The effect of aromatherapy scents on 5 key behaviours of captive pairs of grey wolves (*Canis lupus*).

Name: **Megan Webb**
Student number: **10261727**
Degree: **BSc (hons) Animal Behaviour and Welfare.**
Year: **4**
Faculty of science and environment, university of Plymouth
Project advisor: **Sarah Collins.**

Contents

1: Abstract:

Olfactory enrichment is the administration of scent or a scented material into the environment. The use of olfactory enrichment can improve captive welfare of mammals and has a variety of applications in captive animal management. This study explores the effects of 3 aromatherapy scents (tangerine, peppermint and rosemary) as olfactory enrichment on 5 key behaviours of 4 captive grey wolves housed at the UK Wolf Conservation Trust. The wolves were exposed to each of the scents repeatedly over a period of weeks. Behavioural observations were made every day and scents administered twice a week. The scent was administered via a dilute solution poured over a straw bail. The aromatherapy oils were diluted with water to a ratio of 1:40. The study looks at 5 key behaviours. Vocal, play, rest, sleep and patrol. There was no significant difference between the conditions on sleep, play and patrol behaviours. The results showed a clear significant effect on rest ($P = <0.01$) and vocal ($P = 0.013$) behaviours. Tangerine oil was shown to have a relaxing effect showing a sharp increase in rest behaviours when the scent was administered whereas rosemary showed a slight decrease in rest suggesting a stimulant effect. Exposure to peppermint had little or no effect when compared to control conditions. The results highlight the potential for aromatherapy use for behavioural therapy in captive grey wolves. There is more research required using a larger sample size and a variety of scents in order to gain a definitive picture of what the scents clear effects on behaviour are.

2: Introduction:

Mammals use olfaction in a number of ways for group survival, including communication, feeding, spacing, reproduction, defence and identification and recognition of other individuals (Doty 2012, Doty 1986, Eisenberg and Kleiman 1972). There are a vast number of areas of research involving the effect of scents on behaviour. Studies on the effect of predator scents on captive prey animals are used to understand the induction of fear as a direct effect of scent (Stankowich and Blumstein 2005) and develop stress prevention strategies for captive animals. There are also many studies on physical benefits of scents for humans in order to develop holistic therapies. The benefits of scent have been outlined in a study where the presence of aromatherapy massage showed a significant increase in peripheral blood lymphocytes suggesting that aromatherapy massage could be beneficial in disease states that require augmentation of CD8 lymphocytes (Kuriyama et al 2005).

However, this study will be investigating the effect of aromatherapy scents as environmental enrichment on captive wolf behaviour. Environmental enrichment is described as an animal behaviour principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being (Shepherdson, 1998). The topic of scent enrichment in zoo settings has been thoroughly explored with frequent studies on big cats (Baker,Campbell and Gilbert 1997, Schuett and Frase 2001, Wells and Egli 2004).

Olfactory enrichment is used in many captive scenarios (Ellis 2009, Wells 2004).
This ‘olfactory enrichment’ can stimulate reproduction or naturalistic behaviour, enhance

enclosure exploration, or reduce inactivity (Clark and King 2008). However this 'enrichment' can also increase undesirable behaviours or reduce activity. Reduction in activity can be used to calm very lively animals or reduce stress in transit or medical situations but this can also increase lethargy and reduce visitor enjoyment in zoos (Ryan and Saward 2004). It is important to know what messages these scents are conveying to the animal. Different scents must be chosen carefully taking into account the social and ecological contexts. For example, a study on golden lion tamarins found that scent marking was used to communicate social dominance (Miller et al 2003), whereas another study on alpine marmots showed that scent marking was used in territory defence (Macdonald 1980). Many animals use scent to communicate so it is important to take into account what the animal in question translates the scent as.

Animal sensory systems are typically specialized by species and play crucial roles in their survival. Sensory enrichment is designed to address the animal's sense of smell, touch, hearing, vision, and taste and elicit species-specific response, territorial, reproductive or hunting behaviours. Wolves are driven by their sense of smell, they can smell a scent more than 1 mile away, using olfactory signals to communicate with intra- and inter-specifics, locate prey, attract mates and/or find food (Macdonald and Sillero-Zubiri 2004). A wolfs' sense of smell plays a big part in the behaviour surrounding scent marking both toward other members of the pack and toward neighbouring packs (Asa et al 1990). The scents in their environment communicate information such as what has passed through their territory, how long ago, whether it was male or female. It also aids them greatly when hunting. Wolves tend to smell out their prey and then they can tell if it is injured or sick through olfaction.

Many of the behaviours that wolves express naturally are dependent upon scent (Briscoe et al 2002). However the manipulation of scent in the captive environment is not designed to communicate but to manipulate behaviours.

The use of aromatherapy as a behavioural therapy is used in a number of species in a variety of settings. There are many published sources that explore the effects of essential oils on human behaviour (Roberts and Williams, 1992, Herz 2009), particularly in the treatment of agitation in dementia. A significant improvement in agitated behaviour during aromatherapy compared with placebo has been shown to occur (Ballard et al 2002, Holmes et al 2002). The results of these studies show that aromatherapy for humans can be a safe, well tolerated and highly effective way of treating certain adverse behaviours (Herz 2009). This evidence of aromatherapy effects could give starting points to expand in the use of scent to manage harmful or distressing behaviours in animals. The use of aromatherapy in this case has been shown to calm agitation in humans; there are many captive situations which cause agitation in other, non- human species. These studies are a useful tool in the development of captive animal management systems.

Peppermint, jasmine and rosemary have been reported to improve alertness and enhance cognitive performance in humans (Diego et al 1998). The scent of lavender has repeatedly been shown to decrease motility in laboratory-housed rodents (Buchbauer et al 1992, Shaw et al 2007, lim et al 2005). The same herb has also been shown to reduce activity and vocalisations in dogs housed in rescue shelters (Graham et al., 2005a). These are behavioural changes suggestive of increased relaxation. This could provide a good tool

to offer welfare advantages for individuals in transit or quarantine. Peppermint oil and/ or rosemary (which belong to the same botanical family as catnip) have been found to increase the activity level of many species including dogs (Graham et al., 2005a). These can be useful to stimulate lethargic animals that have been housed in captivity for lengthy periods of time (Wells et al., 2009). Other scents have shown aphrodisiac effects in felids e.g. chilli, cinnamon, cumin, nutmeg and ginger have all shown effects that increase breeding behaviour (Schuett and Frase, 2001, Wells and Egli 2004). This could be useful for captive breeding programmes, particularly with species that do not have high captive breeding success rate.

The two packs observed in this study were very similar in set up and subspecies. The other two packs include different subspecies (arctic) and social groups. They are two packs of three siblings. The behaviour of these differs greatly of the other two. I chose to test the effect on pair packs. This is because there is less complex social behaviours. The behaviour of the two packs I studied was established and settled. The wolves were all of sexual maturity and had established mating relationships. The other two packs are both still in their juvenile years (just under two years). The arctic pack was raised by a surrogate dog which resulted in unpredictable behaviours not ‘typical’ of other captive wolves.

Historically, essential oils are best used in the form of massage or bath oils or inhalations (Thomas 2002). In the setting of captive wolf environmental enrichment the scent will be inhaled. This can provide useful information for husbandry establishments with uses in breeding encouragement, calming, transit, veterinary care and welfare of the animal. The

scents peppermint, rosemary and tangerine have been chosen. There is little or no literature investigating the effects of tangerine oil on captive mammal behaviours. This scent was therefore chosen in order to explore new scents and their effects. The scents rosemary and peppermint were chosen due to the claims against their names as a stimulant.

There is a great deal of literature on the effects of aromatherapy oils as olfactory enrichment on the behaviour of domestic species such as dogs in kennel situations. (Graham al 2005, Bell 2002, Keith 2010 , Wells 2006) or the use of scent as a calming factor for horses (Ferguson et al 2013). However this study aims to explore the effects of captive wolves in a zoo setting. There are many studies in zoo settings (Wells et al 2004 and 2007, Struthers and Campbell 1996) exploring this on a variety of species but there is limited literature on the effects of olfactory conditions specifically on captive grey wolf behaviour.

This study aims to expand the knowledge gained from studies on domestic dogs and captive big cats to the captive wolf. This study will investigate the effect of three aromatherapy oils on captive wolf behaviour measuring their effect on 5 key behaviours: play, sleep, rest, patrol and vocal.

3: Method:

3:1.Test subjects:

Data was collected from four wolves at the UK Wolf Conservation Trust.

Table 1- basic information of the four observed wolves.

Wolf Number	Name	Sex	Age (Years)	Socialised?	Genetic Origin	Place of Birth
1	Mosi	Female	7	Y	*Canis lupus occidentalis,* Northwestern wolf	Dartmoor Wildlife Park, 2006

2	Torak	Male	7	Y	*Canis lupus lupus* X *Canis lupis occidentalis,* European X Northwestern wolf.	Anglian Wolf Society, 2006
3	Mai	Female	7	Y	*Canis lupus occidentalis,* Northwestern wolf	Dartmoor Wildlife Park, 2006
4	Motomo	Male	5	N	*Canis lupus occidentalis,* Northwestern wolf	Combe Martin wildlife and dinosaur park. 2008

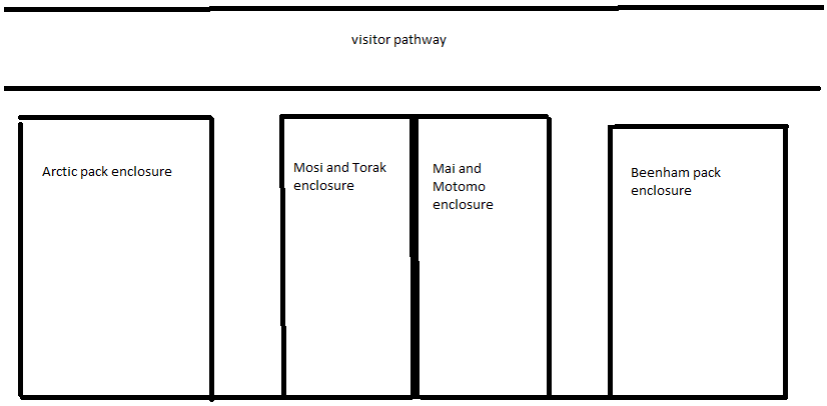
3:2. Study site:

All research for this study was conducted at the UK Wolf Conservation Trust. The 2 enclosures which housed the test subjects were almost identical. Mosi and Torak were housed together as a mating pair in an outdoor enclosure approx. 1.5 acres (approx. 6070 M_2). Mai and Motomo were also housed together as a mating pair in an adjoining enclosure of the same size. Both enclosures feature a viewing mound (a small hill for the wolves to gain visibility of the sight,) viewing tables for the same purpose, water troughs, a small area (approx. 150m2) of dense woodland and a shared indoor kennel for each of the pairs.

There were two more enclosures housing three sibling hybrids born of Mai and Motomo and three sibling Arctic wolves of no relation. These enclosures were in eyes view of the test subjects but not connected to.

The site has a path running along the front of the enclosures where visitors can clearly view the wolves. The enclosures have an area of dense woodland as well as an indoor kennel accessible at night time (see fig. 1).

Figure 1 – diagram of enclosures layout at UK Wolf conservation trust.



2:3. Materials:

- 10ml bottle tangerine pure essential oil (Holland and Barrett)
- 10ml bottle rosemary pure essential oil (Holland and Barrett)
- 10ml bottle peppermint pure essential oil (Holland and Barrett)
- Tap water
- Straw
- Raffia
- 4 different coloured pens
- Behaviour checklist
- Stopwatch

3:4. Olfactory conditions:

Four olfactory conditions were created, including a control, in which the wolves were exposed to no odours other than those arising naturally from their environment (e.g., odours from disinfectants and conspecifics), and three experimental conditions, in which the wolves were exposed to the ambient odours of essential oils including peppermint (*Mentha piperata*) rosemary (*Rosemarinus officinalis*) and tangerine (*Citrus reticulate*).

3:5. data collection

All data was collected over a total of 12 weeks. The three scents were administered each over a four week period:

Period one = peppermint Period two = rosemary Period three = tangerine

Each scent period spans 4 weeks. These weeks were inclusive of only Monday – Friday (5 days). The schedule is shown in table 2.

Table 2- breakdown of weeks within each scent period.

Week 1	Control observations (no scent given)
Week 2	Scent administered on day 2 and 4. Observations every day.
Week 3	Scent administered on day 2 and 4. Observations every day.
Week 4	Control observations (no scent given).

3:6. Scent administration:

A large handful of straw was gathered together and secured with raffia. Approximately 10 drops of essential oil (one of the three scents) were poured into a 400 ml plastic beaker and topped up with water. The solution was then poured all over the straw until saturated. The straw was shaken to allow excess water to drip off. The scented straw was then thrown over the fence into the enclosure. The initial reaction was noted and behavioural observations initiated.

3:7. Data collection/ behavioural observations:

Behavioural observations were done from a ‘visitor viewpoint’. This was done from behind the standoff barrier and at least 3 metres from the fencing. The wolves’ were observed using an interval scan sampling method. Data on wolves was recorded including names of wolves and miscellaneous data e.g. weather, public presence, feeding. Behaviours were then recorded for 30 minutes using a stop watch at 30 second intervals. Behaviours were recorded using a behaviour checklist and a different colour pen for each wolf. See table 3 for ethogram.

Table 3- table to show behaviours observed.

Behaviour	Description
Play	Either alone or with other members of the pack. Includes: darting,
Sleep	Stillness with head resting on ground or some sort of support. Eyes closed. Consistently still.
Rest	Laying or sitting with eyes open or eyes shut with consistent fidgeting or movement. Little or no other behaviours present.
S.A.B. (Scent associated behaviours)	Any behaviours incorporating scent. Scent, marking, sniffing, scratching and smelling, rolling in scent.
O.O.S. (out of sight)	Wolf cannot be observed. Hiding
Vocal	Howling, barking, chuffing, whining any vocal expression with purpose.
patrol	Moving around the perimeter in a repetitive or purposeful manner. Watching out upon raised hills and fixtures, surveying the environment with auditory and visual senses.

3:8: Statistical Analysis:

The data for each of the key behaviours was analysed for each of the olfactory conditions using Kruskal-Wallis non parametric test to test for a significant difference between the groups. Inspection of descriptive statistics tables were used to show extreme groups. Bar graphs were created for each behaviour to show differences between the conditions.

3:9:. Ethical statement:

All scents that were administered have a COSHH report and data safety sheets (see appendix 9:2, 9:3, 9:4). All essential oils were of natural source and non-toxic to the species. The way in which the scent was administered to the environment was an enrichment method the wolves are regularly exposed to and non-invasive.

4: Results

4:1: Play behaviour

There was no significant effect of the different olfactory conditions on play behaviour.

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4:2: Rest behaviour

There is a highly significant effect of olfactory condition on the frequency of rest behaviour *(H*11 = 255.42, *DF* = 3, P = < 0.001). There is a significantly higher percentage of time resting with the administration of tangerine oil into the environment than the other olfactory conditions. Presence of rosemary showed a decrease in percentage of time resting when compared with the control conditions. The results for Peppermint administration differed very little from that of control conditions.

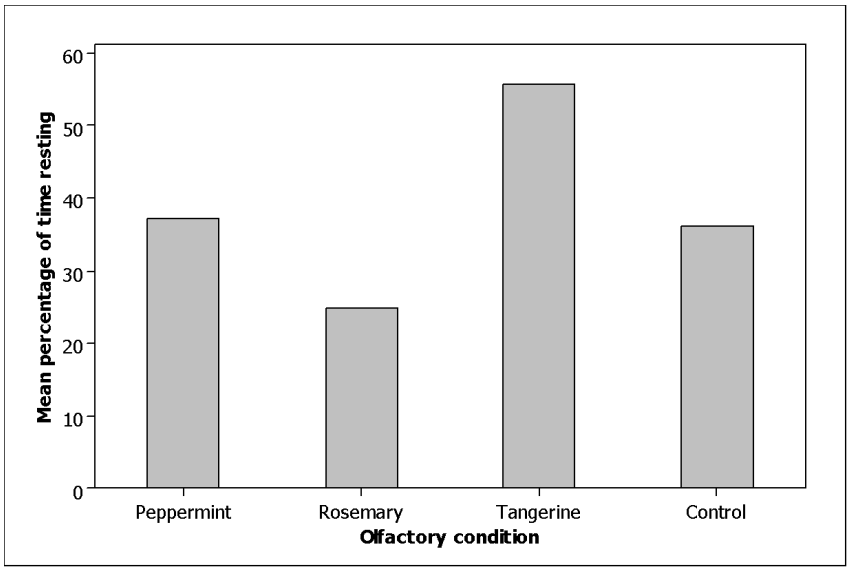

Fig 2: bar graph to show the difference in rest behaviours under different control

4:3: Sleep behaviour

There was no significant difference between olfactory conditions on the effect on sleep behaviour.

4:4: Vocal behaviour

There is a significant result of olfactory condition effects on vocal behaviour.

*H*11 = 117.7, *DF = 3*, *P* = 0.013 see fig 3.

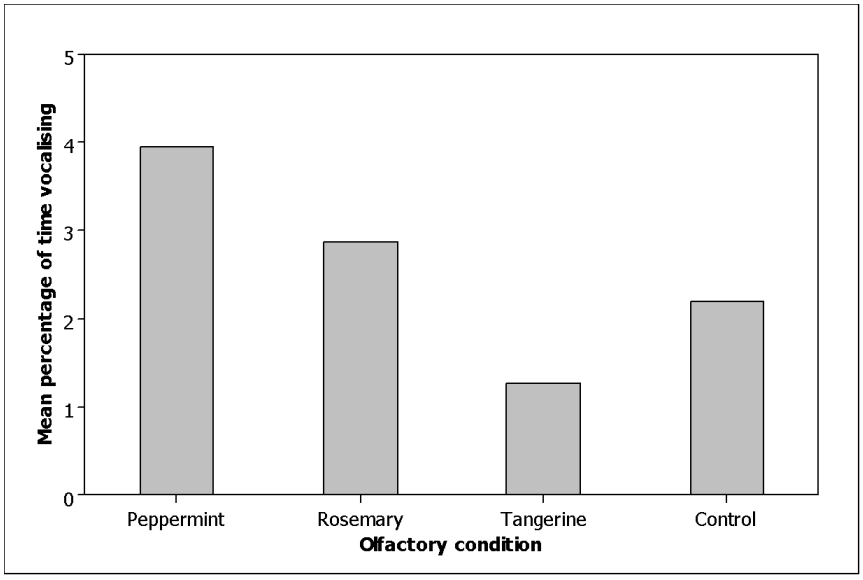

Fig 3: bar graph to show the difference in vocal behaviours under different controls.

Exposure to peppermint showed an increase in vocal behaviour when compared to the other olfactory conditions. Tangerine oil showed a decrease in vocal behaviour.

4:5: Patrol behaviour

There was no significant result regarding to the effect of olfactory condition on patrol behaviour.

5: Discussion:

The findings of this study indicate that the behaviour of the four captive grey wolves was altered after administration of tangerine and rosemary essential oils. The scents showed the most significant effect on rest behaviours with tangerine causing a sharp increase in resting behaviour, Vocal behaviour was also decreased after exposure to Tangerine oil and this scent also showed the lowest values for patrol behaviours. This indicates that tangerine oil has a mild sedative effect.

Exposure to rosemary reduced the amount of time spent resting and showed a slight increase in vocal behaviour. This indicates a stimulant effect of the scent in the environment on captive wolf behaviour. Vocal behaviour increased under peppermint conditions. The results of these two scents corroborate with findings in previous studies on the effects of peppermint and rosemary on active behaviours. Significant effects have been reported in studies on canines (Graham 2004) and big cats (Pearson 2002).

However peppermint conditions had little or no effects on all other observed behaviour's including patrol and play which are active behaviours. This could indicate that other factors could have accounted for the increase in vocalisation. At the UK Wolf Conservation Trust all but one of the wolves has been hand reared by humans and habituated to human presence. This can account for behaviours that are not naturally seen in the wild.

Howling for example is often started by environmental noises. Human influence, birds, dogs. The wolves often howl when the other packs are taken on walks. This is to communicate where the other packs are. They are also prompted to howl by a human howl for vocal

displays to the public. The wolves have also been observed whimpering or barking at human presence. Further investigation into this is needed to establish a definitive result on the effects on vocal behaviour. Stricter control conditions where these factors were removed would help to eliminate the possibility of human contact and activity influencing the results. A recent study on maned wolves showed that wolves that were housed in enclosures that had more human animal contact responded differently to enrichment than wolves that had less contact (Coelho et al, 2012). This indicates that different results should be expected depending on the animals' exposure to human contact.

Three out of the four wolves observed were classed as socialised (hand reared, desensitised to human contact) the fact that one of the wolves out of the sample was un-socialised (raised by wolves) may have affected the results as the behaviour of this wolf is very shy. His reactions to the scents were always delayed and more hesitant so this could mean he did not sniff the scent as much as the others.

The results of effect on sleep behaviours could also have other factors influencing them. Wolves like most species are constantly vigilant to their surroundings and due to the frequent coming and goings of humans in and out of their environment the wolves were observed always keeping watch over territories. This influence would seem to be too strong to override any effects of scent administration. Observations in a more controlled environment would increase validity of results.

Patrol, sleep and play behaviours did not show significant differences between olfactory conditions. A possible reason for this is that the wolves have a natural occurrence of rosemary growing in their enclosures. This could have habituated the wolves to this scent and reduced the experimental effects.

One of the main limitations of the study is the small sample size. The method of data collection along with daily tasks at the trust only permitted one hour of observations per day. Two packs were chosen to observe using scan sampling rather than all four of the UK Wolf Conservation Trust packs as this would only allow 15 minutes of observations a day. This would not have been enough time to get a varied recording of behaviours. The small sample size means that it is of less relevance to the global practice of keeping wolves' in captivity but has a significant relevance to the small number of socialised wolves in the UK and a high level of relevance to the UK Wolf Conservation Trust practice.

The wolves there have been raised there and will live out their lives there. The application of enrichment is explored thoroughly at the trust and new ways of calming or increasing behaviours will be a useful tool for the trust in their activities. The results from this research along with other similar studies such as Graham 2004 can be useful in a variety of captive scenarios. The reduction of rest could be used to motivate lethargic animals and to help reduce weight gain due to inactivity (Schwitzer and Kaumanns 2001). It could also have relevance in increasing activity for displays or visitor enjoyment. When veterinary inspections or capture is necessary it could be a useful tool to have a calmer animal and to reduce the stress on the animals themselves.

The UK Wolf Conservation Trust has a very popular open day three times a year which brings in as many as 3000 visitors throughout the day. Although the wolves are socialised and used to human contact the infrequency of large numbers of people appears to still be intimidating for them. This can cause a minor amount of stress to certain more shy wolves and essential oils enrichment could be administered on these days to help calm the animals. There is also now the introduction of an open day every Wednesday (2014). The frequency of these events will mean the numbers will unlikely reach the large numbers of the 3 annual open days but are still a larger number of visitors passing through than these wolves are used to. Again the administration of a tangerine enrichment could help to calm the wolves. There are also some situations where the wolves need to be transported to or from the vets. This is a stressful situation and could be reduced by giving scent.

There could also be limitations in the method of administering the scent into the environment. The scents were administered in an open obvious way to the wolves to the point where they anticipated its preparation and entry to enclosure. The straw bales were prepared in the wolves view and were administered by throwing it over the fence. The wolves were often waiting at the fence for it. This could prevent the observation of the true effects of the scent. It is a possibility that only the combination of novel objects and scent combined have these effects. A follow up study of administering the scent whilst the wolves were out of sight could give an indication of the effects on results of the visual and physical influence.

The social housing of the test subjects could have an effect on behaviour. The pair groups have the alpha male and female roles but it still differs greatly from a wild pack scenario. Captive research has limitations that increase if the group structure differs greatly from that

which is found in the wild (Erwin et al.1979; Sadler & Ward 1999). The lack of a 'natural pack scenario' means that these wolves are not likely to have the same behaviours as a wild pack or even that of a separate pack in captivity. Wolves' social structure has a strong influence on behaviour so the results of these studies must not be generalised to all captive wolves. The results may only be relevant from this perspective to established breeding pairs.

The statistical analysis methods used are a good indicator of differences between samples. However, the Kruskall Wallis test has limitations (it only shows that there is or isn't a difference in groups, it doesn't show which groups or how much). More specific studies between scents and behaviours would provide a more detailed result.

Future research should focus on a more specific experimental design with a view to testing the application of aromatherapy oils in real scenarios. The development of holistic therapies for captive wolves would be aided by further research on the physiological effects of these scents. To see for example, if tangerine oil reduces adrenaline. This needs to be done to further prove that an effect is made and this would aid further understanding of the true effects of essential oils and why they alter behaviour. This would give rise to a new way of application and give medical reliability.

A larger array of scents should be tested and on a large sample. A collaboration with other UK zoos that house wolves would enable a larger scale study to prove the widespread relevance of the subject. The results could give useful results to all establishments and provide a national standard for enrichment and welfare implications of scent administrations in captive wolves and species with a similar olfactory system and use of scent.

6: Conclusion:

Overall, the results of the study suggest that captive wolves can be influenced by the scents in the environment. And that certain scents have a greater effect than others. The captive environment for a an apex predator such as the grey wolf can be a very challenging environment and enrichment involving calming or relaxing scents should be considered but chosen with reference to the species in question in order to reach a comfortable balance between the effect on behaviour and the species ability to display natural behaviours. The studies objectives were to find ways in which the use of olfactory enrichment can be used to enhance the welfare of captive wolves and to aid management techniques. The study has resulted in some significant effects and given many suggestions for future research in order to better understand the effects and potential use of aromatherapy in captive carnivore management. However the sample size and influencing factors give much limitation to the scope of the study's findings.

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