Department of Physics, Chemistry and Biology

Master Thesis

Behavioral responses of Amur tigers (*Panthera tigris altaica*) and Bush dogs (*Speothos venaticus*) to the blood odor component 2-pentylfuran

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Sammanfattning/Abstract:

Mammalian carnivores use the odor of blood to home in on wounded prey, but it is unknown if a single component of blood odor can elicit the same response as the whole mixture of components comprising the odor of blood. In this study Amur tigers (*Panthera tigris altaica*) and bush dogs (*Speothos venaticus*) were presented with wooden logs impregnated with either the odor of real pig blood, a blood odor component (2-pentylfuran), a fruity odor (isopentyl acetate) or a control odor (diethyl phthalate), and both the frequency of occurrence and duration of interactions with the logs were recorded. The tigers did not show any significant differences in their interactions with the different odors due to the generally low number of interactions. The bush dogs, however, showed a significantly higher interest in the real pig blood compared to the other three odor stimuli. The bush dogs also showed a much higher number of interactions with the odorized wooden logs per animal than the tigers. There were no significant differences for either of the two species on the time they spent per interaction for the four different odors. The high interest that the bush dogs displayed towards the odor of real pig blood suggests that they may perceive this odor as prey-related and it could therefore be beneficial to use real blood as enrichment to these captive carnivores.

Nyckelord/Keyword:

Tigers, Bush dogs, blood odor component, enrichment, olfaction, behavior

Contents

1. Abstract

Mammalian carnivores use the odor of blood to home in on wounded prey, but it is unknown if a single component of blood odor can elicit the same response as the whole mixture of components comprising the odor of blood. In this study Amur tigers (*Panthera tigris altaica*) and bush dogs (*Speothos venaticus*) were presented with wooden logs impregnated with either the odor of real pig blood, a blood odor component (2-pentylfuran), a fruity odor (isopentyl acetate) or a control odor (diethyl phthalate), and both the frequency of occurrence and duration of interactions with the logs were recorded. The tigers did not show any significant differences in their interactions with the different odors due to the generally low number of interactions. The bush dogs, however, showed a significantly higher interest in the real pig blood compared to the other three odor stimuli. The bush dogs also showed a much higher number of interactions with the odorized wooden logs per animal than the tigers. There were no significant differences for either of the two species on the time they spent per interaction for the four different odors. The high interest that the bush dogs displayed towards the odor of real pig blood suggests that they may perceive this odor as prey-related and it could therefore be beneficial to use real blood as enrichment to these captive carnivores.

2. Introduction

Olfaction is used by a variety of carnivore species to localize and hunt prey (Lonnstedt *et al*. 2012), and mammalian carnivores use the odor of blood to home in on wounded prey (Stoddart 1980). The sense of smell is especially important for carnivores, like tigers (*Panthera tigris*), that live in areas of dense vegetation where visual cues are limited to locate prey (Schaller 1967; Brahmachary and Dutta 1987; Barja and de Miguel, 2010). In turn, it has also been shown that blood from conspecifics may serve as a warning signal and elicit vigilance and flight behaviors in mammalian prey species like rats (*Rattus rattus*) (Hornbuckle and Beal 1974; Mackay-Sim and Laing 1981; Stevens and Saplikoski 1973), mice (*Mus musculus*) (Sandnabba 1997) and cattle (*Bos taurus*) (Terlouw *et al*. 1998). It has also been shown that Mongolian gerbils (*Meriones unguiculatus*) avoid areas containing blood from stressed conspecifics, while domestic cats (*Felis domestica*) prefer food that has been contaminated with the same blood (Cocke and Thiessen 1986). March (1980) also found that bears (*Ursus arctos*) displayed aggressive curiosity and attack behaviors when presented with human menstrual blood, while deer (*Capreolus capreolus*) exhibited avoidance. The same response was obtained by Nunley (1981): when he presented male venous blood to domesticated white-tailed deer (*Odocoileus virginianus*) indicating that the deer responds with avoidance behavior to human blood in general.

However, blood from predators or conspecifics is not the only body-borne odor that has been shown to cause prey species to display fear or avoidance behaviors. One study found that foxes (*Vulpes vulpes*) produced a volatile chemical, 2,5-dihydro-2,4,5-trimethylthiazole, that was found in their fur, urine and feces (Ferrero *et al*. 2011), and this chemical caused mice to display aversive and fear-related behaviors.

Despite the research performed on blood odors and the assumed importance that it plays in predator/prey relationships, little is known about the different volatiles that make up the odor of mammalian blood, and even less is known about which of these volatiles cause behavioral responses in predators and prey species. However, recent studies identified components that evoke a "blood-like" smell in humans (Buettner and Schieberle 2001; Konopka and Gorsch1991). One of the components found was 2-pentylfuran. This component has been found in mammalian blood odor (Rachamadugu 2012) and in the odor of meat in cattle (*Bos taurus*) (Boylston *et al*. 1996; Calkins and Hodgen 2007), pig (*Sus scrofa domesticus*) (O'Sullivan et al. 2003), sheep (*Ovis aries*) (Wilches *et al*. 2011), goat (*Capra aegagrus hircus*) (Madruga et al. 2000) and ducks (*Anas platyrhynchos*) (Soncin *et al*. 2007). 2-pentylfuran has been described by humans to have a "blood-like, metallic and meaty" odor and humans have been shown to be highly sensitive to this odorant, having an olfactory detection threshold of this component at 27 ppt (parts per trillion) (van Gemert 2011).

The species under consideration in the present study are Amur tigers (*Panthera tigris altaica*) and bush dogs (*Speothos venaticus*). The sense of smell is highly developed in these carnivores and they use this sense not only for foraging, but also for social communication (Poddar-Sarkar and Brahmachary, 1997; Porton 1983). These two species, as well as many other carnivores, use scent marking as a way of olfactory social communication (Poddar-Sarkar and Brahmachary, 2004; Ralls 1971; Soso *et al*. 2014). A study by Brahmachary and colleagues (1992) suggests that the marking fluid that tigers disperse when scent marking serves as communication signal between different individuals. By smelling these scent marks, conspecifics can gather information about the donor, such as age and sex. Tiger scent marks have also been shown to provide information about the reproductive status of females (Soso *et al*. 2014), and males have been shown to scent mark less when there are no sexually receptive females around (Poddar-Sarkar *et al*. 1994). Scent marking has also been shown to play an important role in the pair formation and maintenance in bush dogs, and it is more frequent in male-female pairs, than in male-male or female-female bonds (Porton 1983).

There has been a limited amount of research done on the effects of different blood odor components on the behaviors of carnivores. The few studies that have been done include two previous master theses that investigated a different blood odor component than in the present study, one on Amur tigers and bush dogs (Sjöberg 2013) and another on African wild dogs and Asian wild dogs (Nilsson 2014). In this study the aim was therefore to:

(1). Assess the behavioral responses of two mammalian carnivore species, Amur tigers (*Panthera tigris altaica*) and bush dogs (*Speothos venaticus*) to the odor of real blood, as well as to the mammalian blood odor component 2-pentylfuran

(2). Compare their behavioral responses towards the blood odor component to those of a plant-derived odor and an odorless control

(3). Compare the behavioral responses between the two carnivore species

(4). Assess the suitability of the odor stimuli as environmental enrichment for captive tigers and bush dogs.

3. Materials and methods

3.1 Animals and housing

The study was conducted on two carnivore species maintained at Kolmården Wildlife Park.

The Amur tigers (*Panthera tigris altaica*) (see figure 1) comprised a group of six individuals, three males and three females of 4-13 years of age. The oldest males were two brothers, Timur and Kazan, who were both thirteen years old. The other individuals were all Kazan’s offspring. The third male, Tsar, was seven years old. The three females comprised two six year old sisters, Olga and Kyra, as well as a four year old named Kalinka. They were housed in a 5000 m^2 outdoor enclosure and also had access to indoor quarters during all hours of the day. The outdoor enclosure consisted of an area of cliffs and rocks, open areas of grass with a few trees and also a waterfall that ran out in a water moat that divided the exhibit in two equally-sized parts. When the outdoor enclosure was cleaned all the tigers were locked inside in the indoor quarters.

The bush dogs (*Speothos venaticus*) (see figure 2) comprised a group of 12 individuals, eight males and four females of 1-8 years of age. The oldest individual was the alpha male, Antonio, and all the other individuals were his offspring from different years. The alpha female, which was also the mother of all the offspring had been euthanized for medical reasons shortly before this study began. During the course of the study the alpha male was also euthanized and two of the females where moved to a zoo in England. After these individuals had been removed the age distribution of the nine remaining animals was 1-5 years. The animals were kept in a 1000 m^2 outdoor enclosure with constant access to indoor quarters. The outdoor enclosure consisted of a cliff and open grass areas with some bushes, and a small pool. During cleaning of the outdoor enclosures, the animals were locked inside the indoor quarters.

Figure 1. One of the Amur tigers kept at Kolmården Wildlife Park

Figure 2. One of the bush dogs kept at Kolmården Wildlife Park

3.2 Odor stimuli

Four different odor stimuli were used in the study.

Real pig blood was used as an odor stimulus to assess whether the whole mixture of odorants that comprise the odor of blood is necessary to elicit behavioral response or whether one single blood odor component may be sufficient. At Kolmården Wildlife Park, the carnivores are not given pig meat as food, so it was interesting to assess how they would react to this type of blood compared to blood from animals that they do receive as food, which mostly consists of horse or cattle. After collecting the blood samples needed for this study they were deep frozen. At the start of each observation day five Eppendorf tubes were thawed and warmed up to approximately 25° C immediately before it was used.

2-pentylfuran (CAS # 3777-69-3) has been identified as a volatile component in pig blood (Rachamadugu 2012) and it has been described to have a “metallic, blood-like” odor. 2-pentylfuran has also been identified to be a component in the odor of meat in cattle (Boylston *et al*. 1996; Calkins and Hodgen 2007) and pig (O'Sullivan *et al*. 2003). 2-pentylfuran was obtained from Sigma-Aldrich (St.Louis, MO) and had a nominal purity of > 99%. It was diluted 1:100 with diethyl phthalate.

Isopentyl acetate (CAS# 123-92-2) has been identified as a volatile component in a variety of fruits and elicits a “banana-like” odor quality in humans (Burdock 2005). Isopentyl acetate was obtained from Sigma-Aldrich (St.Louis, MO) and had a nominal purity of >99%. It was diluted 1:1000 with diethyl phthalate.

Diethyl phthalate (CAS# 84-66-2) is nearly odorless and was therefore used as a control as well as a solvent for the two monomolecular odors.

3.3 Preparation of odors

The two monomolecular odors, 2-pentylfuran and isopentyl acetate, were diluted with diethyl phthalate to concentrations that are readily detected by the human nose. This was done so that the odors are likely to be detectable to the animals, but at the same time not overwhelmingly strong for them. The real pig blood was not diluted.

3.4 Experimental procedures

At the start of each observation day one of the four odors was applied to five wooden logs of 48x7x4.5 cm (length x width x height). 500 µl of the odor was applied on the two largest surfaces of each log with the help of a pipette and then the odor was spread out along the surface of the log with a brush. A separate brush was used for the different odors and also for the different species. Plastic gloves were worn during the whole preparation of the logs to prevent them from being impregnated with human scent. The logs were marked on the short side with different colors depending on the odor that had been applied on them. The logs with the real pig blood were marked with red. The ones with 2-pentylfuran were marked with black. Those with isopentyl acetate were marked green and the logs with diethyl phthalate were marked blue.

At the beginning of each observation day five logs impregnated with the same odor were thrown into the outdoor enclosures of either the tigers or the bush dogs. After that the animals were observed for six hours, three in the morning and three in the afternoon, between 8.00 am and 4.00 pm. During these hours selected behaviors that the animals displayed towards the logs were recorded. The duration of each interaction with a log was also recorded whenever possible. The logs were re-used with the same odor and species if they were still in a good condition; otherwise they were replaced with new logs. A total of twenty observation days were conducted on each species, so that each of the four odors was tested five times. Care was taken to present the odor stimuli in a pseudo-random order and with at least two consecutive days between testing the same species to avoid desensitization. Observations were conducted on non-rainy days from the middle of May to the beginning of October 2014. If it started to rain during an observation, the observation was cancelled. Since it started to rain at the earliest 20 minutes before the morning session was over the results from these sessions were still included in the study.

To be able to compare the results from this study with similar studies from previous years the same ethogram was used (table 1). The recording of the behaviors was done with continuous sampling and the occurrence of each behavior noted in a protocol.

Table 1. The ethogram of all the behaviors towards the odorized logs under consideration in this study, compiled by Sjöberg (2013).

Functional term	**Descriptive term**
Sniffing	Investigating the odor on the log with the nose
Licking	Licking the log
Flehmen	Curling of the upper lip to facilitate the transfer of the odors to the vomeronasal organ (VNO)
Pawing	Scratching the logs with the claws and paws
Vocalizing	Making sounds
Biting object	Using teeth to investigate the logs
Toying	Playing with or carrying around the logs
Impregnating	Rubbing the face or other body parts on the log
Orienting	Turning the head, eyes and ears, to orient themselves, after investigating the log
Scent marking	Urinating on the logs

Figure 3. One of the tigers displaying the behavior toying

Figure 4. One of the bush dogs displaying the behavior sniffing

Figure 5. One of the bush dogs displaying the behavior impregnating

3.5 Statistical analysis

A chi-square test was used to compare the frequency of occurrence of behaviors towards the different odors, morning versus afternoon and also the two species against each other. To compare the durations of the interactions with the different odors and between the two species a Mann-Whitney U test was used. To calculate the variability across the sessions of the different odors a Spearman test was used. The statistical calculations were made in IBM SPSS Statistics 22 with a significance level of 0.05 and a confidence interval of 95%.

4. Results

4.1 Bush dogs

4.1.1 All stimuli

A total of 1178 interactions with the logs were recorded for all four odors in the bush dogs during the twenty days of observation (table 2). Nine out of the ten behaviors described in the ethogram (table 1) were displayed by the bush dogs. The most common behavior displayed was 'sniffing' with a total of 875 interactions, corresponding to 74% of all interactions. Other behaviors performed were 'biting' with 183 interactions (16% of all interactions), 'toying' with 93 interactions (8%), 'impregnating' with 12 interactions (1%), 'scent marking' with 6 interactions (0.5%), 'licking' with 5 interactions (0.4%), 'pawing' with 2 interactions (0.2%) and 'vocalizing' and 'orienting' with one interaction each (0.08%). The only behavior not displayed by the bush dogs was 'flehmen'.

Table 2. A summary of all the displayed behaviors of the bush dogs to the odorized logs as described in the ethogram.

Bush dogs	Sniff	Lick	Flehmen	Pawing	Vocal	Biting	Toy	Impreg	Orient	Scent	Total
Pig blood	326	0	0	1	1	74	44	1	0	2	**449**
2- pentyl-furan	184	0	0	0	0	15	10	3	0	3	**215**
Isopentyl acetate	132	0	0	1	0	31	15	1	0	0	**180**
Diethyl phthalate	233	5	0	0	0	63	24	7	1	1	**334**
Total	**875**	**5**	**0**	**2**	**1**	**183**	**93**	**12**	**1**	**6**	**1178**

4.1.2. Pig blood

Table 2 shows that the bush dogs performed a total of 449 interactions with the logs impregnated with pig blood during all five observation days. Seven of the ten behaviors described in the ethogram (table 1) were displayed by the bush dogs. The behavior that was displayed the most was 'sniffing' with a total of 326 interactions, or 73% of the total number of interactions. The other behaviors were 'biting' with 74 interactions (16% of all interactions), 'toying' with 44 interactions (10%), 'scent marking' with 2 interactions (0.4%) and 'pawing', 'vocalizing' and 'impregnating' with one interaction each (0.2%). The behaviors that were not displayed by the bush dogs when presented with the odor of pig blood were 'licking', 'flehmen' and 'orienting'.

4.1.3. 2-pentylfuran

Table 2 shows that the bush dogs performed a total of 215 interactions with the logs impregnated with 2-pentylfuran during all five observation days. Five out of the ten behaviors described in the ethogram (table 1) were displayed by the bush dogs. The behavior that was displayed most was ‘sniffing’ with 184 interactions or 86% of the total number of interactions. The other behaviors displayed were ‘biting’ with 15 interactions (7% of all interactions), ‘toying’ with 10 interactions (5%) and ‘impregnating’ and ‘scent marking’ with three interactions each (1%). The behaviors that were not displayed when the bush dogs were presented with the odor of 2-pentylfuran were ‘licking’, ‘flehmen’, ‘pawing’, ‘vocalizing’ and ‘orienting’.

4.1.4 Isopentyl acetate

Table 2 shows that the bush dogs displayed a total of 180 interactions with the logs impregnated with isopentyl acetate during all five observation days. The bush dogs displayed five of the ten behaviors described in the ethogram (table 1). The behavior that was performed the most was ‘sniffing’ with a total of 132 interactions or 73% of the total number of interactions. The other behaviors displayed were ‘biting’ with 31 interactions (17% of all interactions), ‘toying’ with 15 interactions (8%) and ‘pawing’ and ‘impregnating’ with one interaction each (1%). The behaviors that the bush dogs did not display when presented with the odor of isopentyl acetate were ‘licking’, ‘flehmen’, ‘vocalizing’, ‘orienting’ and ‘scent marking’.

4.1.5. Diethyl phthalate

Table 2 shows that the bush dogs displayed a total of 334 interactions with the logs impregnated with diethyl phthalate during all five observation days. The bush dogs displayed seven of the ten behaviors described in the ethogram (table 1). The most common behavior displayed was ‘sniffing’ with 233 interactions or 70% of the total number of interactions. The other behaviors displayed were ‘biting’ with 63 interactions (19% of all interactions), ‘toying’ with 24 interactions (7%), ‘impregnating’ with 7 interactions (2%), ‘licking’ with 5 interactions (1.4%) and ‘orienting’ and ‘scent marking’ with one interaction each (0.3%). The behaviors ‘flehmen’, ‘pawing’ and ‘vocalizing’ were not displayed when the bush dogs were presented with the odor of diethyl phthalate.

4.1.6 Comparing the odors

When comparing the four different odor stimuli against each other the number of interactions with the pig blood (449 interactions) was found to be significantly higher than the number of interactions with the 2-pentylfuran (215 interactions) (Chi=82.46; p= 0.0001), the isopentyl acetate (180 interactions) (Chi=115.04; p=0.0001) and the diethyl phthalate (334 interactions) (Chi=16.89;

p=0.0001) (see table 2). There was no significant difference between 2-pentylfuran with 215 interactions and isopentyl acetate (180 interactions) (Chi=3.10; p=0.078). However, the number of interactions with diethyl phthalate (334) was significantly higher than the number of interactions with 2-pentylfuran (215) (Chi=25.79; p=0.0001) and with isopentyl acetate (180) (Chi=46.14; p= 0.0001) (see table 2).

4.1.7 Morning vs afternoon

Table 3 shows that the bush dogs displayed 984 interactions during the morning hours over all 20 sessions. This number was significantly higher than the 194 interactions that were observed during the afternoon hours (Chi=529.80; p=0.0001). With all four odor stimuli considered separately, the number of interactions displayed by the bush dogs in the morning was significantly higher than the number of interactions displayed in the afternoon (Chi: 249.94 with pig blood, 63.67 with 2-pentylfuran, 77.36 with isopentyl acetate, and 144.91 with diethyl phthalate. P=0.0001 in all four cases).

Table 3. The number of interactions displayed by the bush dogs for each of the four odor stimuli, subdivided by morning and afternoon.

Bush dogs	**Pig blood**	**2-pentylfuran**	**Isopentyl acetate**	**Diethyl phthalate**	**Total**
Morning	392	166	149	277	**984**
Afternoon	57	49	31	57	**194**

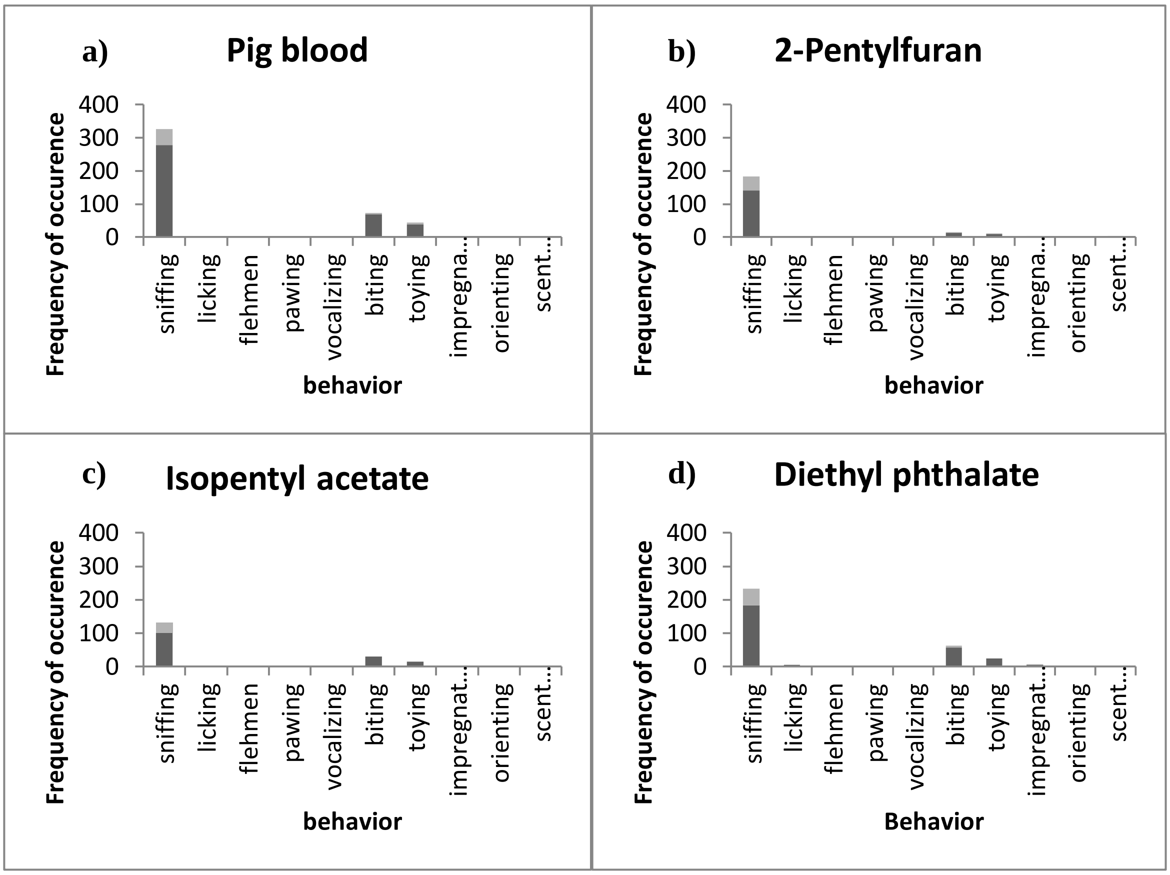

Figure 6. Frequency of occurrence of the different behaviors displayed by the bush dogs during the five sessions with each of the four odor stimuli, subdivided by morning and afternoon. The y-axis shows the number of interactions and the x-axis shows the ten behaviors under consideration. The dark grey bars represent the morning hours and the light grey bars represent afternoon hours.

Across the five sessions with the pig blood all the seven behaviors performed by the animals were displayed more often in the morning than in the afternoon (see figure 6a and table 2). Across the five sessions with the 2-pentylfuran four out of the five behaviors performed by the bush dogs were displayed more often in the morning than in the afternoon (see figure 6b). The only behavior displayed more frequently in the afternoon was impregnating. During the five sessions with the isopentyl acetate all of the five behaviors performed were displayed more often in the morning than in the afternoon (see figure 6c). Across the five sessions with the diethyl phthalate all of the seven behaviors performed were displayed more often in the morning than in the afternoon (see figure 6d).

4.1.8 Duration of interactions

It was not possible to record the duration for all interactions performed by the bush dogs, therefore the duration of the interactions that were possible to record was averaged. The duration of every interaction was recorded from the moment that the animal started interacting with a log and it ended when the animal stopped the interaction. Across all 20 sessions the bush dogs spent on average

2.9 seconds per interaction (mean value from n= 1051) (see table 7). The bush dogs spent on average 3.1 seconds per interaction with the pig blood (mean value of n=380), 2.7 seconds per interaction with 2-pentylfuran (mean value of n=188), 2.6 seconds per interaction with isopentyl acetate (mean value of n=164) and 3.1 seconds per interaction (mean value of n=319) (see table 7). When comparing the different durations against each other no significant difference was found between any of them, (pig blood vs. 2-pentylfuran: U=73.50; p=0.847; vs. isopentyl acetate: U=64.50; p=0.746; vs. diethyl phthalate: U=97.50; p=0.399. 2-pentylfuran vs. isopentyl acetate: U=49.50; p=0.705; vs. diethyl phthalate: U=73.00; p=0.353. Isopentyl acetate vs. diethyl phthalate: U=74.00; p=0.604) (see table 7).

4.1.9 Variability across sessions

The interest that the bush dogs showed towards the odorized logs varied throughout the study in terms of the number of interactions and time spent per interaction. When considering all odors combined the number of interactions towards the logs decreased from 372 interactions during session one to 100 interactions during session four (see figure 7) The average time spent per interaction decreased from 3.5 seconds during session one to 2.36 seconds during session five (see figure 7). The tendency for a decrease in the number of interactions was statistically significant (Spearman r= -0.900; p=0.037), however the decrease in time per interaction was not (r=-0.667; p=0.219).

Across the five sessions with the pig blood the number of interactions decreased from 165 interactions during the second session to 34 interactions during session four (see figure 8). The average time spent per interaction decreased from 3.32 seconds during session one to 1.94 seconds during session five (see figure 8). The tendency for a decrease in the number of interactions was not statistically significant (r= -0.800; p=0.104), however the decrease in time per interaction was (r=-0.90; p=0.037).

Across the five sessions with 2-pentylfuran the number of interactions decreased from 68 interactions during session one to 22 interactions during session five (see figure 8). The average time spent per interaction decreased from 3.38 seconds during session one to 2.13 seconds during session two (see figure 8). The tendency for a decrease in the number of interactions was statistically significant (r=-1.00; p=0.01), however the decrease in time spent per interaction was not (r=-0.30; p=0.624).

Across the five sessions with isopentyl acetate the number of interactions decreased from 79 interactions during session two to 7 interactions during session four (see figure 8). The average time spent per interaction decreased from 3.06 seconds during session two to 1.33 seconds during session fourbut then increased to 2 s in session 5 (see figure 8). There was no statistical significance in the tendency of decrease in the number of interactions (r=-0.600; p=0.285), or the time spent per interaction (r=-0.60; p=0.285).

Across the five sessions with diethyl phthalate the number of interactions decreased from 150 interactions during session one to 21 interactions during session five (see figure 8). The average time spent per interaction decreased from 3.94 seconds during session one to 1.93 seconds during session four bu then increased to 3.8s in session 5 (see figure 8). The tendency for a decrease in the number of interactions was statistically significant (r=-1.00; p=0.01), however the time spent per interaction was not (r= -0.40; p=0.505).

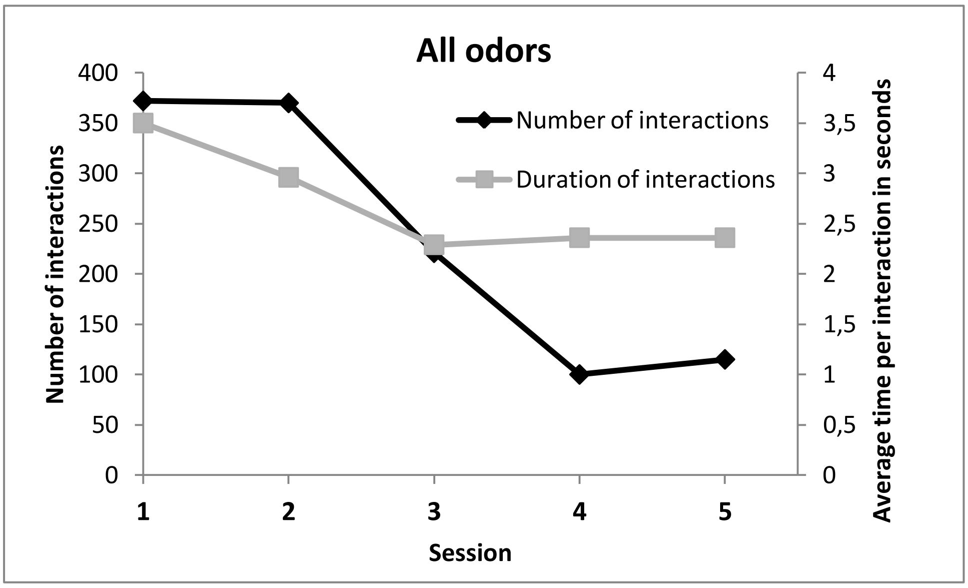

Figure 7. The total number of interactions and time spent per interaction displayed by the bush dogs with all four odor stimuli piled together. Left y-axis shows the number of interactions and the right y-axis shows the average time spent per interaction. The x-axis shows the different sessions.

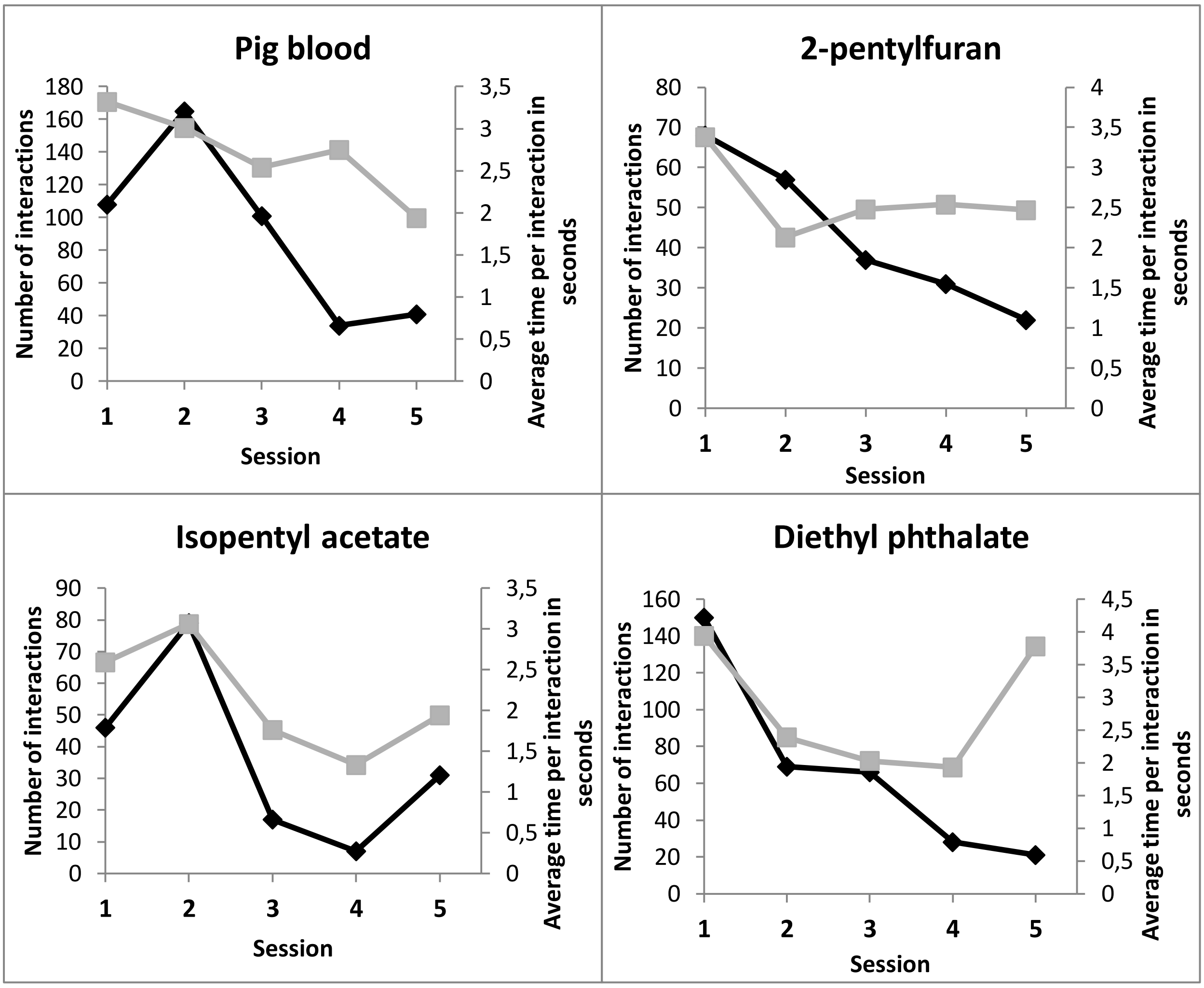

Figure 8. The total number of interactions and time spent per interaction displayed the bush dogs towards each of the four odor stimuli across the five sessions of observation. The black line shows the number of interactions and the grey line shows the duration of the interactions. The left y-axis shows the number of interactions and the right y-axis shows the average time spent per interaction. The x-axis shows the different sessions.

4.2 Amur tigers

4.2.1 All stimuli

Table 4 shows that a total of 61 interactions with the logs were recorded for all four odors in the tigers during the twenty days of observation. Only five out of the ten behaviors described in the ethogram (table 1) were displayed by the tigers. The most common behavior displayed was 'sniffing' with a total of 53 interactions, corresponding to 87% of all interactions. Other behaviors performed were impregnating with three interactions (5% of all interactions), 'biting' and 'toying' with two interactions each (3%) and 'licking' with one interaction (2%).

Table 4. A summary of all the displayed behaviors of the tigers to the odorized logs as described in the ethogram.

Amur tiger	Sniff	Lick	Flehmen	Pawing	Vocal	Biting	Toy	Impreg	Orient	Scent	Total
Pig blood	12	1	0	0	0	0	2	0	0	0	**15**
2- pentyl-furan	19	0	0	0	0	0	0	1	0	0	**20**
Isopentyl acetate	10	0	0	0	0	2	0	2	0	0	**14**
Diethyl phthalate	12	0	0	0	0	0	0	0	0	0	**12**
Total	**53**	**1**	**0**	**0**	**0**	**2**	**2**	**3**	**0**	**0**	**61**

4.2.2. Pig blood

Table 4 shows that the tigers performed a total of 15 interactions with the logs impregnated with pig blood during all five observation days. Three of the ten behaviors described in the ethogram (table 1) were displayed by the tigers. The behavior that was displayed most was 'sniffing' with a total of 12 interactions, or 80% of the total number of interactions. The other behaviors were 'toying' with two interactions (13% of all interactions) and 'licking' with one interaction (7%). The behaviors that were not displayed by the tigers when presented with the odor of pig blood were 'flehmen', 'pawing', 'vocalizing', 'biting', 'impregnating', 'orienting' and 'scent marking'.

4.2.3. 2-pentylfuran

Table 4 shows that the tigers performed a total of 20 interactions with the logs impregnated with 2-pentylfuran during all five observation days. Only two of the ten behaviors described in the ethogram (table 1) were displayed by the tigers. The behavior that was displayed most was 'sniffing' with 19 interactions or 95% of the total number of interactions. The other behavior displayed was 'impregnating' with one interaction or 5% of the total amount number of interactions. When presented with the odor of 2-pentylfuran the tigers did not display 'licking', 'flehmen', 'pawing', 'vocalizing', 'biting', 'toying', 'orienting' and 'scent marking'.

4.2.4 Isopentyl acetate

Table 4 shows that the tigers displayed a total of 14 interactions with the logs impregnated with isopentyl acetate during all five observation days. The tigers displayed three of the ten behaviors described in the ethogram (table 1). The behavior that was performed the most was 'sniffing' with a total of 10 interactions or 72% of the total number of interactions. The other behaviors were 'biting' and 'impregnating' with two interactions each or 14% each of the total number of interactions. The behaviors that the tigers did not display when presented with the odor of isopentyl acetate were 'licking', 'flehmen', 'pawing', 'vocalizing', 'toying', 'orienting' and 'scent marking'.

4.2.5. Diethyl phthalate

Table 4 shows that the tigers displayed a total of 12 interactions with the diethyl phthalate during all five observation days and the only behavior displayed was sniffing. Therefore 'sniffing' represented 100% of the total number of interactions. Accordingly, 'licking', 'flehmen', 'pawing', 'vocalizing', 'biting', 'toying', 'impregnating', 'orienting' and 'scent marking' were not displayed when the tigers were presented with the odor of diethyl phthalate.

4.2.6 Comparing the odors

When comparing the four different odor stimuli against each other the number of interactions with the odorized logs did not differ significantly between any of them. (Pig blood vs. 2-pentylfuran: Chi= 0.714; p=0.398; vs. isopentyl acetate: Chi=0.034; p= 0.853; vs. diethyl phthalate: Chi=0.333; p=0.564. 2-pentylfuran vs. isopentyl acetate: Chi=1.059; p=0.303; vs. diethyl phthalate: Chi=2.0; p=0.157. Isopentyl acetate vs. diethyl phthalate: Chi=0.154; p=0.695) (see table 4).

4.2.7 Morning vs afternoon

Table 5 shows that the tigers displayed 52 interactions during the morning hours over all 20 sessions. This number was significantly higher than the 9 interactions that were observed during the afternoon hours (Chi=30.311; p=0.0001). With all four odor stimuli considered separately the number of interactions displayed by the tigers during the morning hours was significantly higher than the number of interactions displayed during the afternoon (Chi= 8.067; p= 0.005 with pig blood, Chi=9.80; p= 0.002 with 2-pentylfuran, Chi= 7.143; p= 0.008 with isopentyl acetate, and Chi= 5.333; p= 0.021 with diethyl phthalate) (see table 5).

Table 5. The number of interactions displayed by the tigers for each of the four odor stimuli, subdivided by morning and afternoon.

Amur tigers	Pig blood	2-pentylfuran	Isopentyl acetate	Diethyl phthalate	Total
Morning	13	17	12	10	**52**
Afternoon	2	3	2	2	**9**

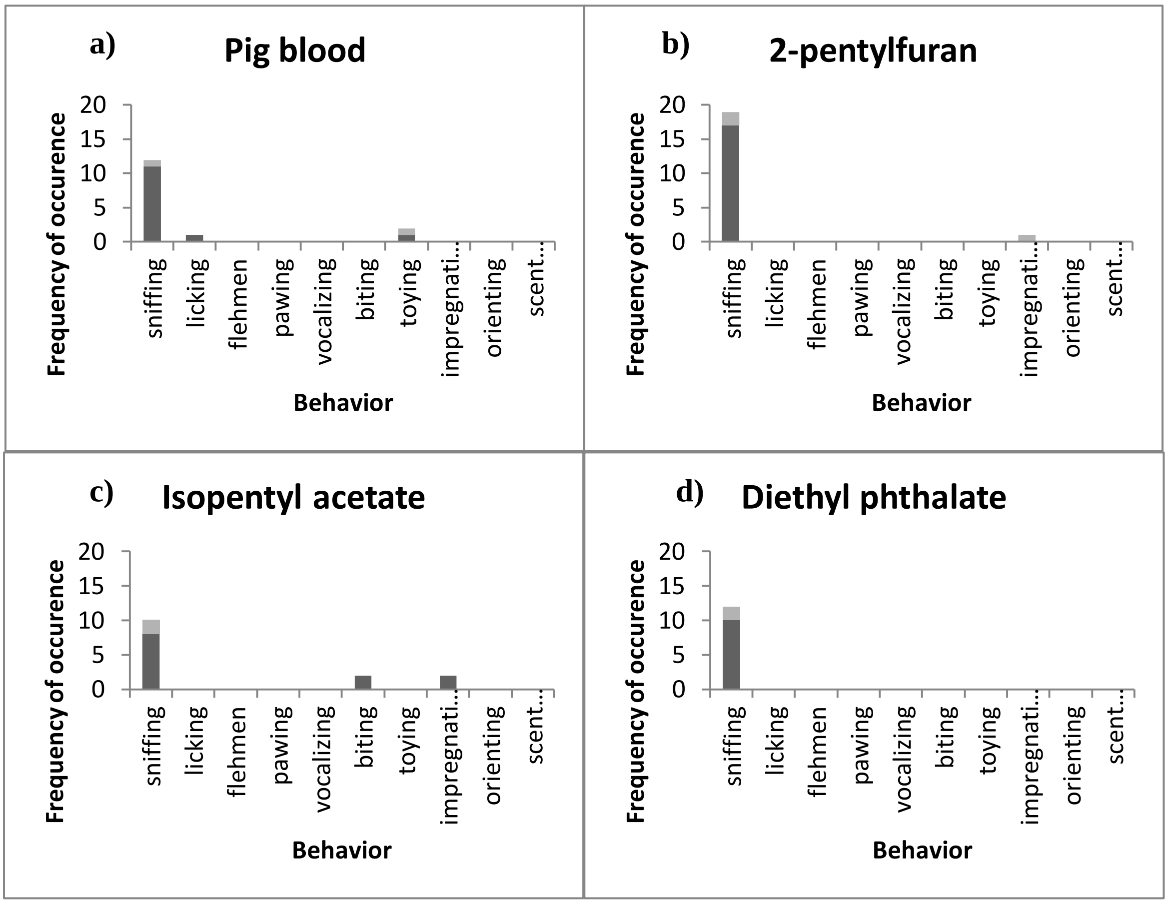

Figure 9. Frequency of occurrence of the different behaviors displayed by the tigers during the five sessions with each of the four odor stimuli, subdivided by morning and afternoon. The y-axis shows the number of interactions and the x-axis shows the ten behaviors under consideration. The dark grey bars represent the morning hours and the light grey bars represent afternoon hours.

Across the five sessions with the pig blood two of the three behaviors performed by the animals were displayed more often in the morning than in the afternoon (see figure 9a). The behavior toying was displayed equally often in the morning and the afternoon. Across the five sessions with the 2-pentylfuran sniffing was performed more often during the morning hours, but impregnating was only displayed in the afternoon (see figure 9b). Across the five sessions with isopentyl acetate all three behaviors performed were displayed more often in the morning than in the afternoon (see figure 9c). Across the five sessions with the diethyl phthalate the only behavior displayed, sniffing, was performed more often in the morning than in the afternoon (see figure 9d).

4.2.8 Duration of interactions

The low number of recorded interactions with the odorized logs made it possible to record the duration for all the interactions in the tigers. The duration of every interaction was recorded from the moment that the animal started interacting with a log and it ended when the animal stopped interacting with the log. Across all 20 sessions the tigers spent on average 2.4 seconds per interaction (mean value of n=61) (see table 7). The tigers spent on average 4.2 seconds per interaction with the pig blood (mean value of n=15), 2.1 seconds with 2-pentylfuran (mean value of n=20), 1.9 seconds with isopentyl acetate (mean value of n=14) and 1.3 seconds with diethyl phthalate (mean value of n=12) (see table 7). When comparing the different durations against each other no significant difference was found between any of them. (Pig blood vs. 2-pentylfuran: U=12.00; p=0.570; vs. isopentyl acetate: U=7.00; p=0.376; vs. diethyl phthalate: U=1.00; p=0.089. 2-pentylfuran vs. isopentyl acetate: U=6.00; p=1.00; vs. diethyl phthalate: U=3.00; p=0.80. Isopentyl acetate vs. diethyl phthalate: U=2.50; p=0.80 (see table 7).

4.2.9 Variability across sessions

The interest that the tigers showed towards the odorized logs varied throughout the study in terms of the number of interactions and time spent per interaction. When considering all odors combined the number of interactions towards the logs decreased from 23 interactions during session two to 5 interactions during session five (see figure 10). The average time spent per interaction decreased from 3.2 seconds during session one to 1.2 seconds during session two, but then increased to 2.9 s in session 3, and decreased to 2.0 s in session 5 (see figure 10). There was no statistical significance in the tendency of decrease in the number of interactions (r=0.671; p=0.215), or the time spent per interaction (r=-0.50; p=0.391).

Across the five sessions with the pig blood the number of interactions decreased from 8 interactions during session two to 0 interactions during session three but then increased to 3 in sessions 5. (see figure 11). The average time spent per interaction decreased from 8.66 seconds during session two to 0 seconds during session three (see figure 11). There was no statistical significance in the tendency of decrease in the number of interactions (r=-0.564; p=0.322), or the time spent per interaction (r=-0.80; p=0.285).

Across the five sessions with 2-pentylfuran the number of interactions decreased from 5 interactions during session one to 2 interactions during session five (see figure 11). The average time per interaction decreased from 4.5 seconds recorded during the second session and 1 second during the fifth session (see figure 111). The tendency for a decrease in the number of interactions was statistically significant (r= -0.949; p=0.014), however the decrease in time per interaction was not (r=-0.40; p=0.505).

Across the five sessions with isopentyl acetate the number of interactions decreased from a maximum of 9 interactions during the second session to 0 interactions during the fifth session (see figure 11). The average time spent per interaction decreased from 2.44 seconds recorded during the second session to 0 seconds during the fifth session (see figure 11). There was no statistical significance in the tendency of decrease in the number of interactions (r=-0.359; p=0.553), or the time spent per interaction (r=-0.671; p=0.215).

Across the five sessions with diethyl phthalate the number of interactions decreased from 5 interactions during session three to 1 interaction during session five (see figure 11). The average time spent per interaction increased from 1 second during session one to 2 seconds during session five (see figure 11). There was no statistical significance in the tendency of decrease for the number of interaction (r=-0.051; p=0.935), or the increase in time spent per interaction (r=0.316; p=0.604).

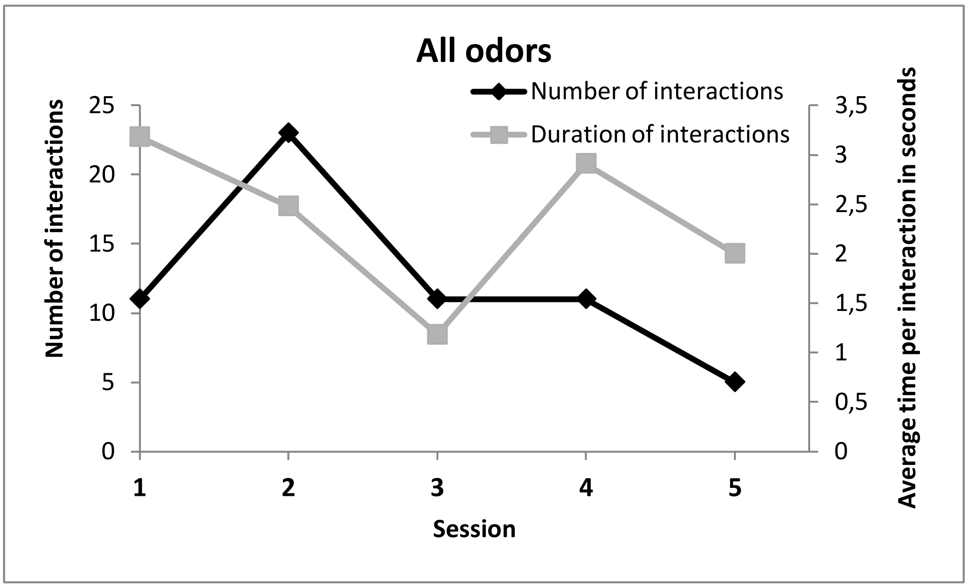

Figure 10. The total number of interactions and time spent per interaction displayed by the tigers with all four odor stimuli piled together. Left y-axis shows the number of interactions and the right y-axis shows the average time spent per interaction. The x-axis shows the different sessions.

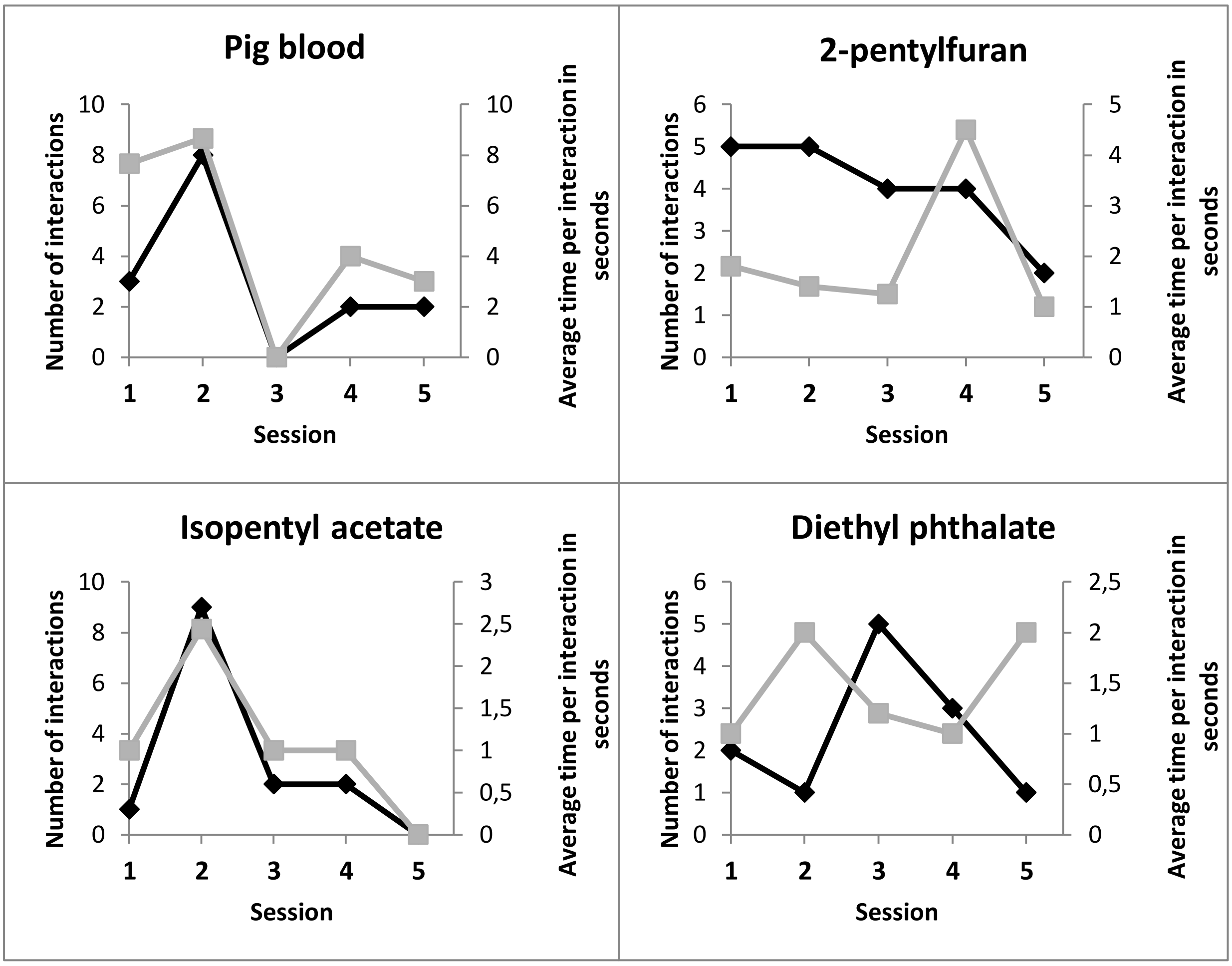

Figure 11. The total number of interactions and time spent per interaction displayed the tigers towards each of the four odor stimuli across the five sessions of observation. The black line shows the number of interactions and the grey line shows the duration of the interactions. The left y-axis shows the number of interactions and the right y-axis shows the average time spent per interaction. The x-axis shows the different sessions.

4.3 Comparison between species

The number of individuals differed between the two species so in order to compare them the number of interactions per individual was calculated (see table 6). When considering all four odors together, the number of interactions displayed by the bush dogs was significantly higher than that of the tigers (Chi=73.283; p=0.0001) (see table 6). The bush dogs also displayed a significantly higher number of interactions than the tigers for each of the four odors separately (pig blood: Chi=30.857; p=0.0001; 2-pentylfuran: Chi=9.783; p=0.002; isopentyl acetate: Chi=8.895; p=0.003; diethyl phthalate: Chi=23.516; p=0.0001) (see table 6).

Table 6. The number of interactions divided by the number of individuals of both species.

Behavior	Pig blood	2-pentylfuran	Isopentyl acetate	Diethyl phthalate	Total
Bush dogs	38.7	18.7	15.9	28.6	102
Tigers	2.5	3.3	2.3	2	10.1

When comparing the average time spent per interaction for all odors combined there was no significant difference between the species (U=45.5; p=0.067) (see table 7). However, when comparing the average time per interaction the tigers spent significantly longer time per interaction than the bush dogs with the pig blood (U=22.5; p=0.020). There was no significant difference in the average time between the species with 2-pentylfuran (U=14.0; p=0.343), isopentyl acetate (U=10.5; 0.469) and diethyl phthalate (U=14.5; p=0.749).

Table 7. Average time spent per interaction for both the bush dogs and the tigers for all odor stimuli.

Time (s)	Pig blood	2-pentylfuran	Isopentyl acetate	Diethyl phthalate	Total
Bush dogs	3.1±2.7	2.7±2.4	2.6±1.9	3.1±3.7	2.9±2.9
Tigers	4.2±3.6	2.1±3.1	1.9±2.6	1.3±0.5	2.4±2.9

5. Discussion

5.1 Main findings

The bush dogs displayed a significantly higher number of interactions across the 20 sessions of observation compared with the tigers. The bush dogs also performed a significantly higher number of interactions with all odors separately. For both species the most frequently displayed behavior was sniffing. This was true both when considering the odors separately and together. The bush dogs performed the most interactions towards the real pig blood and the tigers displayed the most interactions towards 2-pentylfuran. The odor that was least interesting for the bush dogs was isopentyl acetate and for the tigers it was diethyl phthalate. The bush dogs showed significantly more interest towards the real pig blood than for the other odors, but there was no significant difference between the odors for the tigers.

When comparing the time spent per interaction the tigers spent significantly longer time than the bush dogs when presented with the real pig blood. Otherwise there was no significant difference between the species in this parameter, neither when comparing the odors together or separately.

5.2 Bush dogs

The most frequent behavior displayed by the bush dogs was sniffing. This may indicate that the sense of smell is important for the bush dogs when investigating novel objects. A study by Price (2010) showed that a group of captive African wild dogs (*Lycaon pictus*) displayed behaviors like sniffing and locomotion when presented with blood trails. This indicates that the African wild dogs use their sense of smell to explore environments and since bush dogs are also canines it is likely that they may use the same sense. Apart from sniffing, the behaviors that were displayed most frequently were biting and toying. This may indicate that the senses of touch and taste are also important for the bush dogs when investigating new objects. The odor that the bush dogs displayed the highest number of interactions (449) towards was the real pig blood, which may indicate that they associate the odor with food. The bush dogs did not show as strong an interest in the 2-pentylfuran as in the real blood. Possible reasons for this could be that 2-pentylfuran is a component in the odor of meat, for example in chickens (*Gallus gallus domesticus*) (Nonaka *et al*., 1967; Greenberg, 1981) and pigs (*Sus scrofa domesticus*) (Martin et al., 2009) and also in the odor of blood in humans (Forbes *et al*., 2014). Therefore bush dogs might not associate this odor with blood or meat as humans do and it might therefore not be as interesting as the real pig blood. The bush dogs also showed a significantly higher interest in the diethyl phthalate than in 2-pentylfuran and isopentyl acetate. One possible explanation for this is that diethyl phthalate is not completely odorless, but has a slightly sweet odor and it is therefore used in many perfumes (Chingin *et al*. 2009). Hence their high interest in the isopentyl acetate may indicate that they find odors and novel objects interesting in general.

5.3 Amur tigers

Similar to the bush dogs, the most frequently displayed behavior by the tigers was sniffing. This may indicate that the sense of smell is important for the tigers when investigating new objects. Jaguars have been seen to use their sense of smell to localize prey when hunting (Huntrods 2007), and since they belong to the same family as tigers, the feline family, it is possible to assume that the two species use the same sense for this task. With this in mind it is therefore not odd that the most frequent behavior in the present study was sniffing. Apart from sniffing, the only other behaviors displayed were licking, biting, toying and impregnating. These behaviors were displayed at a very low frequency, but it might indicate that the senses of touch and taste are also of some importance for the tigers when investigating new objects. As there was no significant difference in the number of interactions between the different odors it is not possible to draw conclusions if the tigers found one odor more interesting than the other. The number of behaviors displayed by the tigers, however, did differ between the different odors. For the real pig blood and isopentyl acetate the tigers displayed three of the previously mentioned behaviors but for diethyl phthalate the tigers only displayed one, sniffing. One possible reason for the tigers' low interest in the odors could be that they were presented with odorized enrichment on a regular basis. Therefore they might not have found the odors used in the present study interesting.

5.4 Comparison with previous studies

A previous Master's thesis was performed with the same two species but different odor stimuli (Sjöberg 2013). During Sjöberg's study the group of bush dogs consisted of ten individuals and the group of tigers of six individuals, and therefore the number of interactions in table 8 and 9 are given as interactions per individual. The number of interactions obtained with the bush dogs in the present study is compared with the number of interactions from Sjöberg's results in table 8. The odor stimuli used in Sjöberg's study were horse blood, trans-4,5-epoxy-(E)-2-decenal (blood odor component), isopentyl acetate and diethyl phthalate.

Table 8. Comparison between the number of interactions with odorized wooden logs performed per animal by the bush dogs in the present study and in the study by Sjöberg (2013).

Present study	**Odor**	**Pig blood**	**2-pentylfuran**	**Isopentyl acetate**	**Diethyl phthalate**	**Total**
	Bush dogs	38.74	18.74	15.9	28.62	102
Sjöberg's study	**Odor**	**Horse blood**	**Trans-4,5-epoxy-(E)-2-decenal**	**Isopentyl acetate**	**Diethyl phthalate**	**Total**
	Bush dogs	54	52.1	29.7	22.4	158.2

The number of interactions obtained in the present study on the bush dogs was significantly lower than the number of interactions obtained by Sjöberg (2013) on the bush dogs for all four odors combined (Chi=12.062: p= 0.001) and also for the blood odor components (2-pentylfuran vs. Trans-4,5-epoxy-(E)-2-decenal**;** Chi= 15.338; p=0.0001) and isopentyl acetate (Chi= 4.261; p=0.039) (see table 8). There was no significant difference between the number of interactions between the bush dogs in the present study and Sjöberg's on the real pig vs. horse blood (Chi= 2.419; 0.120) or the diethyl phthalate (Chi= 0.692; 0.405).

Table 9. Comparison between the number of interactions with odorized wooden logs performed per animal by the tigers bush dogs in the present study and in the study by Sjöberg (2013).

Present study	**Odor**	**Pig blood**	**2-pentylfuran**	**Isopentyl acetate**	**Diethyl phthalate**	**Total**
	Tigers	2.5	3.3	2.3	2	10.1
Sjöberg's study	**Odor**	**Horse blood**	**Trans-4,5-epoxy-(E)-2-decenal**	**Isopentyl acetate**	**Diethyl phthalate**	**Total**
	Tigers	27.66	27.16	5.66	4.83	65.3

Table 9 shows the number of interactions displayed by the tigers in the present study compared to the number of interactions from Sjöberg's study (2013).

The number of interactions obtained in the present study on the tigers was significantly lower than the number of interactions obtained by Sjöberg on the tigers for all four odors combined (Chi= 40.333; p= 0.0001) and also for the real blood (Chi= 20.161; p= 0.0001) and the blood odor components (2-pentylfuran vs. Trans-4,5-epoxy-(E)-2-decenal; Chi= 18.0; p= 0.0001) (see table 9). There was no significant difference between the number of interactions between the tigers in the present study and the tigers from Sjöberg's study on the isopentyl acetate (Chi= 1.00; p= 0.317) or the diethyl phthalate (Chi= 1.286; p= 0.257).

The high interest that both the tigers and bush dogs in Sjöberg's study showed towards the horse blood odor component Trans-4,5-epoxy-(E)-2-decenal might indicate that they perceive this odor as metallic and blood-like as humans do (Rachamadugu 2012; Buettner and Schieberle 2001). This could explain why they displayed a higher number of interactions towards the Trans-4,5-epoxy-(E)-2-decenal compared to the 2-pentylfuran.

The bush dogs in both the present study and Sjöberg's study displayed a higher number of interactions per individual compared to the tigers. One possible explanation for the differences between the species is that the bush dogs are active more hours of the day than the tigers, and they might therefore interact more with the wooden logs.

5.5 Suitability of odors as enrichment for captive carnivore species

In zoos the enclosures that the animals are housed in are small in size compared to the wild and lack variation in habitat and external stimuli. Therefore environmental enrichment is often used to stimulate these animals (Carlstead and Shepherdson, 1994; Shepherdson, 1998), and to decrease stereotypical behaviors and inactivity and increase species-specific behaviors (Bashaw *et al.*, 2003; Carlstead and Shepherdson, 1994; Hogan et al., 2010). There are several different kinds of enrichment and one of them is based on olfaction. This has been shown to be an effective method of enrichment for felines (Yu *et al.*, 2009). Olfactory enrichment includes presenting scents, herbs or feces from other animals (Clark and King 2008).

Skibiel and colleagues (2007) presented chili powder, cinnamon and cumin to six species of captive felines, including tigers. They found that the spices increased the number of active behaviors displayed by the tigers and decreased the number of stereotypical behaviors. A study on Amur leopards (*Panthera pardus orientalis*) showed that the animals displayed a greater diversity of behaviors when presented with nutmeg, roe deer feces and tiger urine (Zu et al., 2009), than when not presented with any of the odors. The animals displayed more play related behaviors when presented with the nutmeg and roe feces than they did when presented with the tiger urine. A master thesis performed by Rosandher (2009) from Linköping University studied olfactory enrichment in snow leopards (*Panthera uncia*) and found that the animals were more active when presented with objects odorized with cinnamon and lavender compared to when the objects were odorized with lemon balm, cumin, and catnip. Van Metter and colleagues (2008) studied the effects of frozen blood, fresh zebra dung and scented squash as olfactory enrichment on African lions (*Panthera leo*) and Sumatran tigers (*Panthera tigris sumatrae*). They found the largest response to the enrichment in the lions where they observed an increase in active behaviors and a decrease in sleeping. The same results were obtained in the tigers, but not to a lesser extent.

Olfactory enrichment has not only been found to have a positive effect on larger captive felines but also smaller species. The effect of cinnamon and catnip has been studied in oncilla cats (*Leopardus tigrinus*) (Resende *et al*., 2011). The oncilla showed a high interest in the cinnamon which helped to decrease their stereotypical pacing. Wells and Egli (2004) studied black-footed cats (*Felis nigripes*) in response to nutmeg, catnip and body odor from a quail (*Coturnix coturnix*). All of these odors caused an increase in activity and behaviors like moving and grooming, but the odors that elicited the strongest response were catnip and the odor from the quail. Catnip and prey odor from rabbit (Lepus *curpaeums*) has also been tested as olfactory enrichment for domestic cats housed in shelters (Ellis and Wells 2010). The results showed that the cats displayed more exploratory and play behaviors when exposed to these odors.

All of these studies suggest that odors and spices work as enrichment for captive felines. However, in the present study the odors did not have the same effect, mostly because the tigers did not display much interest in them. Therefore the odors used in the present study might not be effective to use as enrichment. However, one reason for their low interest in the odors presented could be that the tigers in this study were presented with odorized enrichment almost every day year round, and therefore there might not be any novelty to the odors used in this study. The different odorized enrichments that the tigers in the present study received was blood in the form of blood trails or frozen blood, different spices, perfume and a variety of odorized toys. These enrichments did not elicit a big response in the tigers either, much like my odors. The tigers did however show a slight interest when these odors or objects were thrown into the enclosure, but that could be because it was the caretakers that presented these objects to the tigers and they might have thought that they would receive food. It has been shown that cats quickly habituate to enrichments so it is therefore important not to present the odors closely together in time (Mellen and Shepherdson 1997).

As mentioned above, olfactory enrichment has not been studied to the same extent in canines as in felines. However, one study has been conducted on domestic dogs (*Canis lupus familiaris*) kept in shelters (Graham *et al*., 2005), where the odors used were lavender, rosemary, chamomile and peppermint. When the dogs were exposed to lavender and chamomile they spent more time resting and performed less vocalization behaviors and they appeared more relaxed than before receiving these spices. It was therefore considered that these spices might have calming effects on stressed dogs in shelters.
In the present study the bush dogs showed a high interest in the odors presented both considered together and separately, but the number of interactions with the

odorized wooden logs decreased quickly across the sessions as can be seen in figure 7 and 8. The high interest that the bush dogs displayed during session one may indicate that odors are an effective enrichment for them. However, the loss of interest may indicate that the animals became habituated to the odors and they should therefore not be presented as frequently as they were, or with other odors so that the interval between each odor gets longer. Thus was however not possible in the present study due to time restrictions. One study reported that the optimal time-span between presenting odors to animals is three weeks to prevent habituation (Clark and King 2008).

5.6 Limitations and further studies

There are factors that may have influenced the results of the present study. One factor was that the activity levels of the species varied across the sessions and also between the morning and afternoon hours of each session. Since this study was conducted between May and October the changes in temperature and weather across these months may also have had an impact. I observed that the bush dogs displayed a strong initial interest in the logs when they were presented in the morning. Also, during colder or cloudy mornings they were more inactive and they lost their interest faster than on warmer mornings. However, during the afternoon hours the bush dogs seemed to have lost their interest in the logs and this could explain why I registered more interactions during the morning hours. The bush dogs were also more active during the warmer months of the study than during late September and October. One explanation for this could be that they are adapted to a warmer climate than we have in Sweden and they therefore spent more time indoors during the colder periods of the year. However, the tigers were more active in general during the morning hours of each session and during September and October when the temperature was low. During the warm months and afternoon hours of each observation they were inactive hidden away in the shadow parts of their enclosure. This could be because they are adapted to a colder climate. It should also be noticed that only three out of the six tigers in this study showed interest in the odors and this could therefore explain the low number of interactions.

Another factor that may have had an effect on the present results is that only one of the stimuli had color. Since the bush dogs showed a significantly higher interest in this odor stimulus it might be interesting to study if color had any effect on the bush dogs’ interest or not. However, since the tigers displayed so few interactions towards any of the stimuli it is difficult to assess if color had any impact on their interest towards the real blood odor stimulus. Color could have an effect on the interest of the species because bush dogs, like many other canine species, are able to perceive color due to the high amount of cones in their retina (Miller and Murphy 1995). Tigers on the other hand, like many feline species, have a large amount of rods in their retina which allows them to see better at night (Meyer *et al.* 1954).

As mentioned previously, similar master's theses have been carried out with different odor stimuli. Sjöberg (2013) used the same two species as the present study but she tested horse blood and Trans-4,5-epoxy-(E)-2-decenal as her blood odor component. The same odors that were used by Sjöberg were also tested by Nilsson (2014) on Dholes (*Cuon alpinus*) and African wild dogs (*Lycaon pictus*). It might therefore be interesting to study these or other carnivore species on the same odors that were used in the present study. It would also be interesting to present different spices and urine marks from different animals to evaluate how efficient they might be as olfactory enrichment for carnivores.

5.7 Social and ethical aspects

The experiments conducted in the present study comply with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 86-23, revised 1996) and also with the current Swedish laws.

6. Conclusions

In conclusion, the bush dogs showed a significantly higher interest in the real pig blood than in the blood-like odor 2-pentylfuran, the banana-smelling odor isopentyl acetate and odor-less diethyl phthalate. This might indicate that they did not perceive the 2-pentylfuran as a prey-related odor. The bush dogs showed a significantly higher interest for the diethyl phthalate than for the isopentyl acetate and 2-pentylfuran. This could be because diethyl phthalate is not completely odorless, but has a faint sweet smell that the bush dogs might have been attracted to, but why this was more attractive than the sweet-smelling banana odor isopentyl acetate is unclear. The tigers, in contrast, did not display a significant difference in their interest between any of the odors presented in this study. However, the high interest that the bush dogs showed for the blood indicates that it could be used as olfactory enrichment for them and maybe also other canine species.

7. Acknowledgment

First I would like to thank my supervisor, Matthias Laska for his help during this whole project, from supplying me with the materials needed to the writing of the thesis. I would also like to thank the carnivore caretakers at Kolmården Wildlife Park for their help with the animals and for making this study possible to begin with.

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