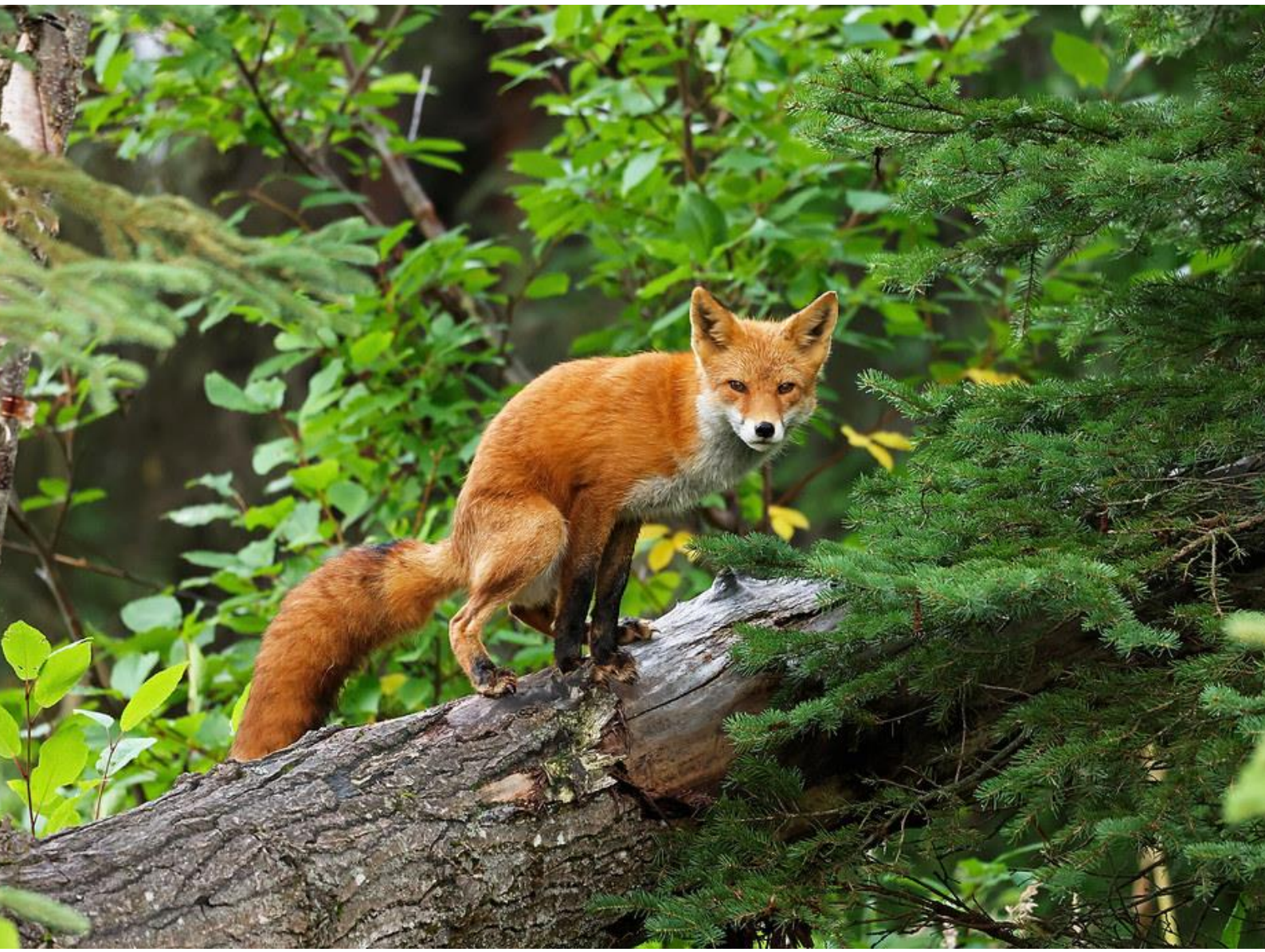
Behavior around grey wolf scats by other vertebrates in the Netherlands.

Study that looks at the occurrences of responses of vertebrate species to grey wolf scats in the Netherlands to infer the possibility of DNA contamination of wolf scats in the field.



Robin Louis.
June 12th, 2023.

Applied Biology BSc.

Title page

Behavior around grey wolf scats by other vertebrates in the Netherlands.

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Photo on cover
Figure shows a red fox (*Vulpes vulpes)* defecating on fallen tree trunk.

Figure 1: Fox defecates on fallen tree trunk. Illustration: (Lackie, 2021*).*

Preface

Before you lies the research report of a study done in May 2023, which looked at the behavior of vertebrates around wolf droppings in the Netherlands. This report is my BSc thesis: the final product of my BSc Applied Biology at Aeres University of Applied Sciences Almere. Kevin Groen PhD from the CML and Leiden University supervised writing this report to answer how other animals responded to wolf scats, as part of ongoing dietary research on the wolf in the Netherlands.

Over the past four years, I had been busy finding my interests in biology. After having explored a dozen of topics (and in doing so, travelling to two continents), I have found out that my heart lies with mammals. As a result, I see this study as the peak of my undergraduate degree and one of many highlights to come.

I would like to thank Kevin Groen for facilitating this research. Without all his suggestions, help, support and guidance, this research would never have been possible. His supervision and support over the last couple of months have been very important and valuable to me. In addition, I would like to thank Pauline Arends and Widmar van der Meer (nature managers and rangers of Hart van Drenthe and het Drenths-Friese Wold for the warm welcome to their nature reserves and all the suggestions given during this research. Their help and hospitality in your nature reserves was extremely valuable for this study.

Lastly, thanks to Martijn Hammers for helping me in the support of this research report. All the clear and prompt answers to my questions, the support with feedback and the general enthusiasm on the topics has done this research and me many good.

Summary

Since the return of the grey wolf (*Canis* lupus) in the Netherlands in 2015, much research has been done on the ecology and distribution of the species. This has led to a dietary study using hair- and DNA-sampling of wolf scats, and preliminary analyses of DNA in wolf scats showed that DNA from non-prey, including red fox (*Vulpes vulpes)* is regularly present in wolf scats. A possible cause for this is an interspecific interaction between other species and wolf scats, since scats serve as olfactory cues for other individuals. However little is known about behavioral responses of other vertebrate species towards wolf scats in the Netherlands. Findings on these interactions could contribute to the knowledge of how other animals respond to wolf droppings and how possible associated contamination of DNA may take place.

For this reason, a one-month pilot study was initiated in May 2023 in Hart van Drenthe and Drents-Friese Wold using camera traps, to find out more on the occurrences of behavioral responses to wolf scats from other vertebrate species. The study also hoped to found out which species show a fear or attraction towards wolf scats, and finally, the study hoped to find out if behavioral responses (such as over-marking of mesopredators) could lead to DNA-transfer on wolf scats. The initial expectations were an attraction and interactive responses including over-marking from mesopredators on wolf scats, possibly leading to DNA-contamination. Additionally, a fear towards wolf scats and increased vigilance was expected from ungulates, and it was expected that birds would be attracted to the scats to forage on invertebrates living on them

Ungulates and lagomorphs showed vigilance to wolf scats 12% of the time, indicating fear towards wolf scats. Mesopredators showed interactive behavior 32% and vigilance 8,5% of the time, indicating both attraction and fear towards wolf scats. Wolves showed little response to wolf scats and birds not engaging with wolf scats, behavior which is possibly habitat-specific. Foxes over-marked on top of wolf scats rather than next to it 75% of the time, European badger (*Meles meles*) 27,3% and dog (*Canis lupus familiaris*) 50% of the time, making DNA-contamination from these species on wolf scats likely. A nation-wide study to the interspecific interactions with the wolf is therefore recommended, and any follow-up study should be done with fresh scats to gather a reliable and population-specific data set on interspecific interactions between the wolf and its nosy neighbors.

Samenvatting

Sinds de terugkeer van de grijze wolf (*Canis lupus*) in Nederland in 2015 is veel onderzoek gedaan naar de ecologie en verspreiding van de soort. Dit heeft onder andere geleid tot een dieetonderzoek en voorlopige DNA-analyses van de gevonden wolven drollen tonen aan dat ook DNA van niet-prooi, waaronder vos (*Vulpes vulpes*) aanwezig is in wolvendrollen. Een mogelijke oorzaak voor deze DNA-besmetting zijn interspecifieke interacties van andere soorten met wolvendrollen, aangezien drollen dienen als geurhints. Er is echter weinig bekend over deze gedragsreacties van mesopredatorsoorten zoals de vos, maar ook prooidieren en vogelsoorten op wolvendrollen in Nederland. Bevindingen over deze interacties zouden kunnen bijdragen aan de kennis over hoe andere dieren reageren op wolvendrollen en hoe mogelijke daarmee samenhangende besmetting van DNA plaatsvind. Om tot deze bevindingen te komen werd in mei 2023 een pilotstudie in Hart van Drenthe en het Drents-Friese Wold gestart, dat met cameravallen hoopte meer te weten te komen over gedragsreacties van andere gewervelde soorten op wolvendrollen. Ook hoopte het onderzoek uit te vinden welke soorten een angst voor of aantrekkingskracht voor wolvendrollen vertonen. Ten slotte hoopte het onderzoek uit te vinden of gedragsreacties (zoals het over-markeren wolvendrollen door mesopredatoren) zou kunnen leiden tot DNA-transfer op drollen. De aanvankelijke verwachtingen waren een aantrekkingskracht en interactieve reacties mesopredatoren op wolvendrollen, die zouden leiden tot DNA-besmetting. Daarnaast werd van hoefdieren een angst voor wolvendrollen verwacht die zou leiden tot een verhoogde waakzaamheid. Ook werd verwacht dat vogels zouden worden aangetrokken tot de wolvendrollen, om te foerageren op ongewervelden die hierop leven.

Hoefdieren en hazen toonden 12% van de tijd een waakzaamheid rond wolvendrollen wat erop wijst dat deze soorten angst tonen voor wolvendrollen. Mesopredatoren vertoonden 32% van de tijd interactieve reacties en 8,5% van de tijd waakzaamheid, wat duidt op zowel aantrekkingskracht als angst voor wolvendrollen. Wolven vertoonden weinig respons op drollen en van vogels werd niet vastgesteld dat ze zich met wolvendrollen bemoeiden, wat mogelijk habitat-gerelateerd is. Vossen over markeerden bovenop de drollen 75% van de tijd in plaats van ernaast, dassen (*Meles meles*) deden dit 27,3% van de tijden en honden (*Canis lupus f.*) 50%, waardoor DNA-besmetting van deze soorten op wolvendrollen waarschijnlijk is. Een landelijke studie naar de interspecifieke interacties met de wolf wordt daarom aanbevolen, en eventuele vervolgstudies moeten worden uitgevoerd met verse wolvendrollen, om een betrouwbare en populatie-specifieke dataset te verzamelen over de interspecifieke interacties tussen de wolf en zijn nieuwsgierige buren.

Contents

1. Introduction.

In 2015, the Netherlands witnessed the return of the grey wolf (*Canis lupus*). After nearly 150 years of being nationally extinct, it has reestablished itself in a place where it has belonged centuries before (Lelieveld et al. 2016). As of 2023, there are four packs in the Netherlands, three in the Veluwe and one in the Province of Drenthe. In addition, there are several pairs in the Veluwe, one in Drenthe and one wolf has settled in Brabant. Furthermore, vagrant wolves are observed in Dutch provinces bordering Belgium and Germany (Bij12, 2023). The reestablishment of the wolf has led to studies and monitoring, to learn more about the species in the Netherlands.

Wolves are opportunistic and generalistic animals, meaning they have great adaptability to change and can live in various environments. They have a large geographic range, and their habitat is described as anywhere where food is sufficient and humans do not kill it (Caniglia, 2008). The wolf is an apex predator and has a large range of prey and its diet is based on local prey availability (Guimarães et al., 2022). Where wolves in northern parts of Europe prey mainly on large ungulates such as moose (*Alces alces*) and reindeer (*Rangifer tarandus*), the dominant prey of wolves further south are the medium sized ungulates, such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*). If ungulates are not abundant, wolves can also prey on small mammals (lagomorphs), fish, birds and livestock. (Guimarães et al., 2022).

As an apex predator, the wolf has many known interspecific interactions with non-prey (Mech & Boitani, 2019). In some parts of their range, wolves compete with bears, tigers and mountain lions for prey and will sometimes kill one another. Wolves also interact with smaller carnivores, such as wolverine (*Gulo gulo*), coyote (*Canis latrans)*, both red fox (*Vulpes vulpes)* and arctic fox (*Vulpes lagopus)*, racoondog (*Nyctereutes procyonoides*) and European badger (*Meles meles)* (Mech & Boitani, 2019). Wolves also have interactions with birds, often playing a role as scavengers of wolf kills. Wolves regularly kill prey to keep themselves and young alive and provide these scavengers such as raven (*Corvus corax*) and White-tailed eagle (*Haliaeetus albicilla*) with a continuous stream of food (Zoogdiervereniging, 2023). This is in contrast to ecosystems without apex predators, where ungulate carcasses are present mainly at the end of a harsh season (Wolveninnederland, 2023).

To locate and follow prey, wolves rely heavily on olfaction (smelling) (Polgár et al., 2016; Conover, 2007), but olfaction is not just used for finding prey. The olfactory system is a major sense system in animals and one of the most evolved systems in the mammalian anatomy (Brenner & Miller, 2001). Communication by scent is therefore very prevalent among mammals. A common use of olfaction in communication is the marking of substrates with urine, feces or other secretions of scent glands. These markings are cues that signal e.g. territory boundaries, resource possession, rank and reproductive status (Ralls, 1971), to both predators and competitors (Cushing, 1984). Much is known about the costs and benefits in olfaction cues for carnivore-herbivore interactions, it is for example how predators find prey.

However, less is known about interspecific interactions of olfactory communication among carnivore species themselves (Wikenros et al., 2017), such as over-marking of scats, where conspecifics place their scent in a way that it partially or completely covers the scent of an individual of another (Ferkin & Pierce, 2007). Over-marking can lead to (1) scent blending, that leads to a new scent that is no longer individual specific, (2) scent masking, where the newest scent mark suppresses the older, and (3) a common ground with multiple scents, that poses as a central area to gather information. Over-marking is a common practice among terrestrial mammals and important for interspecific communication but little is known regarding the role of over-marking in communication between predator species (Ferkin & Pierce, 2007).

A recent study by Wikenros et al. (2017) investigated the behavioral response of red foxes (*V. vulpes*) to an olfactory cue (scat) of lynx (*Lynx lynx*), another apex predator. This study used camera traps to record behavior on scats sites and found that red foxes visited the sites with scats more often than expected, and longer than the sites without scats. They also observed vigilant behavior, sniffing, scent marking, and over-marking at scat sites. The study also observed multiple over-marking, when a red fox over-marked on an already over-marked scat. This study therefore indicates an interspecific interaction of olfactory communication between a mesopredator (red fox) and an apex predator (lynx).

Since its return in the Netherlands in 2015, research to date has focused on the ecology and distribution of the species. Monitoring of the wolf is at the forefront of this, with a focus on the population sizes of wolves across the Netherlands (Klees et al., 2019). In addition, a dietary study was initiated (led by Kevin Groen) using hair- and DNA analyses. Preliminary analyses of DNA in wolf scats showed that scats regularly contain DNA from non-prey. Out of 100 collected scats in Kroondomein het Loo, DNA of prey animals was found, but 23 scats also showed DNA of red fox, 3 scats showed DNA of jay (*Garrulus glandarius*), and 2 scats showed DNA of great tit (*Parus major)* and jackdaw (*Coloeus monedula)* (Groen, personal communication, April 18th, 2023). As previously mentioned, little is known about the behavior of mesopredators around wolf droppings in the Netherlands, but Groen's data indicates that contamination of wolf droppings occurs by the and possibly other species, possibly connected to a behavioral response. However, it is unknown how, why and how often this occurs.

To find answers on the topic, this study collected data on the behavior of animals around wolf scats, to find out how other species respond to wolf scats, and ultimately infer the causes of possible contamination of wolf scats. The following research question was formulated: 'What are the occurrences, types, and frequencies of behavioral responses of other vertebrate species around wolf (*Canis lupus*) scats in the Netherlands?' The study also formulated the following sub-questions to answer the main question:

- What types and frequencies of interactions do vertebrate species show toward wolf scats?
- Which vertebrate species show an increased fear or attraction to wolf scats?
- In what percentage of cases may interactive behavior with wolf scats lead to DNA transfer on wolf scats?

The expectations for the first sub-question were behavioral responses from red fox, that include inquisitive behavior and interactive behavior, including over-marking. Additionally, behavioral responses were expected from mustelids, specifically European badger (*Meles meles*) and possibly pine marten (*Martes martes*) who, like red fox, might benefit from the source of information the wolf scats present, and since interactions between wolves and European badgers are known (Mech & Boitani, 2019). Ungulate responses to wolf scats are known in existing literature (Kuijper et al., 2014) and show

change in vigilant and foraging behavior (Wikenros et al., 2015), so increased vigilance was also an expected behavior. Lastly, visits by bird species were expected since scats attract various invertebrates such as bung beetles, a possible food source for birds. Expectations for the second second sub-question were that red foxes in particular would show an increased interest in wolf scats compared other mammalian species. Expectations for the last sub-question on DNA-transfer were that red fox and birds would exhibit behavior that could cause DNA transfer, this was suggested in data found by Kevin Groen. Finally, the expectations were ungulate responses, possibly leading to contamination of scat.

This research is relevant to ongoing dietary research of the grey wolf in the Netherlands, as the findings contribute to the knowledge of how other animals respond to wolf scats and how possible associated contamination of DNA may take place. Insights about the possible transfer of DNA on wolf scats (Figure 2) can be used to find out to what extent this happens and how this may affect the results of the national dietary study. Answers can lastly bring a different point of view towards results of the DNA-analyses, providing a different perspective on the interpretation of current and future data.

Figure 2. Wolf scats are usually above 20 centimeters in length and above 2,5 centimeters in width, with hairs and bones and a tip near the end. Long black hairs are an indication of wild boar. Illustration by : Kloen, J. (2022*).*

2. Methods.

In this chapter, the methodology used for this study is explained. First, more is told about the survey areas where camera traps were placed. Next, the field methodology of the study is explained, including the setup of the camera trap sites and which materials were used. This is chapter continues by explaining in detail the classification of the recorded behaviors, providing information on the ethogram and what protocol was used. Lastly, this chapter explains which data was recorded, how this was recorded and how this data was analyzed to answer the research and sub-questions.

2.1. Study area.

The Netherlands has 5495 km^2 of protected nature, divided into 162 nature reserves, so called Natura2000 areas (Nu.nl, 2022). Fieldwork for this study happened in two of those nature reserves: Hart van Drenthe and Drenths-Friese Wold in the Drenthe province. These are locations where wolves are currently present, and where wolf scats are found.

Hart van Drenthe is a 4000 hectare region in the Province of Drenthe and is a part of Drentsche Aa National Park. The area is home to varied forests, dozens of fens, expansive moorlands and springs of stream valleys (Staatsbosbeheer, 2023). Hart van Drenthe is home to animals that are important for answering the hypothesis, such as wolf (one pair) (Bij12, 2023), roe deer, badgers, pine martens and fox, with additionally birds, such as jackdaw (https://waarneming.nl/.). Wild boar and red deer are not present, since Drenthe has a zero tolerance policy towards these species (Broekman, 2017).

Drents-Friese Wold is another National Park in Drenthe and is home to a pack of wolves. Drents-Friese Wold is a 6100-hectare region that consists largely of forest, but also contains sand drifts, fens, heath and marsh ([1]Staatsbosbeheer, 2023). One pair with 3 pups and a pack live in the Drenths-Friese Wold and neighboring areas as of May 2023 (Bij12, 2023), and Drents-Friese Wold is additionally home to other species such as roe deer, mustelids, badger, red fox and bovines such as Galloway and Scottish highlander (both *Bos taurus var.) (*https://waarneming.nl/).

2.2. Field methods.

From May to June 2023, 13 camera trap sites (Browning Specops Advantage) with fresh scats recorded the behavior of other vertebrates on wolf scats scattered throughout the two nature reserves. The study used wolf scats that were initially collected for the ongoing dietary studies, and were stored in freezers to preserve the freshness and odor. Setting up the camera trap sites happened in consultation with local wildlife managers, in the forest and out of sight to lower the risk of theft and disturbances. Wolf droppings were placarded (using gloves to avoid human contamination) within sight of the camera, about 10 meters away and in the center of the image, to be able to completely identify an individual. The distance between sites was at least 100 meters, which was the distance used in the study by Wikenros et al. (2017) and to limit individuals visiting different sites.

The camera traps responded to both movement and body heat and automatically switched from color mode during the day to infrared mode at night. The cameras were set to film for 60 seconds once activated, with a short interval period of 10 seconds, to record as much behavior as possible. Camera traps operated for roughly 10 days, after which images were collected, the batteries replaced and a new scat placed. The earlier used scats got recollected to be used for the ongoing diet study. This behavioral study preferred fresh scats since the fresher scats give off a stronger scent, but due to a limiting amount of available fresh scats, older scats had to be used near the end of the study. All used scats came from the nature reserve where they had earlier been found. It was assumed that all recordings within a 5-minute interval were of the same individual and were pooled (Wikenros et al. 2017).

Five camera traps were placed in Hart van Drenthe for a total of 30 days (150 trap nights total). Additionally, 5 camera traps were placed in Drents-Friese wold for 10 days, after which 3 camera traps were added for another 10 days (130 trap nights total).

2.3. Classification of behavior.

Table 1 shows the ethogram with all recorded behaviors and their corresponding classes and codes. Behavior was classified using 4 classes, derived from the study conducted by Wikenros et al. (2017). The first class was 'ignorant', when an individual was at ease, showing ignorant behavior to the scat and either passed by (1a) or showed foraging behavior (1b). Secondly, class 2 behavior was recorded when an individual was vigilant: not at ease, standing still and looking around. In class 3, an animal showed inquisitive behavior toward the scat, either looking at it directly (3a) or sniffing an area around the scat or the scat itself (3b). Finally, behavior in class 4 was any interactive behavior, such as scent marking, (multiple-) over-marking, touching and consuming parts of the scat.

Table 1: Ethogram that shows the recorded behaviors with corresponding classes, codes and descriptions.

Classes	**Code**	**Behavior**	**Description**
Ignorant	1a	Passing by	*when walking or trotting (no other observed behavior), not paying attention to scat*
	1b	Foraging	*when searching for food, head pointed to the ground, not paying attention to scat*
Vigilant	2	Looking around	*when standing still with the head erect*
Inquisitive	3a	Looking at scat	*head pointed to the ground, not foraging*
	3b	Sniffing	*head pointed to the ground, not foraging*
Interacting	4a	Scent marking	*urinating or defecating (not near scat)*
	4b	Over-marking	*urinating or defecating next to/ on top of scat*
	4c	Multiple over-marking	*urinating or defecating next to/ on top of over-marked scat*
	4d	Touching scat	*a body part makes contact with scat*
	4e	Consuming scat	*actively consuming the scat or invertebrates attracted to it*

Focal sampling and behavioral sampling with continuous recording was used for the observation and documentation of behaviors exhibited by each individual. The method imposed a limit of 3 behaviors for focal sampling on each individual, concentrating on a single individual, specifically the one situated nearest to the scat. In instances where an individual demonstrated more than 3 behaviors during one observation or showed behavior in class 4, the method resorted to behavioral sampling with continuous recording, with priority given to the highest classes. The purpose behind this was to curtail irrelevant data, as well as to avert misclassifying vigilant, inquisitive and interactive individuals (classes 2, 3 and 4) as ignorant (class 1) as well. If an individual was ignorant (1a and 1b), no other behavior was written down.

2.4. Data analysis.

Due to limited time given for the research available and conducting analyses, no formal statistical analysis on the data was done. Instead the study recorded only the occurring behavior, which animal species exhibited them, how often these behaviors occurred, and in which cases possible contamination could have occurred (over-marking, multiple over-marking or otherwise touching). This was done by watching all videos from the camera traps and recording all observed behavior in a Microsoft excel sheet. The dataset consisted of the following:

- Species: scientific name of the species and an assigned group, to make some of the collected data more easy to interpret, rather than species specific data:
    - Apex: for this study wolf was the only species to be classified as apex.
    - Mesopredator: medium-sized, middle trophic level predator. In this study, red fox, European badger and mustelids were classified as mesopredator.
    - Ungulates: hoofed mammals, such as Galloway and roe deer
    - Birds.
    - Rodents and lagomorphs: both small prey for grey wolf.
    - Dogs.
- Location and site: camera trap location and site where behavior was recorded.
- Date and time: including number of days from when scat was placed.
- Scat freshness code 1 to 4 (1: soft on both in- and outside, 2: hard on the outside but soft inside, 3: hard both in-and outside, 4: only hairs left).
- Behavioral class: 1a-1b, 2, 3a-3b, or 4a- 4e (see above).

Figures were made to help interpret the collected data correctly for the research- and sub-questions. For the first sub-question, a table showing all occurrences for every species was made. This table helped to make graphs that show interactions within different groups defined as seen above. These graphs could answer how different vertebrates respond to wolf scats, and which species show attraction or fear to wolf scats. For the second sub-question, figures were made that show in what orders behaviors happened, showing if attraction or fear was related to wolf scats. Since making these assumptions is not reliable with little data, these figures were only made for the ungulates, red fox and badger. For the last sub-question, diagrams were made to show percentages between (multiple) over-marking on top of wolf scats and next to them, demonstrating the likelihood of DNA-transfer from different vertebrates species on wolf scat.

3. Results.

Results were collected from May 1 to May 31 2023, from both Hart van Drenthe and Drenths-Friese Wold. Hart van Drenthe had 5 scat sites that were set up to film 11, 10 and 6 days, due to scheduling. Drenths-Friese wold had 2 rounds of camera trapping, also due to scheduling constraints. The first round was done with 5 camera traps, starting May 8, after which 3 camera trap sites were added on May 19, until May 30. The 13 camera traps were set out for 286 trap nights, collecting data of 327 individuals. Because the camera traps had to have their batteries and SD cards replaced every 10 days, data was collected from individual camera traps multiple times (3 for hart van Drenthe: A, B and C), 2 for Drents-friese wold: A and B). This makes that in reality there were 13 camera traps, but 28 trap sites. Of these 28 sites, 24 of them had footage, somewhere in between 5 and 533 videos. Some camera trap sites proved more successful than others, and on average more mesopredators were found at Drents-Friese Wold trap sites, and more ungulates were recorded in Hart van Drenthe, mainly Galloways (*Bos taurus var.).* Out of the 24 sites that had footage, only 10 of them had fresh scats, since especially for Hart van Drenthe, it was difficult to gather fresh scats. From all 327 collected interactions, 206 were done in front of one of these 10 sites with fresh scat.

3.1. Species occurrence data.

Table 2 shows in detail all recorded data from the one-month camera trap survey, sorted by group and species, but the table also shows how many camera traps recorded a certain species was recorded. The table also displays the total number (N) of visits, as well as different types of behaviors (with corresponding category and code), and how often each species showed what behavior. Data shows that ungulates visited the camera trap sites most often, 175 times, but showed mostly ignorant, vigilant and inquisitive behavior. Mesopredator visits were recorded 69 times, of which nearly half (n=32) was red fox. Mesopredators showed interactive behavior 22 times, which is 31,9% of the time. European hare (*Lepus europaeus*) visited a scat site 40 times, but showed ignorant behavior 88% of the time (n=35). Grey wolf visited 10 times and was ignorant 80% of the time (n=8).

Table 2: All occurrence data of recorded behaviors. The table shows species, their corresponding group, number of visits, and all recorded interactions, as well as the N or camera trap sites where a species was found.

				Ignorant		Vigilant	Inquisitive		Interactive				
Group	**Species**	**N of sites found**	**N of visits**	**Passing by**	**Foraging**	**Vigilant**	**Looking**	**Sniffing**	**Scent marking**	**Over-marking**	**Multiple over-marking**	**Touching**	**Consuming**
Apex	*Canis lupus*	*4*	**10**	**8**	**0**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**
Mesopredator	*Martes martes*	*3*	**7**	4		1			1		1		
	Meles meles	*8*	**24**	12	1	1	2	2	1	3	8		
	Vulpes vulpes	*9*	**32**	20		4	7	6		4	4		
	Total		**69**	**40**	**1**	**6**	**10**	**9**	**2**	**7**	**13**	**0**	**0**
Ungulate	*Capreolus capreolus*	*21*	**89**	59	20	16	13	11					
	Bos taurus var.	*5*	**86**	67	23	5	7	5				1	
	Total		**175**	**126**	**43**	**21**	**20**	**16**	**0**	**0**	**0**	**1**	**0**
Bird	*Dendrocopos major*	*1*	**1**	1	1								
	Garrulus glandarius	*3*	**2**	2									
	Motacilla alba	*2*	**1**				1						
	Turdus merula	*3*	**9**	9	4								
	Turdus philomelos	*2*	**8**	7	2								
	Total		**21**	**19**	**7**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Lagomorph	*Lepus europaeus*	*8*	**40**	34	1	4	4	3					
Rodent	*Sciurus vulgaris*	*1*	**1**				1	1				1	
	Total		**41**	**34**	**1**	**4**	**5**	**4**	**0**	**0**	**0**	**1**	**0**
Dog	*Canis lupus f.*	*3*	**11**	**9**	**0**	**0**	**1**	**1**	**0**	**0**	**2**	**0**	**0**

3.2. Group occurrence data.

Figure 3 shows the number of mesopredator responses to wolf scats, showing an occurrence of inquisitive behavior such as looking at the scat (n=10) and sniffing the scat (n=9), and interactive behavior such as scent marking (n=1), over-marking (n=7) and multiple over-marking (n=13) out of 69. The figure also shows that vigilant behavior among mesopredators (n=6) did not happen as much as compared to ungulate responses, seen in Figure 4, which shows vigilant behavior (n=21) as well as inquisitive behavior, looking at the scat (n=20) and sniffing (n=16). Interactive behavior from ungulates was not recorded, except for once, with 1 Galloway touching the scat with its nose while sniffing. Foraging among ungulates happened 43 times.

Figure 3: Number of occurrences of the different mesopredator responses to wolf scats, out of 69 visits.

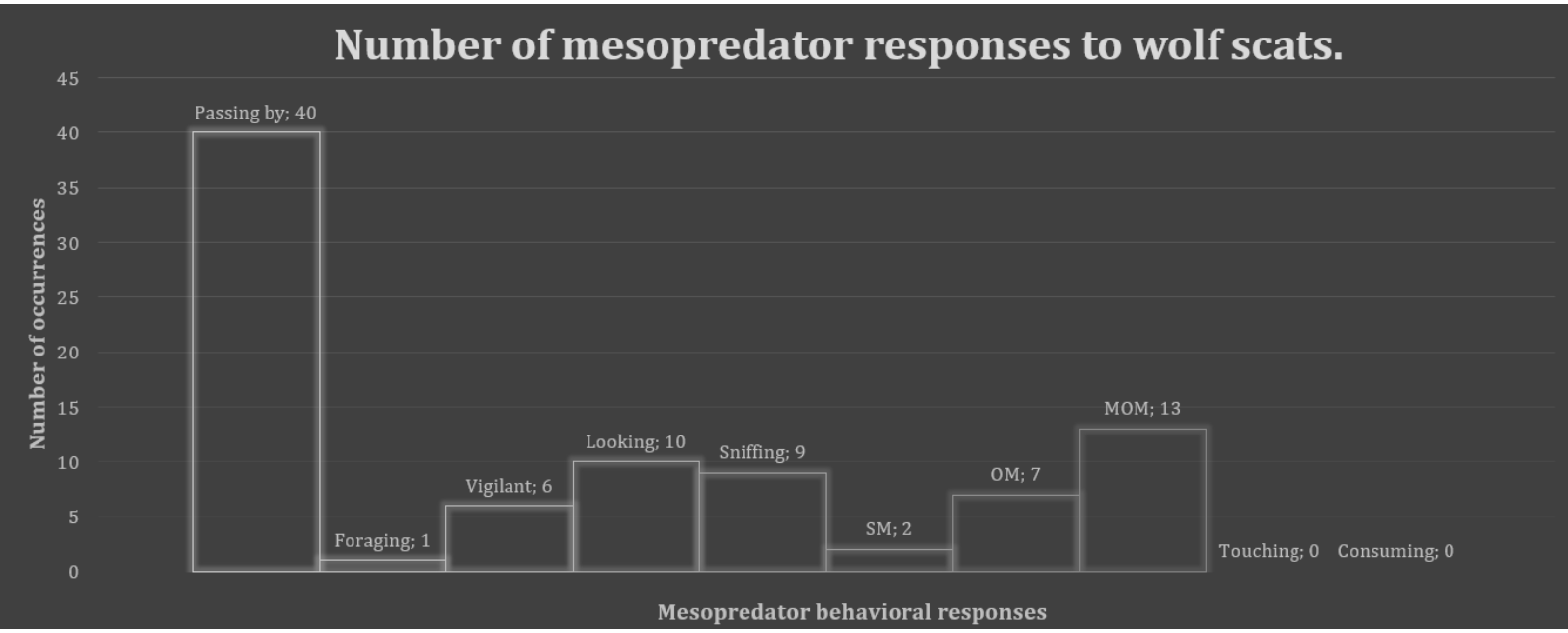
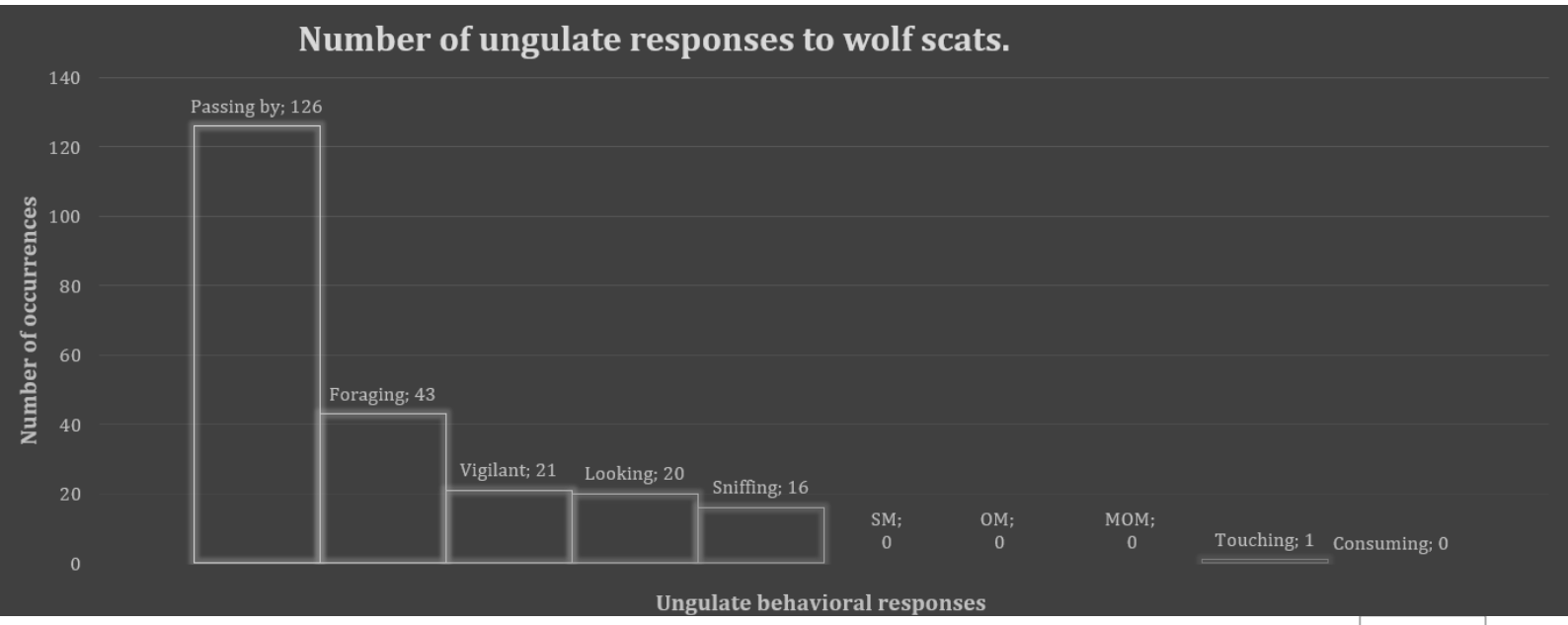


Figure 4: Number of occurrences of the different ungulate responses to wolf scats, out of 175 visits.



For the other groups of species, similar figures were made. Figure 5 shows the recorded behavior for birds, displaying that out of 21 visits, 19 individuals passed by, with only 7 individuals foraging and 1 individual looking at the scat. Figure 6 shows recorded behavior of apex (grey wolf), with out of 10 wolf visits, 8 passing by, and one individual showing vigilant behavior and another individual both looking at and sniffing the scat,

Figure 5: Number of occurrences of the different bird responses to wolf scats, out of 21 visits.

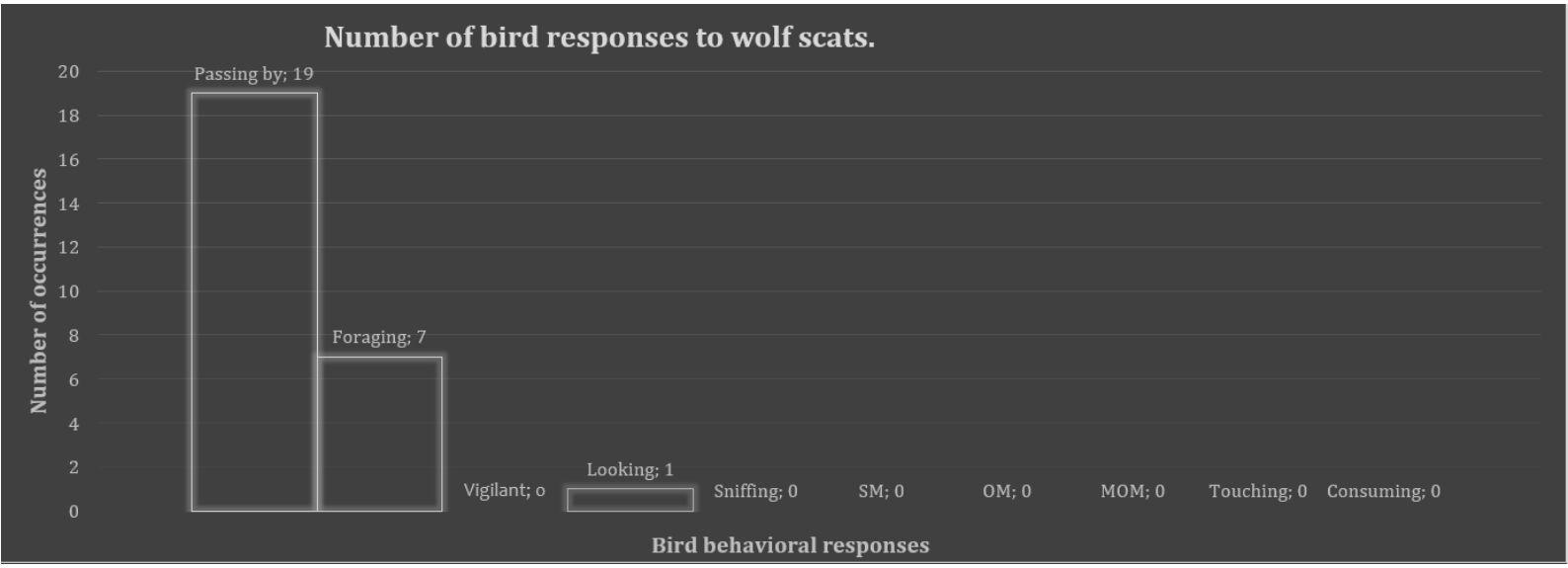


Figure 6: Number of occurrences of the apex (grey wolf), out of 10 visits.

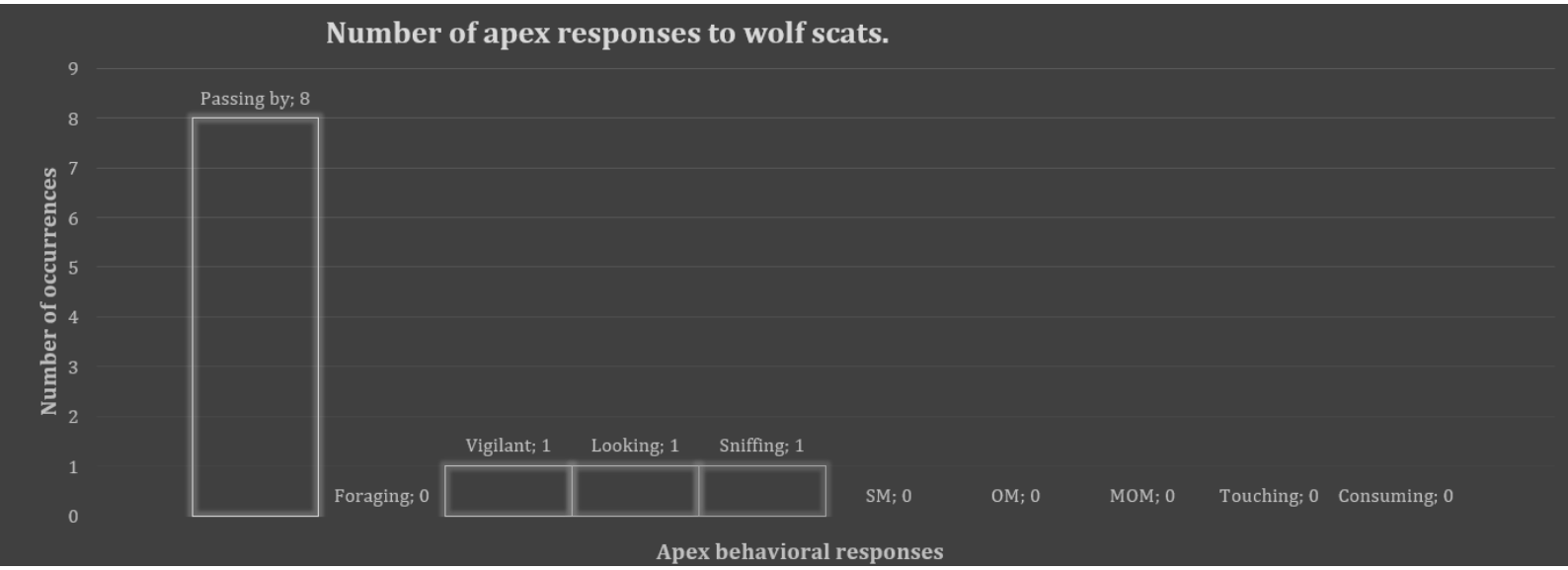


Figure 7 shows recorded data for rodents (only red squirrel was observed) and lagomorphs (only European hare was observed). This figure indicated 51 visits, of which 34 individuals passed by. Some vigilant (n=4) and inquisitive behavior (looking, n=5) was recorded as well. A single red squirrel was seen looking at the scat, sniffing at it, and in doing so, touching it. Finally, Figure 8 shows recorded data for dog with 11 visits, 9 of which were dogs passing by. Multiple over-marking was recorded 2 times for dogs.

Figure 7: Number of occurrences of the different lagomorph and rodent responses to wolf scats, out of 41 visits.

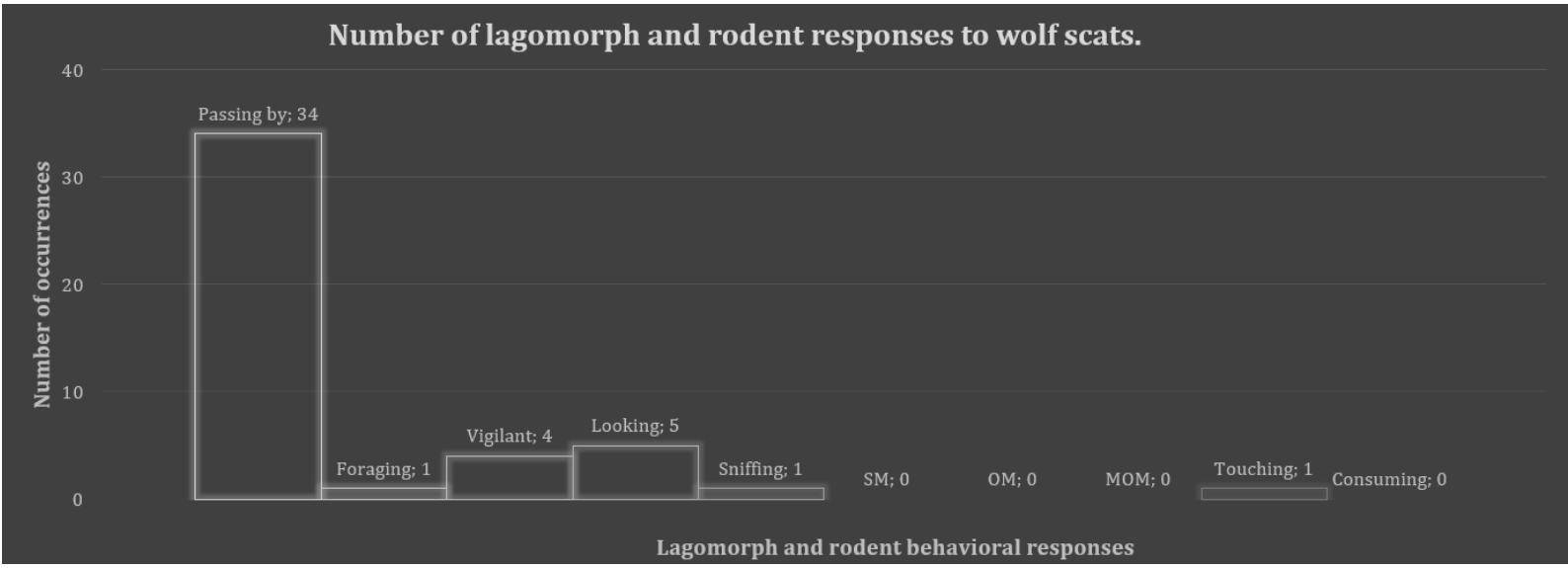
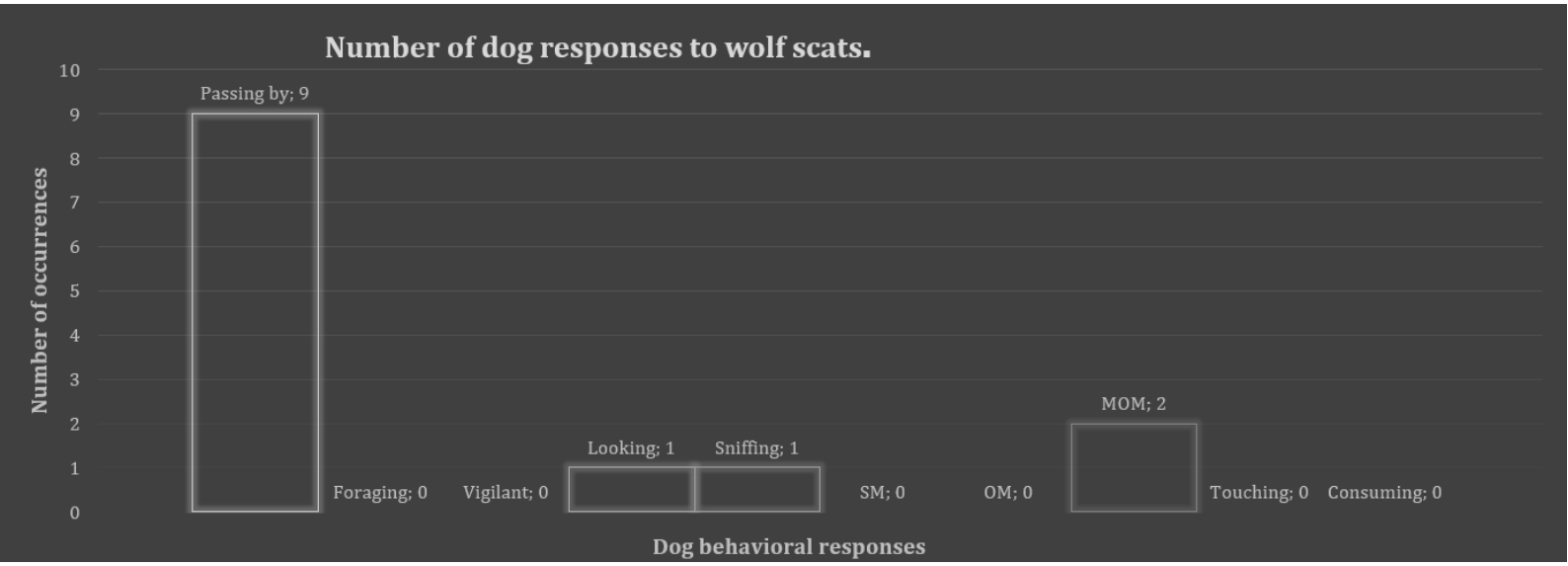


Figure 8: Number of occurrences of the different dog responses to wolf scats, out of 11 visits.



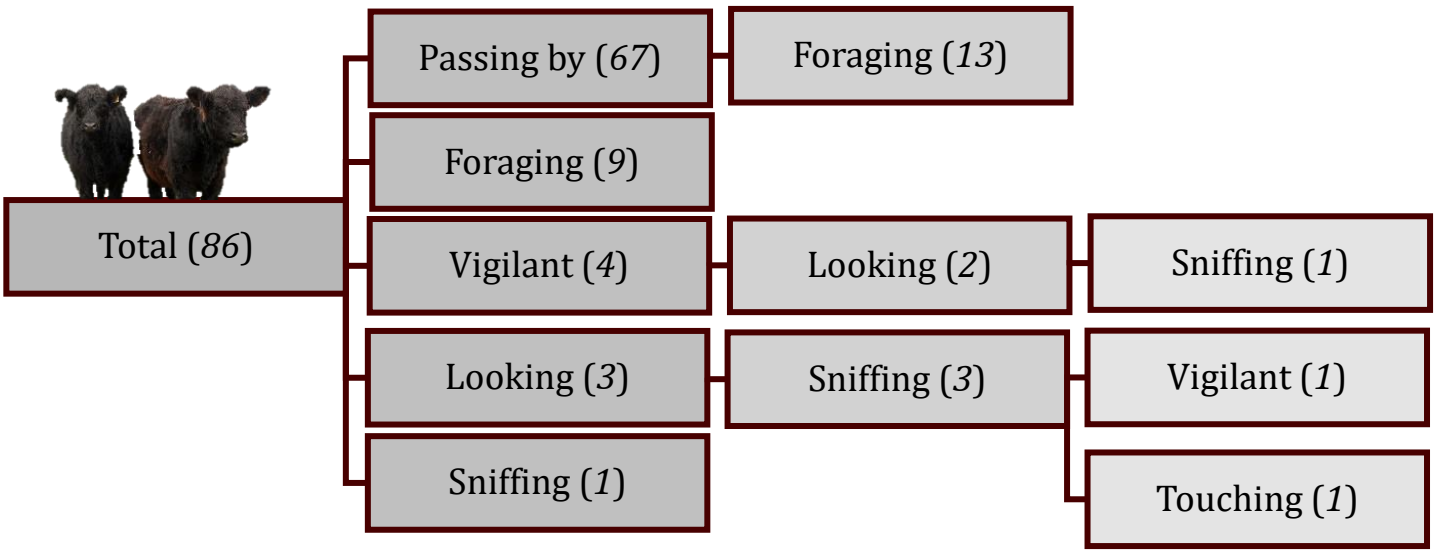
3.3. Fear or attraction to wolf scats.

3.3.1. *Bos Taurus var.*

All Galloway recordings were done in the Hart van Drenthe. Figure 9 shows recorded behavior of Galloways in order of which they happened, in relation to each other. Most individuals passed by, with occasional foraging (13 foraged during walking, 9 individuals foraged while standing still). 4 individuals showed vigilant behavior, even before looking at the scat or sniffing it.

In 3 other cases, a Galloway individual looked at the scat, after which it sniffed the scat. After doing this, 1 individual showed no other response, but another touched it with its nose, and yet another one showed a startled response, jumping up and entering a state of high vigilance.

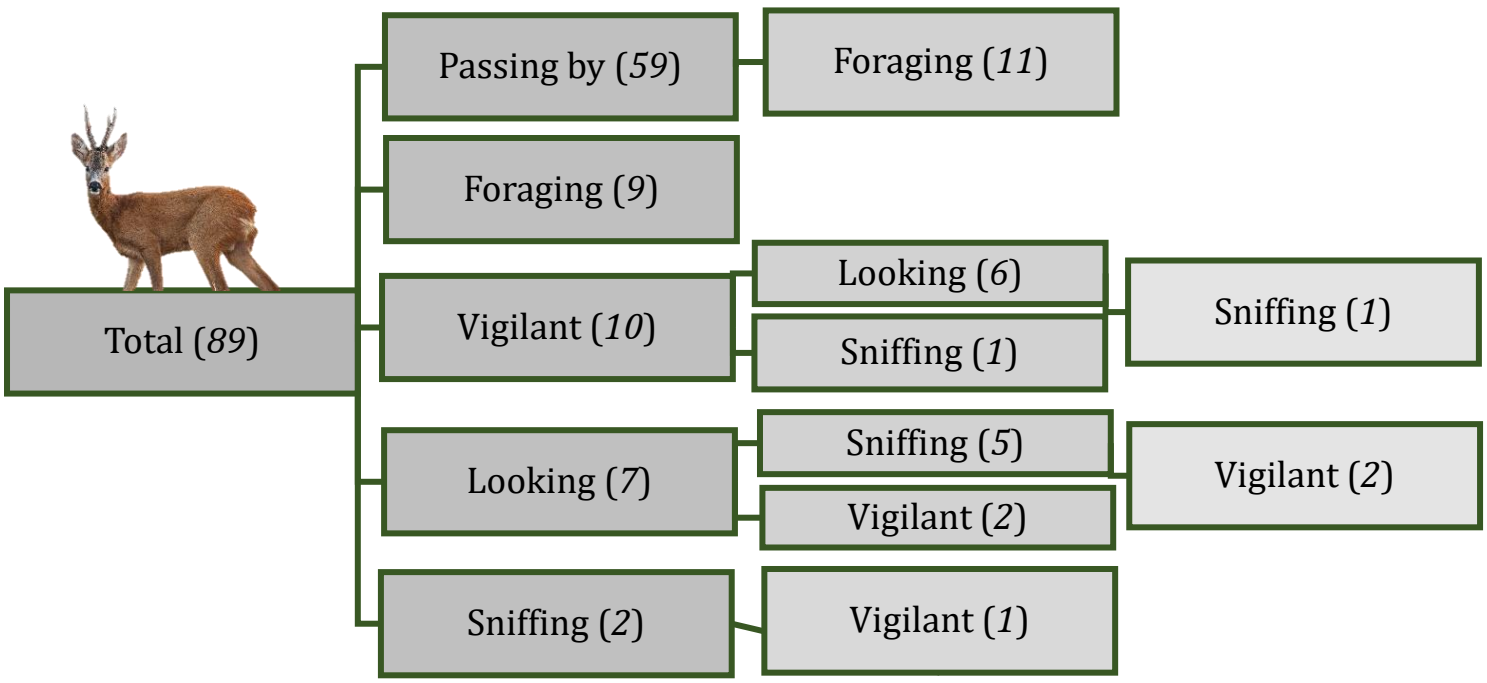
Figure 9: Recorded interactions of Bos taurus var. to wolf scats, in order.



3.3.2. *Capreolus capreolus.*

To indicate if roe deer showed fear or attraction to scats, a similar figure was made for the species. Figure 10 shows that out of 89 visits, 59 passed by and 20 individuals foraging, while passing by or not. Vigilant behavior was recorded 15 times, and roe deer sniffed a scat or near it 9 times, and looked down at it 13 times. 6 out of 15 times, a roe deer individual showed vigilant behavior, only after having sniffed or looked at a wolf scat.

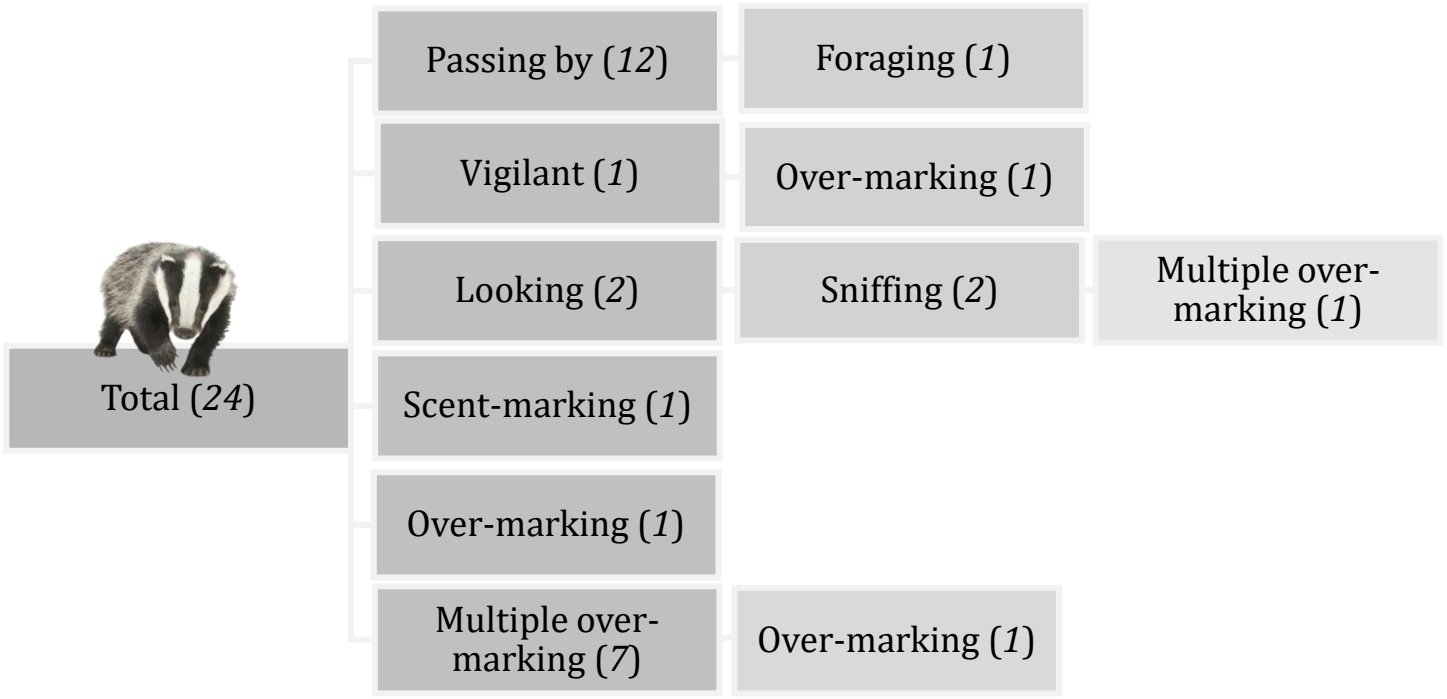
Figure 10: Recorded interactions of Capreolus capreolus to wolf scats, in order.



3.3.3. *Meles meles.*

Figure 11 shows recorded behavior from European badger, showing 24 individuals visiting a scat site, of which half (n=12) passed by and one foraging. Vigilant behavior was recorded once, followed by over-marking. Badgers were seen scent-marking, over-marking and multiple over-marking, usually not in response to a wolf scat. 1 badger was seen multiple over-marking another scent mark, and over-marking the wolf scat in a singular visit visit. Badgers would sometimes sniff or look for a scent, but would usually over-mark right away.

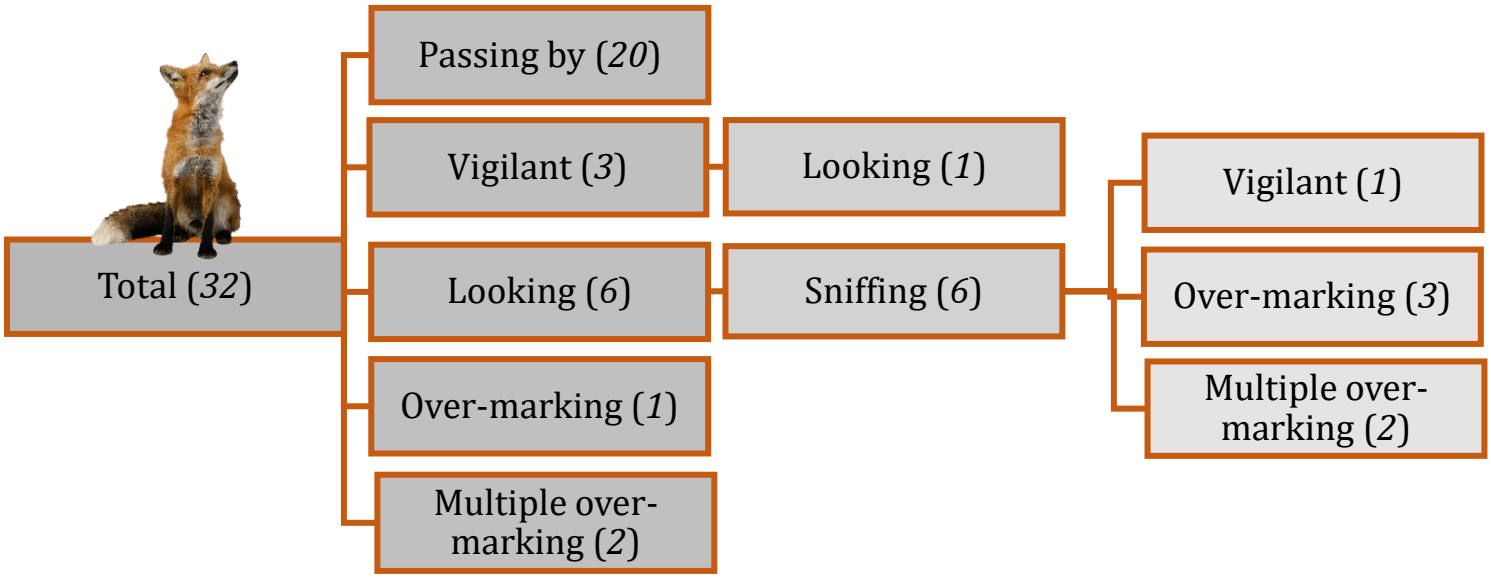
Figure 11: Recorded interactions of Meles meles to wolf scats, in order.



3.3.4. *Vulpes vulpes.*

Lastly, Figure 12 shows recorded behavior from red fox. Out of 32 visits, 20 individuals passed by, with 3 individuals showing vigilant behavior, and one looking at the scat in response to vigilance. In 6 instances foxes would first look at the scat and sniff it, before some over-marked (n=3) or multiple over-marked (n=2) it. One individual showed vigilant behavior after looking at and sniffing the scat. In 3 instances, foxes (multiple) over-marked a scent-mark directly, without looking or sniffing.

Figure 12: Recorded interactions of Vulpes vulpes to wolf scats, in order.

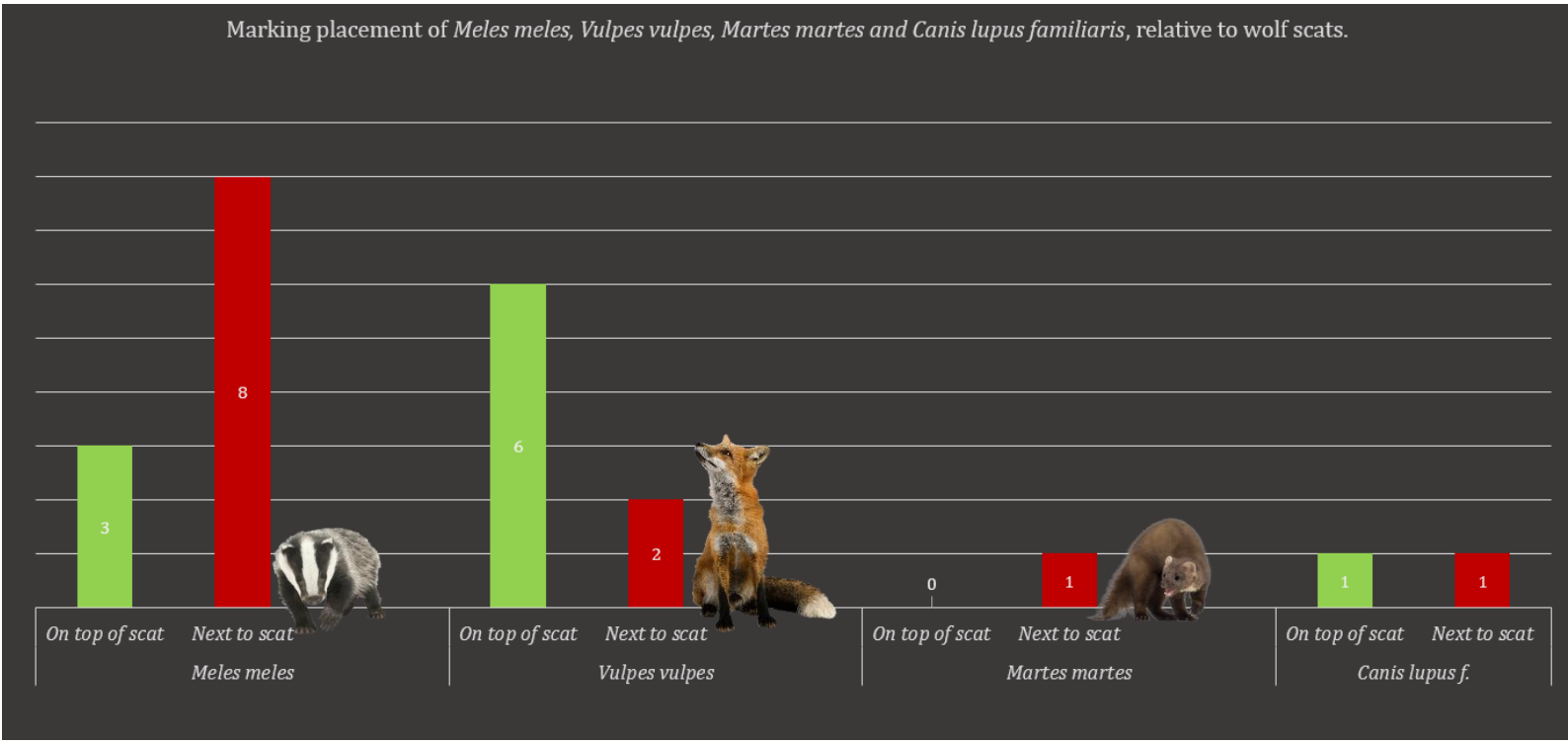


3.4. Mesopredator marking placements.

To determine the likelihood of transfer of mesopredator DNA on top of wolf scats, camera trap footage of red fox, European badger, pine marten and dog were analyzed, to see if over-marking happened on top of, or next to wolf scats, since determining where mesopredators prefer to place their scents could give an idea of the likelihood of a wolf scat being contaminated.

Figure 13 shows the cases of over-marking for red fox, European badger, pine marten and dog. It displays that fox chose to place their mark on top of the wolf scat 6 of the 8 cases (75% of the time), and only twice next to it. European badger (multiple) over-marked most often of all mesopredators: 11 times. Of these 11 over-markings, only 3 occurred directly on wolf scats. Badgers were more often seen to over-mark or multiple over-mark on top of previous badger markings. This study had 27,3% of recorded badgers over-marking on top of a wolf scat, and 82,7% next to the wolf scat. The figure also shows data for pine marten (*Martes martes*); a species that only had 1 case of over-marking on a pine marten scent mark. 2 cases of over-marking were finally present for dog, once on top of wolf scat, and one next to it, on top of a badger scent.

Figure 13: Locations of scent-marking and (multiple) over-marking of 4 species, relative to wolf scats.



4. Discussion.

The results of this pilot study on the behavior of other vertebrate species around wolf scats show a few interesting points:

1. Difference in engagement of vertebrate species between degraded and fresh wolf scats.
2. Inquisitive and vigilant behavior from ungulates and other prey around wolf scats, suggesting these species show a fear towards wolf scats.
3. Inquisitive and interactive behavior from mesopredators and dogs around wolf scats, suggesting these species show an attraction towards wolf scats.
4. Mostly ignorant behavior from birds and grey wolf.
5. A generally high likelihood of (multiple) over-marking from mesopredators on wolf scat, but a difference between marking placement among mesopredators.

In the remainder of this chapter, the results of these 5 points will be further elaborated and interpreted. In addition, some errors and limitations of the study are also mentioned.

4.1. Wolf scat engagement and wolf scat freshness.

Results show that out of 327 recorded interactions, 206 (63%) took place in front of a camera trap site with a fresh scat, a scat that was wet both on the inside and outside, or a scat that was hard on the outside, but still wet on the inside. This is notable, since fresh scats were only present on 10 out of 24 camera trap sites that recorded any data. It means that 41% of camera trap sites recorded 63% of the data, with all species recorded during this study showing more responses to fresh scats, compared to degraded scats. A reason for this could be that degraded scats are drier compared to fresh scats, with sometimes nothing more than hairs left. This results in a loss of odor emitting chemicals such as heterocyclic aromatic organic compounds, aldehydes, low weight fatty acids, and alcohols (Martín et al., 2010), making scats lose their purpose as olfactory cue in chemical communication. That in combination with the fact that camera trap sites were put up in forests such as Hart van Drenthe and Drents-Friese Wold, with relative high ground vegetation coverage, making it difficult for animals to locate a scat, especially if it has lost its scent. The lack of enough available fresh wolf scats for this study may have led to some camera trap sites being less successful than others in collecting data, since sites with degraded scat sites showed mostly ignorant behavior from vertebrates species. This does suggest a hypothesis that responsive behavior possibly leading to DNA contamination is more likely to happen when a scat is fresh.

4.2 Ungulate, lagomorph and rodent response to wolf scats.

Occurrence data for ungulates show an increase in vigilance, 13 times out of 89 (14,6%) site visits for roe deer. This number is relatively low, since a study by Kuijper et al. (2014) suggests a two fold in increased vigilance from red deer (*Cervus elaphus)*. Their study found 22% of red deer show vigilant behavior on control plots without scats, and 46% on plots with one wolf scat. Data shows 10 roe deer were vigilant before looking at or sniffing the wolf scat, meaning vigilant behavior could be unrelated to wolf scents, but 4 roe deer did show an increased vigilance after looking at and sniffing the scat, indicating there is a general fear towards wolf scats.

Galloways showed less vigilance compared to roe deer, only 5 times out of 86 (5,8% of the time). 4 Galloways showed vigilance unrelated to wolf scats. Only once did one solitary Galloway look and sniff at the wolf scat, after which it panicked (Figure 14) and went into a state of high vigilance, making the findings of both ungulate species conform the hypothesis.

Figure 14: Galloway response to sniffing (A) a wolf scat, panicking (B, C) and looking back (D).

The different levels and fear between roe deer and Galloways on wolf scats could be because roe deer were usually recorded as solitary individuals, and Galloways were more than often recorded as a herd, except for some individuals such as the one in Figure 14. Animals in big group are known to show less frightening and vigilant behavior compared to solitary animals (De Boer et al., 2004), which could be an explanation for the generally lower state of vigilance for Galloways. This study used focal and behavioral sampling focusing on one individual, so only behaviors of the individual closest to the wolf scat or the one showing the most interesting behavior were recorded, so no herd behavior was recorded. However, Galloways were the only species seen in groups of more than two and in all these instances, only ignorant behavior was shown.

Lagomorph and rodent responses of this study show mostly ignorant behavior, 34 out of 41 recordings (82,9%). Out of 41 recordings, 40 of them were European hares, with 4 (10%) showing vigilant behavior, 4 looking at the scat and 3 sniffing (7.5%). Mayer et al. (2020) showed an increase of hare vigilance on red fox scents, as well as reduced foraging, and this study raises the hypothesis that European hares also show fear towards wolf scats. European hares were recorded at 8 sites, out of which 5 sites with fresh scats. The 3 camera trap sites with degraded wolf scats recorded 50% (n=20) of European hares, only showing one case of vigilant and inquisitive behavior. Only one red squirrel was recorded looking at the scat, sniffing it, and in doing so touching it with its nose. Since it is only one recording, data is insufficient to argue fear or attraction towards wolf scats.

Data shows that ungulates and lagomorphs show an increased vigilance in response to wolf scats, which is likely because species are predated on by wolves, and a wolf scat might indicate a wolf is in the vicinity. Wild boar was not recorded in this study since it does not occur in Drenthe, but Kuijper et al., (2014) found that wild boars do not show vigilance in response to wolf scats, but attraction, sometimes even interacting with scats.

4.3. Mesopredator and dog response to wolf scats.

Mesopredators visited the camera trap sites 69 times, showing ignorant behavior 41 times (59,4% of visits), with vigilant behavior 6 times (8,6% of visits), Inquisitive behavior 19 times (27,5%) and interactive behavior 22 times (29% of visits). Red fox visited 32 times at 9 camera trap sites, showing interactive behavior 8 times (25% of visits), and showed vigilant behavior 4 times (12,5% of visits), meaning red fox showed both fear and attraction to wolf scats, which is against the expectations that suggested only attraction would be present. The study from Wikenros et al. (2017) suggested that red foxes express both fear and attraction to lynx scats, and data from this study suggests the same for wolf scats. Wikenros et al. (2017) reasons that both attraction and fear is shown because scats are an olfactory cue that serves as a source for information, but that mesopredators also have to stay alert because of a potential risk to have an apex in the vicinity. The fact that interactive behavior was recorded twice as much as vigilant behavior, indicates that in the trade-off between the benefit of information and the risk of interspecific killing, the benefit of gaining information from a wolf scat seems to be important enough to risk an encounter with an apex, if that means a fox has to adjust its behavior to lower the risk of getting killed (Apfelbach et al., 2005).

Red foxes were also seen over-marking and multiple over-marking on badger scent marks, completely ignoring the wolf scat at two sites, implying that red fox is also attracted to badger scent-marks. Since badgers and foxes do not predate on each other (Palomares & Caro, 1999), over-marking between these species gives the benefit of information without the increased risk of predation.

European badgers visited the scat sites 24 times and showed vigilant behavior once, and showed interactive behavior 12 times (50%), scent-marking far away from the scat once, and over-marking and multiple over-marking 3 and 8 times. Badgers were seen over-marking wolf scats 3 times, but were also (multiple) over-marking on badger scent marks, also if they have previously been over-marked by red fox. The lack of vigilance shown on camera footage means badgers do not avoid wolf scats and the over-marking does suggest an attraction towards them, which is conform expectations.

Pine marten visited the scat sites 7 times and passed by 4 times, showing vigilant behavior once, scent marking a couple meters away from the wolf scat, and another individual over-marking this scent mark. The sample size for pine marten is too small to give predictions on attraction toward wolf scats, but data lacks vigilance as well, implying that pine marten does not avoid wolf scats, which was expected. Wikenros et al. (2017) recorded pine marten over-marking lynx scats, expressing interactive behavior, and suggested that responses from pine marten may be similar as that of red fox. Dogs over-marked wolf scats twice out of 11 visits (18.1% of the time), pointing to an attraction from dog to wolf scats possibly related to them being closely related.

4.4. Birds and apex response to wolf scats.

Camera traps recorded 5 bird species a total of 21 times, out of which 17 were from the Turdidae family: *Turdus merula (*n=9), and *Turdus philomelos (*n=8), and 3 other species were recorded another 4 times. Birds passed by 19 out of 21 times (90,4%), with added foraging 7 out of those 19 times (36,8%). No vigilance was shown, neither was interactive behavior, and inquisitive behavior was limited to one case, with one white wagtail (*Motacilla alba*) looking at the scat, before it is scared away by a Galloway (the individual in Figure 14). The data from this study implies that birds rarely interact with wolf scats, which is against the initial expectations, since Kevin Groen's preliminary analyses of DNA of wolf droppings in the Crown Domains (Gelderland Province) showed that DNA of jay, great tit and jackdaw was present (Groen, personal communication, April 18th, 2023).

This hypothesized that birds do in fact interact with wolf scats in the Veluwe. There are two possible reasons for the lack of bird interactions during this study. The first reason for the lack of bird interactions to wolf studies, compared to preliminary data in the Veluwe, is that this study was done in forests. Forests have relative high ground vegetation compared to the Crown Domains, which is a natural area with a relative open landscape. This is due to clearance for farming and mining centuries ago (Van der Heide et al., 2008), turning what was once a forest into sand drifts and heather land. These open landscapes make it easier for species such as birds to spot a scat.

A second reason could be the lack of invertebrates living on wolf scats in the Drenthe areas. Invertebrates are often found on wolf scats in the Veluwe, under which the Crown Domains are a part, especially dung beetles out of the Geotrupidae family. Invertebrates are a big part of the diet of birds in the Veluwe, like the great gray shrike, with a diet consisting 42% of dung beetles (Deuzeman et al., 2015). The great gray shrike is the size of other songbirds such as black bird and (*T. merula*) song thrush (*T. philomelos*), species who also predate on invertebrates. During this study, no dung beetles or other invertebrates were found on wolf scats, so it could be possible that wolf scats are not interesting for birds in Drenthe, because they do not serve as a possible source of nutrition in the form of invertebrates.

Wolf (the only species considered an apex in this study) was recorded 10 times, once in Drents-Friese wold and 9 times in Hart van Drenthe. Vigilance (n=1), looking (n=1) and sniffing (n=1) were the only recorded behaviors other than passing by (n=8), suggesting wolf scats were mostly ignored by wolves. A first reason for this ignorance could be that the scats were degraded, which was the case in 3 out of 4 camera trap sites that recorded wolves. On the one camera trap site with a fresh wolf scat however, a wolf looked at the wolf scat and sniffed it, but did not show interactive behavior. An explanation for this could be that wolves do not over-mark other wolf scats, but data in Canada on over-marking between grey wolf and coyote (*Canis latrans*) disproves that, and shows that wolves do over-mark their scents with urine (Paquet, 1991). A second explanation for the ignorance of wolf scats is that the wolf scats were from the individuals passing by. Hart van Drenthe has one female and male wolf who both and have a territory (Arends, personal communication, May 2023). It could be possible that the wolves ignored the scat because it was familiar to them and was placed within their territory.

4.5. DNA-transfer to wolf scats.

4.5.1. Mesopredators and dogs.

Recorded red foxes displayed a high frequency of behaviors related to attraction, over-marking and multiple over-marking, and chose to place their mark on top of the wolf scat 6 of the 8 cases (75% of the time), and 2 times on top of a badger scent mark (25% of the time). This means red fox over-marked on top of another scent mark 100% of the time. This data shows that over-marking from fox is highly likely on wolf scats, which was expected, but that foxes also over-mark on scents marks of other species. This makes contamination of red fox DNA likely for not only the wolf dietary studies, but hypothesizes that there is also a possibility of it being present with dietary studies of other species as well.

As mentioned earlier, badgers were seen over-marking wolf scats but were also (multiple) over-marking on badger scent marks. A reason for this could be the fact that the badger scent marks were urine scent marks, and while scats are visually more striking, badgers have poor eyesight (Buesching & Macdonald, 2001), and urine is often regarded as the most important scent marking method, especially for wolves (Martín et al. 2010). Another explanation could be that these multiple badger markings were in fact of the same individual, and that this badger was re-visiting a known scent mark. Out of 11 cases of (multiple) over-marking, 27,3 % were on top of a wolf scat, and 82,7% next to wolf scat, which implies that DNA-contamination of European badger on wolf scats is likely to happen, but not as likely as with red fox.

Pine marten did not over-mark on top of wolf scats, which was against the expectations. The sample size for pine marten however is too small to exclude pine marten from being a species that would over-mark on top of wolf scat. The study by Wikenros et al. (2017) had pine marten over-marking on lynx scat, arguing that over-marking from pine marten onto wolf scats could also occur, and that it was not recorded. 2 cases of over-marking from dog show a 50/50% between scent marking on top of wolf scats and next to wolf scats, making DNA-contamination of dog likely as well.

Collected data on DNA transfer of mesopredators are conform the expectations. Mesopredators like red foxes, badgers and pine marten may show over-marking behavior within their species because it plays an important role in intraspecific communication related to competition, mates and group cohesion of individuals between the same

species (Ferkin & Pierce, 2007), but as mentioned in the introduction, little is known about over-marking for interspecific communication. Wikenros et al. (2017) gives a few hypothesis related to over-marking on lynx scats that may apply for wolf scats as well. The first reason why mesopredators such may over-mark on wolf scats is that they see it as an object, such as rocks, trees and bushes, which act as a substrate to increase the effectiveness of their scent mark. This is behavior common amongst red fox (Zaman et al., 2019). Alternatively, over-marking a scat may give a mesopredator information that a certain scat has been investigated earlier (Henry, 1977), or it can be that over-marking a scent is done to mask it, suppressing it and making it less effective (Ferkin & Pierce, 2009).

It was not possible to distinguish individuals in this study, so nothing can be said whether the same or different individuals did multiple over-marking. Multiple over-marking may be done for the same reasons as described above for apex predators, but for unfamiliar conspecifics or mesopredators of the other species within an ecosystem (Leo et al. 2015).

This data suggests that over-marking from fox, badger and dog on top of wolf scats could be common on wolf scats, and that the wolf dietary studies interpret collected samples with DNA of these species carefully.

4.5.2. Others.

Other than mesopredators and dogs, interactive behavior was barely shown, only by one Galloway and one red squirrel. These cases of interactive touching are highly likely to be incidental, because the touching happened out of inquisitive sniffing, with the nose touching the scat. It was earlier discussed that ungulates (Kuijper et al., 2014) and lagomorphs show a fear towards wolf scats, so engagement with a wolf scat is unlikely to happen, and DNA-contamination of roe deer, Galloway, lagomorphs and rodents therefore unlikely as well. Wild boar was not recorded during this study, but Kuijper et al. 2014 did find that wild boar showed an attraction towards wolf scats and were engaging with wolf scats, pressing their belly against the wolf scat. This behavior could be classified as touching and raises the hypothesis whether this behavior could lead to contamination of DNA on wolf scats.

4.6. Limitations of the study.

This study was the first to research the behavioral ecology of other vertebrate species around wolf scats. However, due to the short duration this study, there are a few limitations to the methods and analyses, that impact the reliability of the data. Nevertheless, these limitations provide valuable insights that future studies can draw inspiration from, building upon knowledge offered here.

The first limitation was the fact that the study was relatively short (one month), with a limited amount of camera traps and sites. The study recorded individuals recorded, which is a much considering the short amount of sampling, but is not enough to give accurate numbers for true the likelihood of certain behaviors, also due to the fact that there was no time for a statistical analyses, that could have given more accurate predictions. Results and conclusions in this study are therefore mainly suggestive, backed up by findings in existing literature and collected occurrence data.

The second limitation and maybe the most impactful one was that there were not enough fresh scats available to supply every camera trap site with a fresh scat. In some cases, degraded scats were used, but sites with degraded scats were visited less often and sometimes showed no recordings, making the data biased toward fresh scat sites. Another limitation is that the study limited itself to the Drenthe Province and forests. It was not possible to put camera trap sites in other areas of interest such as the Crown domains and other parts of the Veluwe, where wild boar, red deer, more wolves and birds species of interest live. The fact that all camera-trap sites were in forests, additionally makes the data set biased against birds, who were not recorded showing interactive behavior, while it was hypothesized that they would. Another limitation is that this is a pilot study, meaning there was little literature available on methods, and that data on a temporal and spatial scale was lacking. This made it difficult put collected data on a larger scale.

Finally, DNA samples were taken from each wolf scat prior to its placement and upon collection, so that any interactions resulting in possible over-marking could be assessed for potential DNA transfer. However, this data could not be used due to time constraints, making the conclusions on DNA-contamination limited to the likelihood of it happening, rather than a confirmative answer. The problem with this is that it had to be assumed that (multiple) over-marking on top of a scat always leads to DNA-transfer without margin of error, which is unlikely.

5. Conclusion.

The purpose of this pilot study was to collect the first data on the behavior of vertebrates toward wolf scats, as little was known about this in the literature. The research question was formulated as follows: 'What are the occurrences, types, and frequencies of behavioral responses of other vertebrate species around wolf (*Canis lupus*) scats in the Netherlands?'. To answer this answer this main question, the following 3 sub-questions were formulated:

- What types and frequencies of interactions do vertebrate species show toward wolf scats?
- Which vertebrate species show an increased fear or attraction to wolf scats?
- In what percentage of cases may interactive behavior with wolf scats lead to DNA transfer on wolf scats?

The camera traps recorded a total of 327 interactions. Roe deer, Galloway and European hare together were ignorant to wolf scats 72% of the time, vigilant 12% of the time, sometimes in direct response to wolf scats, indicating these species have a fear towards wolf scats. Foxes, badgers and pine marten combined visited scat sites 69 times, out of which inquisitive behavior (27,5%) was shown, as well as interactive behavior such as scent marking (2,9%), over-marking (10,2%) and multiple over-marking (18,5%). Vigilant behavior (8,5%) indicates that these species show an attraction towards wolf scats, but that there is a trade-off between the benefit of gaining information and the risk of being killed. Wolves showed little response to wolf scats, and bird species were not recorded to engage with wolf scats, possibly related to the birds not being able to see the scats because of dense vegetation and lack of invertebrates living on wolf scats in studies areas. Mesopredators (multiple) over-marked on top of wolf scats 75% of the time for red fox, 27,3% for European badger and 50% for dog, implying that it is common for these species (especially red fox) to over-mark on wolf scat. DNA-contamination from mesopredators on wolf scats is therefore likely, but that this is most common on fresh scats. Data does not show a clear threat of DNA-contamination onto wolf scats from other species, but literature and preliminary data shows it to be possible from wild boar and bird species, which could not be confirmed in this study. This pilot study found relevant data on behavior of vertebrates on wolf scats and suggests further research on DNA-contamination of fox, badger and dog on wolf scats, which seems common, also suggesting that interpreting the DNA of these species in wolf scats should be done carefully.

6. Recommendations.

The interesting findings call for follow-up research, conducted on a larger scale and a longer term, and including statistical analyses, to answer more precisely how often over-marking occurs and under what circumstances this occurs. This follow-up research should be conducted on a national scale to provide a dataset that can be used for the whole ongoing wolf dietary research, which is a nation-wide study in all areas where wolves live, and to give answers for species that were not found in this study, such as wild boar, red deer and bird species of interest. It could also give more information about behavioral responses of different wolf populations, such as the packs in the Veluwe. For such a larger-scale study, cooperation between researchers and nature park managers should be a central point; clear agreements should be established prior to fieldwork.

Secondly, if a follow-up study is planned, a big enough quantity of fresh wolf scats should be collected in all nature reserves of interest prior to conducting fieldwork, because as this research indicated, vertebrates (such as badgers, Figure 15) show much less responses to degraded scats as opposed to fresh scats. Using degraded wolf scats should therefore be avoided, and collecting fresh scats and storing these would be a short-term solution. If finding fresh wolf droppings proves difficult, an alterior recommendation is to conduct a pilot-study on the effects of wolf gland lures as opposed to fresh wolf scats. These lures are made from anal glands (amongst other glands) of wolves and are widely used for hunting and monitoring species in the United States, and could be used instead of or alongside wolf scats.

Figure 15: Camera trap footage of European badger, multiple over-marking near a fresh wolf scat in Drenths-Friese Wold.

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