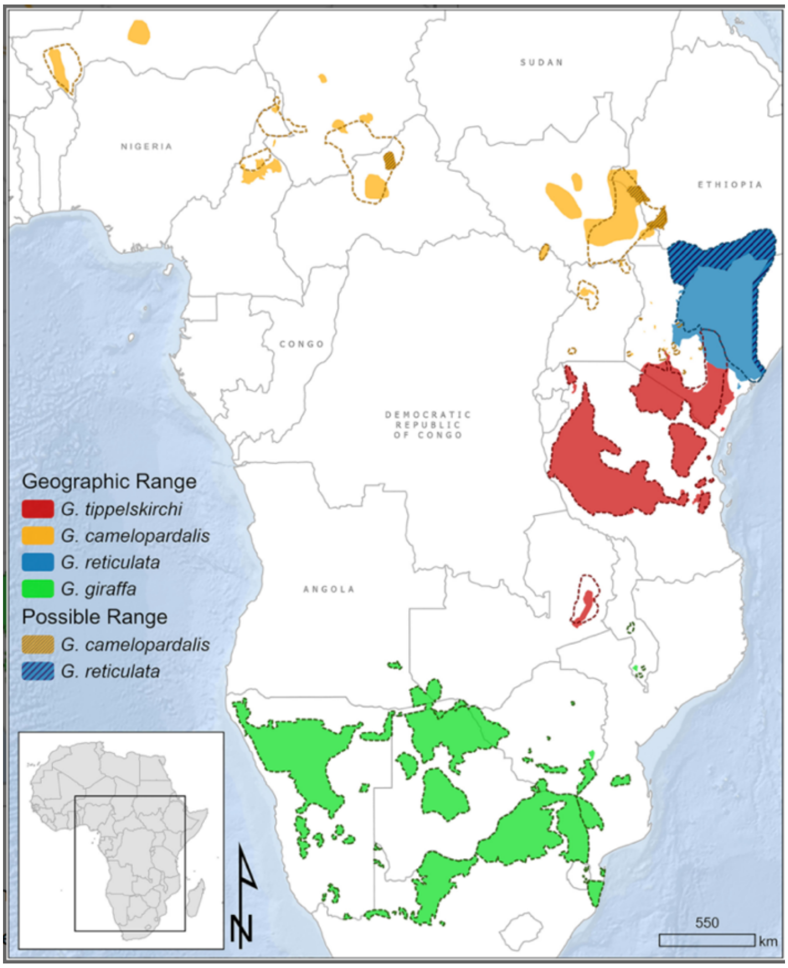

Figure 1.1 -The geographical range of each giraffe species in sub- Saharan Africa. The coloured shapes represent each species known range. The dashed lines represent where the International Union for Conservation of Nature previously thought the species ranges were. The shapes with cross-hatched patterns show areas where giraffe populations have not been identified but may occur (O'Connor *et al.*, 2019).

A giraffe's diet consists of foliage such as leaves, twigs, shoots, fruit, and flowers from various tree and shrub species (Roggenbuck *et al.*, 2014). Giraffes occupy several habitats resulting in their diet containing many different plant species. While a giraffe's diet is relatively diverse, it often contains a few predominant species. *Acacia* is a key species in many giraffe diets, with giraffes feeding on its leaves and flowers. However, *Acacia* is not always the dominant species in a giraffe's diet. For example, giraffes occupying the Serengeti National Park in Tanzania feed primarily on *Grewia bicolor* or G. *fallex* instead of *Acacia* in the dry season (Berry & Bercovitch, 2017). Giraffes are ruminants meaning they digest cellulose through foregut fermentation in the rumen (Van Soest, 2018). This process relies heavily on a symbiotic relationship with the fermenting microbes present in the rumen. The giraffe's rumen contains bacteria from the phyla *Firmicutes* and *Bacteroidetes*, as well as bacteria that are currently undescribed (Roggenbuck *et al.*, 2014).

1.2.1 Giraffe behaviour

Giraffes display a range of behaviours that allow them to access resources, avoid predation, and interact with conspecifics (Seeber *et al.*, 2012; Shorrocks, 2016). Giraffes access nutrients through browsing behaviours where the lips and tongue manipulate foliage. When ruminating, giraffes regurgitate the cud and chew it a second time. When drinking, giraffes often have to lower their mouth to ground level, requiring the front legs to be spread apart. Giraffe locomotion involves two gates: walking and cantering. Walking is a four-beat movement where the giraffe alternates between moving the legs on either side of the body. Cantering is a faster three-beat movement involving a short period of suspension. In this gate, giraffes can reach speeds of 56 km per hour. However, giraffes can only maintain this speed for a few minutes due to elevated blood pressure (Seeber *et al.*, 2012). Giraffes groom themselves using their mouth or by rubbing their bodies against other objects. While giraffes are often standing, they can rest and sleep lying down with their legs bent underneath them (Shorrocks, 2016).

Giraffes have evolved a range of behaviours to avoid and defend themselves from predators such as lions. In synchronisation with other behaviours such as walking and ruminating, giraffes often scan their surroundings for potential threats (Seeber *et al.*, 2012; Shorrocks, 2016). Unlike many species, a giraffe's scanning frequency does not decrease when they are in larger groups. However, females scan more frequently when a mature bull is nearby, and males scan more in the presence of other males. Therefore, alongside predator avoidance, scanning is also directed at conspecifics to avoid conflict and unwanted mating interactions (Cameron & du Toit, 2005). Giraffes can react to a threat by snorting and pawing or stomping the ground with their front legs. Giraffes defend themselves from predators by kicking and rapidly lifting and stomping their front legs. These behaviours can severely injure and kill predators but are rarely directed at conspecifics. Giraffes can also deter birds such as oxpeckers (*Buphagus*) by lowering their head and lifting their back legs in a buck (Seeber *et al.*, 2012; Shorrocks, 2016).

Giraffes display many social behaviours. When seeking to join a group or individual, a giraffe can approach or follow them to remain nearby. When following, giraffes can be close to each other or up to 60 m apart. Giraffes can also engage in tactile interactions with conspecifics. When nuzzling, a giraffe will use its muzzle to touch another giraffe on its face or body (excluding the anogenital area). When allogrooming, a giraffe will lick or bite another giraffe's body (excluding the anogenital area). These behaviours likely strengthen social bonds, and mothers often express them towards their calf (Seeber *et al.*, 2012; Shorrocks, 2016).

Adult male giraffes form social hierarchies. Various behaviours are expressed to form and maintain these hierarchies. Male giraffes commonly display a behaviour called necking. This behaviour can occur in sparing or more serious fighting interactions. When necking, giraffes stand parallel to each other, using their head and ossicones to swing blows at the other individual. During sparing, blows are often light and delivered in turn. Instead of determining social hierarchy, juvenile males can display sparing as a form of play or as an opportunity to practice the necking behaviour. Unlike sparing, fighting is initiated quickly and involves strong blows often delivered simultaneously. Fighting outcomes can determine an individual's hierarchy status. However, direct contact is not always required to reflect an individual's rank. Giraffes can display contactless forms of aggression in dominant gestures such as rigid postures and high head holding. These postures can displace others or lead to submissive individuals avoiding more dominant males (Pratt & Anderson, 1985; Seeber *et al.*, 2012).

Giraffes also express a range of behaviours when courting and mating. Giraffes display a behaviour called investigating, where they sniff or lick another's anogenital area. Males commonly investigate females, but the behaviour has been observed in same-sex sexual interactions. Males display investigating behaviour and touch a female's rump to stimulate urination. The male will then test the urine with his mouth and display the flehmen behaviour where he lifts his head and inhales through his nostrils while exposing the top lip. Males use urine testing and flehmen behaviours to determine if a female is in oestrus (Pratt & Anderson, 1985; Seeber *et al.*, 2012). These behaviours are often more successful in older males. While young males attempt the behaviours, they do not always successfully stimulate or test the urine. Females can also show preferences for older males, sometimes even urinating before stimulation (Pratt & Anderson, 1985). If a female is determined to be in oestrus, a male can display courtship and mate-guarding behaviours for several days before mating. These behaviours can involve following the female, directing her away from others, and repetitive urine testing. A behaviour called laufschlag can also occur during mate-guarding, where the male will touch the female's flank or hind leg with one of his front legs. These behaviours usually intensify in the presence of other males (Pratt & Anderson, 1985; Seeber *et al.*, 2012). Females can respond to a male's courting and guarding attempts by either avoiding him or tolerating his behaviours and presence. When mating, the male will shift his weight onto his hind legs allowing him to mount the female (Seeber *et al.*, 2012; Shorrocks, 2016).

1.2.2 Giraffe social structure

Social relationships can both positively and negatively impact an individual's fitness and reproductive success. Group living can provide benefits such as information transfers and increased predator defense. However, it can also result in costs such as increased competition for resources and mates. Giraffes display a fission-fusion social structure, where herds frequently split and remerge in response to the varying costs and benefits of group living. Understanding these social dynamics can be complicated as the time giraffes spend in herds is highly variable (Wolf *et al.*, 2018a). Giraffes only form herds with individuals who have home ranges overlapping with their own (Deacon & Bercovitch, 2018). A giraffe's home range can vary significantly in size, the average home range is between 68 km^2 and 514 km^2. Resource distribution and availability determine a giraffe's home range; giraffes occupying arid habitats have more extensive home ranges (Shorrocks, 2016). On average, giraffes develop more social connections in the wet season compared to the dry season. Larger herds often form after rainy periods when vegetation growth is the greatest. Competition likely increases in the dry season as food resources become scarce, resulting in smaller herd sizes (Bond *et al.*, 2019; Prehn *et al.*, 2019). However, seasonal fluctuations in herd size are not always consistent between populations. In some cases, herd size increases when resources with a patchy distribution become less abundant as giraffes congregate at the resource locations (Bercovitch & Berry, 2010). Predation risk does not seem to influence herd formation in solo adult giraffes, but it can impact mothers and calves. When predation risk increases, mothers and calves often form smaller herds in dense vegetation where calves can stay hidden (Bond *et al.*, 2019). Giraffes do not have a specific breeding season, resulting in males roaming all year round searching for females in oestrus (Wolf *et al.*, 2018a). However, mating can increase in the wet season, which correlates with an increase in mixed-sex herds and a decrease in all-male herds at this time (Prehn *et al.*, 2019).

The formation of social bonds differs between male and female giraffes. These differences likely occur due to the factors limiting male and female reproductive success. Access to resources determines a female's reproductive success. Therefore, the social interactions between females may be influenced by access to resources. Females form long-tern associations, with connections increasing in adulthood. Females may benefit from group living by gaining information about resource distribution and increased chances of offspring survival. Females are also relatively sedentary which may increase their chances of associating with the same individuals throughout their life (Carter *et al.*, 2013). Females often form larger herds in the wet season when resources are more abundant. However, females can still maintain a few social associations in the dry season. These associations are often between related individuals, with mothers and daughters sharing strong bonds (Wolf *et al.*, 2018a). Access to females determines a male's reproductive success. Therefore, the social interactions between males may be influenced by access to females (Carter *et al.*, 2013). Long-term social associations do not form between male giraffes. As male giraffes mature, they adopt a more solitary lifestyle, spending extensive periods roaming in search of females in oestrus. It is beneficial for adult males to avoid each other as it reduces reproductive competition. Mature males also form social hierarchies that can influence their access to resources, such as females. These hierarchies develop as males mature, with older males frequently dominating younger ones (Pratt & Anderson, 1982, 1985; Shorrocks, 2016). Due to their roaming lifestyle, adult males are often seen alone or in herds with females. All-male herds rarely contain several mature males. However, juvenile males often form all-male herds to practice necking skills and reduce their predation risk. Juvenile males may also associate with a mature male to develop their knowledge about resource and female distribution. While this relationship likely does not benefit the mature male, he might need to expend more energy deterring the younger male than letting him follow (Bercovitch & Berry, 2015).

The presence of human settlements may cause social structures to differ between giraffe populations. Recent research has found that female giraffes living close to human settlements form fewer social associations than those in populations further away. Increased disturbance from anthropogenic activity may cause females to move more frequently. The less sedentary lifestyle may result in these females forming fewer long-term social associations (Bond *et al.*, 2021).

1.2.3 Conservation status and captive giraffes

Unfortunately, anthropogenic expansion across Africa has resulted in giraffe population numbers dropping by 40% in the last 30 years. The International Union for the Conservation of Nature and Natural Resources (ICUN) has classed the four giraffe species as vulnerable and two subspecies of northern giraffe as critically endangered. Giraffe populations face several threats including, habitat conversion, habitat loss, and poaching. Domesticated animals owned by pastoralists can also compete with wildlife such as giraffes for food (O'Connor *et al.*, 2016). Giraffe habitats have experienced significant encroachment from urban and farm development, limiting populations to smaller isolated areas. Limited habitat and subsequent habitat fragmentation are substantial threats to giraffe populations. Populations in fragmented habitats are at risk of inbreeding. Inbreeding reduces a population’s genetic diversity and subsequent ability to adapt to environmental changes such as new diseases and environmental variations (Muller, 2018; O’Connor *et al.*, 2019). Anthropogenic disturbance can also influence metapopulation dynamics. Studies have identified that populations in protected areas with anti-poaching measures and no human settlements act as sources. In contrast, populations in unprotected areas with increased human settlement acted as sinks. If connectivity is lost within the metapopulation sink populations will be at risk of extinction (Lee & Bolger, 2017).

Across Africa there are government-managed protected areas for wildlife such as giraffes. However, studies have indicated that 70% of the giraffe population exists outside these areas. Therefore, as human populations continue to expand, urban development will further restrict and isolate giraffe populations posing a significant threat to the species’ future. Giraffe conservation will likely rely on community and private-sector contributions to ensure populations can remain protected and connected across appropriate habitats (O'Connor *et al.*, 2019). Conservation projects across Africa have already reconnected isolated populations and translocated giraffes between fragmented habitats. Genetic analysis has also determined the genetic diversity and interbreeding within giraffe populations. This information has guided conservation by highlighting vulnerable populations and individuals with appropriate genetics for translocations. Conservation projects like these provide a glimmer of hope for future giraffe populations (Winter *et al.*, 2019).

Ex-situ giraffe populations can also assist in the species conservation (O'Connor *et al.*, 2019). The Zoos and Aquarium Association (ZAA) is an Australasian organization that ensures zoos meet high animal welfare standards. In New Zealand, five ZAA accredited institutions house giraffes, with three of these containing breeding herds (Zoo Aquarium Association, 2021a). The captive giraffe populations in New Zealand cannot assist in species conservation through reintroduction programs. The primary purpose of these captive populations is to help educate people in New Zealand about the species and the challenges they are currently experiencing in wild habitats. People may be more likely to learn about giraffes after viewing them in person. Viewing the animals may also spark empathy in zoo visitors, causing them to make personal donations to in-situ giraffe conservation projects. The majority of funds raised by zoos for in-situ conservation programs come from visitor attendance fees. Therefore, an institution's popularity impacts its ability to contribute to conservation. Alongside native and endangered species, zoos may also benefit from housing large iconic vertebrates as these species can draw attention and excite visitors. Giraffes often captivate the attention of zoo visitors, and many people favor the species. In the right environment, giraffes are also relatively safe, and zoo visitors can engage in closer interactions with them, such as feeding events. Therefore, institutions housing giraffes may increase in popularity, allowing them to educate more people and raise more awareness and funding for the in-situ conservation of many species (Hutchins *et al.*, 1995; Orban *et al.*, 2016; Mooney *et al.*, 2020).

1.3 Animal welfare

The need to assess and maintain animal welfare stemmed from the understanding that they, too, can feel pain and pleasure (Dawkins, 2021). Romanes, a contemporary of Darwin, investigated the mental abilities of animals. His studies were limited, but he did provide evidence that animals experienced conscious emotions. In one example, he described an elephant taking a deep breath before a medical procedure as if it was preparing for the painful experience (Fraser, 2009). Today, awareness of an animal's sentience is likely a key driver in the growing interest in animal welfare (Lawrence, 2008). An animal's ability to experience emotion drives the need to ensure its welfare is maintained. However, it is challenging to determine welfare based on emotion as we do not fully understand conscious feelings and thoughts. We also do not know how different species experience consciousness. For example, do giraffes and chimpanzees feel pleasure in the same way? We can assume that an animal experiences positive and negative emotion, but the inability to determine an animal's consciousness scientifically can make it hard to assess welfare (Fraser, 2009; Dawkins, 2021).

Despite animal welfare being a recognised field of science, a consistent definition for animal welfare does not yet exist. This is likely due to the conflict that often arises when determining what factors influence animal welfare (Dawkins, 2021). There are three common viewpoints about the factors impacting animal welfare. One view focuses on the biological functioning of an animal, suggesting that welfare will be satisfied if an animal is healthy and successfully reproducing. Another states that animals must live in their natural environment to have optimal welfare. The final view focuses on emotions and feelings, stating that animals must be free of suffering to have adequate welfare. These different views may explain the contrasting results that are frequently published in animal welfare science. For example, in 1997, a group of scientists identified that pig (*Sus*) gestation stalls raise welfare concerns. In 2001, another study stated that they met welfare standards. The different results likely arose due to the scientists' different views of animal welfare. One study accessed the pigs' welfare using biological functioning, while the other focused on emotions (Fraser, 2003).

Welfare is complex and challenging to assess. Several guidelines have been created to try and assist in the assessment of animal welfare. In the 1980s, the UK Farm and Animal Welfare Council created a guideline called the Five Freedoms. It stated that animals must have freedom from thirst/ hunger, discomfort, pain/disease, fear, and the freedom to express normal behaviour (Mellor & Stafford, 2001). This guideline was influential in animal welfare science, but it had a few limitations. The keyword 'freedom' suggested that animals should never experience negative states such as hunger and discomfort.

However, these states are necessary to ensure animals know when to remove themselves from unfavorable environments. If an animal does not respond to an unsuitable condition, it will likely suffer health issues or death. The guideline also focuses on avoiding negative experiences instead of promoting positive ones. Over time animal welfare science has emphasized the frequency of positive experiences rather than negative ones (Mellor, 2016).

Key ideas from the Five Freedoms were adapted to create a framework commonly used today called the Five Domains of Welfare (Figure 1.2). A critical factor determining animal welfare is biological functioning. The first three welfare domains, nutrition, environment, and health focus on an animal's biological functioning. An animal's welfare also expands further than its biological functioning. Animals have evolved a range of species-specific behaviours such as foraging techniques, social cues, and predator avoidance strategies that are essential for survival and reproduction. The fourth welfare domain states that an animal must have the ability to express a variety of behaviours in response to its environment. When able to display a range of behaviours, animals will inevitably do what they want and desire in order to meet their fundamental needs. The first four welfare domains feed into the fifth domain of affective states. Affective states refer to emotions or feelings that are pleasant/ positive or unpleasant/ negative. Positive affective states include relaxation, contentment, and security, while negative ones include stress, fear, and pain. We do not understand how animals experience conscious emotions and feelings. Therefore, while we can assume negative and positive affective states occur in specific situations, we cannot fully understand how they are experienced (Fraser, 2008; Mellor, 2015; Dawkins, 2021). The Five Freedoms guideline aimed to avoid negative affective states. In contrast, the Five Domains of Welfare guideline suggests that welfare results from a balance of positive and negative affective states (Mellor, 2016).

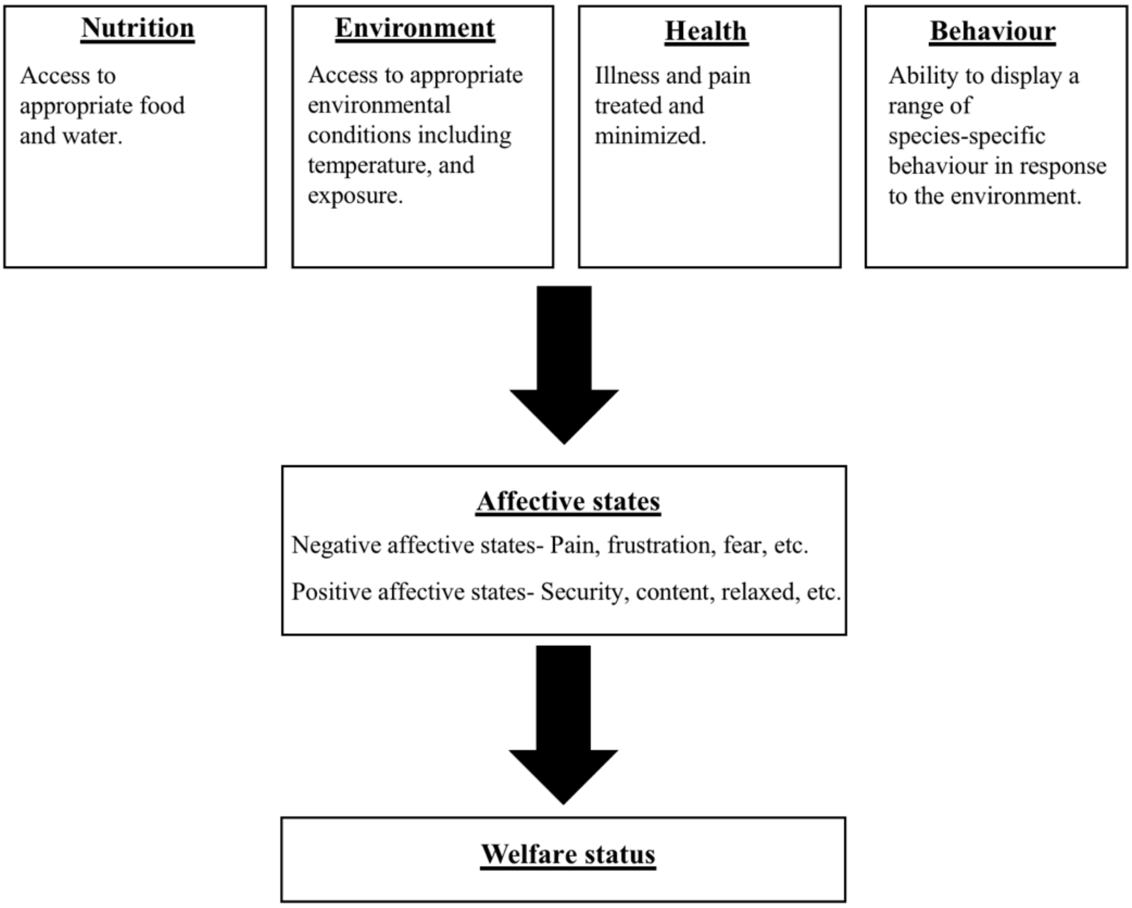

Figure 1.2- The five welfare domains impact an animal's welfare status. The nutrition, environmental, health, and behaviour domains influence an animal's affective states. The balance between positive and negative affective states then determines an animal's welfare status (Mellor, 2016).

Animal welfare applies to all animals, including exotic animals housed in zoos. The Association of Zoos and Aquariums (AZA) is an international organization that aims to advance zoos and ensure institutions meet high animal welfare standards. The AZA and ZAA provide benchmark standards for captive animal welfare. For example, they state that institutions should have welfare standards at a collection level while identifying the specific factors influencing a species' or individual's welfare. They also suggest that institutions should regularly assess animal welfare using frameworks such as the Five Domains of Welfare (Terrell, 2017; Zoo Aquarium Association, 2021b). Zoos that strive to maintain high welfare standards will form ethical arks that contribute to conservation while taking responsibility for their animals' welfare needs (Maple & Perdue, 2013).

1.4 Animal welfare and behaviour

Behavioural observations can help assess animal welfare. Without specialized equipment, scientists can use behaviour to evaluate several factors, including physiological functioning, health, and affective states. An animal's motivational state drives its behaviour. Therefore, behaviour reflects an animal's affective states (Yon *et al.*, 2019; Watters *et al.*, 2021). For example, luxury play and affiliative behaviours are often associated with positive affective states. Luxury behaviours are not required for an animal to meet its fundamental needs. Therefore, these behaviours are likely displayed when an animal is in a favorable position (Whittaker & Marsh, 2019). Behaviour can also reflect negative affective states. For example, piglets squeal when they are separated from their mother, expressing their negative affective state. While we cannot assume that the piglet is experiencing similar emotional distress to a human, we can assume that it is in an unpleasant state. The squealing behaviour has likely evolved to help piglets remove themselves from adverse conditions by getting their mother's attention (Fraser, 2009). While changes in behaviour can reflect changes in affective state, it is essential to note that specific behaviours do not reflect specific affective states. For example, a seemingly frustrated animal may begin displaying aggression, but the display of aggression does not always mean an animal is frustrated (Dawkins, 2021). Behaviours reflecting positive and negative affective states can also seem relatively similar. For example, an animal can freeze when they feel threatened or in danger. An animal can also display immobility when experiencing positive affective states. For example, an animal basking in the sun is likely in a favorable position (Fureix & Meagher, 2015). Species will also respond to environmental conditions and challenges differently. Therefore, there are no universal behavioural indicators of positive and negative affective states. Behavioural responses can also differ between individuals in the same species due to varying experiences and environmental conditions. A species' unique behavioural repertoire needs to be well understood before scientists use it in welfare assessments. This understanding will ensure incorrect assumptions are not made about an animal's behaviour and its reflection on welfare (Hill & Broom, 2009).

Animals have evolved a range of species-specific behaviours that enable them to maintain homeostasis in fluctuating environments. In captivity, animals can experience new environmental challenges. In some cases, captive animals have not evolved behaviours that enable them to overcome these challenges. When captive animals cannot display appropriate behaviour in response to the environment, they may begin expressing stereotypical ones instead (Veasey, 2006; Hill & Broom, 2009). Stereotypical behaviours are abnormal repetitive behaviours that appear to have no function. The underlying cause of these behaviours is often negative affective states such as stress, frustration, and boredom. Therefore, the expression of stereotypes can be a behavioural indicator of suboptimal welfare (Mason *et al.*, 2007). For example, captive cheetahs (*Acinonyx jubatus*) may experience negative affective states similar to frustration when unable to display stalking and killing behaviours. These negative affective states can lead to the expression of pacing stereotypes (Quirke *et al.*, 2012). Enrichment is commonly used in captivity to facilitate the expression of species-specific behaviours and subsequently reduce the display of stereotypical ones. Enrichment can be presented to captive animals in various forms, including puzzle feeders, cognitive games, and olfactory stimulation. The type of enrichment given to an animal depends on the species' behavioural inventory and cognitive ability. For example, altering food location is a form of enrichment used to reduce pacing in captive cheetah as it facilitates the species investigating and stalking behaviours. When facilitating the expression of species-specific behaviour, enrichment can spark positive affective states (Quirke & O'Riordan, 2011). Assessing the expression of stereotypical behaviour and the impact of enrichment on this behaviour may help determine a captive animal's welfare.

1.5 Animal welfare and faecal cortisol metabolite analysis

Alongside behavioural observations, animal welfare assessments can also incorporate physiological measurements. Physiological measurements can involve the evaluation of an animal's heart rate, respiration, and adrenal activity. Variations in these measurements can indicate underlying health issues or negative affective states. However, many physiological assessments are invasive, limiting their ability to monitor the welfare of wild or captive wild species. For example, when assessing heart rate animals are often required to be handled and wear a monitor. This would elicit a stress response in many zoo species impacting the assessment results, and the animal would likely break the monitor (Hill & Broom, 2009). Faecal cortisol metabolite (FCM) analysis is a physiological measurement that scientists can use to assess the welfare of wild and captive exotic animals. This method enables an animal's adrenal activity to be monitored non-invasively (Veasey, 2006; Hill & Broom, 2009).

A stressor is something that does or seems to pose a physical or mental threat to an animal (Wolf *et al.*, 2018b). When exposed to a stressor, animals experience stress. A physicist first defined stress as strain or pressure exerted on an object. In biology, stress can be defined as the biological consequence of an animal being exposed to adverse environments. Animals have adapted to cope with natural and unavoidable fluctuations in stress. Stress responses are necessary as they ensure that animals remove themselves from unfavorable conditions and maintain homeostasis. For example, a stress response can enable animals to escape predators or remove themselves from extreme temperatures (Moberg & Mench, 2000; Broom & Johnson, 2019). When an animal's central nervous system detects an external or internal stressor, various biological responses occur. Responses can be physiological or behavioural, involving the neuroendocrine and autonomic nervous systems. Normal biological functioning will resume when the stressor is resolved or removed. Animals can resolve most stressors quickly, and biological reserves cover the energetic cost of the stress response. However, elevated stress for prolonged periods can overwhelm biological reserves resulting in energy being directed away from other biological processes such as immunity and reproductive ability. When this occurs, stress can negatively impact an animal's long-term welfare (Moberg & Mench, 2000).

Stress responses involving behaviour and the autonomic nervous system are immediate and often short-lived. For example, rapid changes in respiration facilitate an animal's initial fight or flight response. Neuroendocrine responses such as those involving the hypothalamic-pituitary-adrenal (HPA) axis can be delayed up to an hour but are often long-lasting (Moberg & Mench, 2000; Wolf *et al.*, 2018c). The HPA axis regulates the synthesis of glucocorticoid (GC) hormones in a negative feedback system. Cortisol is the predominant GC hormone secreted by most mammals. The reception of a stressor causes neurons in the hypothalamus to produce Corticotropin-releasing (CRF) factors. The CRF factors stimulate the secretion of the adrenocorticotropic hormone (ACTH) in the anterior pituitary. The ACTH then stimulates the uptake of cholesterol by the adrenal gland, where it is converted to GC hormones that get transported in the blood plasma to target tissues around the body. Once in the target tissue, GC hormones help regulate energy levels. For example, they stimulate gluconeogenesis in the liver, where fat and proteins are converted into glucose energy. The additional energy is then used to support an animal's behaviour, metabolism, and immune system during the stress response. The resolution of the stressor results in GC hormones returning to stable levels that maintain homeostasis (Moberg & Mench, 2000; Bashaw *et al.*, 2016; Palme, 2019).

Stress responses often involve changes in behaviour. However, as mentioned in section 1.4, specific behaviours do not always reflect specific affective states. Therefore, it is challenging to use behaviour as an indicator of elevated stress. Monitoring activity in the HPA axis provides a more direct measure of an animal's stress response (Moberg & Mench, 2000; Broom & Johnson, 2019). Measuring the GC hormone levels in blood plasma can determine activity in the HPA axis. However, it is difficult to collect blood samples from captive exotic animals as it is invasive and requires an animal to be handled. Therefore, it is beneficial to investigate non-invasive methods to monitor the HPA axis. The metabolism of GC hormones occurs in the liver, with metabolites excreted in urine and feces. Measuring the levels of GC metabolites in faeces provides a non-invasive way to monitor activity in the HPA axis, as faecal samples can be collected without directly impacting the animal. Therefore, FCM analysis can successfully monitor adrenal activity in wild and captive exotic animals (Palme *et al.*, 2005; Palme, 2019). For example, studies have identified increased adrenal activity in otters (*Lutrinae*) living in transformed habitats and elevated stress in captive fishing cats (*Prionailurus viverrinus*) during habitat construction (Fazio *et al.*, 2020; Majelantle *et al.*, 2020).

A time delay occurs between the secretion of GC hormones and the presence of metabolites in faeces. This delay depends on how long it takes for matter to pass through a species' intestinal passage. When using FCM analysis to monitor an animal's stress response, it is essential to identify the species-specific time lag. Identifying this lag will ensure that faecal samples are collected at appropriate times to monitor the target stressor. Radiometabolism studies have investigated the circulation of GC hormones in numerous species, including sheep (*Ovis aries*). In these studies, an animal is injected with radiolabeled GC hormones. All the following intestinal excretions are collected to determine the time it takes for the radiolabeled GC metabolites to appear in the faeces. It is also essential to identify the type of stressor being analysed. Stressors can be acute, occurring instantaneously, or chronic, occurring for prolonged periods. For example, a dominance interaction can be an acute stressor, while long-term subordination can be a chronic stressor. The time lag between the secretion of cortisol and the presence of metabolites in the faeces can make it challenging to measure quick fluctuations in FCM levels due to an acute stressor. When measuring acute stressors, it may be necessary to collect more faecal samples to increase the chances of capturing the peak in FCM levels. Other factors can also impact FCM levels, such as individual differences in HPA axis activity, sex, reproductive state, and diet (Romero, 2004; Palme *et al.*, 2005; Palme, 2019). It is also essential to note that activity in the HPA axis can support increased energy demands in situations unrelated to stress, including periods of hunting and mating (Broom & Johnson, 2019). Scientists must consider all these factors during FCM analysis.

Faecal cortisol metabolite analysis is often achieved using enzyme immunoassays (EIAs). Enzyme immunoassays incorporate antibodies that bind to the GC metabolites in the faeces. The GC metabolites present in faeces can differ significantly between species. Therefore, unique EIA's are required to carry out FCM analysis depending on the species. Analytical, physiological, and biological validations identify appropriate EIA's. Analytical validation involves tests measuring an assay's precision, sensitivity, and accuracy. Physiological validation often involves an animal being injected with an ACTH injection that directly stimulates the secretion of GC hormones. Several EIAs are then trialed in FCM analysis to determine which ones effectively detect the spike in GC metabolites. Biological validations involve measuring FCM levels using a range of assays before and after a stressful event to identify what ones best identify the increase in metabolites (Palme *et al.*, 2005; Palme, 2019).

1.6 Current research project

As in-situ giraffe populations decline, ex-situ populations play an essential role in the species conservation. Maintaining captive giraffe welfare will ensure ex-situ populations can continue to contribute to conservation. Frequent assessments of captive giraffe welfare will ensure animal management continuously improves and reaches higher standards (Veasey, 2006; Hill & Broom, 2009). Zoos manage reproduction carefully to ensure captive populations are genetically viable. Many male offspring are not required in future breeding programs. Zoos can manage these excess males by housing them together in bachelor herds (Ziarnowski & Fenrich, 2016). However, welfare challenges can arise in bachelor herds. Male giraffes form bachelor herds in adolescence before moving to a more solitary roaming lifestyle as they mature. In adulthood, a social hierarchy often influences the interactions between male giraffe. These hierarchies can also result in subordinate males experiencing higher stress levels (Bercovitch & Berry, 2015; Wolf *et al.*, 2018d). In captive bachelor herds, mature male giraffes cannot display solitary roaming behaviours, which may lead to social conflict. Social rank may also impact a male giraffe's stress levels in captivity. Captive environments can also limit the expression of other species-specific behaviours such as foraging behaviours. When unable to display species-specific behaviour, captive giraffes can display oral and pacing stereotypes (Bashaw *et al.*, 2001). Foraging enrichment is used in many zoos to reduce stereotypical behaviour by facilitating the giraffe's species-specific foraging behaviour. For example, foraging enrichment in the form of mesh hanging baskets significantly reduced the expression of oral stereotypes at the Atlanta Zoo (Fernandez *et al.*, 2008).

At Hamilton Zoo, zookeepers have expressed concerns about the giraffes' behaviour in relation to their social dynamics and expression of stereotypes. Therefore, this thesis aims to use behavioural observations and FCM analysis to identify how these behaviours may reflect their welfare. The giraffes' FCM levels at Hamilton Zoo will also be compared to those of three males housed in a bachelor herd at Gibbs Farm to identify if the giraffes in each bachelor herd experience similar welfare concerns.

The first research aims are-

- To quantify the giraffes' behaviour at Hamilton Zoo using behavioural observations.
    - To investigate the giraffes' social dynamics.
    - To investigate each giraffe's expression of oral and pacing stereotypes.
    - To identify if foraging enrichment impacts the giraffes' behaviour.

The second research aims are-

- To quantify the giraffes' stress levels at Hamilton Zoo using FCM analysis.
    - To identify if subordinate males have higher FCM levels.
    - To identify if FCM levels correlate with the expression of oral and pacing stereotypes.
    - To identify if enrichment impacts the giraffes' FCM levels.
    - To compare the giraffes' FCM levels at Hamilton Zoo to those from three male giraffes in a bachelor herd at Gibbs Farm.

Behavioural observations and FCM analysis will provide an indication of the giraffes' welfare at Hamilton Zoo. The results will provide a deeper understanding of their behaviour and how it influences their stress levels. This understanding could help Hamilton Zoo mitigate behaviours and stressors that may cause suboptimal welfare. Understanding each giraffe's unique behaviour and FCM levels will also ensure husbandry routines support all their welfare needs. Enhancing the giraffes' welfare will help Hamilton Zoo spark a passion for conservation in their community. People may be more likely to engage in education and connect with the giraffes if they appear relaxed and content in their captive environment. Visitors that leave the zoo with a positive experience may also be more likely to contribute to species conservation by supporting the zoo or making changes in their own lives. This research has a broad scope as it will demonstrate how behavioural observations and FCM analysis can help non-invasively determine an animal's welfare. Scientists and zookeepers could use similar methods to assess animal welfare in a range of scenarios. Findings from this study might also help other zoos with bachelor giraffe herds expressing similar social or stereotypical behaviours. While all individuals and groups are unique, these results may provide more understanding about particular behaviours and stressors and how they can be mitigated to enhance animal welfare (Broom & Johnson, 2019).

Chapter 2

Assessing giraffe welfare using behavioural observations

2.1 Introduction

Animals have evolved a range of species-specific behaviours such as foraging techniques, social cues, and predator avoidance strategies that allow them to meet their fundamental needs. Captive animals cannot always display these species-specific behaviours due to enclosure constraints, different environments, and restricted social interactions (Bashaw *et al.*, 2001; Quirke *et al.*, 2012; Lewton & Rose, 2020). Social interactions and relationships are often fundamental to an animal's survival and reproductive success. They can significantly impact an animal's welfare resulting in both positive and negative affective states. For example, social bonds may lead to positive affective states such as security, while competition and aggression may lead to negative affective states such as stress and fear (Bergman *et al.*, 2005; Maag *et al.*, 2019). In captivity, it can be challenging to facilitate appropriate social dynamics in highly social species or species that display regular fluctuations in social activity. For example, it is impossible to facilitate the giraffes' fission-fusion social dynamic in captivity (Veasey *et al.*, 1996; Mellor, 2015; Wolf *et al.*, 2018d).

In captivity, the fixed herd size can result in giraffes displaying different social dynamics to those observed in their wild counterparts. Giraffes often experience fewer environmental pressures in captivity, which may lessen the cost of group living. For example, food availability does not fluctuate significantly between seasons (Lewton & Rose, 2020). In the wild, only male giraffes form social hierarchies. Despite the reduced environmental pressures in captivity, both males and females develop solid linear hierarchies. When herd size is fixed, hierarchies may be necessary to reduce conflict over limited resources such as favoured food items or areas of the enclosure. Like in wild giraffes, age also impacts an individuals' hierarchy status in captive herds alongside how long they have been in the herd (Horová *et al.*, 2015). Both wild and captive giraffes show social preferences resulting in social networks containing non-random associations. In both cases, females are often at the center of these social networks, with males engaging in fewer social interactions (Bashaw *et al.*, 2007; Lewton & Rose, 2020). Captive giraffes can also develop social networks that differ from those displayed by their wild counterparts. In the wild, male and female giraffes rarely form social bonds. However, a study on captive giraffes found that two females displayed behavioural changes thought to reflect stress after a male was removed. This finding suggests that males and females develop long-lasting bonds in captivity (Tarou *et al.*, 2000). In captive bachelor herds, male giraffes have also been observed displaying sexual behaviour towards each other. Many wild animals express same-sex sexual behaviour. However, it may occur more frequently in captive bachelor herds due to the absence of females (Ziarnowski & Fenrich, 2016; Boyle & Richards, 2019).

When unable to display appropriate species-specific behaviours in response to their environment, captive animals can express stereotypical ones instead. Negative affective states are often the underlying cause of these behaviours. Therefore, they can reflect suboptimal welfare (Mason *et al.*, 2007). Captive giraffes commonly display oral stereotypes such as object-licking and locomotory stereotypes such as pacing (Orban *et al.*, 2016). A survey identified that 79.7% of captive giraffes express stereotypical behaviour, with 72.4% displaying object-licking and 29.2% displaying pacing (Bashaw *et al.*, 2001). Wild giraffes spend around 77% of their time foraging using their prehensile tongue. Complex tongue manipulations allow giraffes to access foliage while avoiding things such as thorns and stinging ants (Veasey *et al.*, 1996; Shorrocks, 2016). Captive giraffes often have limited access to browse. Therefore, diets frequently contain lucerne and manufactured pellets presented in open-top feeders. These diets ensure captive giraffes meet their nutritional requirements, but they do not always require giraffes to express complex tongue manipulations to access the food (Duggan *et al.*, 2016). These easy-to-consume diets result in foraging, making up only 26% of a captive giraffe's activity budget (Veasey *et al.*, 1996). The motivation to display extended foraging periods and tongue manipulations may cause captive giraffes to experience negative affective states similar to frustration. These negative affective states are likely the underlying cause of oral stereotypes (Fernandez *et al.*, 2008). Easily consumed captive diets can also reduce saliva production, which affects gastrointestinal pH levels. Perhaps oral stereotypes are also expressed to increase saliva production (Kulkarni, 2020).

When the underlying cause of a stereotype is known, specific enrichment items can help mitigate its expression. Foraging enrichment in the form of puzzle feeders, browse balls, and slatted top feeders have successfully reduced the expression of oral stereotypes in captive giraffes. These devices promote the expression of complex tongue manipulations and extend foraging periods. By facilitating foraging behaviour, these enrichment devices can reduce the negative affective associated with feeding motivation and facilitate positive affective states such as satisfaction (Fernandez *et al.*, 2008; Kulkarni, 2020).

The time captive giraffes spend foraging does not influence the expression of pacing stereotypes. Therefore, it is likely that other environmental factors cause these stereotypes. Several underlying causes have been proposed, including anticipation, enclosure constraints, and social stress (Bennett *et al.*, 2015; Duggan *et al.*, 2016; Orban *et al.*, 2016). The expression of pacing in giraffes and okapi (*Okapia johnstoni*) can increase before feeding events or movement to different enclosures. Therefore, pacing may occur due to anticipation and restlessness before an event (Duggan *et al.*, 2016). Pacing also occurs more frequently in smaller enclosures or enclosures that have had minimal environmental change. Wild giraffes occupy large home ranges between 68 km^2 and 514 km^2, depending on food availability. Confined enclosures, especially night yards and houses, prevent animals from traveling extensive distances. Perhaps pacing is expressed due to a travel motivation or boredom due to a lack of novel stimuli (Bashaw *et al.*, 2001; Shorrocks, 2016). A study on okapi identified that males paced more than females. This finding aligns with the travel motivation hypothesis as wild males travel large distances searching for females (Bennett *et al.*, 2015). Social stress and frustration may also cause pacing stereotypes. A study on captive giraffes found that removing a herd member resulted in the remaining individuals developing pacing stereotypes (Tarou *et al.*, 2000). Elephants (*Loxodonta africana* and *Elephas maximus*) are also known to pace when they experience social separation or regular movement between herds (Greco *et al.*, 2017).

Several studies have investigated the social dynamics of captive giraffes, but only a few have focused on bachelor herds (Ziarnowski & Fenrich, 2016; Boyle & Richards, 2019). In the wild, mature male giraffes exhibit a roaming lifestyle and do not form long-term associations (Pratt & Anderson, 1982). When bachelor herds form, they often consist of adolescent males or mature and adolescent males. Bachelor herds only containing mature males are observed less frequently (Bercovitch & Berry, 2015). In mixed-sex captive herds, males also tend to develop fewer social connections than females (Lewton & Rose, 2020). Therefore, mature males may struggle to display appropriate social behaviour in captive bachelor herds. When unable to exhibit appropriate social behaviour, giraffes in captive bachelor herds may experience high levels of social conflict. Social conflict may result in individuals experiencing negative affective states such as stress and frustration (Dawkins, 2003). Captive giraffes are known to display stereotypical behaviour. Previous studies have predominantly focused on oral stereotypes and the enrichment used to mitigate them (Fernandez *et al.*, 2008; Orban *et al.*, 2016). Pacing stereotypes in giraffes are more complex as multiple underlying causes have been proposed. Few studies have investigated the ability of enrichment to mitigate these stereotypes (Bashaw *et al.*, 2001; Bennett *et al.*, 2015). Investigating pacing stereotypes in a bachelor herd may deepen our understanding of the behaviour and the factors causing it.

Behavioural observations are one of the most common methods used to assess animal welfare. Assessing an animal's behavioural activity budget can help determine its ability to display a range of appropriate species-specific behaviours in response to its environment. An animal's behaviour is driven by its motivational state. Therefore, behavioural observations can also indicate an animal's affective states (Temple *et al.*, 2011; Mellor, 2016; Yon *et al.*, 2019). The first objective of this study was to use behavioural observations to quantify the social dynamics of four male giraffes in a bachelor herd at Hamilton Zoo. I predict that each giraffe's behaviour will reflect social conflict as mature males rarely form bachelor herds in the wild (Bercovitch & Berry, 2015). The second objective of this study is to use behavioural observations to quantify the oral and pacing stereotypes displayed by the four giraffes at Hamilton Zoo. I predict that social behaviour will not influence the expression of oral stereotypes as feeding motivation is often the behaviours underlying cause (Fernandez *et al.*, 2008). In comparison, I predict that social behaviour will affect pacing stereotypes as social stress and frustration may cause these behaviours (Tarou *et al.*, 2000; Greco *et al.*, 2017). The third objective of this chapter is to use behavioural observations to quantify foraging enrichments impact on each giraffe's behaviour at Hamilton Zoo. I predict that enrichment will reduce the giraffes' expression of oral stereotypes as the behaviour is often caused by feeding motivation (Fernandez *et al.*, 2008). I predict that enrichment will have less impact on pacing stereotypes as feeding motivation does not seem to cause this behaviour (Bashaw *et al.*, 2001).

Observing the giraffe's behaviour at Hamilton Zoo will help determine their ability to cope with captive conditions. High levels of social conflict and stereotypical behaviour may indicate suboptimal welfare. Hamilton Zoo could use this study's results to enhance husbandry routines to ensure they mitigate behaviours reflecting negative affective states and facilitate behaviours reflecting positive affective states. The results from this study may also deepen our understanding of male giraffe welfare in captive bachelor herds.

2.2 Methods

2.2.1 Hamilton Zoo

Hamilton Zoo is located in Hamilton, New Zealand. The zoo was initially founded as the Hilldale Game Farm in 1969 and housed a small number of exotic birds and mammals. The farm's purpose was to raise game birds for the Acclimatization Society. In 1976, the zoo experienced financial stress due to competition from other recreational attractions. At this time, the Hamilton City Council brought the 14-hectare site, appointing a zoological trust to run the institution. The zoo almost closed again in 1984 but remained open due to a public petition, and the Department of Recreation and Welfare replaced Hilldale's management. In 1987, the zoo underwent upgrades including the opening of the zoo education center and many new exhibits. Today, the zoo encompasses a 25-hectare site housing 90 species ranging from indigenous birds to exotic mammals (Hamilton Zoo, 2020a).

Hamilton Zoo's purpose is to 'provide a world-class visitor experience that inspires conservation action'. Through education, the zoo inspires and informs those in the community about species conservation. Education is delivered in several formats including static information boards outside enclosures, keeper talks, and animal encounters. The zoo also offers curriculum-based education for primary and secondary school groups, with learning outcomes focused on science, conservation, and sustainability (Hamilton Zoo, 2020b, para. 1). Hamilton Zoo also contributes directly to species conservation. The zoo is involved in many Australasian Species Management Programs. Successful breeding programs have included white rhinos, chimpanzees, and ring-tailed lemurs (Ryan & Saward, 2004; Hamilton Zoo, 2020c). Personal donations and 10% of the revenue from animal encounters and conservation events also allow the zoo to support local and abroad in-situ conservation projects (Hamilton Zoo, 2020b).

2.2.2 Subjects and husbandry

The subjects of this study were four giraffes (*Giraffa camelopardalis*) housed in a bachelor herd at Hamilton Zoo. These males were in a bachelor herd as their genetics were already well represented across the captive population in Australasia. During this study, the bachelor herd consisted of Ndoki, Masamba, Ndale, and Dume. These four males and their parents were all born in captivity. Ndoki was the herd's oldest giraffe born in 1998. He was transported from Wellington Zoo to Hamilton Zoo in 1999. Masamba was born in 2002 and transported from Auckland Zoo to Hamilton Zoo in 2004. Ndale was born in 2004 and transported from Auckland Zoo to Hamilton Zoo in 2005. Dume was born in 2006 and was transported from Auckland Zoo to Hamilton Zoo in 2007. Ndale and Dume were full siblings, and Masamba was their half-sibling sharing the same sire. Based on the zookeeper's observations, it seemed likely that Masamba was the herds dominant male. All four giraffes displayed oral stereotypes, and Dume and Ndoki were known to express pacing stereotypes.

At Hamilton Zoo, the giraffes had a yard and paddock enclosure. They were housed in the yard overnight and moved to the paddock during the day. The giraffes shared the paddock with five zebras (*Equus burchellii*), twelve blackbucks (*Antelope cervicapra*), and five ostriches (*Struthio camelus*). The paddock did not contain giraffe houses and could become muddy and slippery in rainy weather. Therefore, movement to this enclosure was weather-dependent, and the giraffes remained in the yard on rainy days. The giraffes received around 12 shared branches of browse twice a day. The species of browse varied but often included olive, lemonwood, and pittosporum. Diet also included lucerne ad-lib and 50 liters of horse and pony pellets shared between the giraffes throughout the day. The giraffes were given carrots, broccoli, and cauliflower as reinforcers in keeper talks and training sessions. At Hamilton Zoo, giraffe training often focused on voluntary hoof trims. Training sessions were beneficial as they provided cognitive stimulation and positive keeper-animal interactions. As part of their husbandry routine, the giraffes were provided foraging enrichment in the form of puzzle feeders, browse balls, and tunnel feeders filled with various parts of their diet (Figure 2.1). This enrichment stimulated extended foraging periods and complex tongue manipulations.

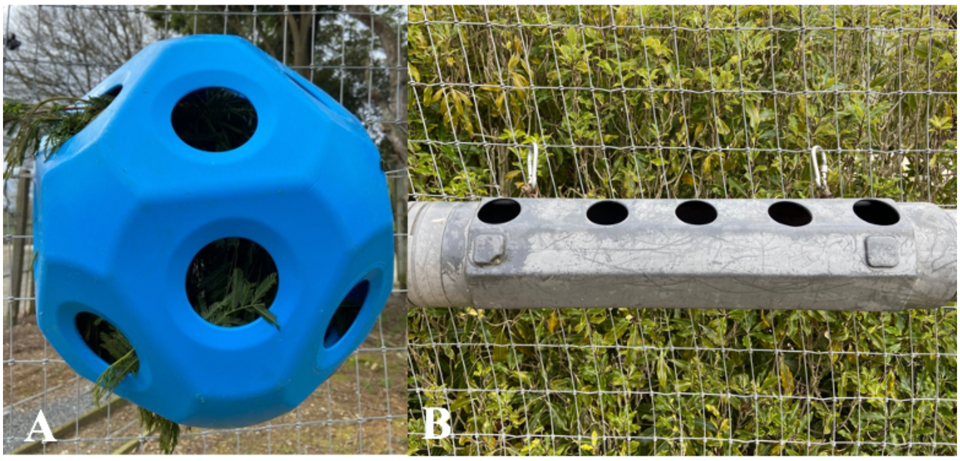

Figure 2.1- Foraging enrichment in the form of a browse ball (A) and a tunnel feeder (B) given to the giraffes at Hamilton Zoo.

Before this study, the dominant male Masamba received a Bopriva vaccine (GnRF neutraliser) to try and reduce his expression of agonistic behaviour towards other herd members. The vaccine has reduced aggression in cattle bulls by lowering testosterone levels. Testosterone production is controlled by luteinizing and follicle-stimulating hormones released in the pituitary gland. Bopriva works by preventing the secretion of these hormones and subsequently testosterone. Studies on cattle have identified that the vaccine is effective for 4-16 weeks depending on the individual (Pfizer Animal Health, n.d). Masamba received the vaccine a month before behavioural observations on 16/9/2020. Therefore, it was taken into consideration when analysing results. It is important to note that research on the vaccine currently focuses on cattle, with little research investigating its effect on other ungulates.

2.2.3 Experimental area

At Hamilton Zoo, the giraffe facilities contained a yard and paddock enclosure (Figure 2.2). The yard enclosure consisted of three interconnecting yards that could be separated with sliding gates. Together the three yards were around 1,300 m^2. The substrate in each yard was lime fines. Yard one contained two browse poles, and yards two and three had one. There was also a lucerne feeder in the middle of yard two, but it was not used. Yards one and three contained giraffe houses that provided shelter from the weather. In each house, there were two lucerne feeders. The substrate in the houses were either sawdust or straw. A seven-metre public viewing platform ran alongside yard two (Figure 2.3). The giraffes typically had access to all three yards. However, zookeepers could shut the giraffes out of singular yards to clean and set up new browse.

The giraffes walked down a 230 m sand raceway to the paddock enclosure (Figure 2.2). The paddock was around 14,280 m^2, and there was a slight incline at the top end. In the top right corner of the enclosure, there were two browse poles and one lucerne feeder. There was also a browse pole and lucerne feeder in the middle of the paddock on the right and left sides. In the top left corner of the paddock, there was a shelter. However, it was only a couple of meters tall, making it inaccessible to the giraffes. The primary substrate in the paddock was grass, with lime fines around the browse poles, the raceway gate, and outside the shelter. A public walkway of around 110 m ran along the right side of the enclosure (Figure 2.4).

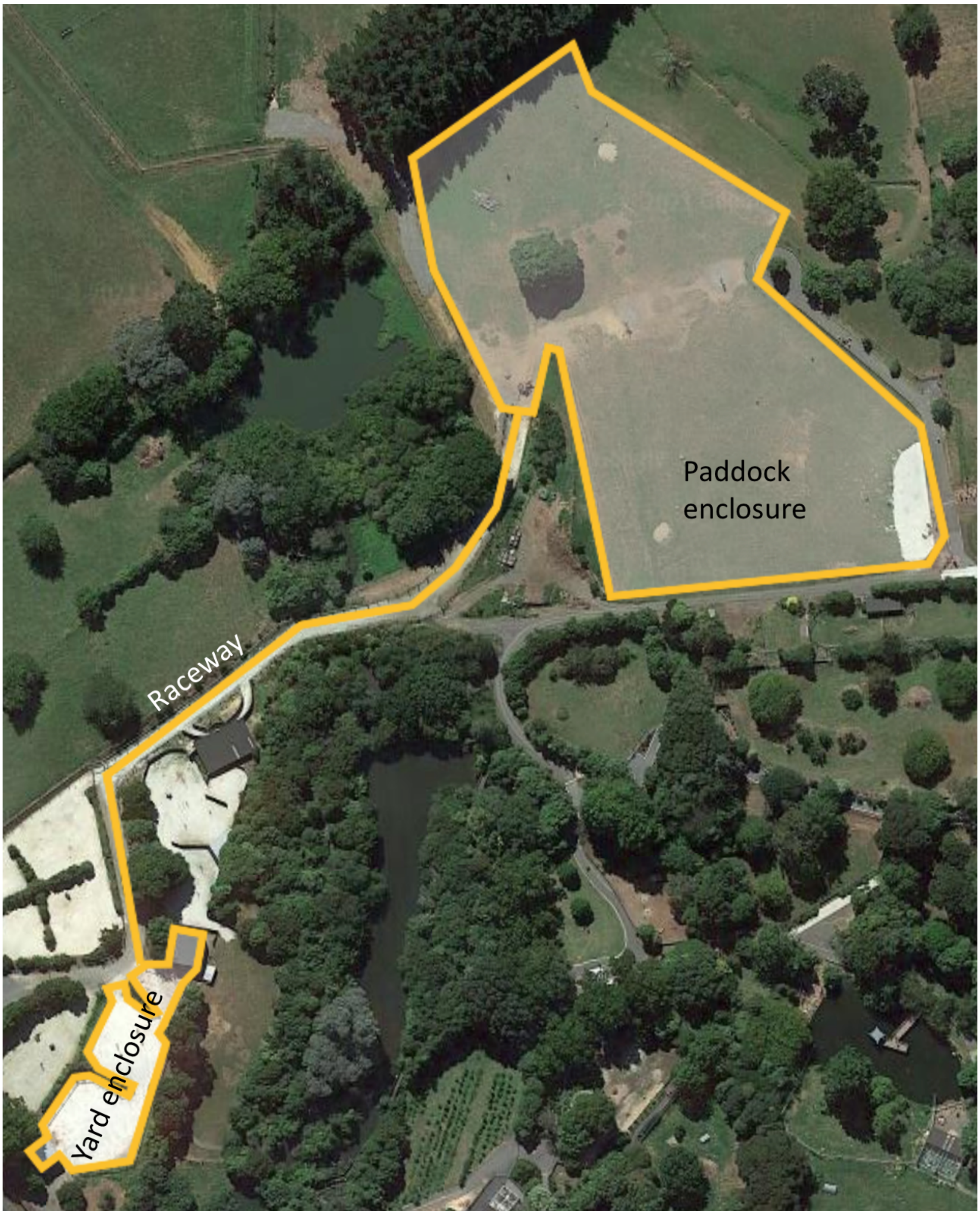

Figure 2.2- Aerial photo of the giraffes' enclosures at Hamilton Zoo. The yard enclosure, raceway, and paddock enclosure are labeled (Google Earth, 2016).

Figure 2.3- Aerial photo of the giraffes' yard enclosure at Hamilton Zoo. The three yards, giraffe houses, raceway gates, and public viewing platform are labeled (Google Earth, 2016).

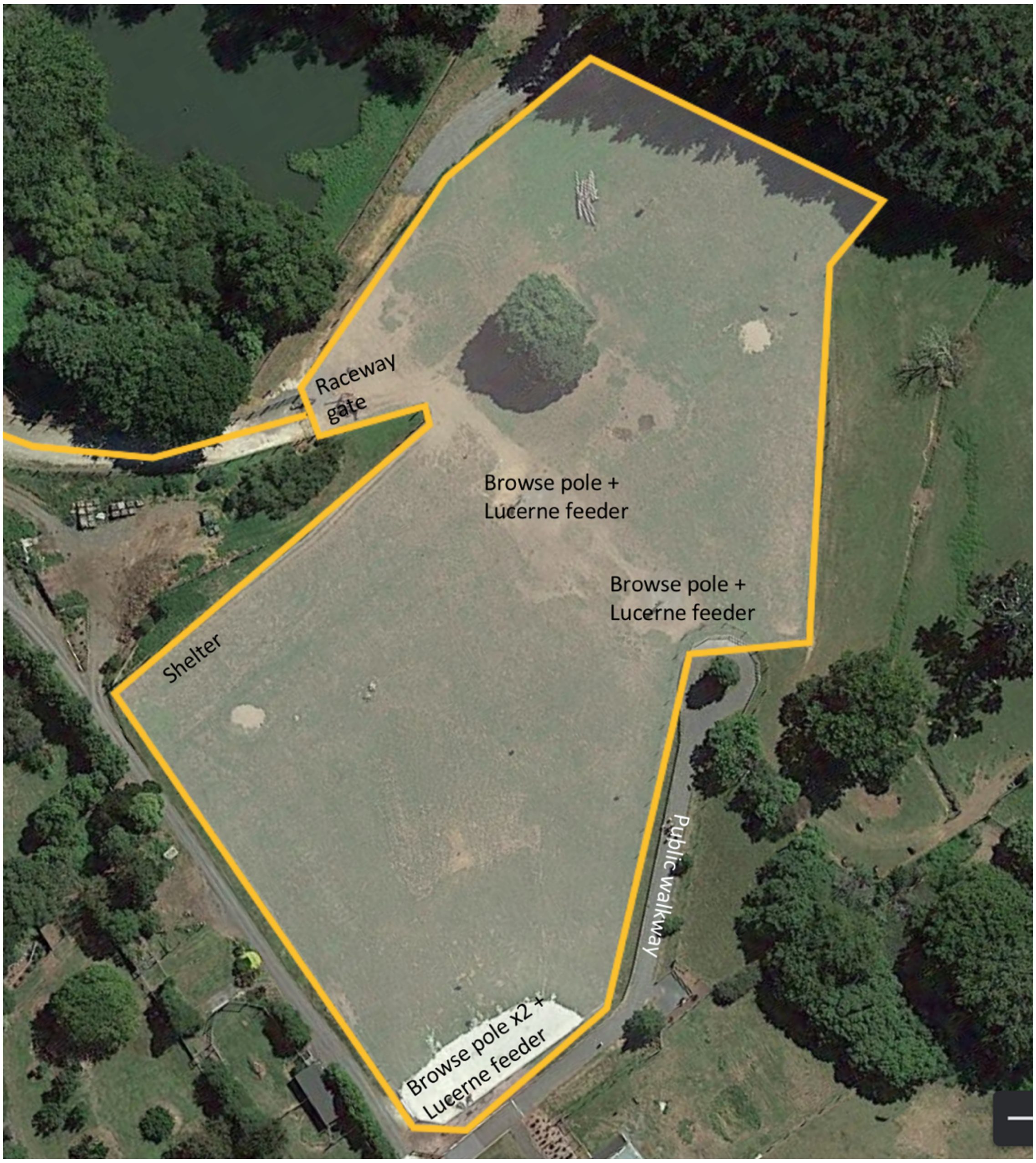

Figure 2.4- Aerial photo of the giraffes' paddock enclosure. The browse poles/ lucerne feeders and public walkway are labeled. This photo was taken in 2016 before the shelter was built, but its current location is labeled (Google Earth, 2016).

2.2.4 Behavioural observations

Behavioural observations occurred between 19/10/2020 - 18/12/2020 and 4/1/21- 15/1/21 on Mondays, Wednesdays, and Fridays. Observations were carried out for 1.5 hr between 8:00-10:00 and 12:00-14:00 to provide a general overview of the giraffes' daily activity budget. The giraffes were always in the yard enclosure during the morning observation period. They were in either the yard or paddock enclosure in the afternoon observation period, depending on the weather. The observation method used was scan sampling with a one-minute interval. This method was beneficial as it enabled all four giraffes' behaviour to be monitored simultaneously. Giraffes often move at a reasonably slow pace; therefore, a one-minute interval was sufficient to record each giraffe's activity budget. Twenty-eight giraffe behaviours were monitored, including general, social, and stereotypical behaviours. An ethogram ensured the behaviour definitions remained consistent throughout data collection (Table 2.1). Observations were made from public viewing areas. The giraffes could be seen in all areas of the yard enclosure except the houses. Despite the paddock's size, the giraffes could be viewed in all areas from the public walkway. If a giraffe or their behaviour could not be seen clearly, 'out of sight' was recorded for that minute. At Hamilton Zoo, the giraffes were provided enrichment as part of their husbandry routine. However, enrichment was not given to the giraffes during many of this study's observation periods. Therefore, the giraffes were experimentally given three foraging enrichment items during the morning observation periods between 4/1/21- 15/1/21 - this enabled the impact of enrichment on giraffe behaviour to be recorded. The enrichment used was not novel and included items mentioned in section 2.2.2.

Observations were recorded on paper before being transferred to an Excel spreadsheet. Overall, each giraffe's behaviour was observed for 93.5 hr. Ethics approval was not required, as behavioural observations had no impact on the giraffes or their husbandry and the provision of enrichment was undertaken by the zoo as part of routine husbandry management.

Table 2.1- Ethogram used during behavioural observations (Seeber *et al.*, 2012; Orban *et al.*, 2016).

Behaviour	**Definition**
Idle	Standing in a stationary position with no oral or locomotory activity.
Resting	Lying down with legs folded under the body.
Locomoting	Walking or cantering.
Eating	Tongue/ mouth in contact with food items during consumption.
Ruminating	The lower jaw moves horizontally up and down successively at least five times. At times the regurgitation or swallowing of the cud can be seen.
Drinking	Mouth or tongue in water.
Grooming	Scratching the body against an object or running the mouth/ tongue over the body.
Urine licking	Licking urine off the ground, often followed with the flehmen behaviour.
Enrichment interaction	Body, mouth, or tongue contacting or manipulating an enrichment item.
Out of sight	Giraffe or their behaviour cannot be observed.
Stereotypical behaviour	
Object licking	Repetitively moving tongue over a non-food object such as an enclosure wall or fence.
Adherent oral behaviour	Lips or teeth are repetitively moving across a non-food object such as the enclosure fence or electric wiring.
Pacing	Repetitive locomotory behaviour occurring across a definite path.
Stargazing	Pacing with head tilted up towards the sky.
Head flick	The head is repetitively flicked to one side while pacing
Social interactions	
Allo-grooming	Rubbing mouth or tongue over another individual.
Nuzzling	Touching another individual with the nose or muzzle. Often on the face but can be any area of the body apart from the flank or anogenital area.
Dominance gesture	Erect posture while standing still or tensed arched neck while moving.
Necking	Standing next to or circling around another giraffe, using the head and neck to swing hits at their body and legs
Avoiding	Moving away from another individual as they approach.
Avoided	As an individual approaches other giraffe move away.
Following	Remaining within five metres of another individual while following their movement patterns

Followed	An individual's movement pattern is followed by another individual who is less than five metres away.
Displaced	An individual is pushed or bumped away by another. Includes being pushed off food.
Displacing	Pushing or bumping another individual away.
Investigating	Sniffing or licking another individual's flank or anogenital area.
Investigated	Flank or anogenital area is sniffed or licked by another individual.
Flehmen	Head raised while exposing the upper lip/teeth and inhaling at the same time. Often occurs after testing another giraffe's urine.
Laufschlag	Standing behind another individual and lifting a front leg to touch their back leg or flank.
Mount	Standing behind another individual while shifting weight onto the back legs and lifting front legs in an attempt to mount them.
Head Toss	Tossing head backward or shaking it side to side.
Keeper interaction	Attention on a keeper's activities or interacting with the keeper.
Training	Interacting with a keeper in a training event.

During observations, I (the observer) remained in public viewing areas and did not interact with the zookeepers. The giraffes are used to people standing in these areas and typically ignore them. The giraffes did not acknowledge my presence during observations. Therefore, I am confident that my presence did not impact their behaviour. Observations took place around normal variations in the giraffes' husbandry routine. For example, when moving to the paddock for the day, the giraffes would spend the morning observation period waiting for the paddock enclosure to be prepared. If the giraffes remained in the yards for the day, zookeepers would set up browse in the yards during morning observations. In the yard enclosure, the giraffes had less space and environmental stimuli. The paddock enclosure was much larger, had several other species, and the giraffes could observe more neighboring enclosures and farmland. These differences in routine and enclosure location may have impacted the expression of behaviours such as eating, locomoting, and avoiding. Factors such as weather could also influence their behaviour as they became less active in the rain. All these variations were noted during each observation period. The 12-week observation period ensured data provided a general overview of the giraffes' behaviour across husbandry and environmental variations.

2.2.5 Data analysis

Behavioural percentages were calculated from the raw data within each observation period. During analysis, each observation period acted as an experimental unit. Datasets were created for each behaviour by collating the behavioural percentages from each experimental unit. These datasets were then used to analyse each behaviour individually. The analysis did not include behaviours displayed infrequently and behaviours that did not link to the study's objectives. The analysed social behaviours were **investigating**, **investigated**, **following**, **followed**, **avoiding**, **avoided**, **displaced**, and **displacing**. When recording the behaviours **investigating**, **following**, **avoiding**, and **displaced**, the other individual in the social interaction was noted. For example, if the **following** behaviour was recorded, the individual being **followed** was also noted. The percentages for the behaviours **investigated**, **followed**, **avoided**, and **displacing** were calculated from these notes. Stereotypical behaviours were grouped into **oral** stereotypes (object licking and adherent oral behaviour) and **pacing** stereotypes (stargazing, flick, pace) for analysis. The general behaviours **eating**, **locomoting**, and **idle** were also analysed, as they made up large percentages of the giraffes' activity budget.

GraphPad Prism version 9.0 (GraphPad Software, LLC) was used to perform statistical analysis and produce graphs. The analysis identified if behavioural percentages differed significantly between the individual giraffes and the morning/afternoon observation periods. Assumptions of normality were tested using the Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test, and Kolmogorov-Smirnov test. Only the **locomoting** dataset met the assumptions of normality. Log10 and logit transformations were unsuccessful in normalizing the other datasets as the data contained many zero values, resulting in it being highly skewed. Therefore, a One-Way ANOVA test (ANOVA) and Tukey's multiple comparison test (TMC) analysed the **locomoting** dataset, and the nonparametric Kruskal-Wallis test (KW) and Dunn's multiple comparison test (DMC) analysed the remaining datasets. The Kruskal-Wallis test identified a significant difference in the giraffes' expression of **pacing** stereotypes. The nonparametric Spearman correlation (SC) test analysed correlations between **pacing** and the social behaviours **avoided**, **followed**, and **investigated**. This analysis aimed to identify if social interactions influenced **pacing** behaviour.

The analysis identified if the giraffes' behaviour differed when enrichment was present. This analysis included the social behaviours **investigated**, **followed**, **displaced**, **avoiding**, and the **oral** and **pacing** stereotypes as they linked to the study objectives. The giraffes were only experimentally given foraging enrichment in seven morning observation periods. Therefore, this test only incorporated behavioural percentages from the morning observation periods. The Kolmogorov-Smirnov test tested for normality. The majority of data was highly skewed and did not meet the assumptions of normality. Again, transformations were unsuccessful due to the data containing many zero values. Therefore, a nonparametric Kruskal-Wallis test and Dunn's multiple comparison test analysed the data. Data analysis also identified if the time spent interacting with enrichment differed between the four giraffes. This analysis only incorporated enrichment interaction percentages from the morning observation periods, when the giraffes experimentally received foraging enrichment. The Kolmogorov-Smirnov test identified that data met the assumptions of normality. Therefore, a One-Way ANOVA and Tukey's multiple comparison test analysed this data. The sample size for enrichment was relatively small and considered in the data analysis.

2.3 Results

2.3.1 General behaviour

The percentage of time spent **eating** differed between each giraffe and the morning and afternoon observation periods (KW, p = <0.0001; Figure 2.5). In the morning, Dume spent more time **eating** compared to the other three giraffes. Masamba, Ndale, and Ndoki all spent more time **eating** in the afternoon compared to the morning (Table A.1). The percentage of time spent in **idle** differed between each giraffe and the morning and afternoon observation periods (KW, p = <0.0001; Figure 2.5). Ndoki spent more time in **idle** compared to Dume and Masamba in the morning. Overall, Ndoki spent more time in **idle** in the morning compared to the afternoon (Table A.1). The percentage of time spent **locomoting** differed between each giraffe and the morning and afternoon observation periods (ANOVA, F = 22.62, p = <0.0001; Figure 2.5). Masamba spent the most time **locomoting** in the morning and Ndoki spent the least. Masamba, Dume, and Ndale also spent more time **locomoting** in the morning compared to the afternoon (Table A.1)

2.3.2 Social behaviour

The percentage of time being **investigated** differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). Dume was **investigated** more than Masamba and Ndale in the morning and afternoon (Table A.2). The percentage of time spent **investigating** also differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). In the morning, Masamba spent more time **investigating** than the other three giraffes. He also spent more time **investigating** than Dume and Ndoki in the afternoon (Table A.2).

The percentage of time being **followed** differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). Dume was **followed** more than Masamba and Ndale in the morning. Overall, Dume was **followed** more in the morning compared to the afternoon (Table A.2). The percentage of time spent **following** also differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). Ndoki spent less time **following** in the morning compared to the other three giraffes. Overall, Masamba spent more time **following** in the morning compared to the afternoon (Table A.2).

The percentage of time spent **avoiding** differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). Ndale spent the most time **avoiding** in the morning and afternoon compared to the other three giraffes. He also spent more time **avoiding** in the morning compared to the afternoon (Table A.2). The percentage of time spent being **avoided** also differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). Masamba was **avoided** more frequently than the other three giraffes in the morning and the afternoon (Table A.2).

The percentage of time spent being **displaced** also differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). Ndoki was **displaced** more frequently in the morning and afternoon compared to the other three giraffes. He was also **displaced** more in the morning compared to the afternoon (Table A.2). The percentage of time spent **displacing** differed between each giraffe and the morning and afternoon observation periods (KW, *p* = <0.0001; Figure 2.6). Dume spent more time **displacing** than Ndoki in the morning and more time than Ndoki and Ndale in the afternoon (Table A.2).

2.3.3 Stereotypical behaviour

The percentage of time spent expressing **oral** stereotypes differed between the morning and afternoon observation periods (KW, p = <0.0001; Figure 2.7). Ndale and Masamba displayed more **oral** stereotypes more frequently in the morning compared to the afternoon (Table A.3). The percentage of time spent expressing **pacing** stereotypes differed between each giraffe and the morning and afternoon observation periods (KW, p = <0.0001; Figure 2.7). In the morning, Dume displayed **pacing** stereotypes more frequently than the other three giraffes. He also expressed more **pacing** in the afternoon compared to Ndale and Masamba. Overall, Dume spent more time **pacing** in the morning compared to the afternoon (Table A.3).

2.3.4 Behavioural correlations

There was a positive correlation between the percentage of time Dume spent **pacing** and the time he spent being **followed** in the morning (SC, r = 0.4778, p = 0.0057; Figure 2.8). There was also a positive correlation between the percentage of time Ndoki spent **pacing** and the time he spent being **followed** in the morning (Sc, r = 0.6273, p =0.0001; Figure 2.8).

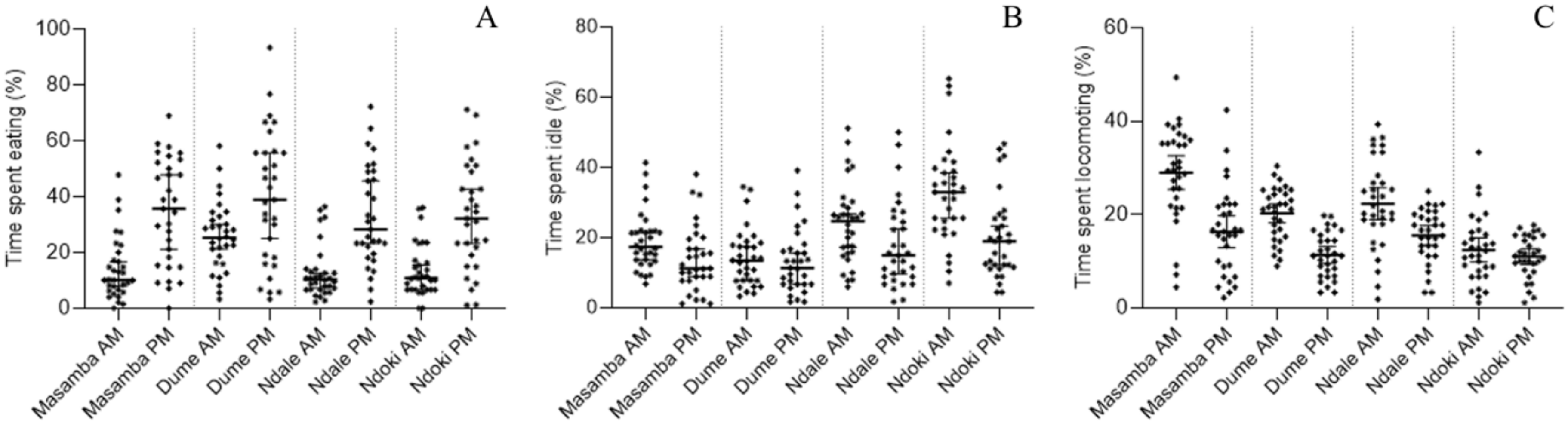

Figure 2.5- The percentage of time each giraffe spent eating (A), in idle (B), and locomoting (C) in the morning (AM) and afternoon (PM) observation periods.

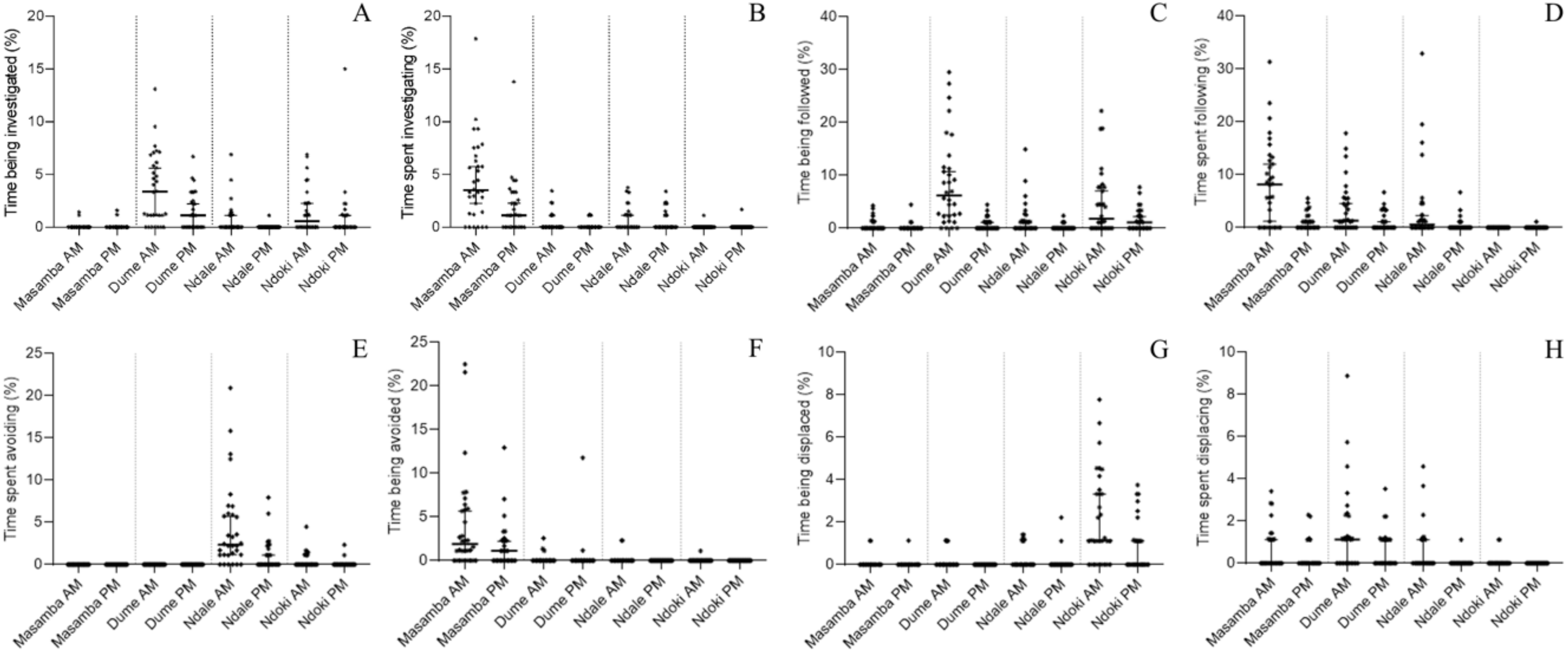

Figure 2.6- The percentage of time each giraffe spent engaging in the analysed social behaviours in the morning (AM) and afternoon (PM) observation periods. Time spent being investigated (A), time spent investigating (B), time spent being followed (C), time spent following (D), time spent avoiding (E), time spent being avoided (F), time spent being displaced (G), and time spent displacing (H).

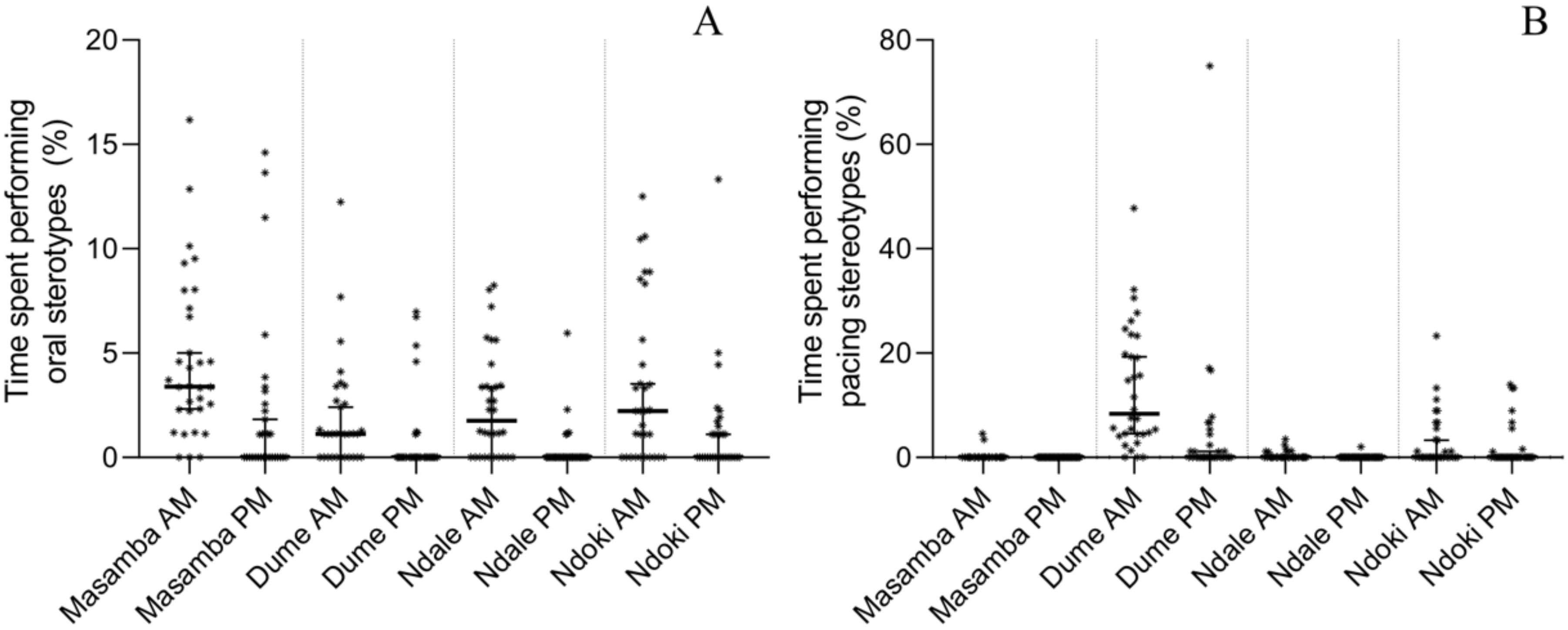

Figure 2.7- The percentage of time each giraffe spent performing oral (A) and pacing (B) stereotypes in the morning (AM) and afternoon (PM) observation periods.

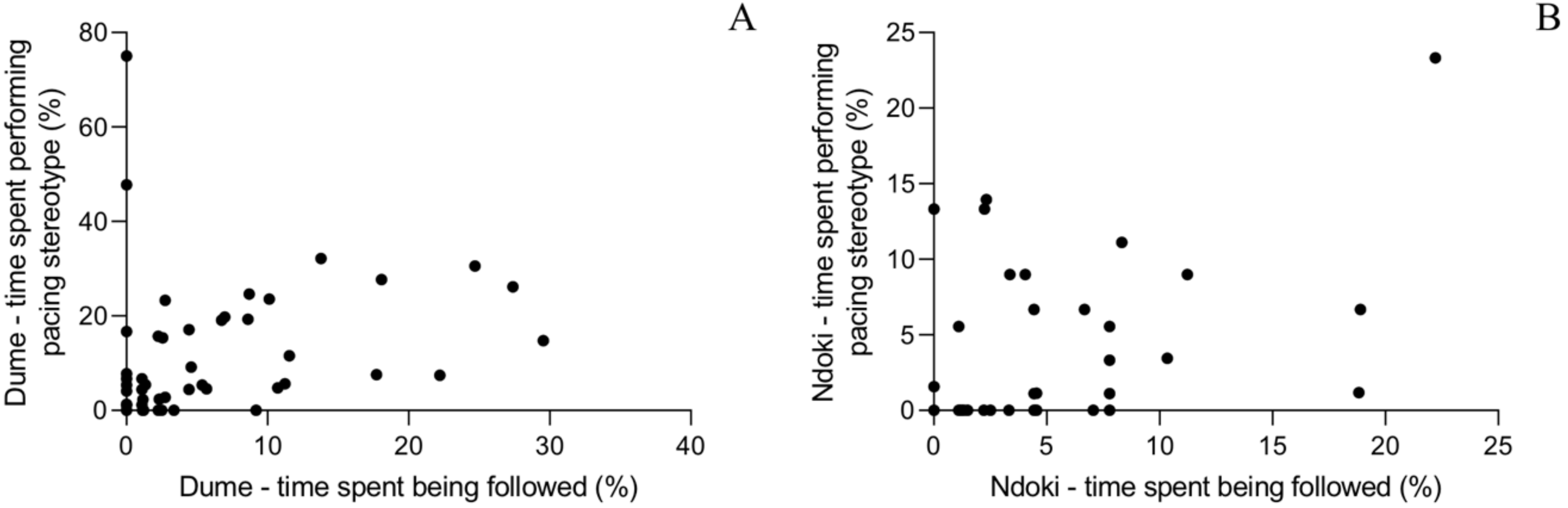

Figure 2.8- The correlation between the percentage of time Dume (A) and Ndoki (B) spent pacing and the percentage of time they spent being followed.

2.3.5 Enrichments effect on behaviour

Foraging enrichment affected the percentage of time giraffes spent being **investigated** (KW, $p = 0.0001$; Figure 2.9). Dume was **investigated** less frequently when enrichment was present (DMC, $p = 0.0032$). The percentage of time spent interacting with foraging enrichment differed between the giraffes (ANOVA, F = 6.28, $p = 0.0076$; Figure 2.10). Dume spent more time interacting with enrichment than Masamba and Ndoki (Table A.4).

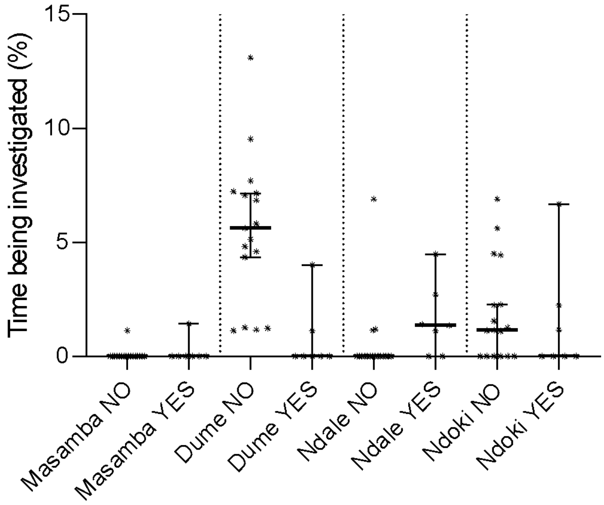

Figure 2.9- The percentage of time each giraffe spent being investigated in the absence (NO) and presence (YES) of foraging enrichment.

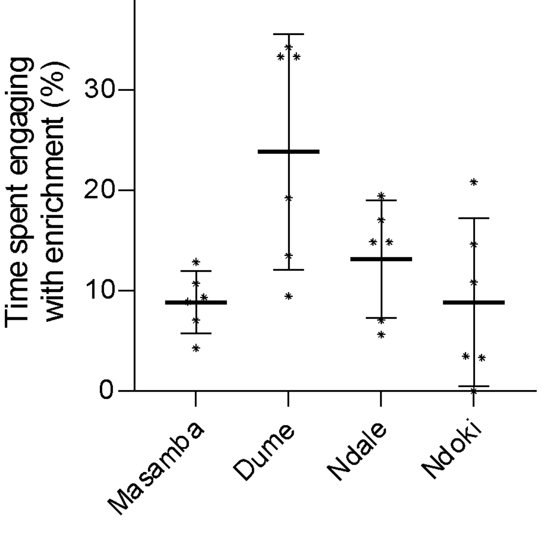

Figure 2.10- The percentage of time each giraffe spent engaging with foraging enrichment.

2.4 Discussion

At Hamilton Zoo, Masamba, Ndale, and Ndoki spent more time **eating** in the afternoon compared to the morning. This result is likely due to the giraffes not always having access to fresh browse during the morning observation period. While **eating** made up the largest proportion of each giraffe's activity budget at Hamilton Zoo, they still spent less time eating than wild giraffes. Wild giraffes spend around 77% of their time **eating**. On average, the giraffes at Hamilton Zoo spent 12.8-25.8% of their time **eating** in the morning and 32.9-39.6% in the afternoon. Previous studies have found similar results suggesting that captive giraffes spend around 26% of their time **eating** (Veasey *et al.*, 1996; Bashaw, 2011). The reduced **eating** time is often due to limited access to browse and easy to consume captive diets (Duggan *et al.*, 2016). When unable to display extended foraging periods, captive giraffes may experience negative affective states due to feeding motivation. These negative affective states may explain why stereotypical behaviours were displayed more frequently in the morning observation period (Fernandez *et al.*, 2008; Dawkins, 2021).

Ndoki was less active in the morning compared to the other three giraffes. He spent more time in **idle** and less time **locomoting**. Wild giraffes only spend 5.6% of their time idling. At Hamilton Zoo, they spent 14.7-32.9% in **idle** in the morning and 13.2-20.5% in the afternoon. Previous studies have found similar results suggesting that captive giraffes spend around 25% of their time idling. Captive giraffes likely spend more time in **idle** due to less time spent foraging and traveling between food resources (Veasey *et al.*, 1996). Ndoki may respond to the limited foraging opportunities in the morning by becoming inactive. Inactivity can occur when an animal is exposed to unfavorable conditions that it cannot control. However, it can also occur when an animal is satisfied, and its fundamental needs have been met. Therefore, it is challenging to determine if Ndoki's inactivity reflects positive or negative affective states (Fureix & Meagher, 2015). Ndoki was also the herd's oldest male, which may have caused him to have lower activity levels.

The results suggested that a social hierarchy influenced the giraffes' social dynamics at Hamilton Zoo. The expression of dominant and subordinate behaviours differed between the giraffes. Ndale displayed the most **avoiding** behaviour, and Ndoki was **displaced** the most, suggesting that these two males were subordinate. The other giraffes **avoided**

Masamba the most, indicating that he was the dominant male. Wild male giraffes also develop social hierarchies and display similar dominant and subordinate behaviours to those observed in this study. For example, subordinate males can express avoidance behaviours in response to another individual's tall dominance gesture. Dominance gestures were not recorded frequently in this study. However, this posture can be relatively subtle (Pratt & Anderson, 1985; Seeber *et al.*, 2012). Captive giraffes are also known to develop social hierarchies. Therefore, it is not surprising that results indicate a social hierarchy at Hamilton Zoo. Older males are often dominant in the wild and captivity (Pratt & Anderson, 1985; Lewton & Rose, 2020). At Hamilton Zoo, Ndoki was older than Masamba. However, during this study, Ndoki was frail, and zookeepers have noticed that he tended to avoid social conflict. Masamba may therefore have assumed a position of social dominance. The expression of subordinate and dominant behaviours suggests that the giraffes experienced some level of social conflict at Hamilton Zoo. However, these behaviours are not abnormal and often occur to reduce the frequency of physical aggression. Resolving conflict without physical aggression is beneficial as physical aggression is energetically demanding and can cause severe injury (Koyama & Palagi, 2006). This study's data analysis could not include aggressive necking behaviours as they were displayed at low frequencies. Therefore, the apparent social hierarchy and subsequent dominant and subordinate behaviours likely reduced physical aggression and social conflict at Hamilton Zoo. There is also a possibility that the Bopriva vaccination before the study successfully reduced Masamba's aggression levels and subsequent social conflict (Pfizer Animal Health, n.d). However, anecdotal evidence from zookeeper observations suggested that the Bopriva vaccination did not reduce Masamba's aggression levels. Necking interactions and **avoiding** behaviour were not observed less frequently after the vaccination (J. DeWaard, personal communication, December, 2020). These results contradicted the prediction that high levels of social conflict would be observed in the bachelor herd at Hamilton Zoo. Limited social conflict is beneficial as it enables animals to spend more time engaging in behaviours that promote positive affective states (Maag *et al.*, 2019).

In the wild, mature male giraffes rarely form long-term associations with other males. Long-term associations have been observed between adult male and female giraffes, but these are often weak. These associations reflect the predominantly roaming lifestyle that adult male giraffes exhibit (Shorrocks, 2016). In captive bachelor herds, mature males cannot display a roaming lifestyle with loose social associations. Despite this, the males at Hamilton Zoo showed low levels of social conflict. Perhaps the male giraffes at Hamilton Zoo display a degree of behavioural plasticity. Behavioural plasticity is the ability to alter behaviour to better suit new environmental conditions. Behavioural plasticity is often observed between wild populations as innovative behaviours can provide adaptive advantages in varying environmental conditions. Captive environments also present species with different climates, diets, and social dynamics. Therefore, behavioural plasticity can enhance a species' success in captive conditions (Mason *et al.*, 2013). In this study, the giraffes may show social plasticity as they have adapted their social behaviour to form long-term associations with other males. These adaptations may have enabled them to optimize their social dynamics in captivity (Gubert & Hannan, 2019).

Despite the low levels of physical aggression, enclosure constraints may impact the welfare of subordinate males at Hamilton Zoo. In the wild, subordinate males have plenty of space to avoid dominant individuals. In captivity, the ability to avoid dominant individuals is limited due to enclosure constraints (Pratt & Anderson, 1982). At Hamilton Zoo, Ndale and Ndoki displayed **avoiding** and **displaced** behaviours more frequently in the morning observation period. During this time, the giraffes were in the smaller yard enclosure. Perhaps the smaller enclosure caused the expression of these behaviours to increase. In hoof stock species, the frequency of social interactions and aggression often increase in smaller spaces (Hogan *et al.*, 1988; Cassinello & Pieters, 2000). Studies on captive primates have also found that subordinate individuals display high levels of social monitoring to ensure they know the dominant individual's behaviour and location (Pannozzo *et al.*, 2007). Therefore, subordinate giraffes likely spend more time monitoring and **avoiding** dominant individuals in captivity and especially in smaller enclosure spaces. When repetitively **avoiding** or being **displaced** by others, Ndale and Ndoki may experience negative affective states such as stress and frustration and have fewer opportunities to engage in behaviours that promote positive affective states.

At Hamilton Zoo, social dynamics were also influenced by the display of sexual behaviour. Results identified that the giraffes, especially Masamba, displayed sexual behaviours such as **investigating** and **following**. Mature males also display these behaviours in the wild. Males use **investigating** and flehmen behaviours to identify females in oestrus. Mate-guarding behaviours, such as **following**, are then used to deter cycling females away from other males (Pratt & Anderson, 1985; Seeber *et al.*, 2012). Sexual behaviour is often directed at individuals of the opposite sex, as this facilitates reproductive success. However, same-sex sexual behaviour has been observed in several species (Monk *et al.*, 2019). For example, 46% of mounting behaviour occurs between individuals of the same sex in Japanese macaques *(Macaca fuscata)* (Vasey *et al.*, 2008). Despite many accounts of same-sex sexual behaviour, its evolutionary purpose is still uncertain. Proposed hypotheses include the strengthening of intrasexual social bonds and the opportunity for young animals to practice courtship behaviour. Same-sex sexual behaviour can occur more frequently in single-sex captive groups than mixed-sex ones. For example, penguins (*Pygoscelis antarcticus*) in captive bachelor groups form sexual pair bonds, and guppies (*Poecilia reticulata*) kept in bachelor groups display same-sex sexual behaviour more frequently than those in mixed-sex groups (Bailey & Zuk, 2009; Boutin *et al.*, 2016). Previous studies on captive giraffe bachelor herds have identified that individuals undergo behavioural changes similar to those observed in wild giraffes. Younger males display sparing and necking interactions, while mature males tend to display same-sex sexual behaviours. These studies also found that an individual's hierarchy position does not influence their expression of sexual behaviour (Ziarnowski & Fenrich, 2016; Boyle & Richards, 2019). The male giraffes at Hamilton Zoo are mature, so it is not surprising that they all display same-sex sexual behaviour. The results from this study contradicted previous ones as hierarchy position did influence each giraffe's expression of sexual behaviour. Masamba displayed **investigating** behaviours more frequently than the other giraffes. In the wild, dominant males gain more access to females (Pratt & Anderson, 1985). Perhaps this is why Masamba displayed higher levels of same-sex sexual behaviour. At Hamilton Zoo, Dume was frequently **followed** and **investigated** by the other giraffes. These behaviours are not abnormal and may be a harmless byproduct of sexual motivation (Ziarnowski & Fenrich, 2016). However, the frequent expression of sexual behaviour towards Dume may have impacted his welfare. Enclosure constraints prevented Dume from avoiding these behaviours which may have caused negative affective states such as stress and frustration.

The giraffes at Hamilton Zoo displayed similar levels of **oral** stereotypes. Therefore, it seems unlikely that the giraffes' differing social behaviours or hierarchy positions impacted the expression of these stereotypes. Negative affective states associated with feeding motivation often cause **oral** stereotypes. Many captive animals experience feeding motivation as they are not required to search, manipulate, or hunt for food resources (Fernandez *et al.*, 2008). All the giraffes at Hamilton Zoo likely experienced feeding motivation as they had limited access to browse, and other elements of their diet did not take long to consume. Masamba and Ndale spent more time displaying **oral** stereotypes in the morning. At Hamilton Zoo, the giraffes often spent the morning observation period without access to fresh browse. The giraffes likely expressed **oral** stereotypes more frequently at this time due to the reduced feeding opportunities. The giraffes' feeding motivation may also increase at this time as they anticipate movement to the paddock enclosure containing fresh browse. **Oral** stereotypes can indicate suboptimal welfare. They suggest that giraffes experience negative affective states such as frustration when unable to display foraging behaviours similar to those seen in wild giraffes.

Dume displayed **pacing** stereotypes more frequently than the other three giraffes at Hamilton Zoo. A range of underlying causes can lead to **pacing**, including social stress and anticipation (Bashaw *et al.*, 2001; Bennett *et al.*, 2015). Results identified a positive correlation between Dume's expression of **pacing** and the time he spent being **followed**. Ndoki also displayed **pacing** stereotypes. Interestingly, there was a similar correlation between his expression of pacing and the time he spent being **followed**. These results support the study's prediction that social behaviour can influence **pacing** stereotypes. Frequently being **followed** by another individual may cause negative affective states such as stress and frustration, especially in captive environments where individuals may struggle to avoid these interactions. Perhaps **pacing** was displayed by Dume and Ndoki as a coping mechanism for these negative affective states. These results align with those in a previous study where removing a herd member caused individuals to display **pacing** stereotypes (Tarou *et al.*, 2000). Both situations demonstrate how social stress can lead to the expression of **pacing**. Therefore, **pacing** stereotypes may indicate that an animal is experiencing suboptimal welfare due to its social environment.

Dume displayed **pacing** stereotypes more frequently in the morning compared to the afternoon. In the morning observation period, the giraffes often waited to move into the paddock enclosure with fresh browse. When animals have set routines, anticipation can lead to the display of stereotypes. At Hamilton Zoo, the routine enclosure moves may cause Dume to display **pacing** stereotypes in anticipation of these events. Okapis can also express **pacing** stereotypes in anticipation before feeding events and enclosure moves (Bennett *et al.*, 2015). Positive and negative affective states can lead to the expression of anticipatory behaviour. On the one hand, anticipation can reflect how much an animal values a specific event. When expecting a positive experience, dopamine can be released from the brain, resulting in positive affective states. On the other hand, anticipatory behaviour for extended periods can result in an animal becoming fixated on one event and missing opportunities to engage in other positive experiences. Intensive anticipatory behaviour can also suggest that an animal has limited positive experiences causing them to fixate on the ones that they do have. Therefore, care must be taken when assessing welfare using anticipatory behaviour (Krebs *et al.*, 2017; Ward *et al.*, 2018). Dume's **pacing** may not indicate suboptimal welfare when displayed for a short period before moving to the paddock enclosure. However, if the behaviour is displayed persistently, it may suggest that he lacks or misses out on other positive experiences.

Contrary to predations, the provision of foraging enrichment had little impact on the giraffe's behaviour at Hamilton Zoo. The enrichment was provided during the morning observation period. Therefore, it was most likely to influence the stereotypical behaviours that may have been expressed more frequently at this time due to limited browse. In previous studies, foraging enrichment reduced the expression of **oral** stereotypes in captive giraffes by facilitating foraging behaviour (Fernandez *et al.*, 2008; Kulkarni, 2020). However, foraging enrichment did not impact the giraffes' expression of **oral** stereotypes at Hamilton Zoo. This finding suggests that the giraffes still experienced excess feeding motivation when enrichment was present. Foraging enrichment also had no impact on the giraffes' display of **pacing** stereotypes. This result aligns with the study's predictions that feeding motivation does not seem to impact these stereotypes (Bennett *et al.*, 2015). Enrichment might have reduced the expression of **pacing** stereotypes if it had reduced the expression of **following** behaviour. In some cases, enrichment can increase social conflict as it is a favoured limited resource (Tarou *et al.*, 2004). While **avoiding** and **displaced** behaviours did not decrease when enrichment was present, they did not increase. Masamba also did not engage with enrichment more frequently than Ndale or Ndoki, suggesting that he did not prevent them from accessing it. Therefore, enrichment did not increase social conflict at Hamilton Zoo. Interestingly Dume was **investigated** less when enrichment was present. This result is surprising as it seems unlikely that giraffes **investigated** Dume due to feeding motivation (Bashaw *et al.*, 2001). Perhaps the giraffes choose to engage with enrichment over sexual behaviour as it is a limited resource and often contains favourable food rewards.

It is not uncommon for enrichment to have limited impacts on captive animal behaviour. A review on enrichment found that it only successfully reduced the expression of stereotypes 50% of the time (Rose *et al.*, 2017). At Hamilton Zoo, the giraffes only spent an average of 8.8-23.9% of their time engaging with enrichment. This engagement period may not have been significant enough to impact the expression of stereotypical and social behaviours (Gray *et al.*, 2018). If the giraffes engaged with enrichment for extended periods feeding motivation may have been reduced, decreasing the expression of **oral** stereotypes. Longer interaction times may have also reduced the expression of social behaviours such as **following**, **avoiding**, and **displaced**, as the giraffes would spend more time engaging in foraging behaviours instead. Despite not decreasing stereotypical behaviour, enrichment would have still facilitated foraging tongue manipulations. The enrichment also did not have adverse effects, such as increased social conflict. Therefore, results suggest that while enrichment did not significantly enhance the giraffes' welfare, it did not reduce it. Behavioural observations were only conducted seven times when enrichment was present. Seven is a small sample size meaning the ability to make conclusions from this data is limited. A larger sample size would have given a better indication of enrichment's impact on the giraffes' behaviour at Hamilton Zoo (Kuhar, 2006).

Enrichment may not have impacted the giraffes' behaviour due to habituation. Habituation occurs when an animal's innate response to an item is reduced over time. Habituation can thus result in animals having a reduced response to familiar enrichment items. Novel enrichment or puzzle feeders with varying reward schedules can help reduce habituation. For example, a remote-controlled enrichment ball with a variable reward schedule was successful when given to rhinos (*Diceros bicornis*). The varied access to rewards resulted in increased engagement times and reduced habituation. The enrichment devices given to the giraffes during this study were not novel, and the giraffes had likely become habituated to them. Familiarity with the items also likely reduced engagement time as the giraffes could consume the food within them relatively quickly. Novel enrichment or enrichment with varying reward schedules may have been more successful.

Such items may result in the giraffes investigating and engaging with enrichment for longer periods. In turn, this may effectively reduce the expression of **oral** stereotypes (Quirke & O'Riordan, 2011; Krebs & Watters, 2017; Gray *et al.*, 2018). At Hamilton Zoo, enrichment may be most effective in the morning while the giraffes wait to move to the paddock enclosure, as this is when **oral** stereotypes are displayed more frequently.

At Hamilton Zoo, mitigating Dume's expression of **pacing** stereotypes would likely enhance his welfare. Multiple factors can cause **pacing** stereotypes. Therefore, techniques used to minimise the behaviour may differ between individuals depending on the specific underlying cause. Novel enrichment or enrichment with varying reward schedules might mitigate Dume's **pacing** in the morning (Quirke & O'Riordan, 2011). Engaging with these items for prolonged periods may redirect Dume's attention away from the anticipation of movement to the paddock enclosure. Diverting Dume's attention would facilitate his engagement in other positive experiences. Eliminating social stress and frustration due to being **followed** may also reduce Dume's expression of **pacing** stereotypes (Tarou *et al.*, 2000). If the giraffes spent extended periods engaging with enrichment, they might engage in social behaviours such as **following** less frequently. However, **following** behaviours are likely expressed due to sexual motivation. Giraffes in captive bachelor herds do not have mating opportunities. Therefore, it may be challenging to reduce sexual motivation and subsequent **following** behaviours. Temporarily separating Dume from the individuals **following** him would eliminate the behaviour for a short period. However, separation may cause the giraffes to experience negative affective states. In captive bachelor herds, males may develop social bonds causing them to experience separation anxiety (Ziarnowski & Fenrich, 2016; Gubert & Hannan, 2019; Lewton & Rose, 2020). After separation, individuals may also feel threatened by the other males, causing the **following** behaviours to increase (Seeber *et al.*, 2012).

The ability to display appropriate social behaviour is essential for an animal's fitness and welfare (Koene & Ipema, 2014). At Hamilton Zoo, the expression of **avoiding** and **displaced** behaviours may impact Ndale and Ndoki's welfare, especially when they are in the smaller yard enclosure. Animal husbandry routines should ensure subordinate individuals are supported. For example, subordinate individuals should not be confined in small spaces with dominant individuals, and they should have equal opportunities to access food and shelter. At Hamilton Zoo, the giraffes have two houses, and both enclosures contain multiple browse stations, ensuring all individuals can assess these resources. Time in the paddock enclosure may also be essential for Ndale and Ndoki's welfare as the large space allows them to spend time away from dominant individuals. Studies on bachelor gorilla (*Gorilla*) groups have also suggested that enclosures should contain multiple pathways for social avoidance and refuges for subordinate males (Stoinski *et al.*, 2004). Ndale and Ndoki will have more opportunities to experience positive affective states when they can easily avoid dominant individuals and assess essential resources. Separating subordinate and dominant individuals would eliminate the expression of **avoiding** and **displaced** behaviours. However, separating individuals may not necessarily enhance their welfare. As mentioned above, separation may also cause negative affective states. Consistent separation and remixing may also disrupt the giraffes' stable social hierarchy, increasing social conflict (Rox et al., 2019).

Behavioural observations can provide a powerful insight into an animal's welfare. Animal behaviour scientists use observations to describe a species' behavioural inventory. These studies also identify how animals respond to their environment using species-specific behaviours. Understanding a species' behavioural inventory will ensure incorrect assumptions are not made about an animal's behaviour and its underlying cause (Hill & Broom, 2009; Seeber *et al.*, 2012). If an animal has the freedom to display a range of species-specific behaviour in response to its environment, it will experience increased fitness and optimal welfare. For example, it will develop appropriate social relationships, access vital nutrients, and avoid risks from exposure to adverse environments. When unable to exhibit appropriate behaviour, animals can display behavioural indicators of suboptimal welfare such as stereotypical behaviour and social avoidance. Abnormal variations in an animal's behaviour can also indicate poor welfare due to factors such as injury, disease, and poor nutrition. Behaviour is also a reflection of an animal's affective states. Therefore, behavioural observations can help monitor the balance between an animal's experience of positive and negative affective states (Koene, 2013; Warwick *et al.*, 2013; Watters *et al.*, 2021).

A strength of behavioural observation is its practicality and ability to assess welfare in several scenarios. Behavioural observations do not require specialized equipment or veterinary/scientific experience. Therefore, anyone knowledgeable of the species' behavioural inventory can use this method to assess welfare. Assessors can also use ethograms to ensure behavioural definitions remain consistent across observations (Grosso *et al.*, 2016). Behavioural assessments are non-invasive and do not require animal training. Therefore, they can assess the welfare of non-contact animals or animals not yet comfortable with human contact. Non-invasive methods are also beneficial as they allow welfare to be assessed regularly without impacting an animal's husbandry routine. Animals experience different welfare challenges throughout their life due to changes in health, life stage, or social structure. Therefore, regular behavioural observations can ensure husbandry routines are continuously adapted throughout an animal's life to maintain optional welfare (Yon *et al.*, 2019).

While behavioural observations can provide insights into animal welfare, the method also has limitations. People can have contrasting views on what factors determine an animal's welfare. These differing opinions can result in conflict around what behaviours reflect optimal welfare. For example, some people believe that natural behaviour (behaviour displayed in in-situ environments) reflects optimal welfare, while others suggest that behaviours reflecting healthy physiological functioning indicate optimal welfare (Lawrence, 2008). In captivity, female giraffes form social hierarchies (Lewton & Rose, 2020). Some people may believe that this behaviour reflects suboptimal welfare as it differs from the behaviour observed in wild females. Others may acknowledge that this behavioural plasticity enhances welfare as it reduces social conflict among captive female giraffes. However, in some cases, deviations from an animal's natural behaviour can cause suboptimal welfare. For example, captive giraffes express **oral** stereotypes when unable to display natural foraging periods (Fernandez *et al.*, 2008). These examples demonstrate how behaviour can be challenging to interpret and cause conflicting conclusions about an animal's welfare.

A challenge commonly faced when assessing animal welfare is our inability to understand how different species experience conscious emotions and feelings. Behavioural studies often rely on the assumption that behaviours reflect positive or negative affective states. We can assume that affective states cause an animal's behaviour, but we must not assume that these affective states are similar to human emotion. For example, in this study, we can assume that the inability to display extended foraging periods and behaviours caused negative affective states similar to frustration. However, we cannot state that the giraffes are experiencing frustration as humans do. We must be careful not to define an animal’s welfare based on the assumption that their behaviour reflects specific human emotions (Lawrence, 2008; Fraser, 2009). It is also important to acknowledge that behaviour may not always reflect an animal’s current affective state. Animals can display some behaviours out of habit. For example, an animal may develop stereotypical behaviours due to negative affective states but continue displaying the behaviour out of habit even when the negative affective state has been resolved. Therefore, an animal displaying stereotypes may not always have poorer welfare than its conspecific who is not (Swaisgood, 2007). Consequently, it may be challenging to determine what behaviours reflect an animal’s current welfare status. These limitations demonstrate how we must be careful when interpreting animal behaviour to ensure we do not make incorrect assumptions about an animal’s welfare status. Acting upon incorrect conclusions can be harmful as it may result in husbandry routines not addressing the actual factors limiting an animal’s welfare. It may even cause husbandry routines to reduce the expression of behaviours that cause positive affective states and enhance an animal’s welfare (Watters *et al.*, 2021).

At Hamilton Zoo, several giraffes displayed behaviours that might reflect stress. However, behavioural studies are unable to confirm this. It would be beneficial to assess the giraffes’ adrenal activity to determine if behaviours such as **avoiding** and **pacing** correlate with elevated stress levels. This would provide a deeper understanding of their affective states and subsequent welfare. Therefore, the following chapter will investigate the giraffes’ stress physiology as a more objective indicator of their welfare.

Chapter 3

Assessing giraffe welfare using faecal cortisol metabolite analysis

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3.1 Introduction

Stress is inevitable and often critical for survival. Stress cannot be avoided as fluctuations in energy are needed to maintain biological functioning in a continuously changing environment. Therefore, stress is an adaptive mechanism that enables animals to remove themselves from unfavorable conditions and maintain homeostasis. As Hans Selye said, ‘complete freedom from stress is death’ (Selye, 1973, p. 693; Moberg & Mench, 2000). Stress sparks several physiological responses throughout the body. One of these is increased activity in the hypothalamic–pituitary–adrenal (HPA) axis. A range of stressors can stimulate the HPA axis, including insufficient nutrients, extreme temperatures, illness, and unfavorable social dynamics Animals can resolve many stressors quickly using energy stored in their biological reserves. When resolved quickly, stressors have little impact on an animal’s welfare. Stress begins to significantly impact welfare when an animal struggles to restore physiological and psychological homeostasis. Stress turns to distress when the cost of the stress response overwhelms biological reserves and energy is detracted away from other biological processes (Figure 3.1). When experiencing distress, an animal enters a prepathological state. In this state, immunosuppression can render an animal vulnerable to environmental pathogens, increasing the chances of illness. Animals will also become susceptible to pathologies such as reproductive issues or stunted development. The longer an animal is in a prepathological state, the more likely it will succumb to pathologies (Moberg & Mench, 2000; Palme *et al.*, 2005).

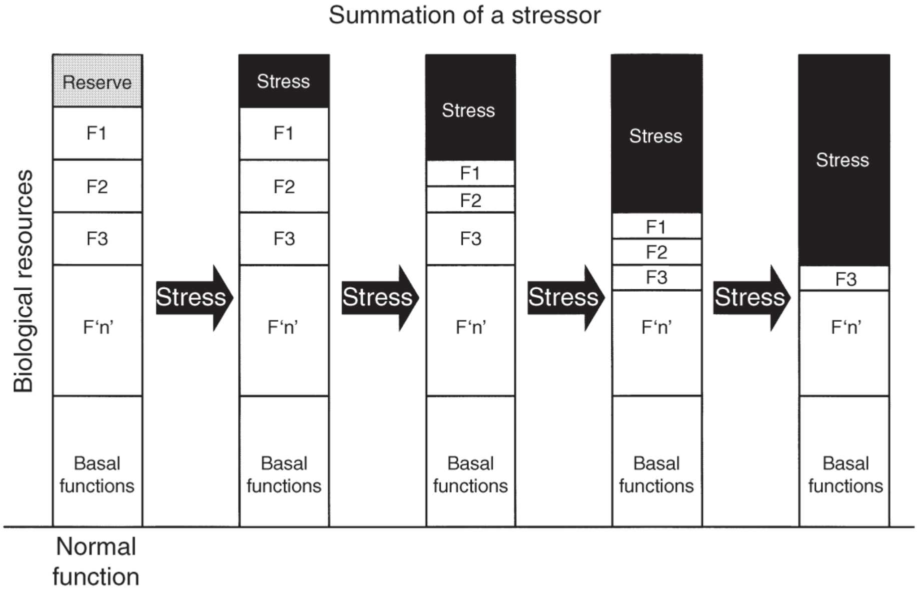

Figure 3.1- When the cost of a stressor increases biological reserves become overwhelmed and energy begins to be detracted away from other biological processes (Moberg & Mench, 2000).

Monitoring the hypothalamic-pituitary-adrenal (HPA) axis allows us to identify when animals are experiencing elevated stress or distress and what stressors may be causing it. The HPA axis controls the synthesis of cortisol, which plays a vital role in the stress response (Moberg & Mench, 2000). The cortisol metabolites are excreted in faeces and can be measured using faecal cortisol metabolite (FCM) analysis. However, FCM analysis is not the only method used to measure activity in the HPA axis. Glucocorticoid (GC) hormones can also be measured in blood samples. This method is beneficial as it provides a more immediate measure of an animal's stress response. However, it can be challenging to collect blood samples from wild animals or uncooperative animals such as those in zoos. The collection of blood samples can also act as a stressor eliciting an animal's stress response. Therefore, FCM analysis is often used to measure stress in wild or captive exotic animals as the collection of faecal samples is non-invasive. Faecal cortisol metabolite levels do not provide an immediate measure of an animal's stress response due to the time lag between the secretion of cortisol and the presence of metabolites in the faeces. Therefore, FCM analysis may not capture rapid changes in cortisol in response to acute stressors. Despite this, FCM analysis provides a powerful tool to assess animal welfare when collecting blood samples is not feasible (Palme *et al.*, 2005; Palme, 2019).

Faecal cortisol metabolite levels can be measured using several methods. Liquid chromatography and mass spectrometry have been used to achieve FCM analysis. However, this method requires the metabolite's chemical identity to be known, and in many cases, scientists do not have this knowledge. Therefore, scientists commonly use immunoassays to perform FCM analysis. There are two types of immunoassays radioimmunoassays (RIA) and enzyme immunoassays (EIA). These immunoassays contain labelled steroids and antibodies; the labelled steroids compete with steroids in the faecal sample for binding spots on the antibody. Radioimmunoassays require expensive equipment and a permit to measure radioactivity. Therefore, EIA's are predominantly used to achieve FCM analysis. The specific cortisol metabolites excreted in faeces can differ between species. Therefore, validation is required to identify species appropriate EIAs. Analytical, physiological, and biological validations determine species appropriate EIA's (Palme, 2019).

Physiological and biological validations have determined appropriate EIAs for FCM analysis in giraffes. Physiological validation involved two giraffes being injected with an adrenocorticotropic hormone (ACTH) injection to stimulate their adrenal glands. Biological validation involved five giraffes being exposed to a stressful event in the form of twenty-minute transportation to an unfamiliar environment. The collection of faecal samples occurred throughout the days leading up to and following these two procedures. Several EIAs were then trailed in FCM analysis. These trials identified two 11-oxoetiocholanolone EIAs that effectively recorded peaks in the giraffes FCM levels. These assays measured metabolites with 11,17-DOA and 3α,11-oxo-CM structures. The physiological and biological validation procedures included giraffes with different housing conditions, ages, diets, and climates. Therefore, these EIAs can effectively analyse giraffe FCM levels in a range of contexts. The most significant peaks in FCM levels were recorded around 24-27 hours after the ACTH injection. Therefore, a time delay of about 24 hours occurs between the secretion of cortisol and the presence of cortisol metabolites in giraffe faeces. The validations also identified that adrenal activity fluctuated alongside the giraffes' circadian cycle, with cortisol metabolite levels peaking at sunrise (Bashaw *et al.*, 2016) (Figure 3.2). These fluctuations are expected as mammalian cortisol levels often cycle in response to the circadian cycle. Cortisol levels usually peak early in the morning before beginning to decline throughout the day. Scientists must account for these fluctuations during FCM analysis (Oster *et al.*, 2017).

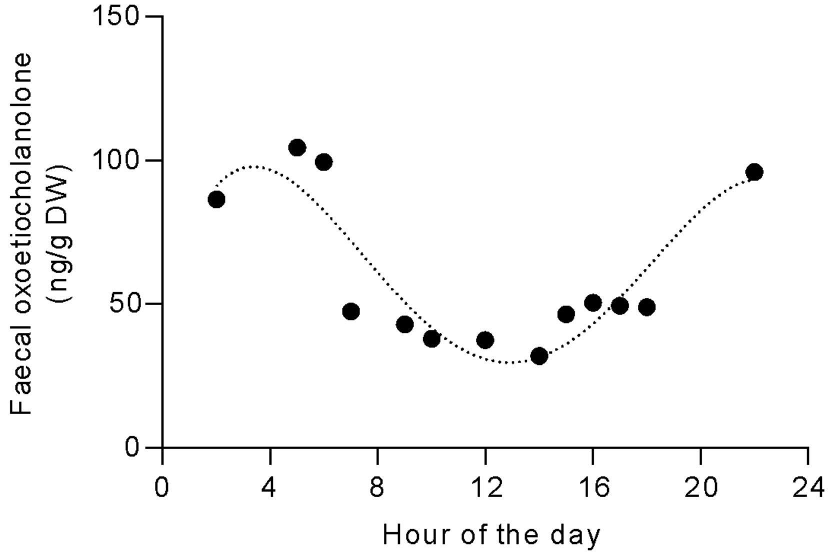

Figure 3.2- Faecal oxoetiocholanolone (ng/g DW) levels across a giraffe's circadian cycle. The data points are averaged values from samples taken over two days from a single captive male giraffe (Bashaw *et al.*, 2016).

Studies involving captive and wild giraffes have used 11-oxoetiocholanolone EIAs to measure FCM levels. The results from these studies have demonstrated how giraffes cope with several environmental stressors. Faecal cortisol metabolite analysis found that captive giraffes stress levels did not differ between seasonal housing rotations despite behavioural changes. Therefore, housing changes may have less impact on giraffe welfare than previously thought (Razal *et al.*, 2017). In wild giraffes, injuries and low body conditions correlated with increased FCM levels. Therefore, injury and poor health likely had a significant impact on these individuals' welfare (Wolf *et al.*, 2018c). Giraffes display complex fission-fusion social dynamics, and FCM analysis has identified how these dynamics impact stress levels in wild male giraffes. Faecal cortisol metabolite levels differ depending on a giraffe's age, with younger males often displaying higher FCM levels. The 'stress of subordination' hypothesis can explain these differences. Older males often have a higher dominance ranking deterring younger males away from favoured resources. Adolescent males may also begin to challenge others to determine their hierarchy status (Wolf *et al.*, 2018b; Wolf *et al.*, 2018d). Therefore, it is likely that younger subordinate males have a more significant allostatic load causing higher FCM levels (Goymann & Wingfield, 2004). Mature male giraffes experience fluctuations in FCM levels, with significant increases occurring in the presence of fertile females. The ‘immunocompetence handicap’ hypothesis can explain this pattern. The change in adrenal activity suggests a trade-off occurs between mating ability and immune function. Males likely increase their adrenal activity around fertile females to support the display of energetically demanding mate guarding and mating behaviour. In the absence of fertile females, the adrenal activity likely decreased to ensure sufficient immune function (Wolf *et al.*, 2018b; Wolf *et al.*, 2018d).

Previous studies have used FCM analysis to investigate the relationship between social dynamics and stress levels in wild giraffes (Wolf *et al.*, 2018b; Wolf *et al.*, 2018d). Similar methods have not yet investigated the relationship between social dynamics and stress levels in captive giraffes. Captive giraffes face different environmental pressures than wild ones. Captivity reduces pressures associated with resource availability but may increase social pressures due to fixed herd sizes (Lewton & Rose, 2020). The different environmental pressures can result in captive giraffes displaying behaviours that differ from those observed in wild giraffes. For example, captive females develop social hierarchies (Horová *et al.*, 2015). Therefore, activity in the HPA axis may also differ between wild and captive giraffes. Giraffes can display pacing and oral stereotypes when unable to exhibit appropriate behaviour in response to captive environments. Studies investigating pacing stereotypes have proposed several underlying causes, including social stress (Bennett *et al.*, 2015; Duggan *et al.*, 2016; Orban *et al.*, 2016). However, studies are yet to use FCM analysis to investigate the relationship between pacing and stress. Enrichment is often used to reduce the expression of stereotypical behaviour by facilitating the expression of species-specific behaviour. Many studies have investigated enrichment’s ability to mitigate stereotypical behaviour in captive giraffes. However, none have identified if enrichment reduces captive giraffes stress levels. Studies on other species have found that enrichment lowers cortisol levels in some cases but not others (Boinski *et al.*, 1999; Liu *et al.*, 2006).

The first objective of this study was to quantify the giraffes’ stress levels at Hamilton Zoo using FCM analysis. The results from FCM analysis will then be compared to the results from behavioural observations in Chapter Two. I predict that individuals displaying subordinate behaviours will have higher FCM levels as this pattern occurs in wild giraffes. I expect that oral stereotypes will not correlate with elevated FCM levels as the behaviour’s underlying cause is often feeding motivation. However, I predict that there will be a correlation between pacing and elevated FCM levels as social stress is a potential underlying cause for this behaviour. Enrichment had little impact on the giraffes' behaviour. Therefore, I predict that enrichment will not significantly impact the giraffes' FCM levels. The second objective of this study was to use FCM analysis to compare the stress levels of the giraffes at Hamilton Zoo to those from three male giraffes housed in a bachelor herd at Gibbs Farm. This comparison provides the opportunity to identify similarities and differences between the stress levels of giraffes in two bachelor herds, strengthening our understanding of how captive environments impact the welfare of male giraffes housed in bachelor herds.

Faecal cortisol metabolite analysis will provide an insight into the giraffes' welfare at Hamilton Zoo and Gibbs Farm. Understanding each giraffe's FCM levels and the potential stressors causing them will enable Hamilton Zoo and Gibbs Farm to develop husbandry routines that mitigate stress. When mitigating stress, animals have more opportunities to experience positive affective states and enhanced welfare. The results from this study will also improve our understanding of how social rank and stereotypical behaviour impact male giraffe welfare in captive bachelor herds.

3.2 Methods

3.2.1 Subjects and husbandry

The subjects for this study were the same as those in Chapter Two; four male giraffes housed at Hamilton Zoo. As mentioned in 2.2.2, Masamba received a Bopriva vaccination on 16/9/2020, a month before this study began. There was a possibility that the Bopriva vaccination impacted Masamba's adrenal activity. While there is currently no research on how the vaccination affects adrenal activity, it was considered during FCM analysis.

This study also included three giraffes (*Giraffa camelopardalis*) housed at Gibbs Farm, a ZAA accredited institution holding a collection of ungulate species in Kaipara, New Zealand. Like Hamilton Zoo, Gibbs Farm housed a bachelor giraffe herd. The three males in this herd were half-brothers born at Orana Wildlife Park in Christchurch, New Zealand. Orly and Flynn were born in 2004, and Kyana was born in 2007. Based on the zookeeper's observations Kyana was the subordinate male, and Flynn was the herd's dominant male. Kyana was known to display pacing stereotypes predominantly before enclosure moves.

All three giraffes were known to express oral stereotypes such as object licking. The giraffes were housed in yard enclosures overnight and paddock enclosures during the day. The movement to the paddock enclosures was weather-dependent as they did not contain giraffe houses. During this study, Flynn was housed separately from Orly and Kyana due to his aggressive behaviour. Orly and Kyana also displayed aggressive behaviour towards each other and had received Bopriva vaccinations before this study, which were considered during FCM analysis. At Gibbs Farm, the giraffes' diet contained around 12 shared large browse pieces, lucerne hay, lucerne chaff, multi-nut pellets, and a carrot/apple/celery mix.

3.2.2 Experimental area

This study took place in the giraffe yard enclosure at Hamilton Zoo. Detailed descriptions of Hamilton Zoo and the yard enclosure are given in section 2.2.3.

At Gibbs Farm, the giraffes moved between yard and paddock enclosures (Figure 3.3). The facility contained two yards located next to each other. Yard one was around $280m^2$, and yard two was around $600m^2$. Each yard had a browse pole and a lucerne feeder. Between the two yards, there was a giraffe house divided into two spaces. Each yard had access to one of these spaces. The substrate in the yards was a mixture of stock rock and limestone, and the substrate in the house was sand. A short raceway connected the yards to two paddock enclosures. Paddock one was around $5790m^2$, and paddock two was around $3150m^2$. Each paddock contained two browse poles and lucerne feeders, and the substrate was grass.

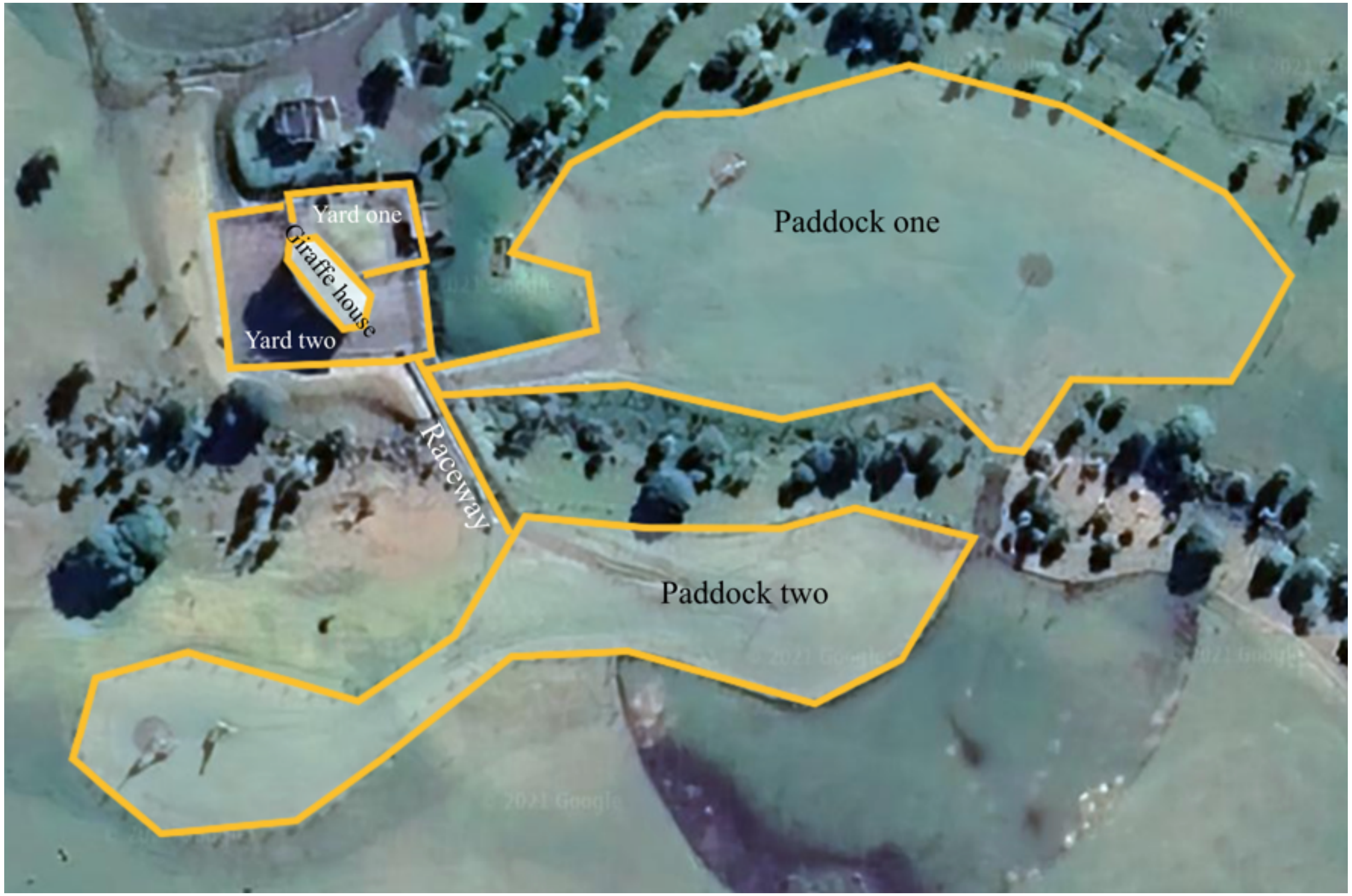

Figure 3.3- Aerial photo of the giraffes' enclosures at Gibbs Farm. The yard enclosures, giraffe house, raceway, and paddock enclosures are labeled (Google Earth, 2019).

3.2.3 Faecal sample collection

At Hamilton Zoo, the collection of faecal samples occurred between 20/10/2020-19/12/2020 and 5/1/2021- 16/1/2021. Sample collection happened on Tuesdays, Thursdays, and Saturdays between 8:00-10:00. Samples were collected at this time, as it was before the giraffes moved to the paddock enclosure. Due to its large size, it would not have been possible to identify and collect faecal samples in the paddock enclosure. Sample collection also occurred at consistent times each day to control for the fluctuations in adrenal activity around the giraffes' circadian cycle (Bashaw *et al.*, 2016; Oster *et al.*, 2017). The collection of faecal samples occurred on alternative days to the behavioural observations described in 2.2.4. It takes about 24 hours for metabolites to appear in giraffe faeces (Bashaw *et al.*, 2016). Therefore, results from FCM analysis can be correlated to data from the previous day's morning behavioural observations. These correlations may be insightful when justifying the results from FCM analysis. Health and Safety regulations prevent zookeepers from entering enclosures with giraffes. Therefore, sample collection occurred from outside the yard enclosures. A two-meter extendable pole and net could be reached through the mesh fence to collect faecal samples deposited near the fence line (Figure 3.4). Faeces deposited away from the fence were collected by temporarily locking the giraffes out of one yard. This method was opportunistic as it relied on giraffes moving out of the yard on their own accord. Each giraffe usually deposited faeces numerous times during collection periods offering multiple opportunities to collect a sample. Keys were required to open and close the gates between each yard. I underwent training before this study that allowed me to carry a set of keys and be around the giraffe enclosures unsupervised. All faecal samples were collected within 20 min of defecation. Studies have identified that FCM levels can begin to change after 20 min. Therefore, FCM levels in samples collected after this time might not accurately represent activity in the HPA axis (Palme, 2019). Previous studies using FCM analysis in giraffes have also collected samples within 20 min of defecation (Wolf *et al.*, 2018b; Wolf *et al.*, 2018d). Sealed plastic bags labeled with the giraffe's name and date held the faecal samples after collection. A chilly bin stored the faecal samples on ice for up to two hours before transferal to a -20°C biohazard freezer at the zoo.

Figure 3.4- The two-meter extendable pole and net used to reach through the enclosure's mesh fence and collect faecal samples.

At Gibbs Farm, zookeepers collected faecal samples from their three giraffes between 2/2/2021- 7/3/2021. Samples were collected opportunistically around the zookeeper's routine schedules. The methods used to collect faecal samples were explained to the zookeepers at Gibbs Farm to ensure they were consistent with those used to collect samples at Hamilton Zoo. For example, sample collection also occurred between 8:00-10:00. Four samples were collected from Flynn, five were collected from Orly, and six were collected from Kyana. Overall, 141 faecal samples were collected across both facilities. Ethics was not required for this study as the collection of faecal samples had no impact on the giraffes or their husbandry.

3.2.4 Faecal cortisol metabolite analysis

The Ministry for Primary Industries (MPI) approved the transfer of faecal samples from Hamilton Zoo and Gibbs Farm to The University of Waikato. Here samples were stored in a -20°C freezer. Validations have shown 11-oxoetiocholanolone EIA's ability to identify peaks in giraffe FCM levels (Bashaw *et al.*, 2016). For this study, two 11-oxoetiocholanolone ELISA kits were purchased from Cayman Chemicals. The MPI identified these kits as risk goods as they contained antibodies derived from rabbits. Therefore, the kits were stored and used in a Physical Containment Level 1 laboratory at Waikato University. I also underwent MPI training for Waikato University's Molecular Biology, Microbiology, and Genetics containment standard.

A protocol suggested by Cayman Chemicals was followed to extract metabolites from the faecal samples in preparation for the assay. To begin, 1.5-2 g of wet matter from each sample was freeze-dried. Any large fibrous material was removed from the freeze-dried samples by pulverizing in a mortar and pestle and sieving through a small metal filter (~1 mm mesh). Next, 0.1-0.11 g of the faecal powder was measured from each sample and put into labelled 14 ml centrifuge tubes. Then 1 ml of pure water, 8 µl of 1.74 M acetic acid, and 1.6 ml of ethyl acetate were added to each sample. Samples were then vortexed and incubated at room temperature for 10 min. After incubation, samples were placed in an Hettich Universal 320/R refrigerated centrifuge for 5 min at 3000 x g. The upper ethyl acetate layer was transferred from each sample into a new labelled 14 ml centrifuge tube using a pipette. Another 1.6 ml of ethyl acetate was added to the original tubes an additional four times with samples incubated and centrifuged between each addition. The layers of ethyl acetate were combined into one centrifuge tube for each sample. The ethyl acetate was evaporated from the centrifuge tubes using a Techne dri-block heater at 36°C under a gentle stream of nitrogen. Finally, the evaporated samples were reconstituted in 500 µl of tris buffer. The tris buffer was prepared by diluting a 10 ml tris buffer vial (supplied in the assay kit) to 10X by adding 90 ml of pure water. Samples were then centrifuged for another 5 min at 3000 x g to ensure no particles were transferred into the assay plate. The 11-oxoetiocholanolone ELISA Standards, 11-oxoetiocholanolone AP Tracer, and 11-oxoetiocholanolone ELISA antibody were then prepared. The eight standards were prepared in 14 ml centrifuge tubes by serial dilution using the standard solution and instructions supplied in the assay kit. The AP Tracer and ELISA Antibody were also provided and prepared according to the kit instructions.

After the samples and assay-specific reagents were prepared, the assay plates were set up. Each plate had 96 wells (12 strips and eight rows), 80 of which could analyse samples. Therefore, two plates were required to analyse the 141 samples. The extra 19 wells were used to analyse replicates. The replicates were prepared by completing the extraction procedure on the same sample twice and running both extractions. For example, 1.5-2 g of the wet matter was measured from the 19 replicate samples twice. The kit instructions suggested that all samples were run in duplicate. However, due to the larger number of samples and cost of the assay kits, most samples were only analysed once. On each plate, the first strip of wells contained two non-specific binding wells (100 µl of tris buffer and 50 µl of AP tracer), three maximum binding wells (50 µl of tris buffer, AP Tracer, and

ELISA Antibody), one total activity well (50 µl of ELISA Antibody), and two blank wells. Each well in the second strip had 50 µl of one of the standard concentrations and 50 µl of the AP Tracer and ELISA Antibody. The remaining ten strips were used to analyse the extracted faecal samples. Each well contained 50 µl from one sample and 50 µl of the AP Tracer and ELISA Antibody. The prepared plates were covered and incubated overnight for 18-24 hr. After incubation, the wells were washed five times using a BioTek 50 TS microplate washer. The wash buffer was prepared by diluting a 5 ml wash buffer vial (supplied in the assay kit) to 150X by adding 750 ml of pure water. After the plate had been washed, 200 µl of pNPP substrate solution (provided in the assay kit) was added to each well, and 5 µl of AP Tracer was added to the total activity well. The plates were then covered and placed on an IKA MS1 minishaker for 120 min. Finally, the plastic covers were removed, and the plates were read using a BioTek 800 TS absorbance reader at a wavelength of 405 nm.

3.2.5 Data analysis

GraphPad Prism version 9.0 (GraphPad Software, LLC) was used to perform statistical analysis and produce graphs. Data analysis identified any significant differences between the giraffes' FCM levels at Hamilton Zoo and Gibbs Farm. The Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test, and Kolmogorov-Smirnov test identified that the two data sets met the assumptions of normality. Therefore, a One-Way ANOVA (ANOVA) and Tukey's multiple comparison test (TMC) was used in analysis. Pearson correlation tests (PC) identified if the giraffes' FCM levels were correlated at Hamilton Zoo. An unpaired T-test (UT) was also used to determine if the presence or absence of enrichment impacted the giraffes' FCM levels at Hamilton Zoo.

Data analysis identified if there were any correlations between the giraffes' FCM levels at Hamilton Zoo and the behavioural data obtained in Chapter Two. The FCM data meet the assumptions of normality. However, the behavioural data did not. Therefore, the non-parametric Spearman correlation (SC) test was used to complete this analysis. This analysis only incorporated behaviours thought to link with stress or the study objectives. Correlations between Masamba's FCM levels and his expression of following, investigating, and oral stereotypical behaviours were investigated. Tests also analysed the relationship between Dume's FCM levels, his frequency of being followed, and display of pacing and oral stereotypical behaviours. Ndale’s FCM levels were compared with his presentation of avoiding and oral stereotypical behaviours. Ndoki’s FCM levels, frequency of being displaced, and expression of oral stereotypical behaviours were also examined.

3.3 Results

3.3.1 Immunoassay results

Calibration curves were constructed from the standards in both assays. Both the linearized logit transformed calibration curves showed a high degree of linearity (Assay 1, R^2= 0.998; Assay 2, R^2=0.999; Figure 3.5). The lowest concentration from assay two was excluded as it fell outside the calibration curve. However, this did not impact analysis as all the samples fell within the range of the remaining standard concentrations. The unknown 3a,11-oxo-CM concentrations from each sample were calculated from the respected linearized logit transformed calibration curves.

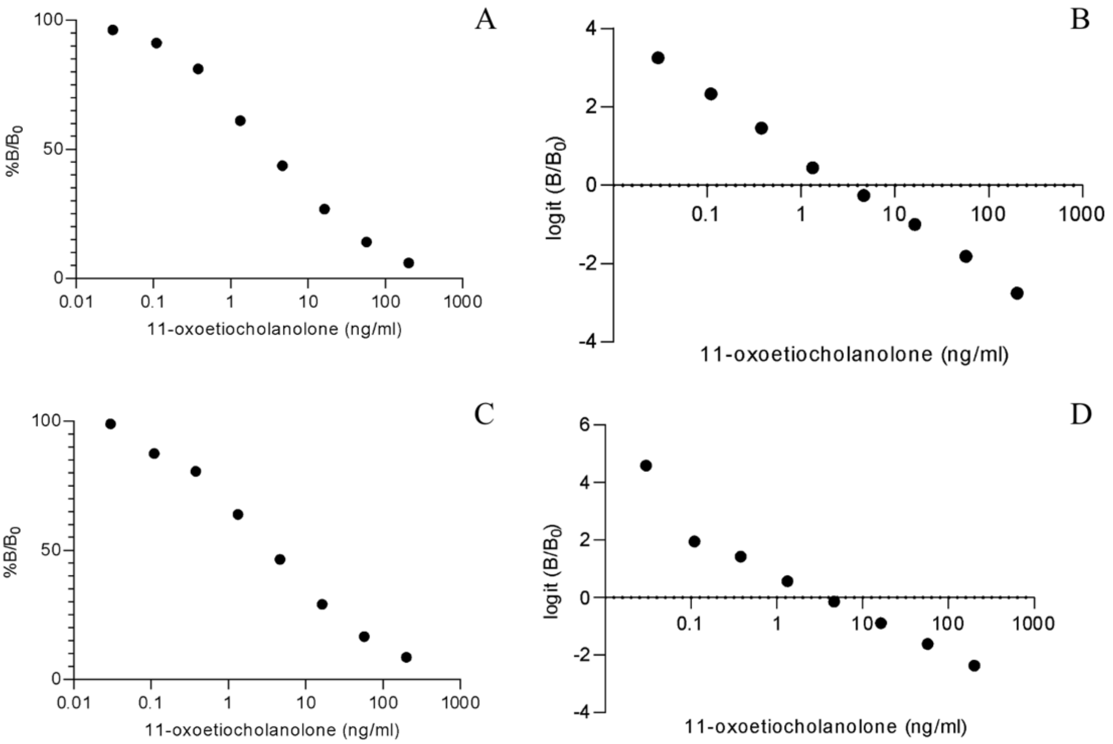

Figure 3.5- Standard calibration curve for assay one (A), and the linearized logit transformed calibration curve for assay one (B). Standard calibration curve for assay two (C), and the linearized logit transformed calibration curve for assay two (D).

The average coefficient of variation for the 19 replicates analysed was 13.7%. Therefore, there is a good level of correspondence between the replicated samples. The average concentration of each replicate was used in the analysis as that value better represented the sample's FCM level.

3.3.2 Faecal cortisol metabolite levels at Hamilton Zoo

Faecal cortisol metabolite levels differed significantly between the four giraffes at Hamilton Zoo (ANOVA, F = 11.54, $p < 0.0001$; Figure 3.6). Masamba had significantly higher FCM levels compared to the other three giraffes. The giraffes' FCM levels showed variations over the 12 week study period (Figure 3.7). Ndoki's FCM levels had the most significant variation (CV = 51.6%) while Dume's had the least (CV = 33.1%). There was also a correlation between Masamba and Dume's FCM levels (PC, r = 0.3738, $p = 0.0383$; Figure 3.8).

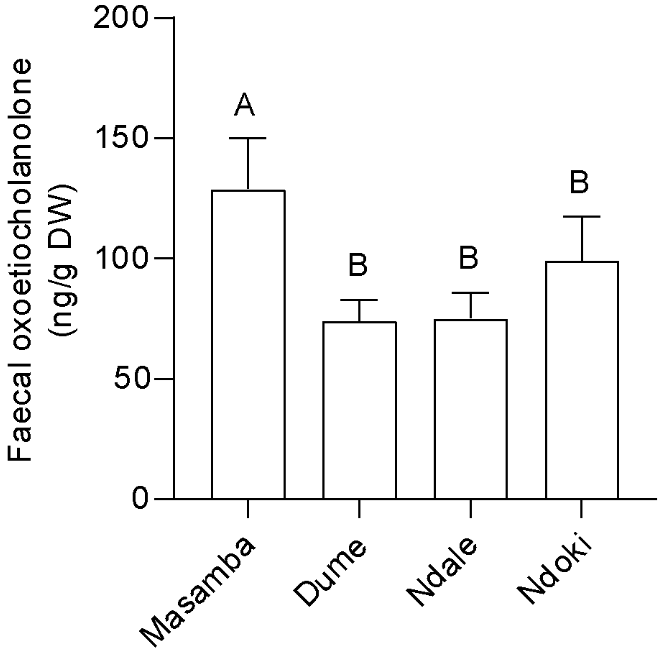

Figure 3.6- Each giraffe's faecal oxoetiocholanolone (ng/g DW) levels at Hamilton Zoo. Individuals with the same letter are not significantly different.

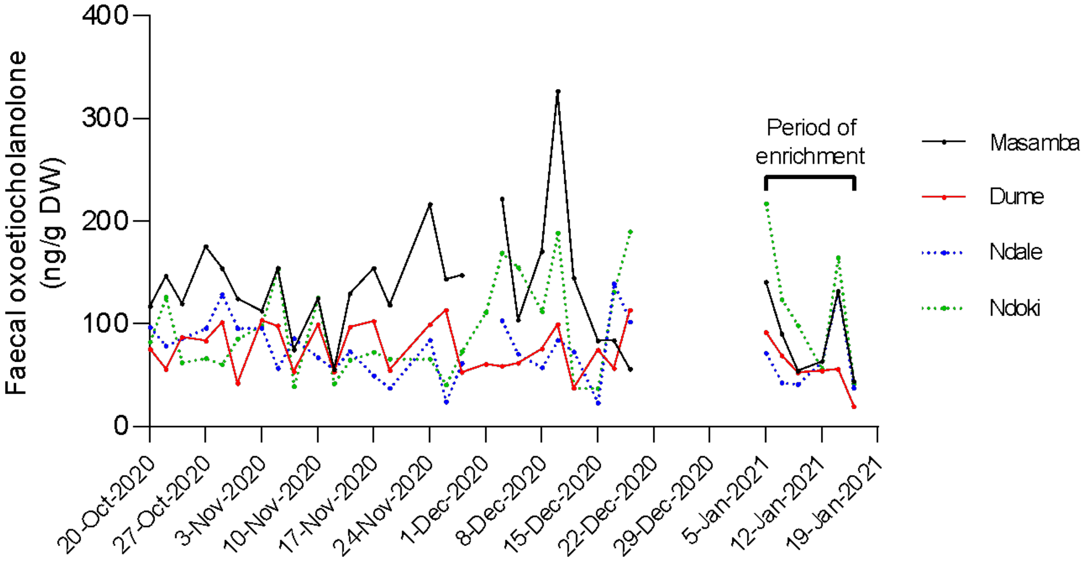

Figure 3.7- Variations in the giraffes' faecal oxoetiocholanolone (ng/g DW) levels at Hamilton Zoo over the 12 week study period.

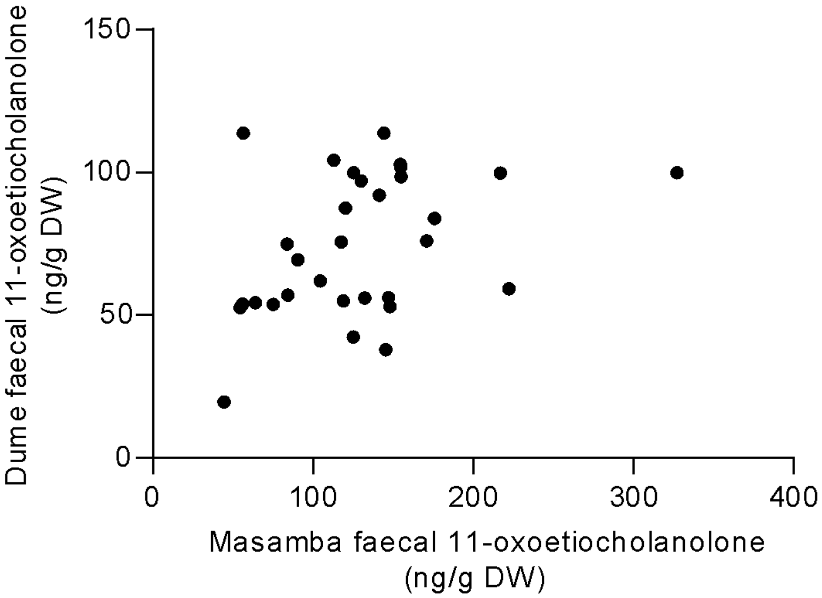

Figure 3.8- The correlation between Masamba and Dume's faecal 11-oxoetiocholanolone (ng/g DW) levels.

3.3.3 Enrichments effect and correlations with behaviour

Masamba's FCM levels were significantly lower in the presence of foraging enrichment (UT, $p = 0.0498$; Figure 3.9). However, the presence or absence of enrichment did not significantly impact the other giraffes' FCM levels at Hamilton Zoo.

There were no correlations between Masamba, Ndale, and Ndoki's FCM levels and their behaviour. There was however a significant correlation between Dume's FCM levels and his expression of oral stereotypes (SC, $r = 0.3634$, $p = 0.0409$; Figure 3.10).

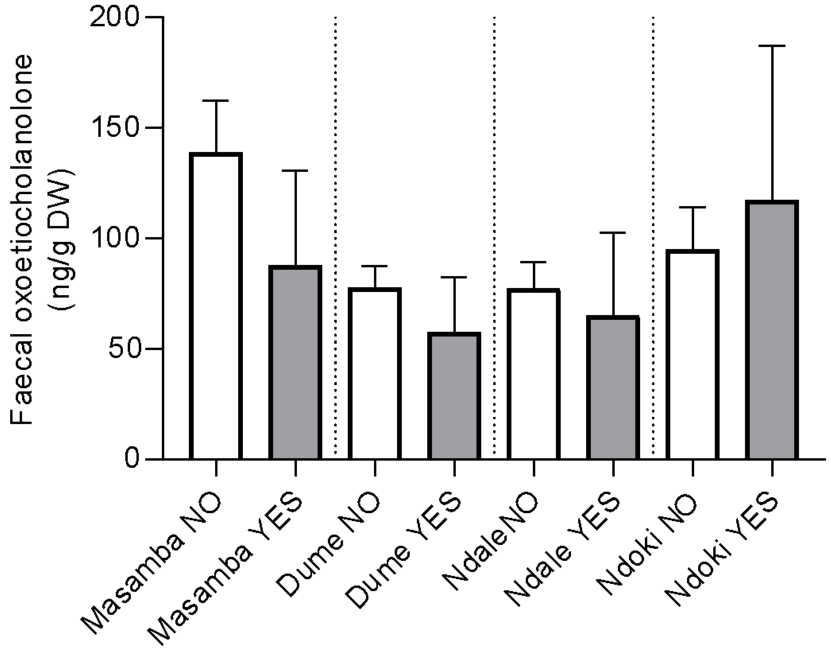

Figure 3.9- Each giraffe's faecal oxoetiocholanolone (ng/g DW) levels in the absence (NO) and presence (YES) of foraging enrichment.

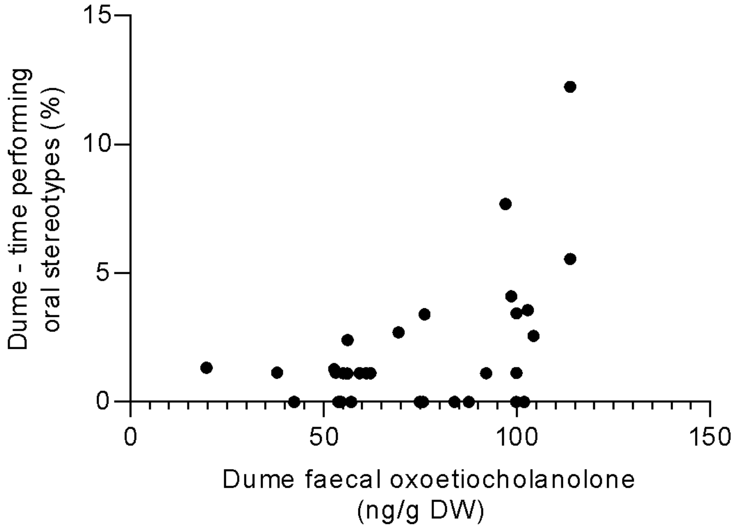

Figure 3.10- The correlation between the percentage of time Dume spent performing oral stereotypes and his faecal oxoetiocholanolone (ng/g DW) levels.

3.3.4 Faecal cortisol metabolite levels at Gibbs Farm

Faecal cortisol metabolite levels differed significantly between the three giraffes at Gibbs Farm (ANOVA, F = 7.96, $p = 0.0063$; Figure 3.11). Kyana had significantly higher FCM levels compared to the other two giraffes. Kyana's FCM levels also had the most variation (CV = 37.6%) while Orly's were the least (CV = 17.7%).

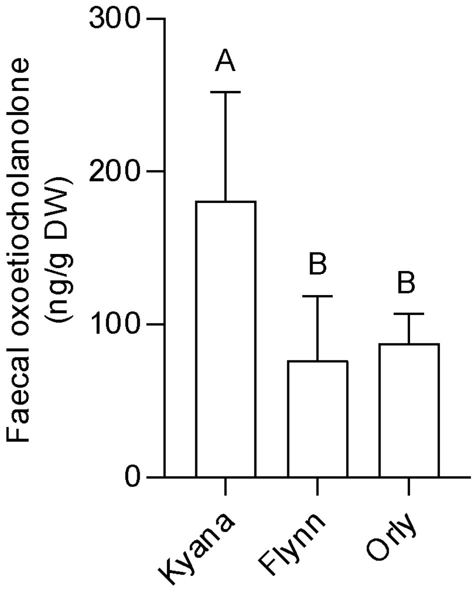

Figure 3.11- Each giraffe's faecal oxoetiocholanolone (ng/g DW) levels at Gibbs Farm. Individuals with the same letter are not significantly different.

3.4 Discussion

Masamba had significantly higher FCM levels than the other three giraffes at Hamilton Zoo. This result contradicted predictions because Masamba is the herd's dominant male. In the wild, older males often have lower FCM levels due to their higher dominance. This pattern reflects the 'stress of subordinate' hypothesis where lower-ranking individuals experience higher stress levels due to reduced access to resources or increased aggression from dominant individuals (Wolf *et al.*, 2018b). However, in some species, such as bison (*Bison bison*), dominant males have the highest cortisol levels. In these cases, the 'stress of dominant' hypothesis suggests that the higher social rank comes at a cost. Dominant males may experience elevated stress if they frequently engage in antagonistic or aggressive interactions. The elevated adrenal activity enhances these individuals' ability to endure energetically demanding periods of aggression (Mooring *et al.*, 2006). The 'stress of dominant' hypothesis often prevails in cooperative/group-living species as this can result in dominant individuals experiencing increased aggression and psychological strain (Creel, 2001; Muller & Wrangham, 2004). The relative allostatic load of subordination or dominance results in one experiencing more stress than the other (Goymann & Wingfield, 2004). In the wild, male giraffes can be solitary for large periods. The solitary lifestyle likely reduces the frequency of aggression and stress in dominant males (Shorrocks, 2016). When housed in a captive bachelor herd, social dominance may come at a higher cost. Enclosure constraints and group living may result in dominant giraffes experiencing increased aggression and physiological strain (Horová *et al.*, 2015). At Hamilton Zoo, the giraffes displayed low levels of aggression and had an established hierarchy, suggesting that dominance may not have caused Masamba's elevated FCM levels. However, results from Chapter Two did identify that Ndale frequently avoided Masamba. Therefore, it cannot be ruled out that antagonistic interactions between these two individuals caused Masamba's elevated FCM levels.

The results of the study presented in Chapter Two also identified that Masamba frequently displayed same-sex sexual behaviour, such as investigating and following. These sexual behaviours were predominantly directed towards Dume. These close interactions may explain why Masamba and Dume's FCM levels were correlated. When reproducing, an individual's allostatic load often increases due to the energetic demands of mating behaviour (Goymann & Wingfield, 2004). In the presence of females and during sexual activity, mature male giraffes experience increased cortisol levels. Increased cortisol reflects the psychological and physiological costs of mate guarding and mating behaviours. For example, a male may expend energy preventing other mature males from accessing a female in oestrus (Wolf *et al.*, 2018b). Other species such as red-fronted lemurs (*Eulemur fulvus rufus*), baboons (*Papio hamadryas ursinus*), and bison also experience increased cortisol levels during the breeding season due to the energetic costs of mate competition and mating (Bergman *et al.*, 2005; Mooring *et al.*, 2006; Ostner *et al.*, 2008). Dominant males often have the greatest reproductive success (Pratt & Anderson, 1982), therefore, in the wild, Masamba would likely have high reproductive success. The drive to reproduce and the energetic demands of same-sex sexual behaviour may result in Masamba experiencing increased cortisol levels. However, there was no correlation between Masamba's FCM levels and his expression of following and investigating behaviours. Therefore, it seems unlikely that the persistent display of same-sex sexual behaviour caused Masamba's higher FCM levels.

Masamba suffers from overgrown hoof-related health issues. Radiographs conducted after this study showed that these issues were likely causing significant pain. Pain can result in an animal experiencing elevated stress. For example, farm animals display elevated cortisol levels when undergoing painful husbandry procedures (Prunier *et al.*, 2005). Giraffes and elephants also show elevated cortisol levels when suffering deep tissue wounds, foot injuries, and poor body conditions (Ganswindt *et al.*, 2010; Wolf *et al.*, 2018c). When experiencing pain, short-term increases in stress can be beneficial as it enhances the immune system and wound healing process. However, elevated stress for prolonged periods can lead to impaired immune function and reduce the body's healing ability (Narayan & Vanderneut, 2019). When experiencing pain, some species display behavioural changes. For example, calves (*Bos taurus*) often lay in abnormal positions after castration (Wolf *et al.*, 2018c). However, many species, especially wild prey species, do not reflect pain in their behaviour. Prey species often display typical postures, locomotion, and facial expressions when experiencing pain to ensure predators do not notice them as vulnerable (Carbone, 2020). Behavioural observations in Chapter Two did not suggest that Masamba was experiencing pain. For example, he did not spend significantly more time idling that the other giraffes. However, giraffes are prey species and likely mask their pain to avoid predation. Masamba may also mask his pain to prevent the other males at Hamilton Zoo from challenging his dominance. Therefore, it is possible that pain is a factor causing Masamba to have the highest FCM levels.

In addition, Ndale's FCM levels did not correlate with his expression of avoiding behaviour, and Ndoki's FCM levels did not correlate with his frequency of being displaced. These results contradicted the study's predictions that subordinate individuals would have the highest FCM levels. As already discussed, the allostatic loads of subordination and dominance may differ in captive conditions. In captivity, a subordinate individual's allostatic load can increase due to higher social densities and the inability to avoid negative social interactions. However, the allostatic load can also decrease due to the reduced pressure from hunger, illness, and predation. In captivity, the allostatic load of subordinate individuals can depend on the social context and whether they experience social support or aggression (Abbott *et al.*, 2003; Lewton & Rose, 2020). At Hamilton Zoo, the giraffe enclosures contain multiple houses and browse stations, enabling subordinate individuals to avoid dominant individuals while still accessing resources. In the wild, hierarchy status can influence a male's ability to access females, with dominant males often deterring lower-ranking males in the presence of females (Pratt & Anderson, 1985). The inability to access females may cause FCM levels to increase in subordinate males. At Hamilton Zoo, subordinate males may have a lower allostatic load than those in the wild due to the absence of females and the ability to assess vital resources.

In the wild, subordinate giraffes are often juveniles/ younger males. These males can experience elevated stress due to hormonal changes during puberty and the pressures faced when establishing a place in the male social hierarchy (Wolf *et al.*, 2018b). The giraffes at Hamilton Zoo are all mature and have an established social hierarchy. An established hierarchy often reduces aggression and conflict over resources (Horová *et al.*, 2015). Studies in baboons have also identified that GC hormone levels are lower when social rankings are stable (Bergman *et al.*, 2005; Engh *et al.*, 2006). Therefore, the subordinate males at Hamilton Zoo may display lower FCM levels than expected as they are not juveniles and have an established social hierarchy.

There was no correlation between Dume's FCM levels and his expression of pacing stereotypes, counteracting the study's predictions. Results from Chapter Two identified a correlation between Dume's expression of pacing and the time he spent being followed by another individual. This correlation suggested that being followed may have caused a negative affective state such as stress. However, the results from this study also identified that Dume's FCM levels did not correlate with being followed. Perhaps, being followed caused other negative affective states such as frustration, leading to pacing stereotypes. Dume may have also paced due to other underlying factors such as anticipation (Bashaw *et al.*, 2001; Bennett *et al.*, 2015; Yon *et al.*, 2019). Following may be displayed as a mate-guarding behaviour (Pratt & Anderson, 1985). When Dume was pacing, other giraffes may have begun following him to remain nearby. This could also explain the correlation between pacing and being followed by another individual.

There is a possibility that stress is the underlying cause of Dume's pacing but that his HPA axis has become habituated. Habituation occurs when the response to a stressor reduces over time. Habituation has an adaptive advantage as it decreases the biological cost of a repeated stressor. Several processes contribute to the habituation of the HPA axis. For example, consistently high levels of GC hormones can reduce the excitability of regulatory sites resulting in a reduced stress response. Receptors in the basolateral amygdala are also essential for habituation. These receptors consolidate memories allowing animals to recognise repeated stressors (Grissom & Bhatnagar, 2011; Gądek-Michalska *et al.*, 2013; Herman, 2013). Habituation is more common in stressors that are unlikely to cause a significant threat to the animal. For example, animals living in a forest may become habituated to the sound of falling branches. However, they will not become habituated to the sighting of a predator (Broom & Johnson, 2019). There is a possibility that Dume became habituated to a repetitive stressor (e.g., being followed) that caused him to pace. Despite the reduced response in the HPA axis, he may still display pacing stereotypes when the stressor is present. Dume may also pace out of habit when the stressor is present despite it no longer causing negative affective states. It is also important to consider that pacing may be a habit that has persisted despite the stressor no longer being present. If this is the case, pacing may not reflect suboptimal welfare.

The expression of oral stereotypes did not correlate with Masamba, Ndale, or Ndoki's FCM levels. These results aligned with the study's prediction that oral stereotypes are caused by negative affective states associated with feeding motivation, such as frustration. Feeding motivation is common in captive giraffes as their food is easy to obtain and does not take long to consume (Bashaw *et al.*, 2001). Interestingly Dume's FCM levels did correlate with his expression of oral stereotypes. Results from Chapter Two identified that Dume spent more time eating than the other three giraffes, suggesting he is highly food motivated. Perhaps a high level of feeding motivation can cause several negative affective states, including stress. Stereotypical behaviour and stress in captive white-naped mangabeys (*Cercocebus lunulatus*) can increase when food is easy to obtain (Waasdorp *et al.*, 2021). Oral stereotypes also increased in captive giraffes after the removal of a herd member. In this case, oral stereotypes may have reflected elevated stress (Tarou *et al.*, 2000). However, it is important to note that FCM analysis did not measure the giraffes' stress levels at a specific point in time. Due to the time lag between the secretion of cortisol and the presence of metabolites in the faeces, FCM analysis provides a more integrated measure of stress over a few hours. During each observation period, the giraffes displayed several different behaviours. Therefore, the results from FCM analysis do not correlate to a single behaviour. A correlation between FCM levels and a single behaviour might miss other behaviours impacting stress levels. Therefore, there is a possibility that another factor or behaviour affected Dume's elevated FCM levels.

The presence of foraging enrichment did not affect Masamba's behaviour but did decrease his FCM levels. Perhaps enrichment provided a distraction from Masamba's hoof-related pain. However, the giraffes did not engage with enrichment for significant periods. Therefore, it seems unlikely that enrichment would have provided a great enough distraction to reduce pain and subsequent stress. This result was only just statistically significant, and enrichment was only presented to the giraffes seven times during this study. More data on the effects of foraging enrichment are needed to form conclusions regarding its overall effect (Kuhar, 2006). Despite Dume having the highest engagement levels, the presence or absence of foraging enrichment absence did not significantly impact his FCM levels. The effect of enrichment on FCM levels possibly depends on its ability to reduce stressors. A study on pandas (*Ailuropoda melanoleuca*) found that bamboo stick enrichment did not reduce FCM levels. This was likely due to enrichment not reducing the pandas' primary stressor of visitor presence. Enrichment such as hideouts may have caused significant reductions in the pandas' FCM levels (Liu *et al.*, 2006). At Hamilton Zoo, foraging enrichment may not have reduced the giraffes' stressors. However, the enrichment did not increase the giraffes' FCM levels, suggesting that it did not negatively impact their welfare.

At Gibbs Farm, the subordinate male Kyana had significantly higher FCM levels than the other two giraffes. This result aligns with previous studies on wild giraffes suggesting the 'stress of subordinate' hypothesis (Wolf *et al.*, 2018b). However, this result differed from those at Hamilton Zoo, where subordinate males did not have the highest FCM levels. The enclosures at Gibbs Farm were smaller than the enclosers at Hamilton Zoo. Kyana and Orly also only had access to one giraffe house in the yard enclosure. The smaller enclosures and single giraffe house might have increased the frequency of antagonistic and aggressive interactions. Kyana might have struggled to access resources while avoiding negative interactions with Orly. Observational data from Chapter Two supports this hypothesis as subordinate behaviours increased in the smaller yard enclosure at Hamilton Zoo. In addition, previous studies have found that social interactions and aggression often increase in smaller enclosures with limited resources (Hogan *et al.*, 1988; Cassinello & Pieters, 2000). The different herd sizes at each institution might have also influenced the subordinate males' FCM levels. Orly only had to dominate and direct aggression towards Kyana. Masamba had to dominate three individuals, which might have caused the subordinates to experience less aggression. Perhaps the difference in enclosure and herd sizes at Hamilton Zoo and Gibbs Farm resulted in the subordinate males having varying allostatic loads. Kyana was also known to display pacing stereotypes. Therefore, the results may suggest that elevated stress is an underlying cause of pacing. Behavioural observations at Gibbs Farm would be necessary to confirm if Kyana's FCM levels correlated with pacing stereotypes or aggressive interactions. There is also a possibility that an unidentified stressor such as an underlying health condition caused Kyana's higher FCM levels.

At Gibbs Farm, the dominant male Flynn did not have the highest FCM levels contrasting the results at Hamilton Zoo. The 'stress of dominant' hypothesis occurs more frequently in group-living species (Creel, 2001; Muller & Wrangham, 2004). Dominance might have had a greater cost at Hamilton Zoo, as Masamba dominated three other giraffes. At Gibbs Farm, the dominant male Flynn was housed alone, which may have caused dominance to come at a lower cost. Orly also only had to dominant one other giraffe. Perhaps herd size also influenced the allostatic load and subsequent stress levels in the dominant males. However, it is essential to note that Masamba's high FCM levels may have resulted from factors other than social stress.

In the wild, dominant males often have lower stress levels. However, Flynn's lower FCM levels also suggest that being housed alone might not be a stressor for captive male giraffes. Perhaps this is because mature males can naturally spend significant periods alone in the wild (Prehn *et al.*, 2019). If individuals have a positive relationship, separation may cause elevated FCM levels (Tarou *et al.*, 2000). However, at Gibbs Farm, separation occurred due to high aggression levels. The giraffe enclosures were also located next to each other, enabling Flynn to interact with the other males. Control over the frequency of his social interactions and no competition for resources probably caused Flynn to have a low allostatic load. However, if reintroductions were to occur, FCM levels may increase due to social instability as a hierarchy is re-established (Bergman *et al.*, 2005; Engh *et al.*, 2006).

Before this study, Masamba, Kyana, and Orly all received a Bopriva vaccine to reduce their testosterone levels. Little research has investigated the impact of this vaccine on other hormones such as cortisol (Pfizer Animal Health, n.d). Both positive and negative associations have been recorded between testosterone and cortisol. In male humans (*Homo sapiens*), there is a negative association between cortisol and testosterone levels during exercise and exercise recovery; when cortisol levels increase, testosterone levels decrease (Brownlee *et al.*, 2005; Daly *et al.*, 2005). In male giraffes, androgen hormones such as testosterone increase alongside cortisol in the presence of fertile females, suggesting that there is a positive association between these two hormones (Wolf *et al.*, 2018b; Wolf *et al.*, 2018d). Therefore, there is a chance that the Bopriva vaccine impacted the giraffes' cortisol levels in this study. However, further research on Bopriva's effect on species other than cattle and its effect on other hormones such as cortisol would be needed to support this hypothesis. Masamba and Kyana had high FCM levels, but Orly did not. Therefore, the results from this study did not show a consistent pattern suggesting that Bopriva had any effect on cortisol.

Many stressors are minor, and the cost of the stress response is covered by biological reserves. However, elevated cortisol for prolonged periods can result in biological reserves becoming overwhelmed, leading to the onset of distress. When experiencing distress, an animal enters a prepathological state and becomes vulnerable to pathologies and suboptimal welfare. Distress occurs more frequently when an animal does not foresee the stressor and cannot control its duration. This prevents the build-up of biological reserves before the stressor's onset. Both chronic and acute stress can result in distress. Acute stressors often have little impact on an animal's biological functioning as reserves cover their cost and are quickly replenished once homeostasis is restored. However, if multiple acute stressors occur simultaneously or sequentially, the biological cost may expand past reserves or prevent them from being restored. For example, exposure to an environmental toxin may result in an animal experiencing low stress levels. Exposure to an additional stressor may cause biological reserves to become overwhelmed. Therefore, even seemingly subtle stressors can impact an animal's biological functioning and welfare (Moberg & Mench, 2000).

Masamba's higher FCM levels might have been caused by a chronic stressor or multiple acute stressors. The higher cortisol levels may mean his ability to replenish biological reserves is limited. Therefore, Masamba may have entered or been at risk of entering a prepathological state. In a prepathological state, welfare is compromised and vulnerable to further declines. While low levels of stress are necessary to maintain homeostasis, stress management can prevent the onset of distress. Stress management aims to limit the biological costs of stress and prevent animals from frequently entering a prepathological state (Moberg & Mench, 2000). Resolving or managing Masamba's hoof pain would likely reduce his FCM levels (Wolf *et al.*, 2018c). Hoof overgrowth is a common health issue in captive giraffes, and general anesthesia is a challenging procedure. Therefore, many institutions have begun focusing on positive reinforcement training to establish behaviours that allow voluntary hoof trims and radiographs (Dadone *et al.*, 2016). After the completion of this study, training enabled zookeepers to obtain diagnostic radiographs of Masamba's hoofs. These radiograms indicated that Masamba would require medication to manage his hoof-related pain alongside hoof trims. In many cases, including human care, medication can effectively manage pain, reduce stress, and improve quality of life (Lindblad *et al.*, 2017; Narayan & Vanderneut, 2019). Therefore, pain medication should lower Masamba's FCM levels, reducing his risk of entering a prepathological state.

Enclosure design and size might help manage the social stressors impacting male giraffes in captive bachelor herds. Multiple opportunities to access resources and space to avoid dominant individuals will likely reduce FCM levels in subordinate animals (Hogan *et al.*, 1988; Cassinello & Pieters, 2000). Larger enclosures may also enhance male giraffe welfare as it would allow them to spend time alone as they would in the wild (Pratt & Anderson, 1982). At Gibbs Farm, a larger yard enclosure and another giraffe house would ensure Kyana could always access resources while avoiding Orly. Equal opportunity to access resources might have reduced Kyana's FCM levels preventing him from entering a prepathological state.

Stress responses occur when an animal perceives an environmental factor to be stressful. Therefore, stress management can also involve convincing an animal that a situation does not pose a physiological or psychological threat. For example, positive reinforcement-based training can reassure animals that routine health checks are not stressful (Dadone *et al.*, 2016; Krebs *et al.*, 2018). Convincing animals like Masamba that husbandry practices such as hoof-trims are not stressful might help manage FCM levels. Previous experiences can also significantly impact an animal's perception of its environment, causing sensitization to specific stimuli. For example, the inconsiderate handling of piglets can cause them to show elevated stress responses later in life. It is beneficial to identify past experiences and associated stimuli that may cause an individual to experience heightened stress. Once identified, these stimuli can be minimized, or training can help reassure the animal that they do not pose a threat. Reducing seemingly minor stressors might also help manage Masamba and Kyana's FCM levels. Biological reserves may replenish faster in the absence of minor stressors, preventing an animal from entering a prepathological state (Moberg & Mench, 2000). While the zookeepers caring for Masamba and Kyana can consider this, identifying and removing all minor stressors is impossible.

Animals vary baseline GC hormone levels in response to daily fluctuations in the circadian cycle. However, in the presence of a stressor, GC hormone levels rise above baseline levels as part of the stress response. Baseline and stress-induced GC hormone levels have different behavioural and physiological effects. Therefore, variations in individuals' baseline GC hormone levels have different consequences than variations between stress-induced levels (Romero, 2004; Oster *et al.*, 2017). During an EIA validation procedure, an ACTH injection was used to stimulate the secretion of stress-induced cortisol levels in two giraffes. After the injection, the giraffes' FCM levels showed an up to 18-fold increase from baseline levels recorded before the injection. This study also exposed five giraffes to a presumably stressful transport procedure, causing

FCM levels to show 2.33-8.0 fold increases (Bashaw *et al.*, 2016). Variation in FCM levels was calculated for the giraffes at Hamilton Zoo by comparing the bottom 20% of values to the top 20%. Masamba's FCM levels had a 3.6-fold increase, Dume's had a 2.4-fold increase, Ndale's had a 4.0-fold increase, and Ndoki's had a 4.3-fold increase. While the fold increases observed in this study are small compared to those observed after the ACTH injection, they are similar to those observed after the transportation event. In this study, variations in the giraffes' FCM levels did not reflect natural fluctuations around the circadian cycle as samples were collected at the same time each day. Therefore, the variations were likely caused by stress responses. Data analysis did not use the fold-increase values to compare the giraffes' stress levels in this study. When comparing stress levels, it is best to analyse the absolute values as individuals with higher values display a more significant stress response (Romero, 2004; Oster *et al.*, 2017).

Studies on wild giraffes have recorded FCM levels much higher than those in this study. For example, in a study examining the effects of injury in wild giraffes, healthy individuals had a median FCM of 2180 ng/g DW, and individuals with significant injuries had a median FCM of 5760 ng/g DW. These results support the hypothesis that hoof-related health issues might cause Masamba's higher FCM levels. However, this study did not state the sex of individuals, and FCM levels may differ between males and females (Wolf *et al.*, 2018c; Palme, 2019). In another study on wild giraffes, older males had a median FCM of 720 ng/g DW in all-male groups, with younger adults and immature males showing higher FCM levels (Wolf *et al.*, 2018d). By comparison, the giraffes in this study had median FCM levels ranging from 75.9 ng/g DW (Dume) to 154.1 ng/g DW (Kyana). In the Bashaw et al. (2016) study, a single captive adult male had median FCM levels of 40 and 63.5 ng/g DW over two days. These results suggest that wild male giraffes express significantly higher FCM levels than those in captivity. Perhaps wild male giraffes face more significant stressors than those in captivity due to resource fluctuations, mate competition, and predation threats. When comparing FCM levels between studies, it is essential to consider whether the FCM metabolites being measured are comparable because different antibodies react to different metabolites with variable specificity (Palme, 2019). In the case of the studies mentioned above, all assays were performed with an antibody targeting the 11-oxoetiocholanolone I molecule (5β-androstan-3α-ol-11,17-dione). This is the same molecule targeted by the Cayman ELISA antibody in this study, although the latter also cross-reacts with 5β-androstan-3.11.17-trione with 54.7%

specificity (Cayman Chemicals, n.d). An antibody's binding ability will influence the FCM levels recorded. Therefore, the same antibody is essential when comparing absolute FCM levels between populations and individuals (Palme, 2019).

Physiological assessments can provide a deeper understanding of an animal's welfare. Faecal cortisol metabolite analysis monitors activity in the HPA axis noninvasively. Monitoring an animal's cortisol levels provides an understanding of its ability to cope with various environmental stressors. Elevated FCM levels for prolonged periods suggest that an animal is struggling to overcome stressors and maintain homeostasis. It is vital to identify when animals are experiencing distress, as in this state, their welfare is compromised and vulnerable to further declines. Monitoring an animal's cortisol levels can also help identify the potential environmental stressors impacting their welfare. Identified stressors can be removed or regulated to reduce cortisol levels and enhance the animal's welfare (Moberg & Mench, 2000; Palme, 2019). When assessing welfare through behavioural observations, researchers often make assumptions about an animal's affective states and how behaviour reflects them. Faecal cortisol metabolite analysis is beneficial as it identifies when animals are experiencing the negative affective state of stress (Palme, 2019).

While FCM analysis can provide powerful insights into an animal's welfare, results can be challenging to interpret. Activity in the HPA axis and the metabolism of GC hormones can differ between individuals due to genetic variations (Palme, 2019). Sensitization and habituation can also cause individuals to respond to specific stressors differently (Veissier & Boissy, 2007; Broom & Johnson, 2019). Human family studies have identified heritable variations in cortisol levels (Mormède *et al.*, 2007). A study on five captive giraffes also identified that FCM levels varied from approximately 100 ng/g DW to more than 1000 under normal (baseline) conditions (Bashaw *et al.*, 2016). Long-term studies where the animal acts as its own control can help identify these individual differences (Palme, 2019). This study only occurred over 12 weeks, making it challenging to identify individual variations in the giraffes' HPA axis activity and secretion of cortisol metabolites.

The HPA axis controls fluctuations in energy. These energy fluctuations can assist in an animal's stress response. However, the HPA axis is not only activated during a stress response. Glucocorticoid hormone levels can also rise during positive experiences such as playing and hunting, as these behaviours often require additional energy (Broom & Johnson, 2019). Activity in the HPA axis can also vary depending on an individual's life stage. For example, pregnant and lactating females often display elevated GC hormone levels. Mature male giraffes also display elevated cortisol during periods of sexual activity. These elevated cortisol levels support an animal's increased energetic demands during these life stages. Therefore, it is essential to understand that high FCM levels do not always mean an animal is experiencing suboptimal welfare (Wolf *et al.*, 2018b; Palme, 2019).

Factors such as sex and diet can also influence activity in the HPA axis and the metabolism of GC hormones. In some species, males and females exhibit different GC hormone levels. It is necessary to consider these differences when collecting anonymous samples from mixed-sex groups. In this study, all the subjects were male, meaning their sex did not impact FCM analysis. Diet can influence an individual's gut bacterial composition and subsequently alter the metabolism of GC hormones. Individuals with a greater ability to metabolize GC hormones will display lower FCM levels. At Hamilton Zoo, the giraffes were on the same diet. However, this differed slightly from the giraffes' diet at Gibbs Farm. These differences might have influenced the giraffes' gut bacterial composition. The gut bacterial composition may also vary between giraffes within each institution due to previous diet changes or medications. Therefore, there is a possibility that the giraffes' gut bacterial composition influenced their FCM levels (Palme, 2019).

Many factors influence activity in the HPA axis, making it difficult to pinpoint the specific stressors affecting an animal's FCM levels. In this study, we can assume that Masamba's elevated FCM levels were caused by pain, but we cannot be sure. Similarly, we cannot rule out the possibility that underlying health issues or unidentified stressors contributed to Kyana or Masamba's higher stress levels. Therefore, it can be challenging to interpret and draw justifiable conclusions from FCM analysis. When analysing FCM levels, it is essential to consider all the potential internal and external factors that may influence activity in the HPA axis and the excretion of GC metabolites (Mormède *et al.*, 2007).

Chapter 4
General discussion

4.1 Combining behavioural and fecal cortisol metabolite analysis

This thesis used behavioural observations and FCM analysis to identify the potential welfare concerns of four male giraffes in a bachelor herd at Hamilton Zoo. Behavioural observations determined that a hierarchy influenced the giraffes' social dynamic. This hierarchy was similar to those displayed by wild males. It was likely a key factor contributing to the low levels of physical aggression and social conflict at Hamilton Zoo (Pratt & Anderson, 1985; Lewton & Rose, 2020). Despite low levels of social conflict, behavioural observations suggested that Ndale and Ndoki may have experienced sub-optimal welfare due to their frequent expression of avoiding and displaced behaviours. However, results from FCM analysis indicated that the display of avoiding and displaced behaviours did not correlate with elevated cortisol levels. Therefore, FCM analysis suggested that being subordinate does not impact the giraffes' welfare at Hamilton Zoo. In comparison, the subordinate male at Gibbs Farm experienced the highest FCM levels. These differences might be explained by the different enclosure and herd sizes at each institution (Cassinello & Pieters, 2000; Creel, 2001).

At Hamilton Zoo, the display of same-sex sexual behaviour also impacted the giraffes' social dynamic. The sexual behaviours displayed were similar to those expressed by wild giraffes (Seeber *et al.*, 2012). Behavioural observations identified that the giraffes frequently directed sexual behaviour towards Dume, which may have impacted his welfare. Dume and Ndoki also paced more regularly when followed, supporting the hypothesis that these behaviours caused negative affective states. However, FCM analysis identified that being followed did not cause Dume to experience elevated cortisol levels. This result suggested that Dume's welfare was not impacted by other giraffes expressing sexual behaviour towards him. In addition, there was no correlation between Masamba's FCM levels and his expression of investigating and following behaviours, suggesting that same-sex sexual behaviour did not reflect suboptimal welfare.

Behavioural observations identified that Dume displayed pacing stereotypes more frequently than the other giraffes at Hamilton Zoo. Dume likely paced due to anticipation or the negative affective states associated with being followed (Tarou *et al.*, 2000; Bennett *et al.*, 2015). Both these causes may have resulted in Dume experiencing suboptimal welfare. However, FCM analysis did not identify a correlation between Dume's pacing and cortisol levels, suggesting that pacing did not reflect suboptimal welfare. However, Dume may have become habituated to a repetitive stressor causing him to pace. Behavioural observations also determined that all four giraffes displayed oral stereotypes at Hamilton Zoo. These stereotypes likely indicated that the giraffes had suboptimal welfare due to excess feeding motivation (Bashaw *et al.*, 2001). Faecal cortisol metabolite analysis revealed a correlation between Dume's elevated cortisol levels and his expression of oral stereotypes. Therefore, FCM analysis also suggested that Dume's display of oral stereotypes reflected suboptimal welfare. However, it is important to remember that behaviour does not occur in isolation, and other factors or behaviours might have contributed to Dume's elevated FCM levels.

Faecal cortisol metabolite analysis identified that Masamba experienced the highest stress levels at Hamilton Zoo. Masamba's elevated cortisol levels were likely due to his hoof-related health issues and consequent pain. Therefore, these results indicated that Masamba experienced suboptimal welfare (Wolf *et al.*, 2018c). However, behavioural observations did not identify Masamba's hoof issues. Masamba's behaviour likely masked his pain as giraffes are a prey species, and visible pain may render them vulnerable to predation (Carbone, 2020). Therefore, behavioural observations were unable to identify this welfare concern.

At Hamilton Zoo, enrichment had little impact on the giraffes' behaviour. Behavioural observations identified that enrichment did not reduce or increase the display of stereotypical behaviour or behaviours related to social conflict. Therefore, results suggested that enrichment neither enhanced nor reduced the giraffes' welfare (Rose *et al.*, 2017). However, faecal cortisol metabolite analysis identified that enrichment did accompany a reduction in Masamba's FCM levels. This finding indicated that enrichment may have improved Masamba's welfare. However, the limited number of days providing enrichment may be too few to draw definitive conclusions, and further study is warranted.

In this thesis, behavioural observations and FCM analysis frequently provided contrasting results about the giraffe's welfare at Hamilton Zoo. A common limitation in animal welfare science is the opposing conclusions produced from different assessment methods. These opposite conclusions can spark conflict over what methods are more valid (Dawkins, 2021). This conflict is often driven by people's varying views on what factors determine welfare. For example, people valuing the display of natural behaviour may believe behavioural observations are more valid, while people valuing physiological functioning may believe FCM analysis is more accurate (Fraser, 2003). Unfortunately, there is no definitive method to assess animal welfare, and all current methods have strengths and weaknesses (Watters *et al.*, 2021). Using multiple methods in conjunction might help overcome limitations and provide a deeper understanding of an animal's welfare. When only using one method, we may not identify all the factors impacting an animal's welfare. As seen in this study, the giraffe's behaviour did not reflect the negative affective state of pain. Faecal cortisol metabolite analysis also only investigated the negative affective state of stress, whereas behaviours such as oral stereotypes may have reflected other negative affective states such as frustration. Using various assessment methods may also help address all five welfare domains. For example, behavioural observations are helpful when assessing the behaviour domain, and FCM analysis is useful when evaluating the health domain (Mellor, 2016).

This thesis demonstrated how individuals in the same group can have varying welfare concerns. For example, Dume's welfare is impacted by his display of pacing stereotypes, while hoof issues affect Masamba's welfare. Subordinate individuals also experienced different environmental pressures than dominant individuals. Individuals can also develop varying coping mechanisms and display negative affective states in alternative ways. For example, some animals may display stereotypical behaviour while others may exhibit abnormal levels of inactivity. Therefore, studies monitoring general group behaviour may miss critical variations between the individual's welfare (Bashaw, 2011). The AZA also acknowledges the importance of assessing welfare at an individual level in its Animal Welfare Standards (Terrell, 2017). Assessing welfare at an individual level is beneficial as it enables zoos to tailor husbandry routines to suit all individuals' needs.

Several factors impacting captive giraffe welfare were identified in this thesis. Enclosure constraints can cause welfare concerns as they limit the giraffes' ability to remove themselves from unfavorable situations. Enclosures designed for bachelor giraffe herds should provide enough space for individuals to avoid each other and still access essential resources such as food and shelter. Husbandry routines should also ensure giraffes are not left in confined spaces with individuals that may spark negative affective states. For example, subordinate individuals should not be confined with dominant individuals (Tilson *et al.*, 1988; Lewton & Rose, 2020). Giraffes may not always reflect injury or illness in their behaviour (Carbone, 2020). Therefore, it is beneficial for giraffe enclosures to contain spaces for animal training and regular health checkups. For example, facilities should enable zookeepers to train their giraffes for voluntary hoof trims. Animal training would also ensure hoof trims or health checkups do not negatively impact an animal's welfare by causing elevated cortisol levels (Dadone *et al.*, 2016). Novel enrichment or enrichment with a varied reward schedule may also enhance the welfare of captive giraffes. When the giraffes engage with enrichment for extended periods, they may experience more positive affective states and display stereotypical behaviour less frequently (Quirke *et al.*, 2012). It is not always feasible to build or make significant adjustments to enclosures when they currently house a group of animals. Therefore, zoos should consider the welfare challenges a species may face before the enclosure construction and animal arrival. This prior planning would ensure the construction of facilities that support a species' specific welfare requirements.

4.2 Limitations

This thesis only collected data from two bachelor herds, one with four giraffes and one with three. It would be beneficial to extend this sample size to include a larger number of captive bachelor herds. When pooling data from several herds, it is impossible to ensure that environmental variables remain consistent across each institution. Individuals in each herd will experience varying conditions such as enclosure size, group numbers, and diet. These environmental differences might cause results to vary between institutions. For example, in this thesis, the different environments might have caused FCM levels to vary between the subordinate and dominant individuals at Hamilton Zoo and Gibbs Farm. However, incorporating a larger number of bachelor herds would have provided a more in-depth analysis of herd-related effects. For example, if dominant males had the highest FCM levels in several herds despite the environmental differences, it would suggest that the social dominance caused the elevated stress. Understanding how environmental differences impact stereotypical behaviour and social dynamics in bachelor herds would also enable more accurate generalizations to be made about the wider population (Swaisgood & Shepherdson, 2005; Kuhar, 2006; Goulart *et al.*, 2009).

Zoo-based studies often have small samples sizes as institutions house a limited number of animals due to enclosure constraints and the species' social dynamic. The importance of sample size depends on a study's objectives. Small sample sizes are more problematic in studies that aim to make generalizations about the entire population. Studies aiming to make generalizations about captive populations should increase the sample size by including a large number of institutions. Small sample sizes may not be as detrimental when a study's aim refers to the specific subjects, not the general population. It is often best to assess welfare at an individual level as unique environments and experiences impact welfare. Therefore, many welfare studies focus on particular individuals/groups instead of the general population. The small sample size in this thesis was not problematic when making conclusions about the giraffes at Hamilton Zoo and Gibbs Farm. However, we must take care when making generalizations from the results in this thesis as they do not have a high level of statistical power (Swaisgood & Shepherdson, 2005; Kuhar, 2006; Bashaw, 2011). However, the results from this thesis can be combined with those from other studies to make broader generalizations. For example, the FCM analysis results were combined with those from several other studies to suggest that captive male giraffes experience lower stress levels than wild male giraffes (Bashaw *et al.*, 2016; Wolf *et al.*, 2018b; Wolf *et al.*, 2018c).

4.3 Future research

Future research could build upon the findings in this thesis by pooling data from several institutions to expand our understanding of the social dynamics in captive bachelor giraffe herds. This research could identify whether subordinate or dominant individuals are more likely to experience elevated stress. It could also determine if subordinate juvenile males are more likely to experience heightened stress when housed with mature males or in herds without an established hierarchy (Wolf *et al.*, 2018b; Lewton & Rose, 2020). Identifying which individuals are more likely to experience elevated stress and the environmental factors that may impact it would enable zoos to better support animals in these situations. These studies could also determine if males often display abnormal or stereotypical behaviour when other males direct sexual behaviour towards them. If so, it would be beneficial to investigate ways to mitigate the expression of these behaviours. However, it may be challenging to reduce the expression of same-sex sexual behaviour as it is likely a by-product of sexual motivation in bachelor herds (Carter *et al.*, 2013).

Additional research could further investigate pacing stereotypes in captive giraffes. Studies could identify if several situations can cause pacing stereotypes due to social stress and frustration. For example, do female giraffes pace when they are unable to develop long-term associations in captivity? These findings would deepen our understanding of the relationship between pacing and social stress, enhancing our ability to manage pacing stereotypes. Future studies could also identify if novel enrichment or enrichment with varied reward schedules can hold captive giraffes' attention for extended periods and subsequently reduce stereotypical behaviour (Quirke *et al.*, 2012). Technology may offer an opportunity for researchers to create new enrichment items. For example, electronic puzzle feeders with varying reward schedules could be designed for giraffes. When investigating the effect of novel enrichment, it may also be beneficial to monitor the expression of social conflict as this may increase over favorable enrichment items (Tarou *et al.*, 2004).

This thesis also brought to light research gaps that future studies could investigate. Future research could identify if pain medication can reduce giraffe FCM levels. Exploring this relationship would deepen our understanding of pain medication's ability to improve a giraffe's quality of life and welfare (Lindblad *et al.*, 2017). This information would be helpful for institutions caring for aged giraffes using pain medication. Future research could also investigate the Bopriva vaccine's effect on male giraffes. These studies could identify if the vaccination can effectively reduce aggressive behaviour in giraffes (Pfizer Animal Health, n.d). It could also determine if the vaccine impacts the secretion of other hormones such as cortisol (Brownlee *et al.*, 2005). These studies would enable zoos to understand how the vaccine affects the welfare of individuals receiving it and the subsequent welfare of others in their herd.

4.4 Conclusions

This thesis successfully used behavioural observations and FCM analysis to assess the welfare of four male giraffes housed in a bachelor herd at Hamilton Zoo. Hamilton Zoo can use this understanding to guide its husbandry routines to ensure they effectively support each giraffe's welfare needs. People often have varying views on what factors contribute to animal welfare and how welfare should be assessed (Dawkins, 2021). Perhaps the best welfare assessments will be achieved when scientists consider all views and use multiple assessment methods in conjunction. Welfare is complex, and a single assessment method may not capture all aspects of an animal's welfare (Hill & Broom, 2009). The methods described in this thesis are non-invasive and could be replicated in other studies to assess animal welfare in several situations. The world is witnessing rapid declines in biodiversity due to anthropogenic pressures and climate change (Leclère *et al.*, 2020). Therefore, ex-situ populations are becoming increasingly critical in species conservation. Regular welfare assessments will ensure zoos continuously strive to enhance animal welfare and improve the success of ex-situ species conservation. Zoos with high animal welfare standards will act as ethical arks and inspire conservation action in their community (Brichieri-Colombi *et al.*, 2019).

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Appendices

Table A.1- p values from the Dunn's multiple comparison test for eating and idle behaviours, and p values from the Tukey's multiple comparison test for locomoting behaviour.

Individuals and AM/PM	*p* Values
Eating	
Masamba AM vs. Dume AM	0.0364
Ndale AM vs. Dume AM	0.0103
Ndoki AM vs. Dume AM	0.0318
Masamba AM vs. Masamba PM	0.0002
Ndale Am vs. Ndale PM	<0.0001
Ndoki AM vs. Ndoki PM	0.0006
Idle	
Masamba AM vs. Ndoki AM	0.0195
Dume AM vs. Ndoki AM	<0.0001
Dume AM vs. Ndale AM	0.0158
Ndoki AM vs. Ndoki PM	0.0120
Locomoting	
Masamba AM vs. Dume AM	0.0001
Masamba AM vs. Ndale AM	0.0091
Masamba AM vs. Ndoki AM	<0.0001
Dume AM vs. Ndoki AM	0.0008
Ndale AM vs. Ndoki AM	<0.0001
Masamba AM vs. Masamba PM	<0.0001
Dume AM vs. Dume PM	<0.0001
Ndale AM vs. Ndale PM	0.0084

Table A.2- *p* values from the Dunn's multiple comparison test for each social behaviour analysed.

Individuals and AM/PM	*p* values
Investigated	
Masamba AM vs. Dume AM	<0.0001
Ndale AM vs. Dume AM	0.0001
Masamba AM vs. Ndoki AM	0.0052
Masamba PM vs. Dume PM	0.0025
Ndale PM vs. Dume PM	0.0007
Investigating	
Masamba AM vs. Dume AM	<0.0001
Masamba AM vs. Ndale AM	0.0003
Masamba AM vs. Ndoki AM	<0.0001
Ndale AM vs Ndoki AM	0.0326
Masamba PM vs. Dume PM	0.0005
Masamba PM vs. Ndoki PM	<0.0001
Followed	
Masamba AM vs. Dume AM	<0.0001
Masamba AM vs. Ndoki AM	0.0093
Ndale AM vs. Dume AM	0.0002
Dume AM vs. Dume PM	<0.0001
Following	
Masamba AM vs. Ndoki AM	<0.0001
Dume AM vs. Ndoki AM	<0.0001
Ndale AM vs. Ndoki AM	0.0016
Masamba AM vs. Masamba PM	0.0080
Avoiding	
Masamba AM vs. Ndale AM	<0.0001
Dume AM vs. Ndale AM	<0.0001
Ndoki AM vs. Ndale AM	<0.0001
Masamba PM vs. Ndale PM	0.0050
Dume PM vs. Ndale PM	0.0050
Ndoki PM vs. Ndale PM	0.0444
Ndale AM vs. Ndale PM	<0.0001
Avoided	
Masamba AM vs. Dume AM	<0.0001
Masamba AM vs. Ndale AM	<0.0001
Masamba AM vs. Ndoki AM	<0.0001
Masamba PM vs. Dume PM	0.0004
Masamba PM vs. Ndale PM	<0.0001
Masamba PM vs Ndoki PM	<0.0001
Displaced	

Masamba AM vs. Ndoki AM	<0.0001
Dume AM vs. Ndoki AM	<0.0001
Ndale AM vs. Ndoki AM	<0.0001
Masamba PM vs. Ndoki PM	0.0074
Dume PM vs. Ndoki PM	0.0022
Ndale PM vs. Ndoki PM	0.0218
Ndoki AM vs. Ndoki PM	0.0281
Displacing	
Dume AM vs. Ndoki AM	<0.0001
Dume PM vs. Ndale PM	0.0301
Dume PM vs. Ndoki PM	0.0132

Table A.3- *p* values from the Dunn's multiple comparison test for oral and pacing stereotypes.

Individuals and AM/PM	*p* Values
Oral stereotypes	
Masamba AM vs. Masamba PM	0.0021
Ndale AM vs. Ndale PM	0.0009
Pacing stereotypes	
Masamba AM vs. Dume AM	<0.0001
Ndale AM vs. Dume AM	<0.0001
Ndoki AM vs. Dume AM	<0.0001
Masamba PM vs. Dume PM	0.0170
Ndale PM vs. Dume PM	0.0385
Dume AM vs. Dume PM	<0.0001

Table A.4- *p* values from the Tukey's multiple comparison test for enrichment interaction.

Individuals	*p* Values
Enrichment engagement	
Dume vs. Masamba	0.0127
Dume vs. Ndoki	0.0127