CHAPTER ONE

1. INTRODUCTION

1.0.1 Environmental enrichment for captive animals

In the wild, animals are exposed to a range of sensory stimuli (Bono, *et al.* 2016). That in captivity are largely absent (Wells, 2009). In an attempt to promote the welfare of captive animals' researchers have begun to explore the advantages of various forms of sensory enrichment (Bono, *et al.* 2016). Varied enrichment strategies encourage more typical patterns of behaviour, increase the ability to cope with challenges, enhance behavioural repertoire, increase positive use of the environment and/or reduce eliminate abnormal patterns of behaviour. For example, stereotypies. (Dawkins, 2012; Hosey, Melfi, and Pankhurst, 2013; Wells, 2009).

Environmental modifications aim to progress welfare by improving physical health (Newberry, 1995). Methods include: occupying animals in harmless activities, reduce incidence of stereotypies, providing opportunities to avoid harmful aggression, and promoting a wide range of movement to improve muscular, skeletal and cardiovascular fitness. (i.e. providing hanging ropes for primates, and providing water holes for tigers) (Young, 2013; Newberry, 1995).

A survey at Edinburgh Zoo created by Reade and Waran (1996) found that 95% of visitors felt it was important that enclosures were made to look as naturalistic as possible. 77% of visitors interviewed also felt animals in the zoo were happy or very happy, it was suggested that as naturalistic enclosures are considered more beautiful and attractive, this generates a positive response to the animals displayed (Claxton, 2011).

The zoo licensing Act (1981) requires the inspection and licensing of all zoos in Great Britain. The Act aims to ensure that animals kept in enclosures have a suitable environment provided to express natural behaviour. The Act was amended by the 2002 Regulations to give effect to the provisions of Council

Directive 1999/22/EC which were not already covered by it (BIAZA, 2016; The Zoo Licensing Act 1981 (Amendment) (England and Wales) Regulations, 2002).

1.0.2 Wolf behaviour and ecology

Wolves are highly social pack animals (Musiani, Boitani and Paquet, 2010). A pack is defined as an extended family group that includes a breeding pair, (the alpha male and female), their subordinate offspring and current pups from one or more years (Fatjo, *et al*. 2007). The alpha wolves decide when the pack will travel and hunt and are normally the first to eat at a kill (Marvin, 2012; Payne, Starks, and Liebert, 2010). Packs containing as many as forty-two members have been recorded but the average is between three and eleven (Marvin, 2012). If a pair of wolves' mate successfully and have pups that survive to early maturity, (between four and ten months) there will be the beginnings of a full pack (Fatjo, *et al.* 2007). The offspring may stay with parents for between one and three years (Marvin, 2012).

Wolves can survive in a variety of habitats, including forests, tundra, mountains, swamps and deserts (Mech, and Boitani, 2010). Their territories vary in size from 18 to 1,000 square miles (Mech, and Boitani, 2010; Nowak, and Jedrzejewski, 2009) depending on prey density, pack size, presence of neighboring packs, and human land use (Sidorovich, et al. 2016; Fatjo, *et al*. 2007). Wolves aggressively defend their territories from other packs and spend about 35% of their time traveling (Marvin, 2012). They often travel 20-30 miles per day, but may cover over 100 miles a day when prey is scarce (Sidorovich, et al. 2016; Mech, and Boitani, 2010).

Wolves communicate through body language (Essler, *et al*. 2016). Specialised behaviors and postures have evolved that help reduce aggression within packs (Fatjo, *et al.* 2007). Facial expressions are often used to express emotions (Marvin, 2012). Wolves indicate dominate behavior by baring teeth, and pointing erect ears forward (Marvin, 2012). Subordinate behavior may be indicated by closed mouths, and ears pulled back and held close to the head

(Marvin, 2012). They also use tail positions to communicate with other pack members (Marvin, 2012). Wolves expressing threatening signs or dominant behaviours, hold their tails high, while submissive wolves lower themselves before dominant pack members with their tail tucked between their legs (Cordoni, and Palagi, 2016).

A wolf's sense of smell is up to 100,000 times greater than humans' and under good conditions they can smell something a mile or more away (Mech, and Boitani, 2010). Using scents is a very effective form of communication for wolves (Mech, and Boitani, 2010). They are highly territorial and scents are used to clearly mark parameters. Scents are used to claim and defend territory, mark food ownership and are used as a map for the pack (Payne, Starks, and Liebert, 2010). Long after moving to different parts within their territory, their presence remains through scents. (Fatjo, *et al*. 2007). Urinating is the most common form of scent marking for wolves, however, they also produce scent from glands between their toes (Fatjo, *et al*. 2007).

1.1 Aims and Objectives

The study aims to discover the effects of three forms of scent enrichment on the behaviour of captive wolves.

Objectives: 1. Review of the relevant literature, 2. Enrichment study designed and implemented. 3. Data analysis and interpretation. 4. Recommendations for further study and zoo management of wolves made.

1.1.1 Null and Alternative Hypothesis

The Null hypothesis was 'no significant difference will be discovered in frequencies of behaviours when comparing wolves of different sex and olfactory enrichment will have positive effects of the wolf behaviour'.

The Alternative hypothesis states ‘the wolves will show a greater interest in various scents, and the frequency of behaviours will vary between male and female wolves’.

1.2 Studies involving enrichment with wolves and what can affect their behaviour in captivity

Environmental enrichment is a process for enhancing captive animals’ environment and care within context of the species behavioural biology and natural history (Young, 2013). It covers a multitude of innovative and imaginative devices, techniques, and practices, that aim to keep captive animals entertained, increasing their diversity and range of behavioural opportunities and providing more responsive and stimulating environments (Shepherdson, Mellen, and Hutchins, 2013; Conn, 2013). It facilitates social interaction, exploratory and cognitive activities (Novaes, *et al.* 2017).

Cummings, *et al.* in 2007 studied the effects of environmental enrichment on behavioural and physiological responses of four adult maned wolves. Responses to enrichment were assessed based on activity levels, exploratory rates, and the level of faecal corticoid metabolites.

There were no significant differences in behavioral responses between the two ‘‘no enrichment’’ periods (P 0.05) so, data was combined and compared to the other treatments. Behavioral responses differed among wolves. Exploratory rate significantly increased (P 0.05).

For the remaining wolves, hiding mice resulted in positive behavioral changes (i.e., increased exploratory rates or activity, P 0.05). When mice were hidden around the enclosure, exploratory rates were significantly higher than when boomer balls were presented (P 0.05). Only male wolves significantly responded to the boomer balls. A significant increase in the rate of scent marking was observed in Wolf Z when a ball was provided (P 0.01).

Enrichment significantly affected peak and baseline levels of faecal corticoids in males only (P 0.05). Baseline and peak levels of faecal corticoids were higher during hidden mice enrichment than introduction of boomer balls (P 0.01). No correlation was present between behavioral responses and corticoid concentrations for any of the wolves evaluated.

Results suggest that environmental enrichment prompts positive effects on the behaviour of captive maned wolves and that genders can be effected differently. A Longitudinal study is required to determine the impact of environmental enrichment in. Only four wolves were used in total to conduct this study, a larger sample size would provide more results.

A study completed by Piffare *et al.* in 2012 investigated the effects of zoo visitors on the behaviour and physiological responses of the Mexican wolf (*Canis lupus baileyi*). Scan sampling during observations, and faecal samples were collected from twelve wolves in three zoos.

The wolves were monitored on Saturdays, Sundays, Mondays, and Tuesdays, over a three-week period. The analysis revealed there was an effect of day on the frequency of time spent lying ($P < 0.01$), eating ($P < 0.01$) and on locomotory behaviours ($P < 0.01$). The wolves spent more time lying on Mondays and Tuesdays (0.3821 ± 0.012 and 0.4075 ± 0.012), and less time lying on Saturdays and Sundays (0.2980 ± 0.012 and 0.3266 ± 0.012). They also spent less time eating on Saturdays (0.1214 ± 0.015) and Sundays (0.0556 ± 0.015), compared to Mondays (0.816 ± 0.015) and Tuesdays (0.0915 ± 0.015). The wolves also had higher faecal cortisol levels on Sundays (242.50 ng/g ± 8.48) than on Saturdays (192.71 ± 8.48), Mondays (170.73 ng/g ± 7.80), Tuesdays (183.82 ng/g ± 8.48).

These findings indicate that the frequency of visitors present can influence the behaviour and adrenal activity of Mexican wolves (Piffare, *et al.* 2012).

1.3 Olfactory Enrichment and Aromatherapy

Olfactory enrichment can stimulate reproduction, naturalistic behaviours and enhance enclosure exploration (Clark, and King, 2008). Olfactory scents include food scent, essential oils, herbs and spices, faeces, urine and other scents (Roberts, *et al*. 2014; Clark, and King, 2008).

Complementary studies are often used alongside mainstream medicine, aromatherapy is a complementary therapy that uses essential oils extracted from plants as the major therapeutic agent to treat several diseases (Ali, et al. 2015). Olfactory enrichment is often integrated into zoo enrichment programs, and differs from aromatherapy as it can be any scent added to an animals' enclosure (i.e. predator/prey scents, urine or faeces) (Clark, and King, 2008)

Introducing olfactory scents into captive animal's environments has desirable and enriching effects (Wells, 2009). For example, increased behavioural diversity in zoo-housed tigers (*Panthera tigris*) and lions (*Panthera leo*). Van Metter, et al. in 2008. utilized stimulus objects in an enrichment program aimed to increase diversity of normal behaviors exhibited in two African lions and four Sumatran tigers (*Panthera tigris sumatrae*).

The enrichment included: frozen blood balls, fresh zebra dung, scented squash (butternut squash injected with cinnamon scent and acorn injected with vanilla scent) and cardboard boxes. Behavioral diversity indices for each trial were calculated using the Shannon Diversity Index.

The data for subjects tested under the same test conditions were pooled, resulting in four subject groups. No significant differences were among the four subject groups in E-C scores ($p = 0.280$) or PE-C scores ($p = 0.767$). Overall, animals spent less observation intervals sleeping in the enrichment conditions than control or post-enrichment trials. A Tukey post-hoc test revealed the E-C scores for the lions were significantly higher than for tigers ($p < 0.05$). No variation was found in PE-C scores ($p = 0.096$).

A Kruskal-Wallis ANOVA found that Behavioural Diversity Indices (BDIs) for object-directed behaviours during enrichment trials differed significantly. The E-C scores for lions was significantly higher than scores for the tiger subject groups ($p < 0.05$).

Lions benefited most from the enrichment, exhibiting increased activity, decreased sleeping, and the greatest increase in discrete behaviors. Responses to enrichment did not change over time, suggesting the animals did not habituate to enrichment. All were successful in increasing behavioral diversity of the lions and tigers. Differences in the study among animals in their response to enrichment suggest that the effects of enrichment on animals of different age, sex, and species, should be further explored and taken into consideration in the design of enrichment programs.

Six subjects were used in total making the sample size too small to provide enough evidence. Also, spending at least one-hour monitoring under each condition would provide more information on the effects.It would be interesting to discover the duration of enrichment effectiveness and if the animals habituate.

1.4 Factors effecting the results of environmental enrichment

1.4.1 The effects of human-animal interactions and the impact the public have on the behaviour of animals

Humans and animals come into contact in various situations: as pets, on farms, in a laboratory, at the zoo and even in wild settings. (Claxton, 2011; Hosey, 2008). Understanding human-animal interactions is important for institutions that display animals to the public (i.e. zoos, safari parks) due to their frequent, interactions with unfamiliar humans (Larsen, et al. 2014).

In 2014 Farrand, Hosey, and Buchanan-Smith investigated what effects the presence and density of visitors had on animal behaviour at a petting zoo display in a safari park. Animals used were mixed-breed goats (*Capra hircus* ssp.), llamas (*Llama glama*), and Vietnamese pot-bellied pigs (*Sus scrofa*). Every 10 minutes, instantaneous scan samples were collected from 5 individuals per species.

No significant change within groups between Autumn and Spring for visitor presence conditions. No change recorded in llama or goat behaviour with or without visitor presence, Pig behaviour was affected. The pigs demonstrated decreased social behaviour, inactivity and were both affiliative and aggressive when visitors were present. The following behaviours decreased significantly: affiliate with conspecifics ($p = .001, .001$), aggression between conspecifics ($p = .001, .002$), and sit ($p = .001, .005$).

The study had similar sample sizes for goats, and llamas, but only six pigs. The pigs were impacted the most by the presence of visitors, therefore, it would be beneficial to repeat the study using a larger sample size. An instantaneous scan sampling method was used, and behaviours were recorded every ten minutes. Consequently, some key behaviour may be missed. Recording using a camera and analysing all behaviours will avoid this and provide more results.

1.4.2 How gender may impact the results of environmental enrichment

Elliott and Grunberg, 2005 examined the separate and combined effects of social and physical enrichment on locomotor activity of male and female Sprague–Dawley rats (*Rattus norvegicus*). Habituation in the open field was used as an index of simple information-processing. Faster habituation indicated greater information-processing.

When all animals were analysed together, there was a significant time of social interaction ($F(3, 486) = 2.96$, $p < 0.05$), that suggests socially reared differ from isolated animals in activity patterns across the different testing periods. Females were the most active (sex: $F(1, 162) = 23.07$, $p < 0.001$), and isolated animals were more active than socially enriched animals (social: $F(1, 162) = 17.12$, $p < 0.001$).

Social and physical enrichment were examined separately for males and females. Both sex, isolated animals were more active than socially enriched animals (M-social: $F(1, 72) = 10.50$, $p < 0.05$; F-social $(1, 90) = 7.05$,

$p < 0.05$). For males, the effects of social enrichment also varied across the experiment (time x social: $F(3, 216) = 2.68$, $p < 0.05$).

When the sexes were considered separately, group differences were present for males and females. For males, both physically and socially enriched animals were less active than non-physically and isolated animals ($F(1, 92) = 19.56$, $p < 0.001$; $F(1, 92) = 5.50$, $p < 0.05$). For females, social enrichment was only effected. Animals in the socially enriched conditions were less active than in isolated conditions ($F(1, 90) = 9.42$, $p < 0.05$).

When measuring all animals together, male and female activity patterns did not differ significantly. Isolated animals were more active than socially enriched animals ($F(1, 168) = 12.46$, $p < 0.001$). Animals in the isolated groups exhibited less activity than animals in the social groups ($F(1, 76) = 12.97$, $p < 0.001$).

Each day a few animals were not used due to equipment failure which's a study limitation. Results showed that social enrichment had the greatest effect on improved performance (i.e. increased habituation) for both sexes. The effects of enrichment overall appeared to be greater in males.

1.4.3 How enrichment type can effect behavioural results

Hogan, *et al.* 2010, investigated stereotypic behaviour in captive southern hairy-nosed wombats (*Lasiorhinus latifrons*) and determined the beneficial effects of enrichment on behaviour and wellbeing.

Four males and eight females, all sexually mature, (>5years) were used in the study. The wombats were housed in four groups (one male and two females) in a separate enclosure and exposed to two different treatments: the first involved enrichment, where the animals received two types of enrichment along with a treatment diet. The second involved no-enrichment. When the animals received no enrichment they were fed the standard diet.

Aniseed, peppermint, vanilla, lemon, coconut and rose odours were introduced on pre-cut logs scented with one odour by applying 20 drops of the scent along its length. Odours were introduced in pairs with the animals being exposed to two different odours on two separate logs. The logs remained in the enclosures for 5 days being freshly scented each day, after which they were removed. Following a transitional period of 2 days with no logs, new odours were introduced. This method continued for 12-weeks.

Behaviours were observed via digital video cameras. Each wombat was observed for twelve 24-hour periods, during each treatment rotation with behaviours recorded at 5-min intervals. Eight of the wombats displayed one stereotypy: straight-line pacing, boundary pacing, figure-8 pacing or wall climbing. Time spent stereotyping varied between individuals ($P < 0.01$) ranging from 61 to 129 min (4–9%), with a mean value of 86.9 ± 6.7 min (6.0 ± 0.5%). Significant ($P \leq 0.02$) increases in foraging (by 333%, from 7 to 30 min/day) and exploration (by 13%, from 70 to 79 min/day) occurred in response to enrichment, however, enrichment had no effect on the time spent stereotyping or being inactive.

Enrichment was unsuccessful at reducing stereotypic behaviour due to the wrong type of enrichment provided, or the expression of this behaviour had become resistant to change. However, it still resulted in improved welfare by providing more stimulus diversity, more choice in behaviour options, more opportunity to interact with their environment and the ability to express a larger amount of natural behaviour.

This study underlines the advantages of using essential oils as a form of environmental enrichment. However, two essential oils provided at once, make it difficult to see the effects of each scent. The sample size was only twelve, and the majority of the wombats were female. Increased research on equal gendered wombats could provide more information on the effects. Additionally, providing essential oils separately rather than together to prevent confusion makes it easier to discover the effects of each scent. A digital video camera recorded the wombats, but the behaviours were recorded at 5-minute intervals, meaning key behaviours could be missed. Analysing all the behaviours recorded would provide additional results and ensure no behaviours are missed.

1.4.4 How different animals' personalities can impact their behaviour

Animal personalities have been reported across different species including; mammals, reptiles, birds and fish (Wolf, and Weissing, 2012; Wilson, 1998). Many species show consistent differences in shyness, sociability, aggressiveness, and activity (Dall, *et al.* 2004).

Svartberg, 2005, investigated the validity of specific traits for predicting behaviours in dogs. A questionnaire relating to a dog's typical behaviour in a range of situations, was sent to owners of dogs that completed the dog mentality assessment behavioural test one to two years earlier. Behavioural factors in everyday life corresponded to the specific personality traits from the dog mentality assessment. The result suggested construct validity for the traits sociability (−0.21, −0.27, 0.36), curiosity/fearlessness (−0.16, −0.26, 0.14), playfulness (0.20, −0.15, 0.36) and distance-playfulness (−0.14, 0.16, −0.19, −0.17, 0.16, 0.29).

These results indicate that using dog mentality assessments is useful to describe certain components of a dogs' personality expressed in everyday life (Svartberg, 2005). Further studies on other species could provide more information on how individual animal personalities can affect their behavioural responses.

1.4.5 Behavioural resistance in animals

Lefevre, *et al.* 2012, investigated monarch butterflies altering their behaviours to protect themselves and their offspring against the protozoan parasite (*Ophryocystis elektroscirrha).*

They discovered that monarch butterflies were unable to avoid infectious parasite spores, and larvae were unable to preferentially feed on therapeutic food plants or increase the ingestion of such plants (logistic regression: odds ratio (OR) = 1·02, 95% confidence interval (CI) = 0·14, 1·9, P = 0·7). Butterflies would favourably lay their eggs on food plants that reduce parasite growth in their offspring (OR = 1·51; 95% CI = 1·1, 2; P = 0·001) suggesting that some animals may use altered behaviours to protect their offspring instead of themselves. For future research, focusing on species in captivity and what encourages them to alter their behaviours may help to promote their welfare and understand their reasons for expressing certain behaviours.

1.4.6 The Animal – keeper bond

Carlstead, 2009, investigated keeper-animal relationships in zoos. Data from zookeepers and animals were collected. Standardised questionnaires rated zookeepers on their daily husbandry routines, behaviour towards animals, response to them and to other people. Additional information was provided about themselves. The subjects included 219 individuals from four endangered species: black rhinoceros (*Diceros bicornis*), cheetah (*Acinonyx Jubatus*), maned wolf (*Chrysocyon brachyurus*), and great hornbill (*Buceros bicornis*).

Zoo keepers were videoed calling to their animals to observe the animals' responses. Responses varied along three dimensions: sociable/curious, fear of people and affinity towards keeper. Fear of people were significantly and positively correlated with independent measure of poor welfare from two later studies: faecal corticoid concentrations for 12 black rhinos (R = 0.73, P = 0.006) and tense-fearful scores for 12 cheetahs (R= 0.61, P = 0.03). The interaction of the two dimensions' affinity towards keeper, and fear of people, appeared to be species-specific. These findings suggest the quality of keeper-animal relationships is influenced by: if the keeper enters enclosure, keeper visibility to animal, time and frequency of feeding.

Among keepers that entered enclosures with animals, a significant negative correlation was found between frequency of feeding/early feed time and average affinity to keeper of their animals (R = 0.31; P = 0.04). A positive correlation between keeper experience and the animals' fear of people (R =

0.33; P = 0.03), suggested certain zoo keeping methods among experienced keepers might be aversive increasing fear in animals. Keepers who moved or made unexpected noises when calling the animals encouraged increased apprehension, or aggression, from cheetahs and maned wolves (P = 0.022, P = 0.008). Wild-born black rhino and parent-reared maned wolf have significantly less affinity to keepers than their captive-born or hand-reared counterparts, but neither differs in fear of people. Keeper–animal relation-ships is also suggested to be reciprocal as evidenced by a negative correlation of job satisfaction with animal fear of people (R = 0.32, P = 0.04).

This study provides evidence on how zoo keepers impact the behaviour of animals in captivity, and how the emotional state of keepers and animals may be reciprocal. This may be key information for improving animal welfare. However, it was questionnaire based for the zoo keepers which questions biased. They also focused on smaller sample sizes of various species, in a number of zoos. In future research, studying larger sample sizes of the same species could avoid species-specific behaviours as an added variable. Observing the keeper’s interactions with the animals over longer periods of time could provide more of an insight into their relationship with the animals.

1.4.7 The effects of health issues and animal personality on their behaviour

Ijichi, Collins and Elwood, 2014, investigated if pain expression is linked to personality in horses. Findings suggested that neuroticism is negatively related to “stoicism” (−.686 .307, clinical lameness = -.156, severity = .711) and extroversion was positively related to levels of lameness (006 −.733, clinical lameness = .583, severity = .174. The results indicate that identifying pain in extroverted horses is easier than in neurotic horses.

This provides evidence on how personalities of animals can affect behavioural expression and responses to pain. There is a need for this to be accounted for during welfare assessments as well as further studies on animal behaviour. For future research, radiograph scoring with objective lameness scoring can be used to provide more accurate results. Also conducting similar studies on other species to gain more of an understanding of how their personality can impact their health and behaviour (Ijichi, Collins and Elwood, 2014).

1.4.8 The effects of age on animal behaviour

Ingram, 1982, conducted an open-field study using an oval runway for mice. Movements on the runway during a 10-minute period were detected by electric contact plates in the floor. Locomotion, or exploratory activity, was organised in terms of counts. Results indicated that exploratory activity declines with age (R=0.56). More than a 50% decline in activity was evident between ages of 6-32 months. It's also clear that individual variability exists. The activity of several mice over 30 months of age was equal to that of mice half their age (Ingram, 2000; Ingram, 1983).

1.4.9 The effects of environmental enrichment on reproductive behaviour

Moreira, *et al.* 2007, studied the effects of different captive housing conditions on the reproductive cycles and adrenocortical activity in three adult female tigrina (*Leopardus tigrinus*) and two female margays (*Leopardus wiedii*). Females were housed individually and subjected to three enclosure conditions: large enriched enclosures (3 months), small, empty enclosures (5.5 months), and small enclosures with branches and nest boxes (6.5 months). Faecal samples were collected for analysis of oestrogen, progestogen and corticoid metabolites.

Ovarian follicular activity decreased and corticoid concentrations increased in tigrinas when transferred to small barren cages ($P < 0.05$). Corticoid concentrations in tigrinas declined after small cage enrichment ($P < 0.05$). Margays showed increased corticoid excretion in empty enclosures, and small enclosures with branches and concentrations remained high even after cage enrichment ($P < 0.05$). Enriching the small enclosures was unable to restore normal ovarian activity within the time frame of the study.

This study suggests environmental enrichment can effect reproductive cycles of female tigrina and margay. However, only five subjects were tested in total, making the sample size too small. Also, all three conditions were recorded over different time frames. For future research, a larger sample size would be required to provide more results, and ensuring they are subjected to each condition for the same time period. Using a camera to record behaviours in each enclosure could also provide more information on behavioural effects.

1.4.10 The impact of changes in weather on animal behaviour

Previous research has suggested certain species behaviour can be affected by changes in the weather. Sergio, 2003, investigated the effects of weather on foraging and breeding performance in the black kite (*Milvus migrans*). He discovered that the frequency of success in prey capture decreased during rainfall and increased when temperature was higher, also nestling provisioning rates declined during rainfall. The majority of the kites were more likely to forage during dry weather and success rates were also higher in good weather conditions. These results provide evidence of when a species will change their behaviour during climate changes. Further research is required to determine the effects of climate change on wolves to discover if behaviour can be impacted by it.

CHAPTER 2

2.0 METHODOLOGY

2.1 Location of study and enclosure sizes

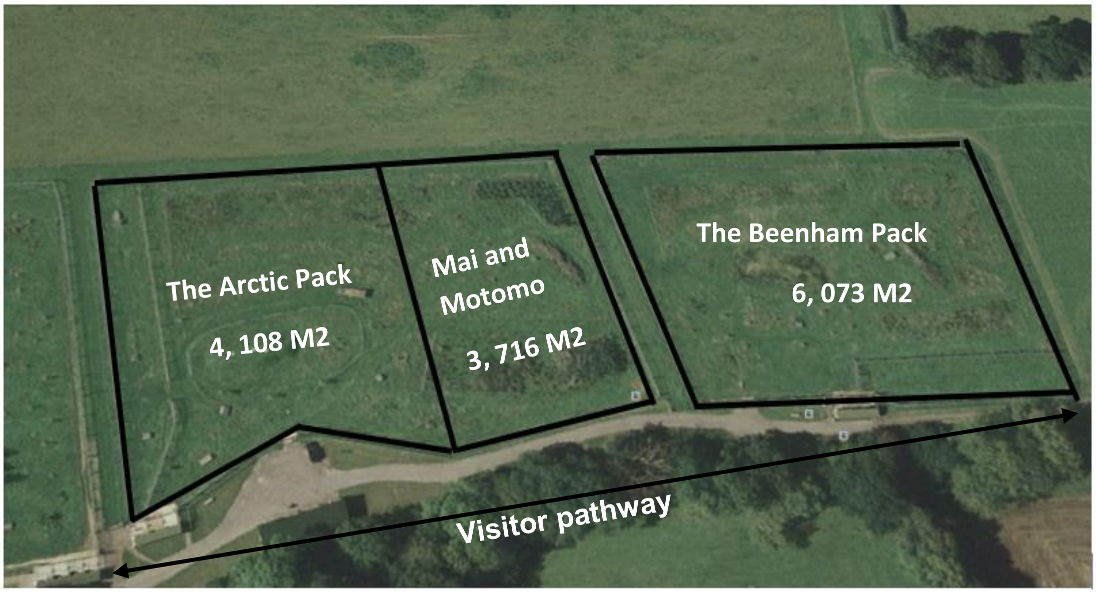

Figure 1: Birdseye view of the three wolf pack enclosures at the UKWCT (Author, 2016).

The wolves were observed at the UK Wolf Conservation Trust (UKWCT). All packs were housed in separate enclosures separated by 12ft. wire mesh fencing. All enclosures featured a viewing mound (a small hill for the wolves to gain visibility of the sight) viewing tables for the same purpose, water troughs, and a small area of dense woodland. The site has a path running along the front of the enclosures where visitors can clearly view the wolves.

2.2 Research Design

All wolves were observed for one hour under control conditions and then one hour subsequent to the introduction of the three olfactory enrichment treatments in turn. Between each of the these being used a control period was used during which animals were again observed. The daily timetable of observations under different treatments for the whole study period is shown in table 1 in appendix F.

Figure 2: Visitors viewpoint of the Arctic pack and Mai and Motomo's enclosures (Author, 2016).

2.2.1 Olfactory conditions and scent administration

The scents were bought from Holland and Barrett in the form of essential oils. The method used to introduce the scents was using hay/straw bundles tied with raffia and soaked in the scent then thrown into each enclosure.

Figure 3: Straw bundles soaked in water and ten drops of essential oil. (Author, 2016).

An ethogram covered all behaviours that could be observed, and a description of each behaviour (Moretti, 2015; Vasconsellos, *et al*. 2009; MacNulty, *et al*. 2007). For every behaviour recorded, the wolf that expressed the behaviour was recorded to enable a more precise, and clear result (refer to appendices E) (Baan, *et al.* 2014). The results were recorded in the form of tables, and each wolf was colour coded (Refer to appendices J). This was to gain a clear perspective of the effects each scent had on the behaviour of each wolf.

They were observed from the viewpoint of a visitor with a distance of three to four metres from the fencing separating the wolves from the observation area. All behaviours recorded stated what wolf expressed the behaviour to provide a clear result. The scents used during each observation were also recorded.

2.2.2 Statistical Analysis

When all data was collected it was put into SPPS version 24 using a non-parametric, Wilcoxon signed-rank test, to compare the overall effects of each olfactory enrichment on the behaviour of the wolf packs. The Wilcoxon signed rank-test was selected after looking at the Hawkins choice test. This test was used because two related samples were being compared (olfactory condition/control condition) to test to for a difference between conditions, on the same wolf packs. The data was also likely to be non-parametric.

2.3 ETHICAL STATEMENT

When working with animals it is important to first ensure that all ethical concerns are covered, and the study is carried out without any risk to the welfare of the animals (Kumar, 2014; Harrington, *et al.* 2013; Dawson, 2009). Addressing any ethical concerns is priority and is worked out before moving forward with the study (Kumar, 2014). Two proposal forms were completed prior to the study, the first was completed for the university, and the second, for the wolf handlers at the UKWCT (appendices A and C).

All resources used during the study were thoroughly inspected for any potential risks to the health of the wolves (Oliver, 2010; Dawson 2009). They were inspected for any substances that could potentially cause harm, or impact their wellbeing. A risk assessment on the area observations were conducted was completed to ensure all hazards and risks were outlined prior to the study (Refer to Appendices B).

The study completed was non-invasive and the scents were administered to the wolves' environment using an enrichment method. All essential oils were of natural source and non-toxic to the species (Artisan Aromatics, LLC, 2016; Essential oils direct, 2016). The wolves had previously been exposed to other forms of essential oil using this method (UKWCT, 2016).

CHAPTER 3

3.0 RESULTS

Wilcoxon signed-rank tests were used to compare the overall effects of each olfactory enrichment on the behaviour of all the wolf packs as one (appendices P, Q, R). Each behaviour under both control and scent conditions was analysed to see if there was a significant difference in results.

3.1 Control and Vanilla results for all three packs

	CONTROL	VANILLA	P VALUE	Z VALUE
Play	2.5	1	.317	-1.000b
Sleep	92.5	58	.064	-1.853b
Explore	46	54.5	.669	-.427b
Sit	4.5	19	.044	-2.015b
Lie	145	94.5	.050	-1.961b
Stand	102	80	.172	-1.367b
Patrol	35	111	.023	-2.278b
No visual	59.5	34.5	.157	-1.415b
SAB (Scent associated behaviours)	0	33	.001	-3.428b
Vocal	0	4.5	.066	-1.841b

Table 1: Frequency of behaviours for all three packs under control and vanilla conditions

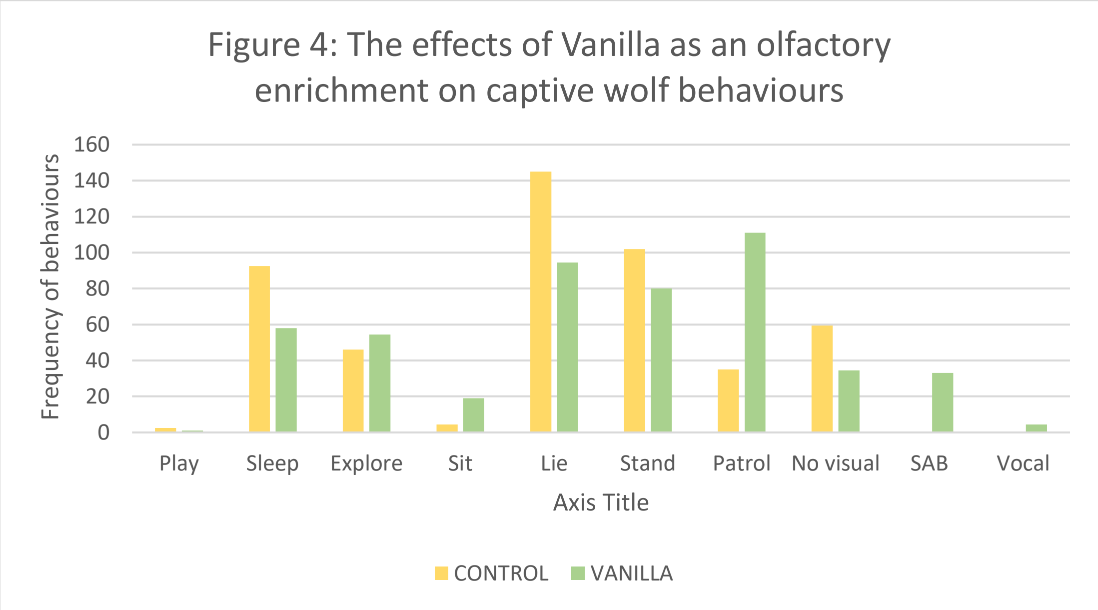

Figure 4: A bar chart of the effects of vanilla as an olfactory enrichment for captive wolves.

The following behaviours did not show a significant difference under the olfactory condition vanilla: play (Z = -1.000b, P = .317), sleep (Z=-1.853b, P=0.64), explore (Z = -.427b, P = .669), stand (Z = -1.367b, P=.172), No visual (Z=-1.415b, P=.157), and vocal (Z=1.841b, P=.066). Therefore, we accepted the null hypothesis (H_0) for these behaviours.

There was a significant difference in the frequency of the behaviours: sit (Z = -2.015b, P = .044), lie (Z=-1.961b, P=.050), patrol (Z=-2.278b, P=.023), and scent associated behaviours (SAB) (Z=-3.428b, P=.001). Therefore, we reject the null hypothesis (H_0) for these behaviours.

3.2 Control and Cinnamon results for all three packs

	CONTROL	CINNAMON	P VALUE	Z VALUE
Play	12	0	.026	-2.226b
Sleep	18.5	40.5	.753	-.314b
Explore	15	13.5	.975	-.031b
Sit	0	13	.035	-2.111b
Lie	52	41.5	.339	-.957b
Stand	30.5	33.5	.224	-1.217b
Patrol	44.5	30.5	.407	-.829b
No visual	8.5	4.5	.046	-1.995b
SAB	0	5	.001	-3.306b
Vocal	2	1	.056	-1.913b

Table 2: Frequency of behaviours for all three packs under control and cinnamon conditions

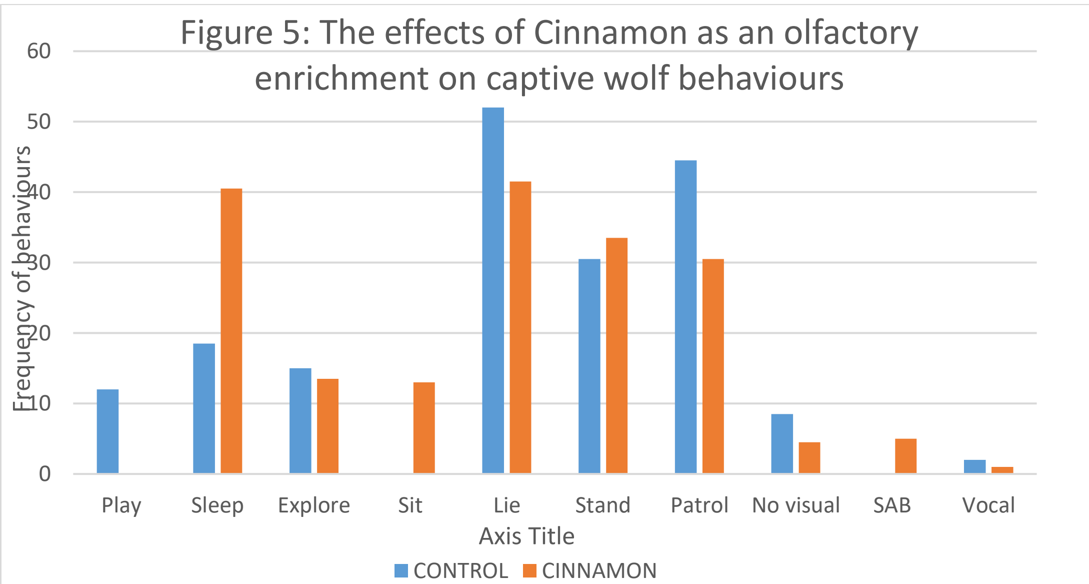

Figure 5: A bar chart of the effects of cinnamon as an olfactory enrichment for captive wolves.

The following behaviours did not show a significant difference under the olfactory condition cinnamon: sleep (Z=-.314b, P=.753), explore (Z=-.031b, P=.975), lie (Z=-.957b, P=.339), stand (Z=-1.217b, P=.224), patrol (Z=-.829b, P=.407), and vocal behaviours (Z=-1.913b, P=.056). Therefore, we accepted the null hypothesis (H_0) for these behaviours.

There was a significant difference in frequency of the behaviours: play (Z=-2.226b, P=.026), sit (Z=*-2.111b, P=.035)*, no visual (Z=*-1.995b, P=.046)*, and scent associated behaviours (SAB) (Z=*-3.306b, P=.001).* Therefore, we reject the null hypothesis (H_0) for these behaviours.

3.3 Control and Eucalyptus results for all three packs

	CONTROL	**EUCALYPTUS**	**P VALUE**	**Z VALUE**
Play	1	0	.109	-1.604b
Sleep	25	8	.050	-1.962b
Explore	12.5	41	.102	-1.634b
Sit	3	5.5	.003	-2.958b
Lie	42	61	.816	-.233b
Stand	45	44.5	.093	-1.678b
Patrol	48	7	.550	-.597b
No visual	6	14.5	.195	-1.296b
SAB	0	2	.005	-2.807b
Vocal	2	0	.011	-2.549b

Table 3: Frequency of behaviours for all three packs under control and eucalyptus conditions

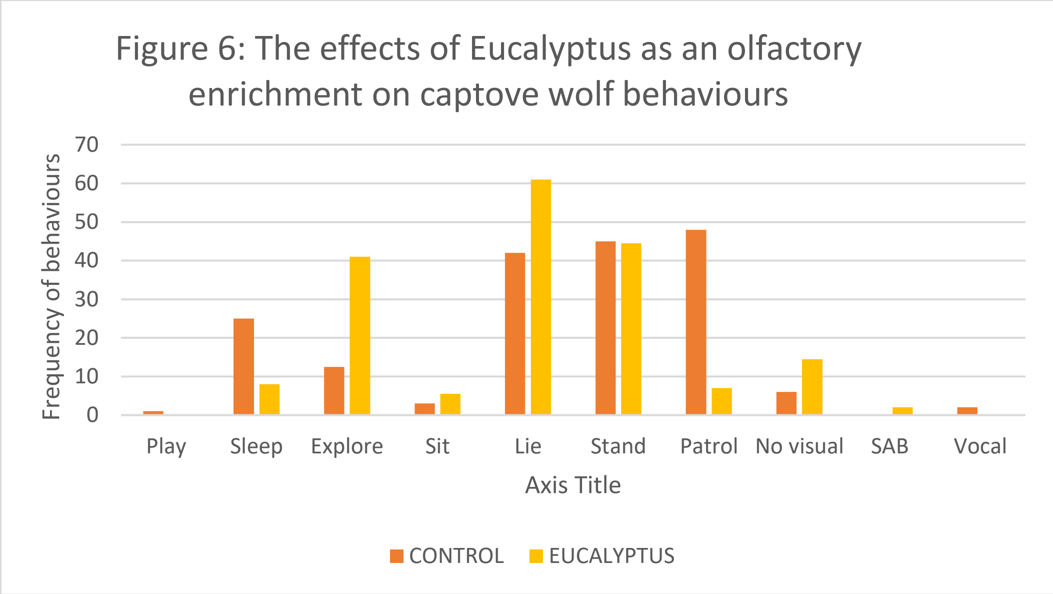

Figure 6: A bar chart of the effects of eucalyptus as an olfactory enrichment for captive wolves.

The following behaviours did not show a significant difference under the olfactory condition eucalyptus: play (Z=-1.604b, P=.109), explore (Z=-1.634b, P=.102), lie (Z=-.233b, P=.816), stand (Z=-1.678b, P= .093) patrol (Z=-.597b, P=.550), and no visual (Z=-1.296b, P=.195). Therefore, we reject the null hypothesis (H_0) for these behaviours.

There was a significant difference in the frequency of the behaviours: sit (Z=-*2.958b, P=.003)*, vocal (Z=-2.549b, P=.011), sleep (Z=-1.962b, P=.050), and scent associated behaviours (SAB) (Z=*-2.807b, P=.005).* Therefore, we accept the null hypothesis (H_0) for these behaviours.

3.4 Results for frequency of behaviours by male and female wolves

A further analysis using the Wilcoxon signed rank-test was used to compare the male and female wolf behaviours under each condition and discover if there was a significant difference between the two (appendices N and O).

3.4.1 Comparing the frequency of behaviours of female and male captive wolves under control and vanilla conditions

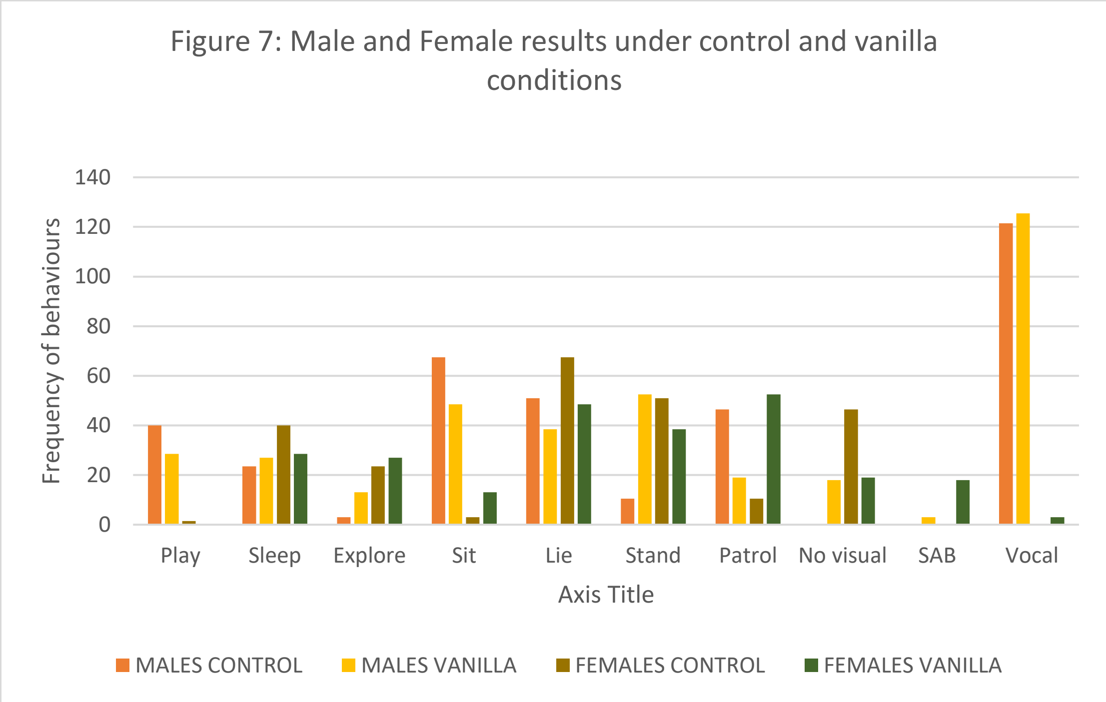

Figure 7: A bar chart comparing the frequency of female and male wolf behaviours under control and vanilla olfactory conditions

All behaviours were analysed for female wolves under control and vanilla olfactory conditions, it was discovered that only scent associated behaviours showed a significant increase (Z = 2.375c, P = .018). All other behaviours under this conditions were non-significant, thus, the null hypothesis was accepted.

The male wolves were analysed and a significant difference was also found in the frequency of scent associated behaviours (Z = -2.536d, P = .011) and the frequency of sleep behaviours (Z = -1.963c, P = 0.50). All other behaviours were non-significant and the null hypothesis was accepted. (Refer to appendices K for table including frequency of male and female wolf behaviours)

3.4.2 Comparing the frequency of behaviours of female and male captive wolves under control and cinnamon conditions

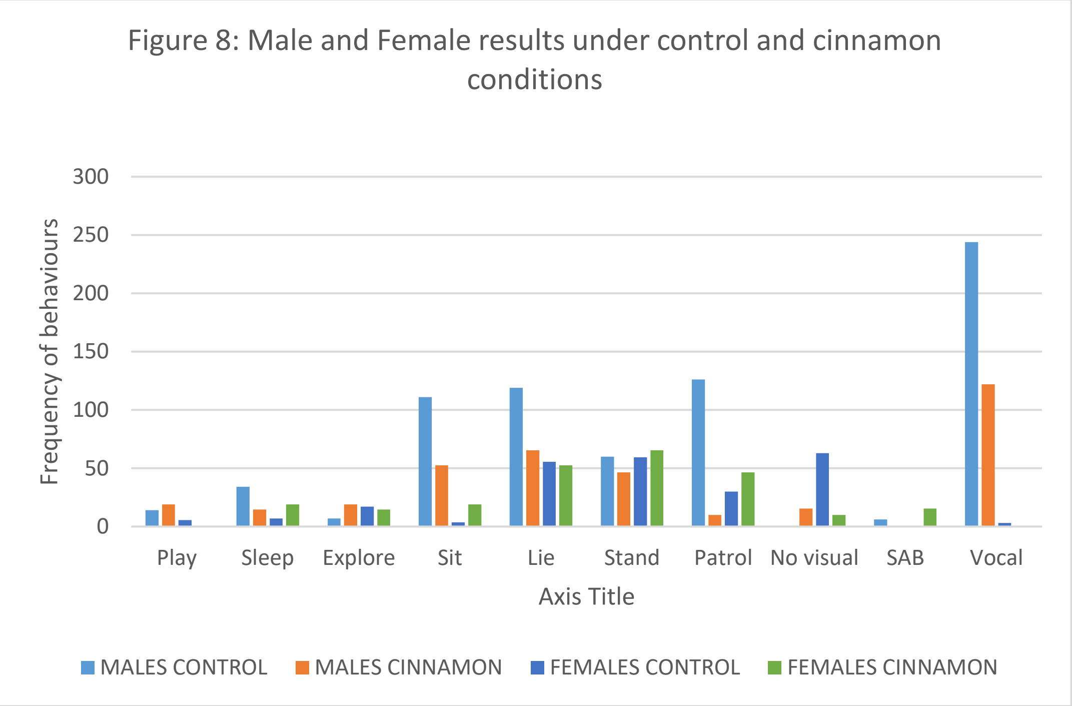

Figure 8: A bar chart comparing the frequency of female and male wolf behaviours under control and cinnamon olfactory conditions

All behaviours were analysed for female wolves under control and cinnamon olfactory conditions, it was discovered that only scent associated behaviours showed a significant increase (Z = -2.207c, P= 027). All other behaviours under this conditions were non-significant, thus, the null hypothesis was accepted.

The male wolves were analysed and a significant difference was also found in the frequency of scent associated behaviours (Z =-2.533c, P = 0.11). All other behaviours were non-significant and the null hypothesis was accepted. (Refer to appendices L for table including frequency of male and female wolf behaviours)

3.4.3 Comparing the frequency of behaviours of female and male captive wolves under control and eucalyptus conditions

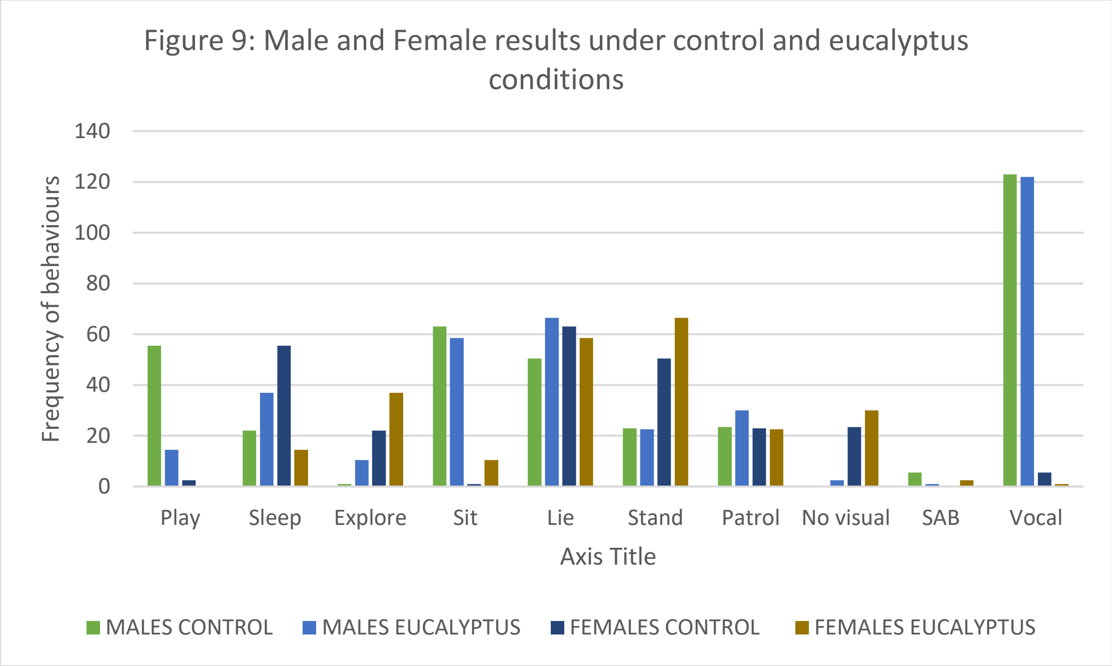

Figure 9: A bar chart comparing the frequency of female and male wolf behaviours under control and eucalyptus olfactory conditions

All behaviours were analysed for female wolves under control and eucalyptus olfactory conditions, it was discovered that only sit behaviours showed a significant increase (Z= -2.214c, P = 0.27). All other behaviours under this conditions were non-significant, thus, the null hypothesis was accepted.

The male wolves were analysed and a significant difference was also found in the frequency of scent associated behaviours (Z =-2.121c, P = 0.34) and sit behaviours (Z =-2.041c, P = .041). All other behaviours were non-significant and the null hypothesis was accepted. (Refer to appendices M for table including frequency of male and female wolf behaviours)

CHAPTER 4

4.0 DISCUSSION

4.1 Outcome

The results suggested certain behaviours are affected by the presence of olfactory enrichment. SAB were analysed and there was a highly significant result for all three (vanilla- P=.001, cinnamon= P=.001, eucalyptus = P=.005), indicating that the wolves were encouraged to behave in a specific way when olfactory enrichment was present. The results are similar to that of a study conducted by Van Metter, *et al.* 2008. They used scented squash as a form of olfactory enrichment. Results suggested that the enrichment increased behavioural diversity when compared to a baseline.

4.1.1 The effects of the olfactory scent vanilla on the behaviour of captive wolves

The Wilcoxon signed rank-test indicated that the behaviours sit (P = .044), lie (P=.050), patrol (P=.023), and SAB (P=.001), all showed a significant effect in comparison with the control conditions.

The wolves spent more time sitting and less time lying during enrichment conditions. There was a significant increase in patrol behaviours which indicates that the enrichment increased the wolves' awareness and made them more active. This does however contradict the theory that vanilla has a relaxing and calming effect on the brain (Bythrow, 2005).

Other variables must also be considered. Tractors drove past the enclosures several times which distracted the wolves. The presence of the observer and keepers may have also impacted the results. Carlstead, 2009, provided evidence on how keepers can impact the behaviour of animals, and that keepers can have both negative and positive effects on behaviour.

The social status within the pack may have influenced certain behaviours (Marvin, 2012). Dominant wolves can influence decisions of other members of the pack (Payne, Starks, and Liebert, 2010). Individual wolf personalities could also be a contributing factor. Ijichi, Collins and Elwood, 2014, linked pain expression to personalities in horses. They suggested that animal personalities should be considered more and that it is a potential factor causing certain behavioural expressions in horses.

4.1.2 The effects of the olfactory enrichment cinnamon on the behaviour of captive wolves

The results of cinnamon conditions showed a significant difference in the frequency of the behaviours: play (P=.026), sit (P=.035), no visual (P=.046), and SAB (P=.001).

The wolves spent significantly less time playing and more time sitting during olfactory conditions. However, their time spent lying and patrolling did not change significantly. This may indicate it was a coincidence rather than an effect caused by the scent.

The public were invited to visit on an open day and school children visited. Two gazebos were pitched near the wolves' enclosures, and the wolf handlers encouraged the public to watch the wolves being fed. These variables may have influenced the wolves to express certain behaviours. Farrand, Hosey, and Buchanan-Smith, 2014 agree with this. they conducted a study to discover the effects the public have on behaviours of animals at a petting zoo. They suggested that visitors can affect the behaviour of pigs.

Pumpkins filled with meat were placed in the Arctic wolves' enclosure as a form of environmental enrichment. The wolves were cautious of the pumpkins and avoided them, it's likely they reacted this way because they were completely new to them. However, many zoos have provided similar forms of enrichment that have had positive effects on species. At San Diego Zoo, cheetah cubs were fed frozen meats in the shape of a birthday cake. This enrichment was successful. The cheetahs responded by licking at the ice to get to the meat in the middle (San Diego Zoo, 2015).

4.1.3 The effects of the olfactory enrichment eucalyptus on the behaviour of captive wolves

During the summer, the wolves were provided with eucalyptus as an alternative to fly repellent. Eucalyptus is often used for its antiseptic and fungicidal properties, so it isn't surprising it was used for this purpose

(Battaglia, 2003). This could be a contributing factor to the results, and is likely that the wolves may have habituated to it (Tarou, and Bashaw, 2007). Habituation influences the effectiveness of enrichment and is defined as response decrement as a result of repeated stimulation (Harris, 1943). Wells 2004, aimed to increase the activity levels of captive black-footed cats. The first day the activity levels of the cats increased and they spent significantly less time resting, by the third day there was no behavioural changes and increased resting behaviour, which suggests that the animals habituated to the treatment.

The results show a significant difference in the frequency of: sit (P=.003), vocal (P=.011), sleep (P=.050) and SAB (P=.005). The frequency of SAB was slightly lower than the previous two scents, suggesting the wolves may not be as interested due to them experiencing the scent previously. The wolves spent significantly less time sleeping and more time sitting which could indicate that they were more alert when the olfactory enrichment was provided. They were significantly less vocal, however, this behaviour is likely to have been encouraged by other wolves within the pack, or nearby enclosures.

4.1.4 Comparing the effects of olfactory enrichment on male and female wolves

The analysis found a significant difference in the frequency of SAB (P = .011) and sleep behaviours (P=.050) for males during the presence of vanilla. All other behaviours were non-significant and the null hypothesis was accepted. Only SAB showed a significant increase in females (P = .018), and all other behaviours were non-significant. This result suggests all wolves were encouraged to behave in a specific way under vanilla conditions. It also indicates that vanilla encouraged male wolves to decrease their time spent sleeping, which suggests it made the male wolves more alert. Other variables must also be considered. The vanilla conditions were conducted on the first two days of the study which could mean the males were more alert due to the presence of an unfamiliar observer. However, this does not explain why the females did not respond similarly. Further research is required to gain more of an understanding if this result is coincidence or a behavioural response.

A significant difference was found in the frequency of SAB (male = P = 0.11, female = P= 0.27) when cinnamon was present. All other behaviours were non-significant and the null hypothesis was accepted. This suggests that cinnamon encouraged SAB when present. However, no significant effects on other behaviours for either sex were found.

A significant difference was found in male wolves' frequency of SAB (P = 0.34) and sit behaviours (P = .041) during eucalyptus olfactory conditions. All other behaviours were non-significant and the null hypothesis was accepted. Female wolves showed a significant increase in sit behaviours (P = 0.27). All other behaviours were non-significant; thus the null hypothesis was accepted. The females were less interested in the scent and showed no significant difference in behaviours when compared to control conditions (P = .725) suggesting females may have habituated to the scent when it was provided as a fly repellent. The males were more interested in the scent even though they had experienced it previously. This could mean that the male wolves did not habituate to the scent when it was previously provided. Personalities of each wolf and social dynamics within packs must also be considered. Longitudinal studies with increased samples sizes could provide more information on the reasons behind this.

4.1.5 Limitations and future research

Observing the wolves using one-minute intervals aimed to avoid missing key behaviours. However, there is a chance that some behaviours were not recorded. The original plan was to use a camera to record the wolves during observations. Unfortunately, there were issues with camera malfunctions and recordings were not taken. For future research, recording the wolves for the full duration under each conditions can avoid missing key behaviours. The wolves could be recorded using a hidden camera and the recordings could then be analysed. Camera trapping is a popular method of catching wild animals when researchers are not present (Foster, and Harmsen, 2011).

Wang and Macdonald, 2009, used camera traps with capture–recapture data analysis to provide the first reliable density estimates for tigers and leopards from the high altitude and rocky terrain in Bhutan's Jigme Singye Wangchuck

National Park. The cameras were collected and the results were used as a basis for conservation planning. This non-invasive method allows conservationists and researchers to improve understanding, monitor threats and explore various species in more detail. (Wong, and Katchel, 2016)

Repeating the study on several packs of captive wolves located in different places, can provide more results on wolves that are unrelated. This could provide more information on if individual wolf personality and family relationships are contributing factors, or if other packs of wolves encourage each other to behave in a certain way.

Another recommendation for further research is to monitor the wolves over a quieter period with no open days or trips. This will prevent members of the public impacting the behaviour of the wolves. Ensuring no other forms of environmental enrichment are present can avoid other objects encouraging behaviours they wouldn't normally express.

Conducting a longitudinal study with increased sample sizes may provide additional information to see if the wolves became habituated, and how long each scent provided stimulus. Using new scents, as seen in a study by Wells, 2004, could encourage extra behaviours and stimulate the wolves for increased durations. It would be interesting to discover what other behaviours can be influenced by other scents.

Finally, we must consider if any factors including age or sub-species, could influence the results provided. This would offer more information into why they react to scents in a specific way. Using olfactory enrichment is recommended to zoos and other locations housing captive animal as a method to encourage behavioural diversity and prevent boredom. In wolves, it is evident that these scents are effective at increasing certain behaviours and encouraging behavioural diversity (i.e. SAB).

CHAPTER 5

5.0 CONCLUSION

The results of this study suggest that using the essential oils vanilla, cinnamon and eucalyptus as forms of olfactory enrichment can encourage certain behaviours in captive wolves. The findings indicate that all three scents encouraged scent associated behaviours when placed into the enclosures. The findings also suggest that the sex of the wolf could be a contributing factor to certain behaviours expressed under the olfactory conditions. Further research is required on a larger sample size, over a longer period of time, to provide more information on how effective olfactory enrichment is on the behaviour of captive wolves. Also recording observations using a camera will ensure no behaviours are missed.

It is recommended that further studies are conducted on other captive species to investigate the effects of olfactory enrichment on their behaviour. Previous studies have provided evidence that environmental enrichment can encourage a range of behaviours in different species and many have investigated the effects of olfactory enrichment on behaviour. However, there is little research on the factors that can contribute towards the behaviours observed, and if the age or sex of the animal can affect how they react to certain scents.

References

Ali, B., Ali Al-Wabel, N., Shams, S., Ahamad, A., Khan, S.A. and Anwar, F. (2015) Essential oils used in aromatherapy: A systemic review. *Asian Pacific Journal of Tropical Biomedicine* [online]. 5 (8), pp. 601-611. [Accessed 30 September 2016].

Artisan Aromatics, LLC (2016) *MATERIAL SAFETY DATA SHEET.* Available from: https://artisanessentialoils.com/msds-essential-oil-safety/ [Accessed 12 March 2017].

Baan, C., Bergmuller, R., Smith, D.W. and Molnar, B. (2014) Conflict management in free-ranging wolves, Canis lupus. *Animal Behaviour* [online]. 90 (1), pp. 327-334. [Accessed 18 February 2017].

Battaglia, S. (2003) *The Complete Guide to Aromatherapy* [online]. International Centre of Holistic Aromatherapy: Perfect Potion. [Accessed 29 February 2016].

BIAZA (2016). *British and Irish Association of Zoos and Aquariums.* Available from: http://www.biaza.org.uk/ [Accessed 26 February 2017].

Bono, L., Mongillo, P., De Boni-Russo, G., Gabai, G. and Normando, S. (2016) Effects of 2 forms of environmental enrichment on a group of captive blackbucks (Antilope cervicapra): A pilot study. *Journal of Veterinary Behaviour: Clinical Applications and Research* [online]. 12 (1), pp. 66-72. [Accessed 26 November 2016].

Bythrow, J.D. (2005) Vanilla as a medicinal plant. *Seminars in Integrative Medicine* [online]. 3 (4), pp. 129-131. [Accessed 01 March 2007].

Carlstead, K. (2009) A comparative approach to the study of keeper–animal relationships in the zoo. *Zoo Biology* [online]. 28 (6), pp. 589-608. [Accessed 07 March 2017].

Clark, F. King, A.J. (2008). A Critical Review of Zoo-based Olfactory Enrichment. In: Hurst, J., Claxton, A.M. (2011) The potential of the human–animal relationship as an environmental enrichment for the welfare of zoo-housed animals. *Applied Animal Behaviour Science*. 133 (1-2), pp. 1-10.

Claxton, A.M. (2011) The potential of the human–animal relationship as an environmental enrichment for the welfare of zoo-housed animals. *Applied Animal Behaviour Science*. 133 (1-2), pp. 1-10.

Conn, M. (2013) *Animal Models for the Study of Human Disease*. Beaverton, Oregon: Elsevier.

Cordoni, G. and Palagi, E. (2016) Aggression and hierarchical steepness inhibit social play in adult wolves. *Behaviour* [online]. 153 (6-7), pp. 749-766. [Accessed 08 January 2017].

Cummings, L., Brown, J.L., Rodden, M.D. and Songsasen, N. (2007) Behavioral and physiologic responses. *Zoo Biology* [online]. 26 (5), pp. 331-343. [Accessed 02 January 2017].

Dall, S.R.X., Houston, A.I. and McNamara, J.M. (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters* [online]. 7 (8), pp. 734-739. [Accessed 04 March 2017].

Dawkins, M. (2012) *Animal Suffering: The Science of Animal Welfare* [online]. Oxford: Springer Science. [Accessed 19 February 2017].

Dawson, C. (2009) Introduction to Research Methods. Fourth Edition. How to Books Ltd

Elliott, B.M. and Grunberg, N.E. (2005) Effects of social and physical enrichment on open field activity differ in male and female Sprague–Dawley rats. *Behavioural Brain Research* [online]. 165 (2), pp. 187-196. [Accessed 24 February 2017].

Essential oils direct, (2016). MSDS. Available from: https://www.essentialoilsdirect.co.uk/msds.html [Accessed 12 March 2017].

Essler, J.L., Cafazzo, S., Marshall-Pescini, S., Viranyi, Z., Kotrschal, K. and Range, F. (2016) Play behavior in wolves: using the '50:50'. *Plos One* [online]. 11 (5), p. e0154150. [Accessed 08 January 2017].

Farrand, A., Hosey, G. and Buchanan-Smith, H.M. (2014) The visitor effect in petting zoo-housed animals: aversive or enriching?. *Applied Animals*

Behaviour Science [online]. 151 (1), pp. 117-127. [Accessed 24 February 2017].

Fatjo, J., Feddersen-Petersen, D., Luis, J., De La Torre, R., Amat, M., Mets, M., Braus, B. and Manteca, X. (2007) Ambivalent signals during agonistic interactions in a captive wolf pack. *Applied Animal Behaviour Science* [online]. 105 (4), pp. 274-283. [Accessed 02 January 2017].

Figure 1: Author, (2016) Birdseye view of the three wolf pack enclosures at the UKWCT

Figure 2: Author, (2016) Visitors viewpoint of the Arctic pack and Mai and Motomo's enclosures

Figure 3: Author, (2016) Straw bundles soaked in water and ten drops of essential oil.

Foster, R.J. and Harmsen, B.J. (2011) A critique of density estimation from camera-trap data. *The Journal of Wildlife Management* [online]. 76 (2), pp. 224-236. [Accessed 21 February 2016].

Harrington, L.A., Moehrenschlager, A., Gelling, M., Atkinson, R.P.D., Hughes, J. and Macdonald, D.W. (2013) Conflicting and Complementary Ethics of Animal Welfare Considerations in Reintroductions. *Conservation Biology*. 27 (3), pp. 486-500

Harris, J.D. (1943) Habituatory response decrement in the intact organism. *Psychol. Bull* [online]. 40, pp. 385-422. [Accessed 02 March 2017].

Hogan, L.A., Johnston, S.D., Lisle, A., Horsup, A.B., Janssen, T. and Phillips, C.J.C. (2010) Stereotypies and environmental enrichment in captive southern hairy-nosed wombats, Lasiorhinus latifrons. *Applied Animal Behaviour Science* [online]. 126 (1-2), pp. 85-95. [Accessed 29 November 2016].

Hosey, G. (2008) A preliminary model of human–animal relationships in the zoo. *Applied Animal Behaviour Science* [online]. 109 (2-3), pp. 105-127. [Accessed 25 February 2017].

Hosey, G., Melfi, V. and Pankhurst, S. (2013) *Zoo Animals: Behaviour, Management, and Welfare* [online]. Oxford: Oxford University Press. [Accessed 18 February 2017].

Ijichi, C., Collins, L.M. and Elwood, R.W. (2014) Pain expression is linked to personality in horses. *Applied Animal Behaviour Science* [online]. 152, pp. 38-43. [Accessed 08 March 2017]. [Accessed 08 March 2017]

Ingram, D.K. (1983) Toward the behavioral assessment of biological aging in the laboratory mouse: concepts, terminology, and objectives. *Experimental Aging Research* [online]. 9, p. 225. [Accessed 08 March 2017].

Ingram, D.K. (2000) Age-related decline in physical activity: generalization to nonhumans. *Medicine and Science in Sports and Exercise* [online]. 32 (9) [Accessed 08 March 2017].

Kumar, R. (2014) *Research Methodology: A step by step guide for beginners.* Fourth Edition. London: Sage Publications Ltd.

Larsen, M.J., Sherwen, S.L. and Rault, J.L. (2014) Number of nearby visitors and noise level affect vigilance in captive koalas. *Applied Animal Behaviour Science* [online]. 154 (1), pp. 76-82. [Accessed 25 February 2017].

Lefevre, T., Chiang, A., Kelavkar, M., Li, H., Li, J., Castillejo, C.L.F.D., Oliver, L., Potini, Y., Hunter, M.D. and Roode, J.C. (2012) Behavioural resistance against a protozoan parasite in the monarch butterfly. *Journal of Animal Ecology* [online]. 81 (1), pp. 70-79. [Accessed 08 March 2017].

Marvin, G. (2012) *Wolf*. London: Reaktion Books Ltd.

Mech, D. and Boitani, L. (2010) *Wolves: Behaviour, Ecology and Conservation*. Chicago: University of Chicago Press.

Moreira, N., Brown, J.L., Moraes, W., Swanson, W.F. and Monteiro-Filho, E.L.A. (2007) Effect of Housing and Environmental Enrichment on Adrenocortical Activity Behavior and Reproductive Cyclicityin the Female Tigrina (Leopardustigrinus) and Margay (Leopardus wiedii). *Zoo Biology* [online]. 26 (6), pp. 441-460. [Accessed 07 March 2017].

Musiani, M., Boitani, L. and Paquet, P.C. (2010) *The World of Wolves New Perspectives on Ecology, Behaviour and Management*. Canada: University of Calgary Press.

Newberry, R.C. (1995) Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science* [online]. 44 (2-4), pp. 229-243.

Novaes, L.S., Santos, N.B.D., Batalhote, R.F.P., Malta, M.B., Camarini, R., Scavone, C. and Munhoz, C.D. (2017) Environmental enrichment protects against stress-induced anxiety: role of glucocorticoid receptor, erk, and creb signaling in the basolateral amygdala. *Neuropharmacology* [online]. 113 (1), pp. 457-466. [Accessed 18 February 2017].

Nowak, S. and Jedrzejewski, W. (2009) Ecology, behavior, and population genetics of wolves (canis lupus) in poland. *Journal of Veterinary Behaviour:Clinical Applications and Research* [online]. 4 (2), p. 67. [Accessed 03 March 2017].

Oliver, P. (2010) The Student's Guide To Research Ethics. Second Edition. Open University Press

Payne, A., Starks, P.T. and Liebert, A. (2010) *Encyclopedia of Animal Behaviour*. Boston: Academic Press, Elsevier.

Piffare, M., Valdez, R., González-Rebeles, C., Vázquez, C., Romano, M. and Galindo, F. (2012) The effect of zoo visitors on the behaviour and faecal cortisol of the Mexican wolf (Canis lupus baileyi). *Applied Animal Behaviour Science* [online]. 136 (1), pp. 57-62. [Accessed 25 February 2017].

Reade, L.S. and Waran, N.K. (1996) The modern zoo: how do people perceive zoo animals?. *Applied Animal Behaviour Science* [online]. 47 (1-2), pp. 109-118. [Accessed 10 November 2016].

Roberts, S.A., Davidson, A.J., Beynon, R.J. and Hurst, J.L. (2014) Female attraction to male scent and associative learning: the house mouse as a mammalian model. *Animal Behaviour*. 97, pp. 313-321.

San Diego Zoo (2015). Birthday cake for breakfast: cheetah cubs at San Diego Zoo turn one. Available from: http://blogs.sandiegozoo.org/2015/07/13/birthday-cake-for-breakfast-cheetah-cubs-at-san-diego-zoo-safari-park-turn-one/ [Accessed 08 March 2017]

Sergio, F. (2003) From individual behaviour to population pattern: weather-dependent foraging and breeding performance in black kites. *Animal Behaviour* [online]. 66 (6), pp. 1109-1117. [Accessed 05 March 2017].

Shepherdson, D.J., Mellen, J.D. and Hutchins, M. (2012) *Second Nature: Environmental Enrichment For Captive Animals* [online].: Smithsonian Institution. [Accessed 10 January 2017].

Sidorovich, V., Schnitzler, A., Schnitzler, C. and Rotenko, I. (2016) Wolf denning behaviour in response to external disturbances and implications for pup survival. *Mammalian Biology - Zietschrift Fur Saugetierkunde* [online]. [Accessed 21 December 2016] (In Press, Accepted Manuscript).

Svartberg, K. (2005) A comparison of behaviour in test and in everyday life: evidence of three consistent boldness-related personality traits in dogs. *Applied Animal Behaviour Science* [online]. 91 (1-2), pp. 103-128. [Accessed 04 March 2017].

Tarou, L.R. and Bashaw, M.J. (2007) Maximizing the effectiveness of environmental enrichment: suggestions from the experimental analysis of behavior. *Applied Animal Behaviour Science* [online]. 102 (3-4), pp. 189-204. [Accessed 02 March 2017].

The Zoo Licensing Act 1981 (Amendment) (England and Wales) Regulations (2002). Available from: http://www.legislation.gov.uk/uksi/2002/3080/contents/made [Accessed 26 February 2017].

UKWCT (2017) *The UKWCT.* Available from: https://ukwct.org.uk/. [Accessed 30 October 2016].

Van Metter, J.E., Harriger, M.D. and Bolen, R.H. (2008) Environmental enrichment utilizing stimulus objects for african lions (panthera leo leo) and sumatran tigers (panthera tigris sumatrae). *Bioscience* [online]. 79 (1), pp. 7-16. [Accessed 20 December 2016].

Wang, S.W. and Macdonald, D.W. (2009) The use of camera traps for estimating tiger and leopard populations in the high altitude mountains of bhutan. *Biological Conservation* [online]. 142 (3), pp. 606-613. [Accessed 21 February 2017].

Wells, D.L. (2004) The influence of olfactory enrichment on the behaviour of captive black-footed cats, felis nigripes. *Applied Animal Behaviour Science*. 85 (1-2), pp. 107-119.

Wells, D.L. (2009) Sensory stimulation as environmental enrichment for captive animals: A review. *Applied Animal Behaviour Science*. 118 (1-2), pp. 1-11.

Wilson, D.S. (1998) Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* [online]. 353 (1366), pp. 199-205. [Accessed 04 March 2017].

Wolf, W. and Weissing, F.J. (2012) Animal personalities: consequences for ecology and evolution. *Trends in Ecology and Evolution* [online]. 27 (8), pp. 452-461. [Accessed 03 March 2017].

Wong, W. and Katchel, S. (2016). Camera Trapping: Advancing the Technology. In: Schaller, G. B. (2016) *Snow Leopards: Biodiversity of the World: Conservation from Genes to Landscapes.* London: Academic Press, pp. 383–394

Young, R.J. (2013) *Environmental Enrichment For Captive Animals* [online]. Australia: Blackwell Publishing. [Accessed 16 February 2017].