1.0 Introduction

The wolf social structure is complex however its basic unit is the breeding pair (Mech and Boitanni, 2003) to which variances have been found. Most commonly the wolves within a pack are related, forming close family groups in order to survive in the wild (Mech, 2012). As such it is important to understand not only how wolves are in the wild but to then understand how humans are viewed by them in captivity.

Davey (2007) described that the perception of humans by nonhuman animals in captivity can either be a negative influence, a form of enrichment, or simply a changing variable that has no effect. Hosey (2008) stated that the keepers and public are seen in a different way, explaining that the keepers are qualitatively and quantitatively different to unfamiliar people. He described the fact that the keepers spend a lot more time with the animals thus far more interactions are possible which includes both positive, such as feeding, and negative, such as catching the animal for veterinary inspection.

Mitchell et al. in 1991 found that the golden-bellied mangabeys viewed the keepers as familiar conspecifics and the observers like familiar neighbours. The public however were perceived as interlopers and are believed to be perceived as an enemy and thus cause stress to the animals, due to their unfamiliarity with this stimulus their reaction to it is generalised across all unfamiliar individuals. However, Davis in 2002 found that a diverse number of species are able to distinguish between different kinds of people and thus the generalised stress response is not always induced. For example the ability to distinguish between familiar and unfamiliar handlers (Rousing et al., 2005); between different handlers dependent on their previous interactions with them (De Pasillé et al., 1996); the identification of their usual caretaker (Boivin et al., 1998); and cues including clothing (Munksgaard et al., 1999; Rybarczyk et al., 2003); facial features (Rybarczyk et al., 2001) and gender (Cheyne, 2006) can help the animal discriminate as to which stimulus is stressful and that which is not.

A positive relationship between keepers and their animals has shown signs that it helps in the alleviation of stress (Carlstead and Brown, 2005) the importance of which is not just for the welfare of the individual but can also have an effect on the reproductive success of animals as discerned by Mellen et al., 1991. In Mellen's study the small felids were found to have greater reproductive success with increased keeper interactions whereby a husbandry style which incorporated the keepers talking to the cats and interacting with them. Mellen concluded that a positive human-animal relationship was desirable for successful reproduction and should start with socialisation with the aim to produce cats with reduced fear of humans.

Socialisation is where the animal subject becomes accustomed to human presence and interaction, by working with them from birth or just after (Woolpy and Ginsburg, 1967; Aitken, 2004) aiming for the critical period of less than 10 days old (Klinghammer and Goodmann, 1985) to attain the best results for fully socialised wolves (Kleiman, 2011). Kelling et al. in 2013 stated that due to our lack of understanding of the effects socialisation has on adult social behaviour the practice is generally avoided however, it is sometimes necessary. Conversely Klinghammer and Goodmann (1985) denoted the advantages of socialising wolves in particular includes a reduction in fear to humans which enables them to behave and interact with each other in a way that allows for the expression of their natural behavioural repertoire, displaying natural hunting and social behaviours. That routine medical care and other handling procedures can be accomplished without trauma which equates to less disruption, especially in regards to the social order of wolves. Klinghammer and Goodmann (1985) acknowledge that there are a number of drawbacks of socialisation such as they are unable to be reintroduced to the wild and they may become too attached to people to the point where they are no longer able to express their normal behavioural repertoire however with a strict socialisation process which acknowledges the critical period and milestones in their development that these are overcome due to the fact that the animals are relaxed and not readily spooked by human activity both inside and outside of their enclosure.

When it comes to assessing welfare in captivity it is important to establish whether the animal is expressing what would be considered its normal behavioural repertoire (Hill and Broom, 2009). A failure to fulfil these needs may lead to the development of abnormal behavioural repertoires, which may include stereotypic or injurious behaviour both of which are indications of poor welfare (Broom and Johnson, 2000).

Odberg (1978) defined stereotypic behaviours as characteristically repetitive, invariant in form, and to have no obvious goal or function (Rushen, 2008). A reduction in the expression of stereotypies can potentially increase the welfare of the animal involved (Markowitz & LaForse, 1987; Forthman et al., 1992). There are a number of different conditions that an animal in captivity must adapt to and can induce a great deal of stress in the animal involved (Seidensticker and Doherty, 1996). Duncan and Fraser (1997) denoted three main ways to conceptualise nonhuman animal welfare: feeling based, functioning-based and abiding by the "nature" of the species to facilitate the expression of the animal's behavioural repertoire. This study will explore these points; looking into the effects that socialisation has on the animal's ability to fully express their natural behavioural repertoire. In this study the identification of stereotypies such as stereotypic pacing (Clubb and Mason, 2006) and those denoted in Mitchell and Hosey's 2005 text will be investigated (Montaudouin and Pape, 2004; Cheyne, 2006; Mason, 2006; Bayazit, 2009). In addition to stereotypies the Grey Wolf's agonistic behaviours will be looked at which according to Neveu and Deputte (1996) are essential for well-being studies (Platt and Novak, 1997; Cheyne, 2006; Veasey, 2006; Molla et al., 2011). Chamove et al. (1988) described that observer presence can increase agonistic behaviour while decreasing grooming behaviour and so in response to this Claxton (2011) expressed that a decrease in aggression would equate to a reduction in stress. Hosey (2005) described that an increase in audience directed and intra-specific aggression occurred when audiences were present however affiliate behaviours were largely unchanged.

By exploring the agonistic behaviours mentioned in the ethogram and looking at the expression of abnormal behaviours, such as stereotypies, this study will explore and help develop our understanding of the effect socialisation has on the complex social structures evident in wolves and as such their ability to express their natural behavioural repertoire. From this their ability to cope with the stressors present in a captive environment can be analysed.

This research aims to address and help further our knowledge into the effects that socialisation has on the frequency and intensity of agonistic and abnormal behaviours of the grey wolf (*Canis lupus*) and as such what this means for their welfare in

captivity. In order to achieve this aim several objectives will be completed through my study:

- 1. To investigate the difference between the frequency and duration of stereotypic behaviour shown by socialised and non-socialised grey wolves.
- 2. To note down and allocate the different expression of agonistic behaviours into the agonistic behavioural categories:
 - a. Elicited aggression
 - b. Food-related aggression
 - c. Sex-related aggression
 - d. All-out attack
 - e. Defence and submission
 - f. Offensive threat
 - g. Ritualised attack.
- 3. To investigate the difference between the frequency and duration between the different agonistic behavioural categories

The way this will be achieved will be to look at the different aspects of agonistic behaviours: elicited aggression, food-related aggression, sex-related aggression, allout attack, defence and submission, offensive threat, and ritualised attack. The data will show whether socialised or non-socialised wolves can be considered to be better able to behave in a way that would signify the expression of a diverse range of natural behaviours or whether there is no significant difference between socialised and non-socialised wolves.

2.0 Literature Review

2.1 Wolf Social Ecology

2.2 Pack Structure

It is important to consider how wolves are in the wild when considering keeping wolves in captivity in order to adhere to their natural behaviours. When discussing the structure of wolf packs in the wild the human family is a good analogy. The basic pack comprises of the mated pair and their offspring which all function as a tightknit unit all year round (Mech and Boitanni, 2003).



Figure 1: Models for Social Structure (*Taken from Mech and Boitani, 2003*)

Wolf packs and pairs are the basic units of wolf packs in the wild. The most basic unit is the breeding pair. There are three main models for wolf social structure (see figure 1) however variations to this were described by Mech and Nelson (1990).

Pups stay with parents between 10-54 months except in special circumstances all offspring will disperse (Gese and Mech, 1991; Mech et al., 1998). As such packs may include as many as 4 years and are variations of the mated pair.

According to current research wolf packs are now considered as family units (Mech and Boitanni, 2003; and Mech, 2012) then arguably packs comprising of unrelated wolves would exhibit statistically greater frequencies of intra-specific agonistic

interactions Mech (2012) showed however that despite the fact that there are many instances where strange wolves have been met with aggression there have also been times when they have actually been adopted into the pack.

2.3 Competition and intra-specific strife

Brown (1964) stated that the predominant and interesting factor increasing aggressiveness through natural selection should be competition. This idealistic concept however is not always the case in captivity as there are many other contributing factors which will be detailed later. It does stand to reason though that in small populations of wolves in an area with abundant prey the frequency and intensity of aggression should decrease. As such Brown (1964) argued that territory defence should decrease however despite this fatal attacks still occur in colonising populations (Frits and Mech, 1981 and Weidaven et al., 1985). For example in Yellowstone National Park post wolf introduction distinct territories were established within a few weeks and fatal attack between packs occurred within 6 months of their release in 1996 despite the fact that the prey abundance numbered in the thousands (Mech and Boitanni, 2003). In addition to this the west pack two in Isle Royale sought out and attacked other wolves despite a surplus of food resources in their own territory (Peterson and Paige, 1988). From this it can be deduced that food availability and abundance has little effect on territorial inter-specific aggression.

There is no limit on the intra-specific competition between wolf kin however it is prevalent when food is short. Adults compete fiercely with yearlings; yearlings with pups; and pups with one another (Packard, (Volume 2 of the Mech and Boitanni 2003 text)). Isle Royale all wolves are related to each other as close as siblings (Wayne et al., 1981) however they have established their own territories and treat each other like any outbreed population and will still kill each other (Peterson, 1977; Peterson and Paige, 1988) however this does not mean that kin would necessarily kill each other under different circumstances or when all competitors are closely related competition will be maximised. The only other documented example of kin killing each other was between two apparent sisters in Yellowstone National Park (McIntyre and Smith, 2000) however gathering such data is difficult (Mech and Boitani, 2003). Alternatively wolf aggression may stem from food or breeding competition.

Analysis on the deaths of wolves due to wolf attacks over a 22 year period in the SNF (Mech, 1994) 9 year period in Dunaly National Park (Mech et al., 1998) such intraspecific strife represents territorial aggression that reduces competing breeders and presents the opportunities for packs to expand their territories whilst indirectly keeping each pack within their own territory. Supported with several lines of evidence including the fact that mature wolves tend to be the territory holders and thus are the ones killed by other wolves; and killings are concentrated in the few months before and after the breeding season (Mech and Boitanni, 2003).

Wolves will also interfere with the reproductive increase of a neighbouring pack thereby reducing the effect that the neighbouring pack has on their food supply and thus on its spatial needs.

2.4 Relatedness Among Pack Members

Pack members from neighbouring packs can be related and is increasingly more likely the closer each pack is to one another. It is a results of the constant budding and splitting process that packs undergo and their attempts to fill in interstices among pack territories. There have been examples of wolf packs getting on however they are usually related. Mech (1987) found that a male wolf that was able to exist between a natal pack and a neighbouring pack over a 20 month period finally moved into the neighbouring pack and paired with a female there. The constant churning of a population resulted from strong competition and intra-specific strife and the immigration of dispersers from distant populations ensures a certain degree of genetic diversity.

2.5 Factors Affecting Sociality

There are a number of factors effecting wolf sociality in the wild that need to be considered when analysing wolf sociality in captivity; Mech and Boitanni (2003) argued that the two main factors affecting wolf sociality are those that are social and physical. Packard and Mech, 1980 argued that we must understand the variation on both aspects of their environment. Wild wolf packs like in a stimulating environment subject to environmental variation due to ecological cycles due to the changes in climate and predator to prey populations dynamics (Mech and Boitani, 2003). Social aspects include conspecifics such as the members of their own pack, other territorial packs and lone wolves in the pack. Physical are abiotic factors such as weather and

landscape as well as biotic factors that are not other wolves in their environment for example prey species and animals that threaten wolves (Mech and Boitani, 2003)

2.6 What is Socialisation?

Socialisation is where the animal subject becomes accustomed to human presence and interaction, by working with them from birth or just after (Woolpy and Ginsburg, 1967; Aitken, 2004) aiming for the critical period of less than 10 days old (Klinghammer and Goodmann, 1985) to attain the best results for fully socialised wolves (Kleiman, 2011). This will help to speed up the taming process by abiding by the conditions of filial imprinting (Kleiman et al., 1996). Hediger (1984) explained how wild or older animals will be harder to tame by comparison to younger ones due to their previous negative experiences or due to the absence of humans during their sensitive period for socialisation (Woolpy and Ginsburh, 1967; Kleiman et al., 1996).

Mech, 2012 stated that the "affectional" tie wolves have with one another is the same as a dog and their owner and is a trait that dogs have retained. In addition to this Mech argued that Woolpy and Ginsburg's work on socialising wolves to comparing it to two strange wolves forming a bond

Why do we not socialise all animals in a zoo environment?

Hediger (1964) defined taming as to have no flight tendency with respects to man. In 1965 he then went on to say how humans could be significant to animals in five different ways: as an enemy (that is, to be avoided), as prey, as a symbiont (that is, as a partner working towards a common goal), as of no consequence (that is, part of the background to be ignored), as a conspecific (for example, a rival or a sexual partner, both of which would cause problems) (Hosey et al., 2013). Whether the animal perceives us as an enemy is the one that impacts most on welfare (Hosey, 2013)

Kelling et al. in 2013 stated that due to our lack of understanding of the effects socialisation has on adult social behaviour the practice is generally avoided however, it is sometimes necessary for example when a mother abandons their young and they have to be hand raised.

One of the major arguments against socialisation is that it prevents the reintroduction of that individual back into the wild. Zidon et al., (2009) did a study in zoos that were reintroducing Persian fallow deer (*Dama mesopotamica*) to wild. They found that despite the fact that deer from zoos with high or low visitor numbers both acquired

predator-avoidance behaviours post release those that came from zoos with higher visitor numbers did not survive past 200 days after release whilst 80 per cent of the animals from the zoo with low visitor numbers survived

Alternatively if Hedigar's idea 'as of no consequence' is true, that in fact the animals are simply habituated to zoo visitors. For example Margulis et al., 2003 studied six species of felid in seven different enclosures and found that visitors caused no significant difference on any of the cat's behaviour. If this is the case then the whole socialisation process is meaningless. However this response could be taxon-specific as primate studies have attained different results Margulis et al., 2003. Animals that are able to understand human cues are more likely to react which has been shown to be true in wolves (Udell et al., 2008). Anderson et al., 2002 showed how sheep and goats in a petting zoo show aggression or avoidance due to their perception of us as predators.

2.6.1 Non-human animal perception of humans

Humans are necessary for zoos as they arguably help to fund conservation projects around the world. Early studies on primates have shown that activity increase with visitor numbers (Hosey and Druk, 1987). Mitchell et al. (1992) argued that it could simply be the more active animals attract large groups of zoo visitors. Hosey (2000) consequently has referred to the visitor effect hypothesis and visitor attraction hypothesis as alternative explanations of zoo animals and their audiences. Much of the research on primates fits in well with the visitor effect hypothesis. Margulous et al., 2003 showed that small cats attracted more human audiences with increased activity and appeared to be unaffected by the human presence which coincides with Hosey's second hypothesis detailing visitor attraction.

2.6.2 Negative effect of humans on zoo animals

Most research done in this area has been done on primates and an overwhelming amount of evidence points to the fact that the effect is generally negative, exhibiting behaviours associated with the stress response posing a welfare concern (Hosey, 2000; Hosey, 2013; Hosey et al., 2013). Berk (2002) found that Orang-utans (*Pongo spp.*) would physically react to noisy visitors by covering their heads with paper sacks. In addition to this stress from handling can potentially effect the mother-infant relationship (Kleiman et al., 1996). Assessing physiological responses have shown that

urinary cortisol increases correlate across to increases in visitor numbers (Davis et al., 2005); faecal cortisol increases with visitor number (Rajagopal et al., 2011); and there are correlations between cortisol in the saliva and stress induced by zoo visitors (Monatanha et al., 2009).

2.7 What are the positives to the socialisation of zoo animals?

Hosey et al., 2013 argued that keepers who have a permanent positive relationship with the individual animal would in kind prove to produce positive levels of welfare. Klinghammer and Goodmann (1985) denoted the advantages of socialising wolves in particular includes a reduction in fear to humans which enables them to behave and interact with each other in a way that allows for the expression of their natural behavioural repertoire, displaying natural hunting and social behaviours. That routine medical care and other handling procedures can be accomplished without trauma which equates to less disruption, especially in regards to the social order of wolves. This was seen as well where abyssinian colobus monkeys (*Colobus guereza*) received positive reinforcement training. Their interactions with zoo visitors declined once they were trained to undergo oral examination. This could be a sign that they have simply become habituated to the stimulus (Melfi and Thomas, 2005).

2.7.1Can zoo visitors be enriching?

It is important to try and make the interaction between zoo visitors and the non-human animals less stressful (Fernandez et al., 2009). The idea that zoo visitors might be enriching was most likely first posed by Desmound Morris in 1964 (Hosey et al., 2013). Morris argues that zoo animals were probably bored most of the time and zoo visitors probably acted as a form of enrichment in what was otherwise a monotonous environment. However, evidence in favour of this theory is sparse. Fa (1989) failed to find any increases in agonistic behaviours on days when the zoo was open by comparison to when it was closed in green monkeys (*Chlorocebus sabaeus*). Instead they spent most of the time trying to get food from the visitors which they argues could mean that they were enriched rather than stressed (Cook and Hosey, 1995; Claxton, 2011). However Fa (1989) disputed that these behaviours were expressed at the expense of others such as social behaviours. Orang-utans (*Pongo Pygmaeus*) showed no stress-related behaviours in response to visitor presence, but did start begging when visitor numbers reached about forty at the exhibit (Choo et al., 2011). Inter-specific interaction for example in Miller et al., 2011 found that the bottlenose dolphins exhibited a greater diversity of behaviours that included diversity of swimming styles, and play behaviours after taking part in shows and interaction events which implied that the opportunity to take part in these shows was enriching for the animals. Unfortunately however the effect that interactive shows has on the welfare on animals has not been so thoroughly investigated (Hosey et al., 2013).

2.8 How can we overcome the drawbacks of socialisation?

Klinghammer and Goodmann (1985) acknowledge that there are a number of drawbacks of socialisation such as they are unable to be reintroduced to the wild and they may become too attached to people to the point where they are no longer able to express their normal behavioural repertoire however with a strict socialisation process which acknowledges the critical period and milestones in their development (denoted in their 1985 text on the socialisation of wolves) that these are overcome due to the fact that the animals are relaxed and not readily spooked by human activity both inside and outside of their enclosure.

2.9 What are abnormal behaviours?

A captive environment can cause a range of behaviours not found in the wild (Kleiman et al, 1996). Appleby et al., (2010) defined abnormal behaviours as the performance of behaviour patterns or sequences that differ fundamentally from the behaviour of free-living animals. The term abnormal literally means to stray from the norm and can be associated with a reduction in the animal's well-being (Mason and Latham, 2004), however, as Dawkins (1980) rightfully pointed out the word 'abnormal' is an emotional loaded term and is usually used to denote suffering which is not always the case. When recognising abnormal behaviours the differences from the desired 'norm' could be to do with the form of the behaviour (intensity, variability or orientation). The most commonly described abnormal behaviour are those that are stereotypic (Clubb and Mason, 2003).

2.10 Stereotypies

There are three main causal factors of stereotypic behaviours (Mason and Turner, 1993; Würbel, 2003): the first is sustained eliciting stimuli and is possibly in combination with the mechanisms of habit formation (Mason and Turner, 1993), the second is progressive pathological changes in the neural substrates underlying behavioural control which leads to 'perseverative behaviour' (Garner, 2006), and

finally possible reinforcement through reward in the form of some coping effect (Würbel, 2006).

2.11What can effect abnormal behaviours?

Genetic aspects such as inner ear damage in mice that resulted in circling behaviours (Lee et al., 2002)) and physical such as lesions that can cause limping (Weary et al., 2006). Abnormal behaviours can also result from the animal simply trying to adapt to their environment where their natural behaviours have been compromised or thwarted as is the case with pacing in a captive environment. Pacing itself can be seen as an attempt to express territorial behaviours however by looking at its variability you can explore whether it can be defined as stereotypic (Clubb and Mason, 2007).

2.12 What effect does socialisation have on abnormal behaviours?

There are a number of studies that have explored visitor effect on zoo animals however in terms of abnormal behaviours Mallapur et al., 2005 found up to 20% increase in the abnormal behaviours in the lion-tailed macaques (*Macaca silenus*) which shows that visitors have an effect on the frequency of abnormal behaviours. However it is important to consider individual animals and how their behavioural reactions to zoo visitors may differ from what these studies found (Hosey, 2008 and Koohe, 2008).

2.13 What are agonistic behaviours?

Agonisitc behaviours are to do with social competition (Mech, 2012) and includes the forms of aggression (Goodmann et al., 2002), displayed as subcategories in the ethogram (Appendix A). Schenkel described three levels of expression: first the peripheral structure of the body such as the face and scent organs; second the "nondirected" behavioural changes e.g. erection of hair, changes in breathing rate and reaction of the pupils; and finally the third level where social behaviours are clearly directed toward another wolf, and that may involve reaction, social exploration and social impression all at the same time for example threat, bluff attack, and invitations to play Mech (2012). Schenkel's first level can be utilised as an indicator of social rank when observing wold packs (see figure 2 and 3).

The wolf's most important visual expression centre is the head highlighting that the colouring of the face and function of the facial muscles and, also, the activity of the eyes, are the bearers of extremely important and variable expression phenomena

during wolf social interactions (Schenkel, 1947) for example it can be utilised to express the varying degrees of dominance (see figure 2).



Figure 2: facial expression in the wolf (taken from Mech, 2012)



Figure 3 Tail position communication (taken from Mech's 2012 text)

2.14 What can effect agonistic behaviours?

There are a number of different factors that can affect the expression of agonistic behaviours of grey wolves to which have been identified via the behavioural categories found in the ethogram in appendix A. First of all with elicited aggression it typically occurs when a subordinate, often young, wolf spontaneously submissively approaches a more dominant animal and is met with aggression. The aggression appears to us to try and cause the submitting wolf to flee however this just causes the submitting wolf to appease harder as if to try and stem the aggression. This results in the dominant wolf intensifying their aggressive display resulting in a positive feedback loop (Goodmann et al., 2002). Food-related aggression simply enough is an agonistic interaction initiated over the ownership of food resources (National Geographic Explorer, 1988; Mech and Boitanni, 2003) while sex-related aggression is much the same however it is usually between two males for a female in heat (Derix et al., 1993; Mech and Boitani, 2003). Ritualised aggression is characterised by fights that are restrained, or "ritualised" (Ridley, 1995). Fights are restrained and conventionalised, rather than allout fights with tooth or claw. Dominance aims at controlling another by the means of ritualised behaviour, without harm or injury to either involved (Abrantes, 2005). In contrast to this an all-out attack is the opposite to a ritualised attack, resulting in injury or harm to the subordinate wolf and is an example of aggression without dominance (Abrantes, 2005). An offensive threat is where an individual initiates an interaction so as to threaten to which no submissive or fearful behaviours are exhibited. If they are, they will be at a very low intensity (Ridley, 1995; Abrantes, 2005). Defence and submission pertains to the complex interactions exhibited between wolf individuals in terms of their standing in the pack described in great detail in the texts by Mech and Boitini (2003) and Mech (2012). This category aims to acknowledge pack status as well as variation in individual temperament before it can be identified as being ritualised in any sense of the term.

2.15 What effect does socialisation have on the agonistic behaviours?

Mitchell et al., 1992 found that human visitors induced gender-specific aggression directed at the zoo visitors themselves (golden-bellied mangabeys of Sacramento Zoo) and arguably perceive human audiences as rival conspecifics. Chamove et al., 1988 found that agonistic behaviours increased while affiliative behaviours decreased in three different primate species when human audiences were present and Glatston et

al., 1984 and Mitchell et al., 1991 attained similar results. A far more recent study by Wells (2005) discovered that the gorilla (*Gorilla gorilla*) intra-specific aggression and abnormal behaviours increased with increased visitor numbers in the summer by comparison to lower visitor numbers in the winter which shows that despite the fact that a lot of the research done in this area is old it still holds some validity in terms of that humans do have an effect on the agonistic behaviours of animals in captivity.

3.0 Methodology

The study was carried out at are the UK Wolf Conservation Trust for the examples of socialised wolves and Longleat Safari Park for the non-socialised wolf examples. The total observation period for each institution was dependent on the number of wolves that they have.

Institution Observation Period

 $= \frac{(Mean Focal Period \times Number of Wolves in Institution)}{Time for Observation Period per Day}$ + 1 day $= \frac{(6 \times Dependent on Institution)}{8}$ + 1 day (Habituation Period) UK Wolf Conservation Trust = $\frac{6 \times 10}{8}$ + 1 = 8.5 = 9 days observation period Wildwood Trust = $\frac{6 \times 5}{8}$ + 1 = 4.75 = 5 days observation period

Cotswold's Safari Park = $\frac{6 \times 4}{8} + 1 = 4$ days observation period

Based on the approaches to the observation of animal behaviour described by Martin and Bateson (2007) the wolves were observed using a focal sampling method, utilising continuous sampling as the recording method to help alleviate the chance for missed behaviours (Birkett and Newton-Fisher, 2011).

The observation period was 2 weeks; 1 week for socialised and 1 week for nonsocialised. It occurred between 9am and 5pm as these are the typical opening hours for zoos (Mallapur and Choudhury, 2003) which has the dual benefit that it is easier for the institutions to facilitate this approach and the wolves behaviour can be observed whilst being induced to a stressful stimulus (Hosey, 2000). Similar to the methodology presented in the study on lion-tailed macaques by Mallapur et al. (2005) the day was divided into four two hour segments (Figure 4), observing all of the wolves in the institution over the two hour segment.



Each two hour segment was divided by the total number of wolves in each institution thereby identifying the focal period for each wolf.

$$Focal Period = \frac{Segment Time}{Total number of wolves in institution}$$
$$= \frac{2 hours}{Dependent on Institution}$$

As described in Mallapur et al.'s study the order was chosen at random at the start of each day, the order remaining consistent for all four segments in a day, they were simply reselected at the start of each day. By collecting the data in this way the total focal period for each wolf will be from fours parts of each day, which will help decrease the effect that time of day has on behaviour (Maia et al., 2012). Each focal sample looked at the wolves individually for a set amount of time with a handheld camera to follow them around as they move (O'Connor, Robertson and Kleindorfer, 2010). For example if there were 4 wolves then each wolf will be observed for half an hour over the two hour period (figure 5).

$$Focal Period = \frac{Segment Time}{Total number of wolves in institution} = \frac{2 hours}{4} = 0.5$$



Figure 5 Focal Period Example

This was then repeated four times in that day making the total of 120 minutes of data for each wolf in this example for that day.

As described by Martin and Bateson the advantages of observing the wolves in this way include the fact that it provides an exact visual record; behaviour can be analysed many times in different ways; for analysing code behaviour i.e. transcribe into quantitative measurements relating to specific behavioural categories and finally it is a good medium for fast or complicated behaviours. Prior to each focal sample details of which enclosure and wolf were noted down, as well as the conspecifics present in the enclosure at the time. A full description of each enclosure and wolf were written separately prior to the observation at each institution. For the enclosure the size and enrichment devices were all described and for each individual wolf their age, gender and subspecies were noted. At the start of each observation each enclosure, environmental factors such as weather and temperature, wolf and segment were identified and spoken as an oral documentation to label what has been filmed. This can then be referred to when analysing each wolf and when acknowledging any of the possible variables present such has environmental enrichment (shown to be directly proportional to standards for welfare (Mollá et al., 2011)) or environmental conditions (see Morgan and Tromborg, 2007).

Once the data has been obtained the filmed data for each behavioural category (described in the ethogram) will be analysed manually, allowing for each behaviour observed and the length of time that it was expressed to be noted down. The time budgets for each data set will be calculated and due to the fact that it is continuous data and that there are 2 samples the t-test can be used (Townend, 2002). This parametric statistical test can be used so long as the data meets the requirements of parametric

data of a normal data distribution (illustrated by the bell curve after data transformation). Conversely the Mann Whitney non-parametric test can be used if the data does not coincide with the parameters of normal data distribution.

The approach to this study is non-invasive in nature due to the fact that the wolves were simply observed through a fence in a similar manner to how they would be during open hours by the public. However, as has already been stated, there are a number of studies that show that public presence can induce stress in animals. In light of this in accordance to the parameters set by Mitchell and Hosey (2005) an extra day was added to the observation period for each institution to allow for an habituation period. This allowed for the animals to have the opportunity to desensitise to observer presence thereby lowering the stress invoked (International Society for Applied Ethology, 2013). As such this approach to the topic meets the parameters of both the 3Rs (Replacement, Reduction and Refinement (2004)) and the Animal Welfare Act of 2006 for the UK.

4.0 Results

4.1 Agonistic Behaviours

A Kolmogorov-Smirnov test was conducted on all of the data to be statistically analysed to test for normality (Townend, 2003) for which it was deduced that the data is parametric (see each behavioural statistical assay appropriately mentioned below). The normal distribution illustrated by the bell-curve and the unrelated nature of the two samples socialised and non-socialised meant that the statistical test that would best fit the data would be the independent T-test.



Figure 6 Mean Defence/Submissive Behavioural Differences between Socialised and Non-Socialised Wolves

Due to insufficient data for the behavioural categories Elicited Aggression, Food-Related Aggression, Sex-Related Aggression, All-Out Attack, Offensive Threat, and Ritualised Aggression a behavioural comparison between socialised and nonsocialised wolves could not be conducted. The behavioural category Defence and Submission however contained sufficient data for statistical analysis which are illustrated by the descriptive statistics seen in figures 6 and 7 drawn from the mean values from that raw data found in Appendix B. Figure 6 illustrates the duration of time spent on each of the behaviours actually expressed during the observation period for this behavioural category. According to this chart socialisation does not appear to have any kind of strong overall effect on this behavioural category of agonistic behaviours. Socialisation appeared to have no real effect on creeping behaviours (P = 0.452 (Appendix C), the duration the wolves spent with their ears back during intraspecific agonistic social interactions between conspecifics (P = 0.762 (Appendix D), the submissive behaviour of licking the muzzle of a dominant wolf (P = 0.366(Appendix E), the dominance behaviour to stand tall while interacting with a submissive wolf (P = 0.452 (Appendix F), the T1 tail position (P = 0.366 (Appendix G), T2 (P = 0.366 (Appendix H), and whimper (P = 0.927 (Appendix I). Figure 9 shows the mean value of the T3 tail position for socialised and non-socialised wolves however despite this large difference these results were inconclusive (P = 0.366(Appendix J).



Figure 7 Mean Difference for T3

4.2 Abnormal Behaviours

Of all the abnormal behaviours denoted by this category pacing behaviours were the only ones that were able to be statistically analysed due to insufficient data from the other behavioural types. As displayed by the error bars in figure 8 however despite a clear difference between the duration of pacing between non-socialised and socialised wolves the results are inconclusive (P = 0.09 (Appendix K)).



Figure 8 Mean Differences in Abnormal Pacing

5.0 Discussion

With consideration to the results attained the alternative hypothesis will be rejected and the null hypothesis describing that socialisation will have no effect on the agonistic and abnormal behaviours of the grey wolf in captivity can be accepted.

Despite the fact that the abnormal stereotypic behaviour pacing displayed substantially different results (illustrated by figure 10) the t-test showed that there was no significant difference between the two data sets.

Sands and Creel (2004) showed that subordinate wolves exhibited greater levels of glucocorticoid secretion which has been shown to relate to levels of stress. The fact that most data was found in the behavioural category defence/submission could signify that these wolves are being induced to high levels of stress. This could be due to a number of causal factors such as interspecific pressures from the general public observing the animals or intraspecific factors such as conflicts between conspecifics.

Insignificant results could be due to the small sample size and observation period however it could also indicate that as described by Hedigar that wolves are an example of 'as of no consequence'. That is regardless whether the individual is a member of the public or a keeper working with a fully socialised animal that they have no real effect on the behaviour of the wolf in captivity. If socialisation has no effect on the agonistic and abnormal behaviours of the grey wolf in captivity then this in itself could be considered neutral in terms of the animal's welfare as it neither increases or decreases time budgets for agonistic or abnormal behavioural expressions.

5.1 Limitations

There were a number of limiting factors such as financial constraints that restricted the size of the study. With funding the number of institutions included would increase thereby increasing the sample size and perhaps yielding more conclusive results. Increasing the sample size could also help to control other variables such as gender, subspecies and age. With the bigger sample size the wolves could be divided into groups according to these factors to help better control them and assure that socialisation is the only variable that can affect the results.

The structure of the ethogram itself requires some experience as distinguishing as to which category a behaviour falls into can be difficult at times which can skew the results. For example the tail positions one to four can express the spectrum of confident to submissive respectfully however all of them fit into each behavioural category. Tail position itself can also only really be analysed when comparing it to the tail position of a conspecific. A T2 tail position usually signifies confidence especially if next to a wolf that is expressing a tail position T3 or lower however if the wolf expressing T2 is interacting with a wolf expressing T1 then the T1 wolf could be viewed as far more confident.

5.2 Improvements

Incorporation of inter-observer reliability can help to prevent the effect of observer bias and combat the interpretative nature of the ethogram. Increasing the number of zoos into the study will increase sample size however respectively funding will need to be granted. Alterations to the methodology would include having an individual observer per wolf so as to not miss any significant behaviours which would enable each focal sample to last the entire duration of the observation period rather than having to be shared across all wolves.

6.0 Conclusion

With respects that the results attained over the course of the study it would appear that socialisation does not have an effect on the duration and intensity of agonistic and abnormal behaviours of the grey wolf (*Canis lupus*) in captivity. As such due to the fact that it does not seem to incur a change with regards to the specific behaviours studied the socialisation of wolves does not appear to cause any real welfare concerns. However, in order to attain a truly comprehensive and conclusive analysis of the effects that socialisation has on the behaviours and complex social structures of the grey wolf further research would need to be conducted with increased sample size and control over the confounding variables in order to attain a truly comprehensive understanding into the effects of socialisation.