

LANDSCAPE OF FEAR IN AN ANTHROPOGENIC ENVIRONMENT

The response of roe deer *Capreolus capreolus* and red deer *Cervus elaphus* to the presence of wolves *Canis lupus* in a Danish wolf territory

Master's thesis of Rebecca Lyhne

Section for Ecoinformatics and Biodiversity

June 2021



AARHUS
UNIVERSITY



English title: Landscape of fear in an anthropogenic environment – The response of roe deer *Capreolus capreolus* and red deer *Cervus elaphus* to the presence of wolves *Canis lupus* in a Danish wolf territory

Dansk titel: Frygtlandskab i et menneskepåvirket miljø – Rådyr *Capreolus capreolus* og kronodyrs *Cervus elaphus* respons på tilstedeværelsen af ulve *Canis lupus* i et dansk ulverevir

60 ECTS Master's project

Rebecca Lyhne, 201607599

Section for Ecoinformatics and Biodiversity

Department of Biology, Aarhus University

Handed in on the 9/6 2021

Defended on the 30/6 2021

Supervised by:

Jens-Christian Svenning

Section for Ecoinformatics and Biodiversity

Department of Biology, Aarhus University

Peter Sunde

Section for Wildlife Ecology

Department of Bioscience, Aarhus University

Kent Olsen

Head of Research

Natural History Museum Aarhus

Front page:

Three young wolves crossing heathland, Stråsø autumn 2019

Photograph by Helge Schulz www.helgeschulz.de

Table of Contents

<i>Preface</i>	3
<i>Part A – General Introduction</i>	6
Recolonising Predators in Europe.....	6
Ecological Role of Predators	6
Antipredator Behaviour	7
Perceived Predation Risk	8
Landscape of Fear and Trophic Cascades	10
Wolves in Denmark.....	12
Wolf Biology and Ecology	12
Danish Deer Populations	15
Wolf-deer Interactions	17
Ecological Impact of Returning Wolves in Europe.....	19
Camera Traps in Ecological and Ethological Studies	20
This Study.....	21
References	23
<i>Part B – Article manuscript</i>	32
Abstract.....	32
Introduction	32
Methods.....	35
Study species	35
Study area	36
Data collection and trail cameras	36
Data processing.....	37
Wolf activity index	39
Habitat variables	39
Sun angle and light periods	40
Statistical analysis.....	40
Deer presence model selection.....	40
Calculating overlap in diel activity	41
Comparing vigilance behaviour	41
Results	42
Deer presence	42
Diel activity of deer and wolves	46
Vigilance behaviour of deer	48
Discussion.....	51
Synopsis	51
Habitat selection including spatial wolf avoidance	51
Temporal overlap	52

Vigilance behaviour	53
Ecological Implications	54
Conclusion.....	55
Acknowledgements.....	56
References	56
<i>Appendix 1 - Wolf activity heatmaps.....</i>	<i>62</i>
<i>Appendix 2 - Control camera project</i>	<i>64</i>
<i>Appendix 3 – R code.....</i>	<i>66</i>

Preface

In 2012, after 200 years of absence, the first sighting of a grey wolf *Canis lupus* took place on Danish soil. The wolf, later named the “Thy wolf”, marked the beginning of a recolonization of wolves in Denmark, which would prove that large predators can thrive in intensely cultivated landscapes if we let them. The reappearance of wolves in Denmark was immediately a controversial topic, as some feared what having a large predator roaming the country would entail, while others celebrated the reintroduction of natural predatory dynamics to Danish nature. This complete split in attitudes towards the Danish wolves highlight the need for studies on what impact they might have on Denmark and the Danish nature.

After having had a life-long fascination with wolves, evident in both art and school projects of mine, the return of wolves to Denmark was a dream come true. So when the time came to choose a subject for my master’s thesis, there was never any doubt that the wolf would have to be involved somehow. After getting Jens-Christian Svenning, Peter Sunde and Kent Olsen involved as supervisors, it was decided that the subject would be how wolf presence affects the behaviour of roe deer *Capreolus capreolus* and red deer *Cervus elaphus*. This project took advantage of the large collection of camera trap data from the Ulfborg wolf territory that, as part of the National Danish Wolf Monitoring Project, has been monitored using camera traps since November 2016. I was very excited to get the chance to incorporate both ethology and ecology, as well as the much-debated *landscape of fear* concept, in my project. Throughout the entire project, I have been fortunate enough to receive excellent and thoughtful supervision from all three supervisors, whose expertise has been of great value to me.

Concurrently with my project, Sofie Amund Kjeldgaard studied the effect of wolves on red fox *Vulpes vulpes* using the same data, and we were therefore able to work together when processing data and conducting field work. We are glad that our work will contribute to the database on fauna activity in the Ulfborg territory. During the four months of photo processing, we minimised inter-observer bias by consulting with each other and also quality checked older entries in the database. Since we would both use

wolf activity as a variable in our analysis, computing of this variable using GIS-software was done collaboratively. To accurately estimate the spatial distribution of wolf activity in the area across the study period, we used the complete record of verified wolf observations from camera traps and scat finds in the study area, the coordinates of which we manually verified prior to use. During data handling, we divided parts of the coding work between us, with Sofie locating entries that needed to be corrected for daylight savings time and me converting the time to UTC and estimating the sun angle for each observation.

Sofie and I also collaborated on a side project funded by a grant from Fonden Frands Christian Frantsens Legat. The purpose of that project is to test if photos captured using the “wolf strategic camera placement” give an accurate representation of the general fauna activity on the location (see **Appendix 2**). To study this, we installed 14 randomly placed control cameras in the Ulfborg wolf territory in November 2020. Unfortunately, the time frame for our thesis projects did not allow us to analyse the resulting data, however, we hope to do so after we graduate. Through our field work, we gained great insight into the advantages and limitations of using camera traps for ecological and ethological research, and I am very grateful to have had this opportunity. The field work underlying our side project would not have been possible without the help and guidance from Thomas Boesdal and Søren Krabbe. From the very beginning, you have been nothing but welcoming and willing to teach us all you know about camera trapping and the local wildlife, and we owe you our deepest gratitude.

Throughout the entire project, Sofie has been an invaluable sparring partner and companion, and this project would not have achieved its current form without her. Thank you for the great teamwork during our field work, and the insightful discussions during the data analysis and writing process. Lastly, I would like to thank my friends and family for putting up with me through all these years, even when I must surely have begun boring you with all my wolf talk. A special thank you to Nina Pirker Dalsgaard, Tina Egestrøm Jensen and Morten Hoppe for the many hours of mutual support and fun during our time at Aarhus University.

This thesis consists of a general introduction to the ecological role of predators, the characterization of predation risk, the landscape of fear concept and the wolf-deer system, as well as an introduction to the three study species – grey wolf, roe deer and red deer. Following that is the article manuscript “Landscape of fear in an anthropogenic environment – how deer respond to the presence of wolves in a Danish wolf territory”, which is the result of my project on how wolves may affect the habitat selection, diel activity and vigilance behaviour of deer in a Danish wolf territory.



Part A – General Introduction

Recolonising Predators in Europe

During the last decades large predators have been recolonising the European continent, proving that even landscapes highly modified by humans can sustain predator populations (Chapron et al., 2014). Even though recolonising carnivores avoid the most densely populated areas, they have successfully recolonised areas with moderate human densities (Milanesi et al., 2017). Studies on the movement of predators in anthropogenic landscapes show that their habitat selection is the result of a trade-off between prey availability and human disturbance (Basille et al., 2009; Carricondo-Sanchez et al., 2020; Lesmerises et al., 2012). Predators have historically been persecuted in most areas to protect livestock, and the recent recolonisation is therefore both due to legal protection of predators and a parallel rise in ungulate abundances (Randi, 2011). Ecological dynamics and species interactions are missing extensively in Europe at the moment due to trophic downgrading and over exploitation of species by humans (Estes et al., 2011; Worm & Paine, 2016). Predation from recently colonised and expanding carnivore populations could reintroduce missing dynamics resulting ecological changes on multiple trophic levels. However, prey behaviour might be more affected by human disturbance in areas where predators return to (Ciuti et al., 2012). To properly understand the impact of returning predators, studies are needed on the effect of predation in areas with large human populations.

Ecological Role of Predators

Predators play a fundamental role in ecosystems by exerting top-down effects on lower trophic levels through prey consumption (Ripple et al., 2014; Wilkinson & Sherratt, 2016). Predators also affect population dynamics, adaption and behaviour of their prey through both direct predation and the indirect non-lethal effect they have on prey animals (Lima, 1998). These non-lethal effects stem from the efforts of prey to avoid predation, by spending energy on fleeing, increasing vigilance behaviour at the cost of foraging and avoiding high quality foraging areas if these are associated with a higher predation risk. Preisser *et al.* (2005) argue that the indirect effects of predation are equal to or may even surpass effects of direct killings, as defensive strategies lead to reduced energy income, increased energetic investment in defensive structures, lower mating success and increased vulnerability towards other predators. The high price of these defensive strategies force prey to make the optimal trade-off between foraging and antipredator behaviour at any given moment

(Brown & Kotler, 2004). To do so prey need to assess the riskiness of any given situation, known as the *perceived predation risk* (Bleicher, 2017). In theory, the spatial variation in perceived predation risk can affect top-down effects of herbivory, which might be reduced in areas of high risk and increased in safe areas (Ripple & Beschta, 2004). The presence of predators can therefore have far-reaching effects on environmental heterogeneity and species composition.

Antipredator Behaviour

Antipredator behaviour encompasses all behavioural choices that aids prey in avoiding detection and capture by predators. In the most basic form, prey might react to presence of predator cues by activating the acute stress response resulting in “fight or flight” (Bonnot et al., 2017; Osada et al., 2015). Behavioural immobility is another response to predator cues, indicated by decreased foraging and hypervigilance (Anholt et al., 2000; Roseboom et al., 2007). Vigilance lets an animal scan for predators at the cost of movement and foraging and should therefore increase with predator encounter rate and lethality and decrease with forage quality (Brown & Kotler, 2004; Lima & Bednekoff, 1999). Antipredator behaviour is triggered by the presence of predator cues in the environment (Apfelbach et al., 2005). Studies showing that prey respond to olfactory cues of regionally extinct predators, prove that some components of antipredator behaviour is innate (Osada et al., 2015; Sahlen et al., 2016).

If animals begin associating certain areas, habitat types or times with increased risk of predator encounter, they will exhibit antipredator behaviour on a much broader scale. This can take the form of avoidance, either of areas generally or at certain times (Kohl et al., 2018; Palmer et al., 2021; Sahlen et al., 2016), changed diel activity (Rossa et al., 2021), increased group size (Creel et al., 2014) and altered behaviour in specific habitats, e.g. increased vigilance in risky areas (Blanchard et al., 2018). The *predation risk allocation hypothesis* (Lima & Bednekoff, 1999) postulates that antipredator behaviour of an animal depends not only on the immediate level of predation risk, but also on the background level, that is established through an animals association between places and predator presence. As risky encounters become more frequent, prey will have to allocate more time to foraging when risk is lower. Consequently, antipredator behaviour should be strongest in high-risk situations when these are rare, and lowest in low-risk situations when risk is generally high. This clashes with earlier studies on antipredator behaviour, where risk was often assumed to be constant (the *risky places hypothesis*) and the responding antipredator behaviour of prey to be

proportional to the amount of risk (Anholt et al., 2000). Instead, the *predation risk allocation hypothesis* emphasizes the need to take temporal and spatial variability in risk into consideration as well as the condition of the animal when evaluating the strength of antipredator behaviour.

Perceived Predation Risk

The decision making of prey is affected by the perceived predation risk, as they have to decide when and how to exhibit antipredator behaviour, when to forage and when to engage in social behaviour (Lima & Dill, 1990). In a review, Moll *et al.* (2017) made a framework for predation risk metrics which divided predation risk into *risky places* and *risky times* (see **fig. 1**). When defining *risky places* only background risk associated with an area is considered. In the most basic interpretation of the *risky places* concept, areas with many predators will be seen as riskier than areas with fewer predators, which again is riskier than areas with no predators at all (Anholt et al., 2000; Laundré et al., 2001). Habitat characteristics such as openness (Blanchard et al., 2018; Sahlen et al., 2016) and elevation (Mao et al., 2005) may affect the general risk associated with an area. Estimating the probability of predator encounter or kill occurrence in an area rather than simply noting the presence of predators, is another way to characterise background risk (Hebblewhite et al., 2005). In some cases, habitat characteristics are used as a proxy for riskiness, by assuming certain habitats have a higher predator density, probability of predator encounter or kill occurrence. In these cases, risk can be measured e.g. as degree of openness (Barja & Rosellini, 2008) or elevation (Festa-Bianchet, 1988). The clearest example of a *risky time* is situations where prey interact directly with real or simulated predators. However, the definition can be broadened to include situations where predator and prey are not directly interacting, but where prey are assumed to be aware that predators are close by. By this definition predation risk can be measured as the presence of olfactory or auditory predator cues (Kuijper et al., 2014), distance to nearest predator (Bonnot et al., 2017; Middleton et al., 2013) or presence/absence of predators on a shorter temporal scale (e.g. hour, day) (Creel et al., 2005). Habitat characteristics can affect the perceived riskiness of a situation by providing an animal cover pre-detection or hindering its escape post-detection (Bonnot et al., 2017).

The two definitions of risk are not necessarily independent, as the riskiness of a time can be affected by the general riskiness of the place, and the riskiness of a place can be defined by how often *risky times* happen. How an animal perceives risk and how it chooses to modify its behaviour

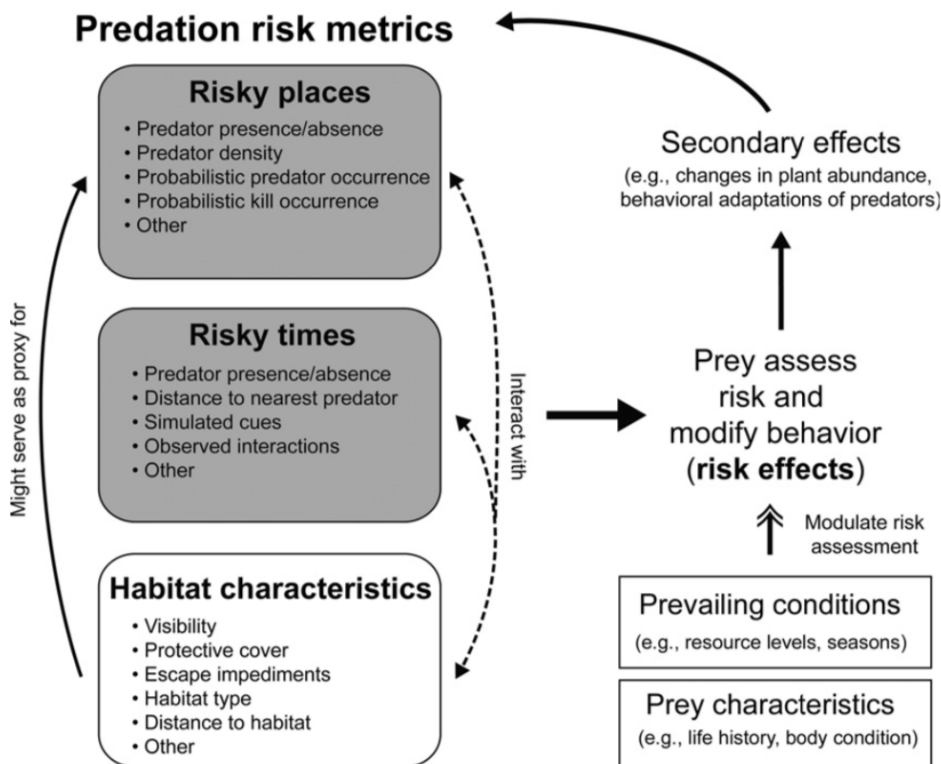


Figure 1 – from Moll *et al.* 2017: “A framework for predation risk metrics and risk effects. Risk metrics fall into one of three categories (rounded boxes) and 1 of 16 subcategories (bullet points). Light gray rounded boxes include measures of predator space use or behaviour and the white rounded box contains habitat-based metrics. Prey modify behaviour after assessing risk emanating from the left rounded boxes and modulated by both prevailing conditions and prey characteristics (lower right boxes, double arrow). Modified behaviours (risk effects) might have secondary effects on both lower (e.g., plants) and higher (e.g., predators) trophic levels. These secondary effects can then feedback into risk metrics by modifying predator behaviour and/or habitat characteristics.”

as a result of its risk assessment is affected by its condition as well as the condition of the environment (Moll *et al.*, 2017). Characteristics such as social status, group size, age, sex are known to affect antipredator behaviour (Childress & Lung, 2003; Festa-Bianchet, 1988), as well as the prior experience of the animal (Dickman, 1992). As the trade-off between risk and foraging is affected by resource availability and the condition of the animal, there can be large seasonal differences in antipredator behaviour (Brown & Kotler, 2004). In a study on big horn sheep *Ovis canadensis*, pregnant ewes migrated to higher elevations during lambing season despite the lower quality forage there (Festa-Bianchet, 1988). This shows that the risk at lower elevation increased during lambing season, due to high lamb mortality, as non-pregnant ewes only migrated later when forage had increased in quality. Likewise, adult female elk allocate more time to vigilance during calving season (Lung & Childress, 2006). However, risk might even vary across the diel period,

with areas only being risky at certain times of the day where predator encounter is assumed to be high (Blanchard et al., 2018; Kohl et al., 2018; Palmer et al., 2021).

Moll *et al.* (2017) notes that there is high variability in how predation risk is characterised and measured. If we take the wolf-deer system as an example, some studies focus on short-term predation risk measured by presence of wolves on the day (Creel et al., 2005), distance to nearest wolf (Liley & Creel, 2008; Middleton et al., 2013) and presence of wolf urine or scat (Kuijper et al., 2014; Palmer et al., 2021). Others focus on long-term predation risk defined as presence or absence of wolves (Laundré et al., 2001), density of wolves in certain areas (Creel et al., 2008), wolf encounter rate (Lung & Childress, 2006; Rossa et al., 2021), kill densities (Kohl et al., 2018), or wolf home range estimates (Anderson et al., 2005; Flagel et al., 2016; Kuijper et al., 2013). A concern with defining predation risk for longer periods is that risk is not static, and prey would waste a lot of energy, if they did not use fine-scale cues to assess predation risk (Lima & Bednekoff, 1999). Consequently, studies might have overlooked the effect of predators because they do not see prey responding to long-term risk.

Landscape of Fear and Trophic Cascades

If the distribution of a prey population in a habitat is affected by the perceived predation risk, it can be said that the prey inhabit a *landscape of fear* (LOF), which Bleicher (2017) defines as “the spatially explicit distribution of perceived predation risk as seen by a population”. LOFs can be visualised by creating a map of perceived predation risk experienced by prey in a specific area. A simple way to do this is to interpolate data on predator density (either from observation on static locations or GPS movement data) to create a map of estimated predator encounter rate across the entire area. However, assuming that a higher predator encounter rate equals a higher predation risk might be inaccurate, as successful predation events rely on landscape features, prey type, time of day, season etc. (Hebblewhite et al., 2005; Merrill et al., 2010). It might therefore be more accurate to visualize perceived predation risk by estimating the probability of kill occurrences. By using models to estimate kill probability, researchers can combine predator presence with fine-scale spatial measures such as habitat characteristics affecting prey vulnerability and hunting success (see **fig. 2**). Furthermore, knowledge on activity patterns of both predator and prey as well as species specific antipredator behaviour can be incorporated. Using this approach recent studies on wolf-mediated LOFs have shown that LOFs can vary between sympatric species (Gervasi et al., 2013)

and across the diel period (Kohl et al., 2018). The basic LOF model predicts that when an animal moves from an area associated with low predation risk to an area associated with high predation risk, it will alter its behaviour in order to minimize risk. The response can be complex and recent studies emphasize the need to look at multiple behavioural responses to LOFs (Blanchard et al., 2018; Creel et al., 2014). The response of prey to LOFs can lead to behaviourally mediated trophic cascades if foraging patterns are affected (Miller et al., 2012; Ripple & Beschta, 2004).

A direct change in one trophic level might indirectly affect lower trophic levels with cascading effects. Such *trophic cascades* include release in herbivory due to predator absence which limits primary production (Terborgh et al., 2001). The best known example of this is how the reintroduction of grey wolves *Canis lupus* (hereafter referred to as wolves) in the Greater Yellowstone Ecosystem (GYE) between 1995 and 1997 through predation on American elk *Cervus canadensis* indirectly affected woody plant communities and riparian systems (Ripple & Beschta, 2004). The increased recruitment of aspen *Populus spp.* and willow *Salix spp.* was thought to be caused by a decrease in elk numbers due to wolf predation (Beschta & Ripple, 2015; Ripple & Beschta, 2012). However, the recovery of riparian systems and subsequent increase in beaver

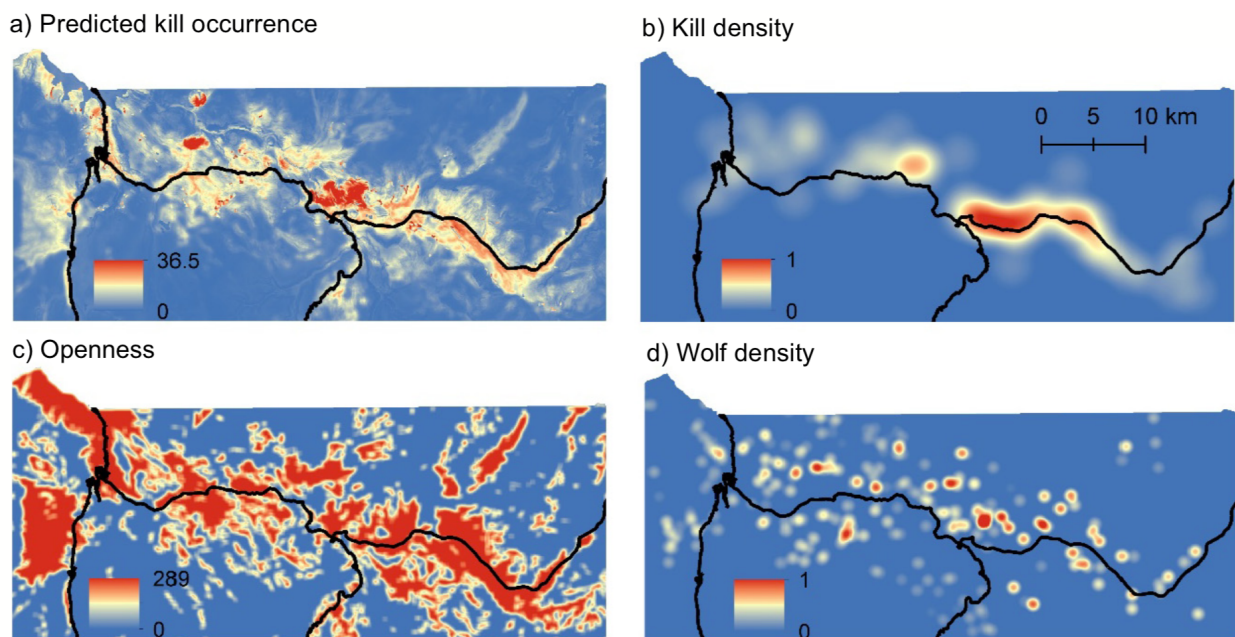


Figure 2 – figure from Kohl *et al.* 2018 showing the spatial variation in wolf predation risk during winter in northern Yellowstone National Park. a) predicted kill occurrence of elk using a model incorporating wolf pack distribution, relative elk density, proximity to streams/roads, openness, slope and snow depth, b) density of wolf-killed adult female and calf elk, c) openness, and d) density of wolves. Black lines denote roads.

Castor canadensis numbers was explained by the fear of predation causing elk to avoid foraging in open areas, where predation risk was higher, leading to a decrease in herbivory in the riparian zones (Ripple & Beschta, 2004). Later studies have challenged the cascading effects of wolf predation on both vegetation structure and riparian recovery (Marshall et al., 2013; Mech, 2012). A large concern is that earlier studies failed to take climatic and landscape factors as well as other trophic forces into account (Marshall et al., 2014). Furthermore, the effect of reduced elk browsing might be reduced by a concurrent rise in bison *Bison bison* populations, whose foraging pressure is now 10 times that of elk in the GYE (Beschta et al., 2020). Dobson (2014) emphasizes the need to take a system-wide approach to study the effect of species interactions in complex natural systems.

Wolves in Denmark

Wolves were absent in Denmark until 2012 when a wolf was observed in Thy almost exactly 200 years after the last Danish wolf was shot in 1813 (Madsen et al., 2013). Wolves were almost entirely eradicated in Europe during the 18th and 19th century, with only small fragmented populations remaining in Italy, Iberia and Northern Scandinavia (Randi, 2011). The “Thy wolf” as it was later named, was to be the first of many wolves to cross the Danish border from Germany (Sunde & Olsen, 2018). The reappearance of wolves in Denmark is just one part of a larger recolonization of wolves in Europe, which is the result of legal protection, increasing ungulate populations and socio-ecological changes in rural areas (Randi, 2011). The return of wolves immediately sparked discussions as some thought Denmark too small to sustain a wolf population without it impacting livestock farming, hunting and recreational activities negatively.

Wolf Biology and Ecology

Wolves are large, highly social predators that function as apex predators in ecosystems across the entire Holarctic region (Castelló, 2018). Wolves hunt collaboratively and have an evolved social behaviour. Pack composition is highly variably across geographic regions, with the basic social unit being the mated pair who might be joined by old offspring or even unrelated adult wolves (Mech & Boitani, 2003). Wolf packs tend to be largest where wolves prey on the largest ungulates, but whether pack size is an adaption to larger prey is still debated (MacNulty et al., 2012). Offspring are born in late spring (in Denmark late April to early May), correlating with prey abundance (Mech & Boitani, 2003). Pups dispersing in their first year often do so between January and May and may stay near their parents territory or undergo long dispersal immediately (Mech & Boitani, 2003). In

Denmark all offspring dispersed as yearlings (Ulveatlas.dk, upupl. data). Long dispersal to the edge of species range is not unusual (Wabakken et al., 2007). The Danish wolf population is a result of such long dispersals, with wolves having dispersed between 450-850 km from central Germany and Poland (Sunde & Olsen, 2018). The Danish wolves are part of the central European lowland wolf population (Sunde & Olsen, 2018), which have been steadily expanding during the last decades (Deinet et al., 2013; Reinhardt et al., 2015).

Established wolves move within a home range, the size of which varies considerably across the geographical distribution of wolves (Mech & Boitani, 2003). Home range size depends on resource availability, habitat characteristics, intraspecific competition and human disturbance (Kusak et al., 2005; Mattisson et al., 2013; Rich et al., 2012). In Denmark, lone established males have patrolled areas up to 500 km², whereas the territory of the first mated pair was 78 km² (Sunde & Olsen, 2018). During pup rearing in spring and early summer, the activity area of wolf packs shrink as the den becomes the centre from which adults radiate out from, and increases in size again when pups begin following the parents (Mech & Boitani, 2003). Yet the home range of the mated pair in Denmark only shrunk further in the following fall and winter (September 2016 – March 2017: 77 km², April-September 2017: 30 km², October-December 2017: 4.2 km²). However, as wolf territory size is dependent on prey abundance, the small home range size could be a reflection of a large prey population in the area (Kittle et al., 2015). In late summer, pups are left at rendezvous spots, which are often placed in the middle of the territory (Mech & Boitani, 2003). Wolves are highly mobile and can travel up to 8 km/h, with 27 km being the average distance travelled in Italy (Ciucci et al., 1997). Wolves take advantage of easy travel routes, often using trails and roads to move effectively through the landscape (Ciucci et al., 2003; Gurarie et al., 2011; Kusak et al., 2005; Zimmermann et al., 2014). After many years of being hunted by humans, wolves generally avoid anthropogenic features in the environment such as major roads and settlements (Carricondo-Sanchez et al., 2020; Eggermann et al., 2011; Kaartinen et al., 2005; Lesmerises et al., 2013). Some studies find that wolves in anthropogenic environments are more nocturnal than wolves in more natural areas (Kusak et al., 2005), whereas others do not find an effect of human density on nocturnality (Theuerkauf et al., 2007).

The presence of European wolves is generally correlated with high prey abundance, high forest cover and low human density (Ciucci et al., 2018; Gurarie et al., 2011; Jedrzejewski et al., 2004;

Massolo & Meriggi, 1998; Zlatanova & Popova, 2013). However, wolves are able to tolerate moderate human disturbance if prey availability is high and refuge is provided (Eggermann et al., 2011; Grilo et al., 2018; Llaneza et al., 2018; Reinhardt et al., 2019). Based on this, wolves were expected to establish territories in the remote western parts of Denmark where forest and heath cover is high, human density is low and prey is abundant (Madsen et al., 2013). As expected wolves in Denmark established territories in these areas (Sunde & Olsen, 2018). As with other European wolves, Danish wolves have been shown to be nocturnal (Thorsen et al., 2019).

Wolves are flexible and opportunistic carnivores and have highly adaptable feeding habits, both in terms of the large differences in feeding ecology between geographic ranges and in the large seasonal changes seen in some populations (Mech & Boitani, 2003). Wolves have been known to feed on wild and domestic ungulates, arctic hare *Lepus arcticus*, beaver *Castor* spp., fish, seal carcasses and even human garbage (Mech & Boitani, 2003). It appears that wolves can thrive of almost anything and that at any given period the preferred food is determined by a number of factors such as prey abundance, environmental conditions and temporally dependent prey vulnerability (Jędrzejewski et al., 2012; Nowak et al., 2011). Studies on the diet of European wolves find that wolves primarily feed on large ungulates, such as red deer *Cervus elaphus*, moose *Alces alces*, wild boar *Sus scrofa* and roe deer *Capreolus capreolus* (Jędrzejewski et al., 2002; Nowak & Mysłajek, 2016; Sand et al., 2008; Wagner et al., 2012). Where red deer are present, they seem to be the favoured prey of wolves, who selectively hunted red deer regardless of density in Poland (Jędrzejewski et al., 2012). Furthermore, roe deer were only preferred in the French Alps when wolves had not yet established themselves in the area (Randon et al., 2020) and when red deer density was low due to recent introduction (Marucco et al., 2008). However, other German and Polish studies have found that wolves select roe deer in favour of red deer (Nowak et al., 2011; Wagner et al., 2012). Importantly, European wolves mostly target wild ungulates, with only negligible proportions of domestic ungulates in their diet (Capitani et al., 2004; Figueiredo et al., 2020; Octenjak et al., 2020; Wagner et al., 2012), apart from in highly human-dominated areas with low abundances of wild ungulates (Torres et al., 2015).



Figure 3 - the three study species captured on the same camera location. Top left: red deer *Cervus elaphus*, top right: grey wolf *Canis lupus*, bottom: roe deer *Capreolus capreolus*. Photo credit: Thomas Boesdal, Søren Krabbe & NHMA/AU.

Danish Deer Populations

Denmark houses five deer species (family: Cervidae); red deer *Cervus elaphus*, roe deer *Capreolus capreolus*, fallow deer *Dama dama*, sika deer *Cervus nippon* and muntjac *Muntiacus reevesi*, of which only the red deer and roe deer are regarded as native (Asferg et al., 2004). Fallow deer, sika deer and muntjac have established naturalised populations from intentionally released individuals as well as individuals escaped from captivity (Miljøstyrelsen, 2017a, 2017b). However, fallow deer occurred naturally in Denmark during the last interglacial, reflecting the general widespread distribution of the species in Europe during warm climate periods during the Middle and Late Pleistocene (Baker et al., 2017; Pfeiffer-Deml, 2018). Only red deer, roe deer and rarely fallow deer were observed in the study area during the study period and this project is therefore focused on red deer and roe deer (see **fig. 3**).

Red deer is the largest of the Danish deer species (female weight: 90 kg, male weight: 140-150 kg) and one of the largest free-ranging herbivores in Europe (Asferg & Madsen, 2012). They are gregarious animals with groups being divided by sex outside of the mating season (Asferg & Madsen, 2012). In Denmark favoured habitats are large, forested areas near open land (e.g. heathland and agriculture), with occurrence being positively related to forage availability, tree cover and mean terrain height (Müller et al., 2017). Red deer will primarily use open pastures with

abundant forage and little cover for active foraging during night-time and use forested areas with little forage and more cover during the day, where activity is lower (Asferg & Madsen, 2012; Godvik et al., 2009). However, this activity pattern is highly dependent on predation, which in the absence of large carnivores is attributed to human hunting (Müller et al., 2017). Red deer are grazers, who supplement their diet with herbs and tree shoots, and even heath and tree bark during winter months (Asferg & Madsen, 2012). Foraging on agricultural crops and saplings in cultured forests puts them in conflict with farmers living in areas with large red deer population. Red deer have large home ranges and may roam an area as big as 200 ha on a single day (Asferg & Madsen, 2012). During the mating season (rut), which runs through September to October, males will establish territories with the aim of obtaining and defending a harem of females (Asferg & Madsen, 2012). A single calf is born in June and will, like other “follower” species, start following the female shortly after birth (Jensen & Jørgensen, 2017). The Danish population of red deer was almost completely decimated in the middle of the 1800s in order to decrease damages to crops and timber (Asferg & Madsen, 2012). The population size of red deer has increased significantly since 1970 when annual hunting recording began, and is still increasing at present (Noer et al., 2009). The annual hunting season in Denmark runs from 1st September (stags)/ 1st October (hinds and calves) to 31st January (Asferg & Madsen, 2012). In 2019 a total of 10.000 red deer were shot in Denmark (Vildtudbyttestatistikken).

Roe deer are the smallest native deer species in Denmark with a weight that ranges from 15 to 30 kg (Olesen et al., 2002). After having been almost completely eradicated in the 1800s, roe deer are now one of the most common mammals in Denmark and are found in every part of the country (Asferg & Madsen, 2013). Their impressive recovery was probably facilitated by the concurrent advance in agriculture that improved access to high quality forage especially in winter months (Asferg & Madsen, 2013). Roe deer are selective concentrate feeders with a diet that includes shoots from grass and trees, mushrooms, nuts, roots and crops (Duncan, 1998; Olesen et al., 2002). Their small size and flexible forage strategy make them quite well adapted to the agricultural landscape in Denmark which consists of mosaics of forests and fields. Roe deer have relatively small home ranges (8-41 ha) and are generally solitary, but may form groups during winter months (Asferg & Madsen, 2013). Males form territories in spring and actively defend them until the rut ends in early fall (Asferg & Madsen, 2013). During July and August males follow females around to secure a mating. Females give birth to 1-3 fawns between May and June, who will stay with them

until the following spring (Asferg & Madsen, 2013). The diel activity of roe deer is dependent on predation risk and in areas with high human activity roe deer often change their activity to be less diurnal (Bonnot et al., 2020). Contrary to red deer, the habitat selection of Danish roe deer do not seem to shift between diel periods (Olesen et al., 2002). In a Swedish study roe deer activity peaked in April-May and again in October, with individuals being least active in February (Cederlund, 1989). The decreased activity in winter was attributed to energy conservation and increased time spent ruminating, as their diet consists of less readily digestible vegetation during winter. The annual hunting season in Denmark runs from 1st October to 31st January and again from 16th May to 15th July (only bucks). In 2019 nearly 88.000 roe deer were shot in Denmark (Vildtudbyttestatistikken).

Wolf-deer Interactions

The wolf-deer system is one of the most widely studied mammalian predator-prey systems, especially the American wolf-elk system in the GYE (Say-Sallaz et al., 2019). Multiple studies have found that wolf predation has a negative effect on both energy intake and reproduction in elk (Christianson & Creel, 2010; Creel et al., 2007). It is therefore not surprising that elk exhibit antipredator behaviour in response to wolf presence to decrease fitness costs. In response to immediate wolf presence elk will move into protective cover (Creel et al., 2005) and increase vigilance (Creel et al., 2008; Middleton et al., 2013). On a broader scale elk permanently changed vigilance behaviour (Laundré et al., 2001; Lung & Childress, 2006), shifted activity patterns (Kohl et al., 2018) and habitat (Fortin et al., 2005; Mao et al., 2005) as a result of wolf reintroduction in the GYE. In a study Creel *et al.* (2008) wanted to see if elk vigilance in GYE was best explained by the *risky places hypothesis* or the *predation risk allocation hypothesis*. To do this they compared general elk vigilance in three drainages which varied in wolf density (no wolves, 5-17 wolves and 54-84 wolves), as well as vigilance on days with and without wolves. If the *risky places hypothesis* was supported general elk vigilance should increase from no wolves present to most wolves present on days with no wolves. What they found was that general vigilance did not increase with number of wolves present. Instead, the drainage with few wolves had the highest vigilance. They interpreted the result as support for the *predation risk allocation hypothesis* where vigilance is expected to decrease as predator encounter increase. Elk in both areas with wolves were more vigilant on days where wolves were present, supporting that elk only allocate time to vigilance behaviour when risk is high.

Habitat characteristics may affect how deer assess risk of wolf predation. GYE elk moved into protective cover when wolves were present (Creel et al., 2005), and increased vigilance when distance to cover grew (Liley & Creel, 2008). This supports that in the presence of wolves open areas are seen as riskier. A study on moose found that wolf predation risk was indeed highest in open areas (Gervasi et al., 2013). In Białowieża Primeval Forest (BPF) browsing pressure decreased when the amount of obstructions (within a 5 meter radius) increased, but only in high wolf use areas (Kuijper et al., 2013). This shows that even on a fine-scale habitat characteristics can affect the perceived predation risk of deer. However, in another study by Kuijper *et al.* (2014), vegetation structure did not affect the risk assessment of ungulates, when these were exposed to wolf scent. Wolves are cursorial predators and might therefore not provide as persistent cues of predation risk as ambush predators (Moll et al., 2016; Preisser et al., 2007). In a study on the response of roe deer and red deer to Eurasian lynx *Lynx lynx* scat, vigilance remained the same, but visitation rates were reduced (Wikenros et al., 2015). Compared to that, red deer was shown to increase vigilance twofold when being exposed to wolf scat, but did not reduce visitation (Kuijper et al., 2014). The difference in response illustrates how ambush predators create *risky places*, which prey can avoid spatially, whereas cursorial predators are less predictably distributed and thus harder to avoid. Furthermore, it is easier to spot a cursorial predator coming towards you, whereas the point of ambush hunting is that prey has very little chance of detecting the predator even if vigilant. As a result, the likelihood of vigilance leading to predator detection and avoidance is higher for cursorial predators than ambush predators. Vigilance is therefore a favourable adaptation to cursorial predators, as vigilance should increase with predator lethality in the absence of vigilance (Brown & Kotler, 2004).

For a long time wolves were thought to strictly prey on large ungulates, which have led to them being attributed an exceedingly large role in regulating specific deer species (Mech & Boitani, 2003). Say-Sallaz *et al.* (2019) notes that (especially American) wolf-ungulate studies are overrepresented in studies on the non-consumptive effect of predation. In a review, Mech (2012) notes that especially conservation biologists have become obsessed with the ecological effects of wolf predation to the point of blindness. This is exemplified by the overwhelming interest from both the scientific community and the public in the proposed existence of a behaviourally mediated trophic cascade in the GYE, the support for which is now challenged (Kauffman et al., 2010; Marshall et al., 2013; Mech, 2012; Winnie, 2012). Wolves have also been attributed to cause a

trophic cascade on Isle Royale, US, affecting moose browsing pressure on aspen and fir *Abies spp.* (De Jager et al., 2017; McLaren & Peterson, 1994). However, as Isle Royale is an island, the effect of predation and herbivory might be exaggerated compared to more open systems (Mech & Boitani, 2003). Even so, newer studies have found that wolves do affect ungulate browsing pressure, e.g., white-tailed deer *Odocoileus virginianus* foraging in the Great Lakes forest, US (Flagel et al., 2016), and ungulate browsing patterns in BPF, Poland (Kuijper et al., 2013).

Ecological Impact of Returning Wolves in Europe

The return of wolves to Europe could potentially restore important ecosystem dynamics by affecting prey behaviour and habitat selection. However, the carrying capacity of predators in many anthropogenic areas might be too low for top-down effects of predation to have ecological implications (Mech, 2012; Worm & Paine, 2016). Human disturbances such as traffic, forestry and hunting activities have been shown to affect habitat selection (Theuerkauf & Rouys, 2008) and antipredator behaviour (Ciuti et al., 2012) more than wolf predation. Nevertheless, Kuijper *et al.* (2013) found that wolves create a LOF in BPF that affect the spatial patterns of ungulate browsing on multiple scales. Additionally, a new study found that returning wolves in Wisconsin, USA, reduced deer-vehicle collision by modifying deer behaviour in ways that human hunters did not. This suggests that risk associated with wolves and human hunters might differ, and that the return of wolves therefore could have behaviourally mediated cascading effects on ecosystems. If risk effects associated with humans and natural predators are additive, predators might affect prey populations more in anthropogenic areas, where prey is already limited by human activities (Kuijper et al., 2016). Many species have adapted to human presence by becoming nocturnal (Gaynor et al., 2018). As most natural predators are nocturnal, prey may therefore face a dilemma regarding when to be active, resulting in fitness costs (Bonnot et al., 2020). Furthermore, as wolves are returning to areas where they have previously been absent, prey might have lost antipredator adaptations making them more vulnerable towards wolf predation (Sih et al., 2010). Naïve prey may be more vulnerable if antipredator behaviour is not innate, which can lead to lower survival rate (Dickman, 1992). In Scandinavia recently colonised wolves had a higher moose hunting success than wolves in North America, where wolves have never been absent (Sand et al., 2006). Similarly, roe deer populations in the French Vercors mountain range had a lower recovery rate following severe winters in areas where wolves had recently colonised (Randon et al., 2020).

Camera Traps in Ecological and Ethological Studies

The data from this study came from the National Danish Wolf Monitoring project, which has had cameras in the Ulfborg Territory since November 2016 in an effort to track the wolves residing in the area (Thorsen et al., 2019). In recent years remote-sensing camera traps have become increasingly popular in ecological and ethological studies, as they offer a non-invasive and cost-efficient way to quantify animal activity and behaviour (Caravaggi et al., 2017). It is easy to see the advantages of camera traps compared to direct observations which often suffers from small sample sizes and is most suited to studying larger animals located in relatively open areas (Caravaggi et al., 2017). Observer bias is limited as observations can be verified through recurrent analysis. The presence of observers might also disturb animals, which is especially problematic in ethological studies. During direct observation studies, observers must be able to see the animals directly and do this reliably multiple times, which excludes more elusive animals from this kind of survey. In a study, Galaverni *et al.* (2011) found that data from genetic monitoring and camera trapping of elusive wolves were comparable. Camera traps make it possible to monitor areas that would otherwise be inaccessible, like nuclear exclusion zones (Lyons et al., 2020; Schlichting et al., 2019). Furthermore, camera traps can collect data on multiple species simultaneously making it especially useful for studying species interaction and niche partitioning in both space and time (Frey et al., 2017; Ikeda et al., 2016).

However, it is important to be aware that camera traps come with their own set of limitations. In a paper reviewing the current state of camera-based ethological studies, Caravaggi *et al.* (2017) consider the main limitation to be a lack of standardising between studies, as the wide variety of camera models, setting and choice of survey site decreases repeatability (Burton et al., 2015). Furthermore, detectability of species is not equal as it depends on both morphology (e.g. size, height) and behaviour of the species (e.g. elusiveness, habitat choice, movement patterns) (Tourani et al., 2020). Choice of ideal placement is also highly variable across species, as species move very differently in the environment. Capture rate is often assumed to be comparable across the diel period, however sensor sensitivity can be affected by temperature and light conditions, and observations might be less clear at night due to slow shutter speed or over- or underexposed due to the flash used, leading to species misidentification. In Denmark, low quality of night pictures decreased species identification certainty (Thorsen et al., 2019). Habitat and light conditions may also affect the vulnerability of animals to photo capture, meaning that the spatial and temporal

distribution of observations do not accurately represent the habitat choice and diel activity of a species. In a study on a collared coyote *Canis latrans* population, individuals were often photographed during night-time, but rather than reflecting the diel activity pattern it was due to coyotes being more vulnerable to photo capture at this time (Larrucea et al., 2007). If the data is used for ethological studies, it is important to keep in mind that animal behaviour might be affected if animals are aware of the camera or find the noises and lights associated with it aversive.

This Study

As wolves return to Europe there is a need to study if and how they affect local prey populations to understand the ecological implications of returning apex predators (Kuijper et al., 2016). The first Danish wolf pair settled in the Ulfborg territory in November 2016 and since then camera traps have been used to monitor wolf activity in the area (Thorsen et al., 2019). The resulting database gives an unique opportunity to study the behaviour and activity patterns of wolves and their prey in an environment with a large degree of human disturbance (e.g., hunting, commercial forestry and recreational activities). The spatial and temporal scale of the data collection makes it possible to study if deer have adapted their use of the area to the presence of wolves. In short, we want to see if deer live in a LOF where they avoid wolves in space and time.

This study focused on two sympatric deer species, red deer and roe deer, who differ in both biology and behaviour. Red deer are closely related to the american elk, and it is therefore possible that they will respond similarly to the presence of wolves. Compared to that, roe deer are generally not preyed on by wolves when larger ungulates (e.g. red deer and wild boar) are present (Mattioli et al., 2004). To study how the spatial variation in wolf activity affected deer wolf activity heatmaps were created for six months periods where level of wolf activity was interpolated from verified wolf observations. As the resulting wolf activity index was estimated for six months periods it gave an indication of background wolf predation risk. Rather than avoid predators in space prey may avoid predators in time instead, which results in a shift in diel activity in areas with high predation activity (Rossa et al., 2021). To see if deer adapt their behaviour to avoid areas associated with a higher risk of wolf predation, their presence and diel activity was analysed in relation to wolf activity. Additionally, we wanted to see how the vigilance behaviour of the deer related to the level of wolf activity. Here, we wanted to test if vigilance was correlated with wolf activity in a way that met the assumptions of the *risky places* hypothesis where vigilance is only dependent on background risk,

the *risky times* hypothesis where vigilance is only dependent on immediate risk, or the *predation risk allocation* hypothesis where vigilance depends on both background and immediate risk.

Accordingly, we predicted that **1)** the observation frequency of deer correlated negatively with wolf activity (spatial avoidance) and/or that **2)** deer expressed lower overlap in diel activity with wolves in areas with high wolf activity than in areas with lower wolf activity (temporal avoidance). If the *risky places* hypothesis was supported, we predicted that **3)** deer alertness was positively correlated with wolf activity as vigilance was expected to increase with predator encounter rate. If the *predation risk allocation* hypothesis was supported, we predicted that **4)** deer alertness was negatively correlated with wolf activity as deer responded to a higher background risk by decreasing vigilance and/or that **5)** the proportion of deer exhibiting alertness was higher on days where wolves were present than on days where wolves were locally absent.

The results and perspectives of this project is the subject of the article manuscript “Landscape of fear in an anthropogenic environment – how deer respond to the presence of wolves in a Danish wolf territory”.

References

- Anderson, D. P., Turner, M. G., Forester, J. D., Zhu, J. U. N., Boyce, M. S., Beyer, H., . . . Green. (2005). Scale-Dependent Summer Resource Selection by Reintroduced Elk in Wisconsin, USA. *Journal of Wildlife Management*, 69(1), 298-310. doi:10.2193/0022-541x(2005)069<0298:Srsbr>2.0.Co;2
- Anholt, B. R., Werner, E., & Skelly, D. K. (2000). Effect of Food and Predators on the Activity of Four Larval Ranid Frogs. *Ecology*, 81(12), 3509-3521. doi:10.1890/0012-9658(2000)081[3509:Eofapo]2.0.Co;2
- Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A., & McGregor, I. S. (2005). The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neurosci Biobehav Rev*, 29(8), 1123-1144. doi:10.1016/j.neubiorev.2005.05.005
- Asferg, T., & Madsen, A. B. (2012). *Kronstyr i Dansk Pattedyratlas*. Retrieved from <https://pattedyratlas.lex.dk/Kronstyr> on 30/04/21
- Asferg, T., & Madsen, A. B. (2013). *Rådyr i Dansk Pattedyrsatlas*. Retrieved from <https://pattedyratlas.lex.dk/Rådyr> on 30/04/21
- Asferg, T., Olesen, C. R., & Andersen, J. P. (2004). Kronstyr, dådyr og sika i Danmark: Forekomst og jagtlig udnyttelse i jagtsæsonen 2001/02. *Danmarks Miljøundersøgelser, Aarhus Universitet, Faglig rapport fra DMU Nr. 512*.
- Baker, K. H., Gray, H. W. I., Ramovs, V., Mertzaniidou, D., Akin Peksen, C., Bilgin, C. C., . . . Hoelzel, A. R. (2017). Strong population structure in a species manipulated by humans since the Neolithic: the European fallow deer (*Dama dama dama*). *Heredity (Edinb)*, 119(1), 16-26. doi:10.1038/hdy.2017.11
- Barja, I., & Rosellini, S. (2008). Does habitat type modify group size in roe deer and red deer under predation risk by Iberian wolves? *Canadian Journal of Zoology*, 86(3), 170-176. doi:10.1139/z07-129
- Basille, M., Herfindal, I., Santin-Janin, H., Linnell, J. D. C., Odden, J., Andersen, R., . . . Gaillard, J.-M. (2009). What shapes Eurasian lynx distribution in human dominated landscapes: selecting prey or avoiding people? *Ecography*, 32(4), 683-691. doi:10.1111/j.1600-0587.2009.05712.x
- Beschta, R. L., & Ripple, W. J. (2015). Divergent patterns of riparian cottonwood recovery after the return of wolves in Yellowstone, USA. *Ecohydrology*, 8(1), 58-66. doi:10.1002/eco.1487
- Beschta, R. L., Ripple, W. J., Kauffman, J. B., & Painter, L. E. (2020). Bison limit ecosystem recovery in northern Yellowstone. *Food Webs*, 23. doi:10.1016/j.fooweb.2020.e00142
- Blanchard, P., Lauzeral, C., Chamaille-Jammes, S., Brunet, C., Lec'hvien, A., Peron, G., & Pontier, D. (2018). Coping with change in predation risk across space and time through complementary behavioral responses. *BMC Ecol*, 18(1), 60. doi:10.1186/s12898-018-0215-7
- Bleicher, S. S. (2017). The landscape of fear conceptual framework: definition and review of current applications and misuses. *PeerJ*, 5, e3772. doi:10.7717/peerj.3772
- Bonnot, N. C., Couriot, O., Berger, A., Cagnacci, F., Ciuti, S., De Groeve, J. E., . . . Hewison, A. J. M. (2020). Fear of the dark? Contrasting impacts of humans versus lynx on diel activity of roe deer across Europe. *J Anim Ecol*, 89(1), 132-145. doi:10.1111/1365-2656.13161
- Bonnot, N. C., Hewison, A. J. M., Morellet, N., Gaillard, J.-M., Debeffe, L., Couriot, O., . . . Vanpé, C. (2017). Stick or twist: roe deer adjust their flight behaviour to the perceived trade-off between risk and reward. *Animal Behaviour*, 124, 35-46. doi:10.1016/j.anbehav.2016.11.031

- Brown, J. S., & Kotler, B. P. (2004). Hazardous duty pay and the foraging cost of predation. *Ecology Letters*, 7(10), 999-1014. doi:10.1111/j.1461-0248.2004.00661.x
- Burton, A. C., Neilson, E., Moreira, D., Ladle, A., Steenweg, R., Fisher, J. T., . . . Stephens, P. (2015). REVIEW: Wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. *Journal of Applied Ecology*, 52(3), 675-685. doi:10.1111/1365-2664.12432
- Capitani, C., Bertelli, I., Varuzza, P., Scandura, M., & Apollonio, M. (2004). A comparative analysis of wolf (*Canis lupus*) diet in three different Italian ecosystems. *Mammalian Biology*, 69(1), 1-10. doi:10.1078/1616-5047-112
- Caravaggi, A., Banks, P. B., Burton, A. C., Finlay, C. M. V., Haswell, P. M., Hayward, M. W., . . . Sollmann, R. (2017). A review of camera trapping for conservation behaviour research. *Remote Sensing in Ecology and Conservation*, 3(3), 109-122. doi:10.1002/rse2.48
- Carricondo-Sanchez, D., Zimmermann, B., Wabakken, P., Eriksen, A., Milleret, C., Ordiz, A., . . . Wikenros, C. (2020). Wolves at the door? Factors influencing the individual behavior of wolves in relation to anthropogenic features. *Biological Conservation*, 244. doi:10.1016/j.biocon.2020.108514
- Castelló, J. R. (2018). *Canids of the World*. Princeton, New Jersey: Princeton University Press.
- Cederlund, G. (1989). Activity patterns in moose and roe deer in a north boreal forest. *Ecography*, 12(1), 39-45. doi:10.1111/j.1600-0587.1989.tb00820.x
- Chapron, G., Kaczensky, P., Linnell, J. D., von Arx, M., Huber, D., Andren, H., . . . Boitani, L. (2014). Recovery of large carnivores in Europe's modern human-dominated landscapes. *Science*, 346(6216), 1517-1519. doi:10.1126/science.1257553
- Childress, M. J., & Lung, M. A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour*, 66(2), 389-398. doi:10.1006/anbe.2003.2217
- Christianson, D., & Creel, S. (2010). A nutritionally mediated risk effect of wolves on elk. *Ecology*, 91(4), 1184-1191. doi:10.1890/09-0221.1
- Ciucci, P., Boitani, L., Falco, M., & Maiorano, L. (2018). Hierarchical, multi-grain rendezvous site selection by wolves in southern Italy. *The Journal of Wildlife Management*, 82(5), 1049-1061. doi:10.1002/jwmg.21440
- Ciucci, P., Boitani, L., Francisci, F., & Andreoli, G. (1997). Home range, activity and movements of a wolf pack in central Italy. *Journal of Zoology*, 243(4), 803-819. doi:10.1111/j.1469-7998.1997.tb01977.x
- Ciucci, P., Masi, M., & Boitani, L. (2003). Winter habitat and travel route selection by wolves in the northern Apennines, Italy. *Ecography*, 26(2), 223-235. doi:10.1034/j.1600-0587.2003.03353.x
- Ciuti, S., Northrup, J. M., Muhly, T. B., Simi, S., Musiani, M., Pitt, J. A., & Boyce, M. S. (2012). Effects of humans on behaviour of wildlife exceed those of natural predators in a landscape of fear. *PLoS One*, 7(11), e50611. doi:10.1371/journal.pone.0050611
- Creel, S., Christianson, D., Liley, S., & Winnie, J. A., Jr. (2007). Predation risk affects reproductive physiology and demography of elk. *Science*, 315(5814), 960. doi:10.1126/science.1135918
- Creel, S., Schuette, P., & Christianson, D. (2014). Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community. *Behavioral Ecology*, 25(4), 773-784. doi:10.1093/beheco/aru050
- Creel, S., Winnie, J., Maxwell, B., Hamlin, K., & Creel, M. (2005). Elk Alter Habitat Selection as an Antipredator Response to Wolves. *Ecology*, 86(12), 3387-3397. doi:10.1890/05-0032

- Creel, S., Winnie, J. A., Christianson, D., & Liley, S. (2008). Time and space in general models of antipredator response: tests with wolves and elk. *Animal Behaviour*, *76*(4), 1139-1146. doi:10.1016/j.anbehav.2008.07.006
- De Jager, N. R., Rohweder, J. J., Miranda, B. R., Sturtevant, B. R., Fox, T. J., & Romanski, M. C. (2017). Modelling moose-forest interactions under different predation scenarios at Isle Royale National Park, USA. *Ecol Appl*, *27*(4), 1317-1337. doi:10.1002/eap.1526
- Deinet, S., Ieronymidou, C., McRae, L., Burfield, I. J., Foppen, R. P., Collen, B., & Böhm, M. (2013). Wildlife comeback in Europe: the recovery of selected mammal and bird species. *Final report to Rewilding Europe by ZSL, BirdLife International and the European Bird Census Council*. London, UK: ZSL.
- Dickman, C. R. (1992). Predation and Habitat Shift in the House Mouse, *Mus Domesticus*. *Ecology*, *73*(1), 313-322. doi:10.2307/1938742
- Dobson, A. P. (2014). Yellowstone wolves and the forces that structure natural systems. *PLoS Biol*, *12*(12), e1002025. doi:10.1371/journal.pbio.1002025
- Duncan, P. (1998). Feeding Strategies and the Physiology of Digestion in Roe Deer. In *The European Roe Deer: The Biology of Success*: Scandinavian University Press.
- Eggermann, J., da Costa, G. F., Guerra, A. M., Kirchner, W. H., & Petrucci-Fonseca, F. (2011). Presence of Iberian wolf (*Canis lupus signatus*) in relation to land cover, livestock and human influence in Portugal. *Mammalian Biology*, *76*(2), 217-221. doi:10.1016/j.mambio.2010.10.010
- Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., . . . Wardle, D. A. (2011). Trophic downgrading of planet Earth. *Science*, *333*(6040), 301-306. doi:10.1126/science.1205106
- Festa-Bianchet, M. (1988). Seasonal range selection in bighorn sheep: conflicts between forage quality, forage quantity, and predator avoidance. *Oecologia*, *75*(4), 580-586. doi:10.1007/BF00776423
- Figueiredo, A. M., Valente, A. M., Barros, T., Carvalho, J., Silva, D. A. M., Fonseca, C., . . . Torres, R. T. (2020). What does the wolf eat? Assessing the diet of the endangered Iberian wolf (*Canis lupus signatus*) in northeast Portugal. *PLoS One*, *15*(3), e0230433. doi:10.1371/journal.pone.0230433
- Flagel, D. G., Belovsky, G. E., & Beyer, D. E., Jr. (2016). Natural and experimental tests of trophic cascades: gray wolves and white-tailed deer in a Great Lakes forest. *Oecologia*, *180*(4), 1183-1194. doi:10.1007/s00442-015-3515-z
- Fortin, D., Beyer, H. L., Boyce, M. S., Smith, D. W., Duchesne, T., & Mao, J. S. (2005). Wolves Influence Elk Movements: Behavior Shapes a Trophic Cascade in Yellowstone National Park. *Ecology*, *86*(5), 1320-1330. doi:10.1890/04-0953
- Frey, S., Fisher, J. T., Burton, A. C., Volpe, J. P., & Rowcliffe, M. (2017). Investigating animal activity patterns and temporal niche partitioning using camera-trap data: challenges and opportunities. *Remote Sensing in Ecology and Conservation*, *3*(3), 123-132. doi:10.1002/rse2.60
- Galaverni, M., Palumbo, D., Fabbri, E., Caniglia, R., Greco, C., & Randi, E. (2011). Monitoring wolves (*Canis lupus*) by non-invasive genetics and camera trapping: a small-scale pilot study. *European Journal of Wildlife Research*, *58*(1), 47-58. doi:10.1007/s10344-011-0539-5
- Gaynor, K. M., Hojnowski, C. E., Carter, N. H., & Brashares, J. S. (2018). The influence of human disturbance on wildlife nocturnality. *Science*, *360*(6394), 1232-1235. doi:10.1126/science.aar7121

- Gervasi, V., Sand, H., Zimmermann, B., Mattisson, J., Wabakken, P., & Linnell, J. D. (2013). Decomposing risk: landscape structure and wolf behavior generate different predation patterns in two sympatric ungulates. *Ecol Appl*, 23(7), 1722-1734. doi:10.1890/12-1615.1
- Godvik, I. M., Loe, L. E., Vik, J. O., Veiberg, V., Langvatn, R., & Mysterud, A. (2009). Temporal scales, trade-offs, and functional responses in red deer habitat selection. *Ecology*, 90(3), 699-710. doi:10.1890/08-0576.1
- Grilo, C., Lucas, P. M., Fernández-Gil, A., Seara, M., Costa, G., Roque, S., . . . Revilla, E. (2018). Refuge as major habitat driver for wolf presence in human-modified landscapes. *Animal Conservation*, 22(1), 59-71. doi:10.1111/acv.12435
- Gurarie, E., Suutarinen, J., Kojola, I., & Ovaskainen, O. (2011). Summer movements, predation and habitat use of wolves in human modified boreal forests. *Oecologia*, 165(4), 891-903. doi:10.1007/s00442-010-1883-y
- Hebblewhite, M., Merrill, E. H., & McDonald, T. L. (2005). Spatial decomposition of predation risk using resource selection functions: an example in a wolf-elk predator-prey system. *Oikos*, 111(1), 101-111. doi:10.1111/j.0030-1299.2005.13858.x
- Ikeda, T., Uchida, K., Matsuura, Y., Takahashi, H., Yoshida, T., Kaji, K., & Koizumi, I. (2016). Seasonal and Diel Activity Patterns of Eight Sympatric Mammals in Northern Japan Revealed by an Intensive Camera-Trap Survey. *PLoS One*, 11(10), e0163602. doi:10.1371/journal.pone.0163602
- Jędrzejewski, W., Niedziałkowska, M., Hayward, M. W., Goszczyński, J., Jędrzejewska, B., Borowik, T., . . . Wojtulewicz, M. (2012). Prey choice and diet of wolves related to ungulate communities and wolf subpopulations in Poland. *Journal of Mammalogy*, 93(6), 1480-1492. doi:10.1644/10-mamm-a-132.1
- Jędrzejewski, W., Niedziałkowska, M., Nowak, S., & Jędrzejewska, B. (2004). Habitat variables associated with wolf (*Canis lupus*) distribution and abundance in northern Poland. *Diversity & Distributions*, 10(3), 225-233. doi:10.1111/j.1366-9516.2004.00073.x
- Jędrzejewski, W., Schmidt, K., Theuerkauf, J., Jędrzejewska, B., Selva, N., Zub, K., & Szymura, L. (2002). Kill Rates and Predation by Wolves on Ungulate Populations in BiaŁowieża Primeval Forest (Poland). *Ecology*, 83(5), 1341-1356. doi:10.1890/0012-9658(2002)083[1341:Krapbw]2.0.Co;2
- Jensen, J. K., & Jørgensen, O., F. (2017). *Pattedyr i Norden*: Gyldendal.
- Kauffman, M. J., Brodie, J. F., & Jules, E. S. (2010). Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade. *Ecology*, 91(9), 2742-2755. doi:10.1890/09-1949.1
- Kittle, A. M., Anderson, M., Avgar, T., Baker, J. A., Brown, G. S., Hagens, J., . . . Fryxell, J. M. (2015). Wolves adapt territory size, not pack size to local habitat quality. *J Anim Ecol*, 84(5), 1177-1186. doi:10.1111/1365-2656.12366
- Kohl, M. T., Stahler, D. R., Metz, M. C., Forester, J. D., Kauffman, M. J., Varley, N., . . . MacNulty, D. R. (2018). Diel predator activity drives a dynamic landscape of fear. *Ecological Monographs*, 88(4), 638-652. doi:10.1002/ecm.1313
- Kuijper, D. P., Sahlen, E., Elmhagen, B., Chammille-Jammes, S., Sand, H., Lone, K., & Cromsigt, J. P. (2016). Paws without claws? Ecological effects of large carnivores in anthropogenic landscapes. *Proc Biol Sci*, 283(1841). doi:10.1098/rspb.2016.1625
- Kuijper, D. P., Verwijmeren, M., Churski, M., Zbyryt, A., Schmidt, K., Jędrzejewska, B., & Smit, C. (2014). What cues do ungulates use to assess predation risk in dense temperate forests? *PLoS One*, 9(1), e84607. doi:10.1371/journal.pone.0084607

- Kuijper, D. P. J., de Kleine, C., Churski, M., van Hooft, P., Bubnicki, J., & Jędrzejewska, B. (2013). Landscape of fear in Europe: wolves affect spatial patterns of ungulate browsing in Białowieża Primeval Forest, Poland. *Ecography*, 36(12), 1263-1275. doi:10.1111/j.1600-0587.2013.00266.x
- Kusak, J., Skrbinišek, A. M., & Huber, D. (2005). Home ranges, movements, and activity of wolves (*Canis lupus*) in the Dalmatian part of Dinarids, Croatia. *European Journal of Wildlife Research*, 51(4), 254-262. doi:10.1007/s10344-005-0111-2
- Kaartinen, S., Kojola, I., & Colpaert, A. (2005). Finnish wolves avoid roads and settlement. *Ann Zool Fennici*, 42(5), 523–532.
- Larrucea, E. S., Brussard, P. F., Jaeger, M. M., & Barrett, R. H. (2007). Cameras, Coyotes, and the Assumption of Equal Detectability. *Journal of Wildlife Management*, 71(5), 1682-1689. doi:10.2193/2006-407
- Laundré, J. W., Hernández, L., & Altendorf, K. B. (2001). Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, U.S.A. *Canadian Journal of Zoology*, 79(8), 1401-1409. doi:10.1139/cjz-79-8-1401
- Lesmerises, F., Dussault, C., & St-Laurent, M.-H. (2012). Wolf habitat selection is shaped by human activities in a highly managed boreal forest. *Forest Ecology and Management*, 276, 125-131. doi:10.1016/j.foreco.2012.03.025
- Lesmerises, F., Dussault, C., & St-Laurent, M.-H. (2013). Major roadwork impacts the space use behaviour of gray wolf. *Landscape and Urban Planning*, 112, 18-25. doi:10.1016/j.landurbplan.2012.12.011
- Liley, S., & Creel, S. (2008). What best explains vigilance in elk: characteristics of prey, predators, or the environment? *Behavioral Ecology*, 19(2), 245-254. doi:10.1093/beheco/arm116
- Lima, S. L. (1998). Nonlethal Effects in the Ecology of Predator-Prey Interactions. *BioScience*, 48(1), 25-34. doi:10.2307/1313225
- Lima, S. L., & Bednekoff, P. A. (1999). Temporal Variation in Danger Drives Antipredator Behavior: The Predation Risk Allocation Hypothesis. *Am Nat*, 153(6), 649-659. doi:10.1086/303202
- Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68(4), 619-640. doi:10.1139/z90-092
- Llaneza, L., Sazatornil, V., & López-Bao, J. V. (2018). The importance of fine-scale breeding site selection patterns under a landscape-sharing approach for wolf conservation. *Biodiversity and Conservation*, 27(5), 1239-1256. doi:10.1007/s10531-017-1491-9
- Lung, M. A., & Childress, M. J. (2006). The influence of conspecifics and predation risk on the vigilance of elk (*Cervus elaphus*) in Yellowstone National Park. *Behavioral Ecology*, 18(1), 12-20. doi:10.1093/beheco/ar1066
- Lyons, P. C., Okuda, K., Hamilton, M. T., Hinton, T. G., & Beasley, J. C. (2020). Rewilding of Fukushima's human evacuation zone. *Frontiers in Ecology and the Environment*, 18(3), 127-134. doi:10.1002/fee.2149
- MacNulty, D. R., Smith, D. W., Mech, L. D., Vucetich, J. A., & Packer, C. (2012). Nonlinear effects of group size on the success of wolves hunting elk. *Behavioral Ecology*, 23(1), 75-82. doi:10.1093/beheco/arr159
- Madsen, A. B., Andersen, L. W., & Sunde, P. (2013). Ulve I Danmark – Hvad kan vi forvente. *Notat fra DCE – Nationalt Center for Miljø og Energi, Aarhus Universitet*.
- Mao, J. S., Boyce, M. S., Smith, D. W., Singer, F. J., Vales, D. J., Vore, J. M., . . . Hudson. (2005). Habitat Selection by Elk before and after Wolf Reintroduction in Yellowstone National Park. *Journal of Wildlife Management*, 69(4), 1691-1707. doi:10.2193/0022-541x(2005)69[1691:Hsbeba]2.0.Co;2

- Marshall, K. N., Cooper, D. J., Hobbs, N. T., & Bellingham, P. (2014). Interactions among herbivory, climate, topography and plant age shape riparian willow dynamics in northern Yellowstone National Park, USA. *Journal of Ecology*, *102*(3), 667-677. doi:10.1111/1365-2745.12225
- Marshall, K. N., Hobbs, N. T., & Cooper, D. J. (2013). Stream hydrology limits recovery of riparian ecosystems after wolf reintroduction. *Proc Biol Sci*, *280*(1756), 20122977. doi:10.1098/rspb.2012.2977
- Marucco, F., Pletscher, D. H., & Boitani, L. (2008). Accuracy of scat sampling for carnivore diet analysis: wolves in the Alps as a case study. *Journal of Mammalogy*, *89*(3), 665-673. doi:10.1644/07-mamm-a-005r3.1
- Massolo, A., & Meriggi, A. (1998). Factors affecting habitat occupancy by wolves in northern Apennines (northern Italy): a model of habitat suitability. *Ecography*, *21*(2), 97-107. doi:10.1111/j.1600-0587.1998.tb00663.x
- Mattioli, L., Capitani, C., Avanzinelli, E., Bertelli, I., Gazzola, A., & Apollonio, M. (2004). Predation by wolves (*Canis lupus*) on roe deer (*Capreolus capreolus*) in north-eastern Apennine, Italy. *Journal of Zoology*, *264*(3), 249-258. doi:10.1017/s095283690400576x
- Mattisson, J., Sand, H., Wabakken, P., Gervasi, V., Liberg, O., Linnell, J. D., . . . Pedersen, H. C. (2013). Home range size variation in a recovering wolf population: evaluating the effect of environmental, demographic, and social factors. *Oecologia*, *173*(3), 813-825. doi:10.1007/s00442-013-2668-x
- McLaren, B. E., & Peterson, R. O. (1994). Wolves, moose, and tree rings on isle royale. *Science*, *266*(5190), 1555-1558. doi:10.1126/science.266.5190.1555
- Mech, D. L. (2012). Is science in danger of sanctifying the wolf? *Biological Conservation*, *150*(1), 143-149. doi:10.1016/j.biocon.2012.03.003
- Mech, L. D., & Boitani, L. (2003). *Wolves: Behavior, Ecology and Conservation*. Chicago IL: The University of Chicago Press.
- Merrill, E., Sand, H., Zimmermann, B., McPhee, H., Webb, N., Hebblewhite, M., . . . Frair, J. L. (2010). Building a mechanistic understanding of predation with GPS-based movement data. *Philos Trans R Soc Lond B Biol Sci*, *365*(1550), 2279-2288. doi:10.1098/rstb.2010.0077
- Middleton, A. D., Kauffman, M. J., McWhirter, D. E., Jimenez, M. D., Cook, R. C., Cook, J. G., . . . White, P. J. (2013). Linking anti-predator behaviour to prey demography reveals limited risk effects of an actively hunting large carnivore. *Ecol Lett*, *16*(8), 1023-1030. doi:10.1111/ele.12133
- Milanesi, P., Breiner, F. T., Puopolo, F., & Holderegger, R. (2017). European human-dominated landscapes provide ample space for the recolonization of large carnivore populations under future land change scenarios. *Ecography*, *40*(12), 1359-1368. doi:10.1111/ecog.02223
- Miljøstyrelsen. Muntjak (*Muntiacus reevesi*). Retrieved from <https://mst.dk/natur-vand/natur/artsleksikon/pattedyr/muntjak/> on 16/05/21
- Miljøstyrelsen. (2017a). Faktaark for invasive arter – Dådyr (*Dama dama*). Retrieved from www.mst.dk on 16/05/21
- Miljøstyrelsen. (2017b). Faktaark for invasive arter – Sikahjort (*Cervus nippon*). Retrieved from www.mst.dk on 11/01/21
- Miller, B. J., Harlow, H. J., Harlow, T. S., Biggins, D., & Ripple, W. J. (2012). Trophic cascades linking wolves (*Canis lupus*), coyotes (*Canis latrans*), and small mammals. *Canadian Journal of Zoology*, *90*(1), 70-78. doi:10.1139/z11-115

- Moll, R. J., Killion, A. K., Montgomery, R. A., Tambling, C. J., & Hayward, M. W. (2016). Spatial patterns of African ungulate aggregation reveal complex but limited risk effects from reintroduced carnivores. *Ecology*, *97*(5), 1123-1134. doi:10.1890/15-0707.1
- Moll, R. J., Redilla, K. M., Mudumba, T., Muneza, A. B., Gray, S. M., Abade, L., . . . Montgomery, R. A. (2017). The many faces of fear: a synthesis of the methodological variation in characterizing predation risk. *J Anim Ecol*, *86*(4), 749-765. doi:10.1111/1365-2656.12680
- Müller, A., Dahm, M., Bocher, P. K., Root-Bernstein, M., & Svenning, J. C. (2017). Large herbivores in novel ecosystems - Habitat selection by red deer (*Cervus elaphus*) in a former brown-coal mining area. *PLoS One*, *12*(5), e0177431. doi:10.1371/journal.pone.0177431
- Noer, H., Asferg, T., Clausen, P., Olesen, C. R., Bregnballe, T., Lauersen, K., . . . Haugaard, L. (2009). Vildtbestande og jagttider i Danmark: Det biologiske grundlag for jagttidsrevisionen 2010. *Danmarks Miljøundersøgelser, Aarhus Universitet, Faglig rapport fra DMU nr. 742.* , 288 s.
- Nowak, S., & Mysłajek, R. W. (2016). Wolf recovery and population dynamics in Western Poland, 2001–2012. *Mammal Research*, *61*(2), 83-98. doi:10.1007/s13364-016-0263-3
- Nowak, S., Mysłajek, R. W., Kłosińska, A., & Gabryś, G. (2011). Diet and prey selection of wolves (*Canis lupus*) recolonising Western and Central Poland. *Mammalian Biology*, *76*(6), 709-715. doi:10.1016/j.mambio.2011.06.007
- Octenjak, D., Pađen, L., Šilić, V., Reljić, S., Vukičević, T. T., & Kusak, J. (2020). Wolf diet and prey selection in Croatia. *Mammal Research*, *65*(4), 647-654. doi:10.1007/s13364-020-00517-8
- Olesen, C. R., Asferg, T., & Forchhammer, M. C. (2002). Rådyret - fra fåtallig til almindelig. *Danmarks Miljøundersøgelser, Aarhus Universitet. TEMA-rapport fra DMU, 39/2002.*
- Osada, K., Miyazono, S., & Kashiwayanagi, M. (2015). The scent of wolves: pyrazine analogs induce avoidance and vigilance behaviors in prey. *Front Neurosci*, *9*, 363. doi:10.3389/fnins.2015.00363
- Palmer, M. S., Portales-Reyes, C., Potter, C., Mech, L. D., & Isbell, F. (2021). Behaviorally-mediated trophic cascade attenuated by prey use of risky places at safe times. *Oecologia*, *195*(1), 235-248. doi:10.1007/s00442-020-04816-4
- Pfeiffer-Deml, T. (2018). The fossil fallow deer *Dama geiselana* (Cervidae, Mammalia, upgrade to species level) in the context of migration and local extinctions of fallow deer in the Late and Middle Pleistocene in Europe. *PalZ*, *92*(4), 681-713. doi:10.1007/s12542-018-0417-5
- Preisser, E. L., Bolnick, D. I., & Benard, M. F. (2005). Scared to Death? The Effects of Intimidation and Consumption in Predator–Prey Interactions. *Ecology*, *86*(2), 501-509. doi:10.1890/04-0719
- Preisser, E. L., Orrock, J. L., & Schmitz, O. J. (2007). Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. *Ecology*, *88*(11), 2744-2751. doi:10.1890/07-0260.1
- Randi, E. (2011). Genetics and conservation of wolves *Canis lupus* in Europe. *Mammal Review*, *41*(2), 99-111. doi:10.1111/j.1365-2907.2010.00176.x
- Randon, M., Bonenfant, C., Michallet, J., Chevrier, T., Toigo, C., Gaillard, J. M., & Valeix, M. (2020). Population responses of roe deer to the recolonization of the French Vercors by wolves. *Population Ecology*, *62*(2), 244-257. doi:10.1002/1438-390x.12043
- Reinhardt, I., Kluth, G., Nowak, C., Szentiks, C. A., Krone, O., Ansoerge, H., & Mueller, T. (2019). Military training areas facilitate the recolonization of wolves in Germany. *Conservation Letters*, *12*(3). doi:10.1111/conl.12635

- Reinhardt, I., Kluth, G., Nowak, S., & Mysłajek, R. W. (2015). *Standards for the monitoring of the Central European wolf population in Germany and Poland*. Bonn: Bundesamt für Naturschutz.
- Rich, L. N., Mitchell, M. S., Gude, J. A., & Sime, C. A. (2012). Anthropogenic mortality, intraspecific competition, and prey availability influence territory sizes of wolves in Montana. *Journal of Mammalogy*, *93*(3), 722-731. doi:10.1644/11-mamm-a-079.2
- Ripple, W. J., & Beschta, R. L. (2004). Wolves and the Ecology of Fear: Can Predation Risk Structure Ecosystems? *BioScience*, *54*(8). doi:10.1641/0006-3568(2004)054[0755:Wateof]2.0.Co;2
- Ripple, W. J., & Beschta, R. L. (2012). Trophic cascades in Yellowstone: The first 15 years after wolf reintroduction. *Biological Conservation*, *145*(1), 205-213. doi:10.1016/j.biocon.2011.11.005
- Ripple, W. J., Estes, J. A., Beschta, R. L., Wilmers, C. C., Ritchie, E. G., Hebblewhite, M., . . . Wirsing, A. J. (2014). Status and ecological effects of the world's largest carnivores. *Science*, *343*(6167), 1241484. doi:10.1126/science.1241484
- Roseboom, P. H., Nanda, S. A., Bakshi, V. P., Trentani, A., Newman, S. M., & Kalin, N. H. (2007). Predator threat induces behavioral inhibition, pituitary-adrenal activation and changes in amygdala CRF-binding protein gene expression. *Psychoneuroendocrinology*, *32*(1), 44-55. doi:10.1016/j.psyneuen.2006.10.002
- Rossa, M., Lovari, S., & Ferretti, F. (2021). Spatiotemporal patterns of wolf, mesocarnivores and prey in a Mediterranean area. *Behavioral Ecology and Sociobiology*, *75*(2). doi:10.1007/s00265-020-02956-4
- Sahlen, E., Noell, S., DePerno, C. S., Kindberg, J., Spong, G., & Cromsigt, J. P. (2016). Phantoms of the forest: legacy risk effects of a regionally extinct large carnivore. *Ecol Evol*, *6*(3), 791-799. doi:10.1002/ece3.1866
- Sand, H., Wabakken, P., Zimmermann, B., Johansson, O., Pedersen, H. C., & Liberg, O. (2008). Summer kill rates and predation pattern in a wolf-moose system: can we rely on winter estimates? *Oecologia*, *156*(1), 53-64. doi:10.1007/s00442-008-0969-2
- Sand, H., Wikenros, C., Wabakken, P., & Liberg, O. (2006). Cross-continental differences in patterns of predation: will naive moose in Scandinavia ever learn? *Proc Biol Sci*, *273*(1592), 1421-1427. doi:10.1098/rspb.2005.3447
- Say-Sallaz, E., Chamaillé-Jammes, S., Fritz, H., & Valeix, M. (2019). Non-consumptive effects of predation in large terrestrial mammals: Mapping our knowledge and revealing the tip of the iceberg. *Biological Conservation*, *235*, 36-52. doi:10.1016/j.biocon.2019.03.044
- Schlichting, P. E., Dombrowski, V., & Beasley, J. C. (2019). Use of abandoned structures by Przewalski's wild horses and other wildlife in the Chernobyl Exclusion Zone. *Mammal Research*, *65*(1), 161-165. doi:10.1007/s13364-019-00451-4
- Sih, A., Bolnick, D. I., Luttbeg, B., Orrock, J. L., Peacor, S. D., Pintor, L. M., . . . Vonesh, J. R. (2010). Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, *119*(4), 610-621. doi:10.1111/j.1600-0706.2009.18039.x
- Sunde, P., & Olsen, K. (2018). Wolves (*Canis lupus*) in Denmark 2012-2017 - Review and analysis of available population information. *Videnskabelig rapport fra DCE-Nationalt Center for Miljø og Energi. Institut for Bioscience. Aarhus University, Aarhus, Denmark*.
- Terborgh, J., Lopez, L., Nunez, P., Rao, M., Shahabuddin, G., Orihuela, G., . . . Balbas, L. (2001). Ecological meltdown in predator-free forest fragments. *Science*, *294*(5548), 1923-1926. doi:10.1126/science.1064397

- Theuerkauf, J., Gula, R., Pirga, B., Tsunoda, H., Eggermann, J., Brzezowska, B., . . . Radler, S. (2007). Human impact on wolf activity in the Bieszczady Mountains, SE Poland. *Ann. Zool. Fennici*, *44*, 225–231.
- Theuerkauf, J., & Rouys, S. (2008). Habitat selection by ungulates in relation to predation risk by wolves and humans in the Białowieża Forest, Poland. *Forest Ecology and Management*, *256*(6), 1325-1332.
doi:10.1016/j.foreco.2008.06.030
- Thorsen, H., Olsen, K., & Sunde, P. (2019). Danske ulves (*Canis lupus lupus*) døgnaktivitets- mønster studeret med brug af vildtkameraer. *Flora og Fauna*, *125*(4), 1-11.
- Torres, R. T., Silva, N., Brotas, G., & Fonseca, C. (2015). To Eat or Not To Eat? The Diet of the Endangered Iberian Wolf (*Canis lupus signatus*) in a Human-Dominated Landscape in Central Portugal. *PLoS One*, *10*(6), e0129379.
doi:10.1371/journal.pone.0129379
- Tourani, M., Brøste, E. N., Bakken, S., Odden, J., Bischof, R., & Hayward, M. (2020). Sooner, closer, or longer: detectability of mesocarnivores at camera traps. *Journal of Zoology*, *312*(4), 259-270. doi:10.1111/jzo.12828
- Vildtudbyttestatistikken. Online vildtudbyttestatistik af DCE - Nationalt Center for Miljø og Energi. Retrieved from <https://fauna.au.dk/jagt-og-vildtforvaltning/> on 25/05/21
- Wabakken, P., Sand, H., Kojola, I., Zimmermann, B., Arnemo, J. M., Pedersen, H. C., & Liberg, O. (2007). Multistage, Long-Range Natal Dispersal by a Global Positioning System–Collared Scandinavian Wolf. *Journal of Wildlife Management*, *71*(5), 1631-1634. doi:10.2193/2006-222
- Wagner, C., Holzapfel, M., Kluth, G., Reinhardt, I., & Ansorge, H. (2012). Wolf (*Canis lupus*) feeding habits during the first eight years of its occurrence in Germany. *Mammalian Biology*, *77*(3), 196-203.
doi:10.1016/j.mambio.2011.12.004
- Wikenros, C., Kuijper, D. P. J., Behnke, R., & Schmidt, K. (2015). Behavioural responses of ungulates to indirect cues of an ambush predator. *Behaviour*, *152*(7-8), 1019-1040. doi:10.1163/1568539x-00003266
- Wilkinson, D. M., & Sherratt, T. N. (2016). Why is the world green? The interactions of top–down and bottom–up processes in terrestrial vegetation ecology. *Plant Ecology & Diversity*, *9*(2), 127-140.
doi:10.1080/17550874.2016.1178353
- Winnie, J. A. (2012). Predation risk, elk, and aspen: tests of a behaviorally mediated trophic cascade in the Greater Yellowstone Ecosystem. *Ecology*, *93*(12), 2600-2614. doi:10.1890/11-1990.1
- Worm, B., & Paine, R. T. (2016). Humans as a Hyperkeystone Species. *Trends Ecol Evol*, *31*(8), 600-607.
doi:10.1016/j.tree.2016.05.008
- Zimmermann, B., Nelson, L., Wabakken, P., Sand, H., & Liberg, O. (2014). Behavioral responses of wolves to roads: scale-dependent ambivalence. *Behav Ecol*, *25*(6), 1353-1364. doi:10.1093/beheco/aru134
- Zlatanova, D., & Popova, E. (2013). Habitat variables associated with wolf (*Canis lupus* L.) distribution and abundance in Bulgaria. *Bulgarian Journal of Agricultural Science*, *19*(2), 262–266.

PART B – Article manuscript

Landscape of fear in an anthropogenic environment – how deer respond to the presence of wolves in a Danish wolf territory

Abstract

Predators can affect the spatial distribution and behaviour of prey species by creating a *landscape of fear* (LOF), where prey avoid areas associated with high perceived predation risk and increase vigilance in risky areas. LOFs created by returning predators have been theorised to cause behaviourally mediated trophic cascades in areas where predators have historically been absent. However, the evidence for these cascading effects have been disputed especially in areas with a high degree of human disturbance. Grey wolf *Canis lupus* populations are presently recovering in Europe, which emphasizes the need for studies on predator-mediated changes in prey behaviour in anthropogenic landscapes. This project sought to study how spatial variation in wolf activity affects the habitat selection, diel activity patterns, and vigilance behaviour of two sympatric deer species, roe deer *Capreolus capreolus* and red deer *Cervus elaphus*, in a Danish wolf territory. To do this, we analysed camera trap data (7613 active camera days) spanning across three years at multiple locations inside the first wolf territory in Denmark with an established wolf pair. The presence of both deer species was negatively correlated with wolf activity, which suggests that deer avoid areas associated with high wolf activity. Temporal overlap in activity between deer and wolves did not vary between areas of high and low wolf activity signifying that deer did not respond to increased wolf activity by avoiding wolves in time. We found no indication that an increase in wolf activity increased deer vigilance, rather, roe deer lowered vigilance as a response to increased wolf activity. Our study shows that wolf presence can lead to predator-mediated changes in prey behaviour even in areas with a high human presence.

Introduction

The risk of predation is a key factor in determining how animals are distributed in space, as prey animals seek to avoid predator encounters and reduce the risk of detection and capture (Lima, 1998). Avoiding predators can be costly for prey as they may reduce foraging, lose access to mates, increase energetic investment in antipredator behaviour and forego high quality foraging in risky areas (Brown & Kotler, 2004; Lima & Dill, 1990). It has been argued that the non-consumptive

effects of predators may affect prey more than direct predation (Preisser et al., 2005). Therefore, prey must be able to accurately assess predation risk to minimize costs. The perceived predation risk is determined by how high the perceived chance is of encountering a predator in an area as well as the perceived vulnerability of the individual (Moll et al., 2017). If the distribution of a prey population in a habitat is affected by the perceived predation risk, it can be said that the prey inhabit a *landscape of fear* (LOF) which is defined as the spatially explicit distribution of perceived predation risk as seen by a population by Bleicher (2017). Perceived predation risk has been characterised using vastly different techniques focusing on either constant background risk and habitat characteristics, *risky places*, or immediate risk, *risky times* (Moll et al., 2017). Recent studies show that LOFs may be more dynamic than earlier thought due to the risk of an area being highly dependent on temporal variability in risk (Kohl et al., 2018; Palmer et al., 2021). These results align with the *predation risk allocation hypothesis* by Lima and Bednekoff (1999) which postulates that the temporal variability of risk plays an important role in the decision making of prey species. According to them, prey should allocate less time to antipredator behaviour in high-risk situations as these become more frequent which is the opposite of what is expected in the *risky places hypothesis* which assumes a simple relationship between an increase in predation risk and an increase in vigilance (Creel et al., 2008). Studies on North American elk *Cervus canadensis* (hereafter referred to as elk) have found vigilance behaviour to be highly complex and not easily explained by models assuming risk to be static in space and time (Creel et al., 2008; Kohl et al., 2018)

Laundré et al. (2001) originally defined the LOF concept in the Greater Yellowstone Ecosystem (GYE), USA, where female elk responded to the reintroduction of grey wolves *Canis lupus* (hereafter referred to as wolves) in 1995 by increasing their vigilance level and changing habitats (Fortin et al., 2005; Mao et al., 2005). Other wolf-mediated behavioural responses in GYE elk include: shifting from open grassland into coniferous forest (Creel et al., 2005), adjusting vigilance behaviour based on the presence of wolves (Creel et al., 2008) and selecting habitats based on the spatiotemporal variability in wolf predation (Kohl et al., 2018). It has been theorised that the wolf-mediated changes in prey behaviour could lead to trophic cascades (Flagel et al., 2016; Ripple & Beschta, 2004). However, the ecological effect will be weakened if prey continue to use risky places during safe times (Kohl et al., 2018; Palmer et al., 2021). Later studies on riparian systems and aspen stands in GYE did not observe a recovery following wolf reintroduction, casting doubt

on the existence of a trophic cascade (Kauffman et al., 2010; Marshall et al., 2013). Although the limited decrease in browsing pressure may be due to a concurrent rise in bison *Bison bison* numbers (Beschta et al., 2020). Additionally, while some find that wolf predation risk impacts nutritional and reproductive fitness negatively in elk (Christianson & Creel, 2010; Creel et al., 2007), others doubt the severity of the fitness cost (Middleton et al., 2013). To get a clearer picture of the interaction between wolves and ungulates, it is crucial to study the system in other parts of the world (Say-Sallaz et al., 2019). Studies in anthropogenic areas such as Europe are especially needed as it is not yet known whether human disturbance exacerbate or reduces the effect of predators (Kuijper et al., 2016; Worm & Paine, 2016).

Recent advances have emphasized the potential of using predators to restore ecosystems through top-down effects, however, the effects are highly dependent on factors such as ecosystem productivity, species diversity and human interference (Ritchie et al., 2012). Beginning in the late 20th century, wolves have seen a recovery in many European countries (Chapron et al., 2014), which has sparked interest in the ecological effect they and other predators could have on local ungulate populations (Kuijper et al., 2013; Sahlen et al., 2016). Wolves are flexible predators whose diet depend on local prey abundances and seasonal variations (Mech & Boitani, 2003). Cervids, such as red deer *Cervus elaphus* and roe deer *Capreolus capreolus* have recently increased in both abundance and distribution throughout Europa (Gill, 1990) and are the primary prey for much of the continental wolf population (Jędrzejewski et al., 2002; Marucco et al., 2008; Nowak et al., 2011; Randon et al., 2020; Wagner et al., 2012). How the expansion of wolf populations will affect prey populations in Europe is unclear, but has been linked to a reduction in hunting harvest of moose *Alces alces* in Sweden (Wikenros et al., 2015) as well as population decline and low recovery rate of roe deer following wolf recovery in the French Vercors mountain range (Randon et al., 2020). There is a lack of studies on the effect of wolf predation on red deer populations. European deer have been found to react behaviourally to wolf presence: roe deer increased herd size in open areas in response to wolf presence on the Iberian Peninsula (Barja & Rosellini, 2008), red deer changed diet composition in high wolf use areas in Poland (Churski et al., 2021) and fallow deer *Dama dama* reduced their temporal overlap with wolves in high wolf use areas in Italy (Rossa et al., 2021). However, in human-dominated landscapes, the effect of wolf predation may be overshadowed by disturbances related to human activities (Ciuti et al., 2012; Mech, 2012). For instance, hunting and forest exploitation influenced the habitat selection of red deer and roe deer

more than wolf predation did in the Białowieża Primeval Forest in Poland (Theuerkauf & Rouys, 2008).

The aim of this study was to investigate whether or not the presence of wolves affects the avoidance and vigilance behaviour, and diel activity of their prey in Denmark as is expected according to the *landscape of fear* hypothesis. To do this, we evaluated the response of deer to the spatiotemporal distribution of wolf predation risk using camera trap data from a wolf territory in Denmark. We assessed whether deer within a wolf territory avoid wolves in space and time and/or show heightened vigilance in areas associated with high wolf activity. If deer avoid wolves, we predict that **1)** the observation frequency of deer correlates negatively with wolf activity (spatial avoidance) and/or that **2)** deer express lower overlap in diel activity with wolves in areas with high wolf activity than in areas with lower wolf activity (temporal avoidance). If the *risky places* hypothesis is supported, we predict that **3)** deer alertness is positively correlated with wolf activity as vigilance is expected to increase with predator encounter rate. If the *predation risk allocation* hypothesis is supported, we predict that **4)** deer alertness is negatively correlated with wolf activity as vigilance should decrease with long-term predator encounter rate, and/or that **5)** the proportion of deer exhibiting alertness is higher on days where wolves are present than on days where wolves are locally absent.

Methods

Study species

This study focused on the behavioural response of roe deer and red deer to wolf activity. As is the case for most of Europe, both red deer and roe deer have experienced strong population growth during the last 50 years in Denmark (Noer et al., 2009; Olesen et al., 2002). Roe deer are found throughout the country, whereas red deer are concentrated in distinct geographic areas (Asferg & Madsen, 2012, 2013). Red deer are the largest deer in Denmark (males: 140-150 kg, females: 90 kg) and gregarious grazers that prefer open pastures with abundant forage and little cover for active foraging during the night and forested areas with little forage and more cover during daytime (Asferg & Madsen, 2012; Godvik et al., 2009). The smaller, and more elusive, roe deer (weight: 15-30 kg) prefer to forage alone or in small groups and are concentrate selective browsers, i.e., prefer forage with a high soluble carbohydrate and a low fibre content (Asferg & Madsen, 2013; Duncan,

1998; Olesen et al., 2002). Based on the previously mentioned European studies, both red deer and roe deer should be preyed on by Danish wolves.

Grey wolves are both the largest extant wild canid and the largest terrestrial predator in Denmark (weight: ~32 kg, Smith et al. (2003)). As a cursorial predator, wolves actively patrol for prey, followed by chase and capture (Mech & Boitani, 2003; Preisser et al., 2007). The first confirmed wolf observation in Denmark in 200 years occurred in 2012 in the north-western part of Jutland (Sunde & Olsen, 2018). Since fall 2016, wolves have been more or less continually present in our study area in Western Jutland (Thorsen et al., 2019).

Study area

The study area is approximately 40 km² of plantation and heathland (of which most is managed by the Danish Nature Agency) located near Ulfborg (Holstebro municipality) in western Jutland, Denmark. The area contains the Ulfborg wolf territory which held the first and second established wolf pair in Denmark in recent times and is presently the only confirmed location in Denmark where wolves have reproduced (in 2017 and 2019 respectively) (Thorsen et al., 2019). Red deer and roe deer are numerous in the area, along with a smaller population of fallow deer. The habitat is highly heterogenous and consists primarily of plantations (*Pinus sylvestris*, *P. contorta*, *P. nigra* and *P. mugo*) and large areas of heathland with heather (*Calluna vulgaris*, *Erica tetralix*, *Empetrum nigrum*, *Vaccinium uliginosum*, *V. vitis-idaea*) and juniper *Juniperus communis*. The remaining area includes managed and unmanaged conifer forest (*Pinus*, *Larix*, *Abies* and *Picea*) and broadleaved deciduous forest (*Fagus sylvatica*, *Quercus*, *Betula*, *Salix*, *Populus*, *Prunus serotina* and smaller trees and shrubs). Cultivated fields with e.g., Christmas trees and grass are also present. Hunting on the state-owned areas is limited to a few joint hunts held between October and January (Naturstyrelsen). Meanwhile, the annual hunting season on neighbouring private land stretches from 1st September (red deer stags)/1st October (red deer hinds and calves, roe deer) to 31st January and again from 16th May to 15th July (only roebucks) (Asferg & Madsen, 2012, 2013).

Data collection and trail cameras

The data used in this study came from the National Danish Wolf Monitoring Programme, which has had cameras in the area since November 2016 in an effort to monitor the wolves present (Thorsen et al., 2019). The dataset consists of data from 2017 – 2019. Over the years, different models of trail

cameras have been used in the monitoring programme; including Bushnell cameras (Trophy Cam Aggressor, Bushnell Core DS, trigger speed: 0.2 seconds, recovery rate: 0.6 seconds) and Reconyx Ultrafire XS8 (trigger speed: 1 second, recovery rate: 5 seconds). In recent years, all cameras have been no glow and the infrared flash used at night should thus not be discernible to animals. The cameras were all wolf targeted and were therefore placed near roads, paths, or natural tracks in the landscape at an angle to improve wolf capture probability. The cameras were placed 80-120 cm above ground on trees or poles. Cameras have most commonly been set to take three pictures followed by a 15-30 second video. As the primary purpose of the cameras is to monitor wolf activity, the cameras have been moved around at irregular intervals to locate the wolves. Each location is given a unique ID when deployment begins, and the coordinates are recorded. The practical part of the monitoring is carried out by volunteers, who inspect and collect data from the cameras approximately once a month. The time between checks amounts to one deployment period which is named after the location and the start and end date.

Data processing

For every observation (consisting of both photos and a video) date, timestamp, person coding and species (including humans) was noted. The certainty of the species identification was noted as either certain, probable, or possible. If less certain than “possible” the animal was either categorized on a higher taxonomic level (e.g., “ungulate sp.,” “carnivore sp.” etc.) or as an “unidentified mammal”. Whether the picture was taken in daylight or at night (using infra-red light) was noted as well as the unique location ID and the deployment period ID. In cases when a camera had malfunctioned or lost power before the end date, the date of the last observation was used as the end date for the deployment period instead. The behaviour of the animal was scored using an ethogram based on Kuijper *et al.* (2014) (**table 1**). In cases when the animal exhibited multiple behaviour types the dominant one (the one exhibited the longest time) was chosen. However, some brief behaviour types (e.g., “marking” and “sudden rush”) are relevant to record, and in instances where they were performed by the animal, these were chosen instead of the dominant behaviour. The amount of alertness of each individual was also noted from the pictures and videos. Alertness was scored by counting the number of photos where the animal was characterized as vigilant (head held still and parallel to the body, with ears pricked forward) and adding the number of seconds spent being vigilant in videos divided by two. To include even brief expressions of vigilance, a binary behaviour category was created where 0 signifies that the animal did not express alertness at all, and

1 signifies that the animal was either recorded as performing “vigilance” or had been alert for at least 1 second. We did this since the number of pictures and length of video per observation were not consistent throughout the study. It was therefore impossible to compare the alert value of different observations.

Table 1 - ethogram used to define observed animal behaviour. Behaviour was averaged over time and between individuals within the same observation, and the dominant behaviour chosen, unless brief (but important) behaviour such as “marking” or “sudden rush” was exhibited.

Behaviour	Definition
Vigilance	Standing still with its head held parallel to body or higher, looking around and/or twitching the ears. Hares can also press their body close to the ground
Foraging	Includes grazing and browsing for herbivores. Includes stalking and active hunting for carnivores
Walking	Walking without eating
Running	Quick movements in the observation
Sudden rush	When an animal shifts from standing still to running – demand more than one picture or a video
Sniffing/exploring	Sniffing to scent source, animal has its head near the ground or objects without chewing or eating
Social behaviour	All social interactions between conspecifics, touching/rubbing heads, aggression/fighting, social play, mating
Marking	Marking with urine, scent canals and/or scratching/rubbing on the ground or on trees
Checking camera	Walking towards camera, sniffing the camera
Other behaviour	All other behaviour which is not included above

To limit pseudo replication, observations of the same species within 5 minutes where it was not possible to distinguish one individual from the other, was judged to be the same individual and given one observation using the first timestamp. If there was more than one observation of the same species within one hour and the individuals could not be distinguished from each other, then each instance was recorded, but only the first observation was counted. In this study, it was later decided to count every observation and include observations regardless of species certainty. Prior to analysis, data was checked for errors, obvious outliers were taken out, and the time was corrected for daylight savings when needed. For some analyses, the data needed to be in the form of a presence/absence table, which was constructed by generating all days in every deployment period using start and end dates. The final table shows the number of each species observed per day. To calculate the diel activity, the time of day was converted into radians.

Wolf activity index

We used a Kernel Density Estimator in QGIS (v. 3.16.3) to estimate the spatial distribution of wolf observations in the area for “wolf periods” of 6 months (summer: May-November, winter: November-May). Both wolf observations from camera traps and scat finds were included in the estimation as the two measures correlated strongly (Pearson’s correlation coefficient, r between 0.46 and 0.96, see **Appendix 1.1**). For details on how wolf observations were validated, see Sunde and Olsen (2018) and Thomsen *et al.* (2020). 6 months “Wolf period” intervals were chosen as they correspond with different stages in the wolf’s reproductive cycle (pups are born in early May and by November they are accompanying their parents on hunts (Mech & Boitani, 2003)), and because shorter periods did not have sufficiently large sample sizes. A smoothing factor of 1000 meters was chosen, which means that when estimating the number of expected wolf observation at each point, the estimator took account of wolf observations within a 1000-meter radius. The resulting heatmap visually illustrated the continuous spatial variation in estimated wolf observations (resolution 10x10 meters) as a gradient from cool to warm colours (see **Appendix 1.2**). Each observation could then be assigned an index value of “wolf activity” for that specific location in the 6 months period the observation fell under. The extracted values were scaled by dividing them with the total amount of wolf observations in the specific period to make comparison between periods possible. The variable was log-transformed to account for non-normal distribution. A second variable was generated separating locations into areas of high and low wolf activity based on the distribution of the continuous wolf activity variable in each period. High activity zones were defined by having a wolf activity above the mean activity value for the given period, while low activity zones had a value below.

Habitat variables

To account for variation caused by difference in habitat a number of habitat variables were computed in QGIS. Using shapefiles of roads, fields and buildings in the area the precise distance between these features and the different camera locations could be measured. For buildings a centroid was generated and the distance between each camera and the nearest house was found using a distance matrix. To find the distance to the nearest road (≥ 3 meters wide) and nearest field the tool v.distance (GRASS plugin) was used. All distances were measured in meters. To account for vegetation structure variables for mean, minimum, maximum and variation in vegetation height was calculated. To do this a Canopy Height Model (CHM) was generated by subtracting the Digital

Terrain Model (DTM) of the area from the Digital Surface Model (DSM) of the area. Both the DTM and DSM was based on data from 2007, had a 0.4-meter grid and were downloaded from kortforsyningen.dk. Afterwards, the mean CHM raster value, the minimum and maximum raster value, and variation in raster value for buffer zones with a 25-meter radius around each location could be calculated. All vegetation variables were measured in meters.

Sun angle and light periods

To take account of the amount of daylight for each observation the sun angle was calculated. This was done using the sunAngle function from the oce package in R (RStudio v. 1.3.1093), which calculates the sun angle from the precise time (in UTC) and location (Kelley et al., 2021).

Afterwards, the observations were divided into three light periods defined by the sun angle (daytime: sun angle $> 0^\circ$; nighttime: sun angle $< -6^\circ$; twilight: $0^\circ \leq \text{sun angle} \leq -6^\circ$). -6° is the limit for “civic twilight” and was chosen as it is approximately an hour-long year-round and because using “nautical twilight” (which ends at -12°) would result in “nightless” summers as the sun never goes below -11° in Denmark near solstice. To calculate the sun angle, we had to calculate the time in UTC for each observation while also taking daylight savings into account (Denmark is UTC+1 in winter and UTC+2 in summer).

Analysis

Deer presence model selection

The correlation between fixed predictors were evaluated using a Pearson’s correlation matrix using a cut-off of $r = 0.70$. Mean vegetation height was highly correlated with both vegetation height variance ($r = 0.91$) and maximum vegetation height ($r = 0.80$) (**table 2**) and these were therefore excluded. Distance to nearest house and distance to nearest field were moderately correlated ($r = 0.58$), but we decided to keep both predictors in the analysis. To evaluate which covariates best explained number of deer observations per active camera day (24-h units), models containing different combinations of ecologically relevant predictors were compared with the model having the lowest AIC_C being considered best (Burnham et al., 2010). The log-transformed number of deer observation per day was modelled with generalized linear mixed models (GLMMs) using a negative binomial distribution and the log-link function (glmmTMB package (Magnusson et al., 2020)). To account for seasonal variation, all evaluated models included season as a fixed effect. Observations were grouped into seasons of three months where winter was the three darkest months (November,

December and January). To account for habitat variation, we included mean vegetation height and the distance to roads, houses and fields. The unique location ID was included as a random effect to account for random variation between observation sites. Year and wolf period were included as factors to account for difference in monitoring effort and difference between different wolf periods, but both factors were excluded through the evaluating process.

Table 2 – a Pearson’s correlation matrix showing the correlation between spatial habitat covariates. Correlations greater than 0.7 are shown in parentheses.

	House distance	Road distance	Field distance	Mean vegetation height	Maximum vegetation height	Vegetation height variance
Road distance	0.04					
Field distance	0.58	-0.03				
Mean vegetation height	0.10	0.19	0.35			
Maximum vegetation height	0.10	-0.01	0.37	(0.80)		
Vegetation height variance	0.13	0.12	0.40	(0.91)	(0.89)	
Wolf activity	-0.34	0.25	-0.11	-0.03	-0.13	-0.07

Calculating overlap in diel activity

We visualised the diel activity of the different species using kernel density estimation of the radian values. To determine shifts in activity caused by wolf activity, the overlap between wolf activity and roe deer and red deer activity was compared for low and high wolf activity zones using the overlap package (Meredith & Ridout, 2020). The value of “Dhat4” was used as an estimate of the overlap. The value of “Dhat4” ranges from 0 to 1 with 0 signifying no overlap in activity and 1 signifying a total overlap in activity. Confidence intervals for each overlap estimate were calculated using 500 bootstrap samples.

Comparing vigilance behaviour

To determine if wolf presence had an effect on deer vigilance in a broader sense, we used a GLMM model with a binomial distribution to see if wolf activity was a significant predictor of deer alertness. Season, mean vegetation height, and light period were included to account for seasonal, habitat and daily variability. To determine if wolf presence had an effect on deer vigilance on a fine scale, we compared the proportion of alert and non-alert deer on days with and without wolves present using a χ^2 -test. To do this, the presence of wolves, alert deer, and non-alert deer on each day was treated as binary variables.

Results

Deer presence

A total of 1353 roe deer observations and 917 red deer observations collected over 7613 active camera days was included in the study. Roe deer, but not red deer, were observed less frequently in zones with high wolf activity (**fig. 2**). Red deer were observed least frequently in spring and most frequently in winter, while roe deer were observed most frequently in summer and fall and least frequently in fall and spring (**fig. 3a**).

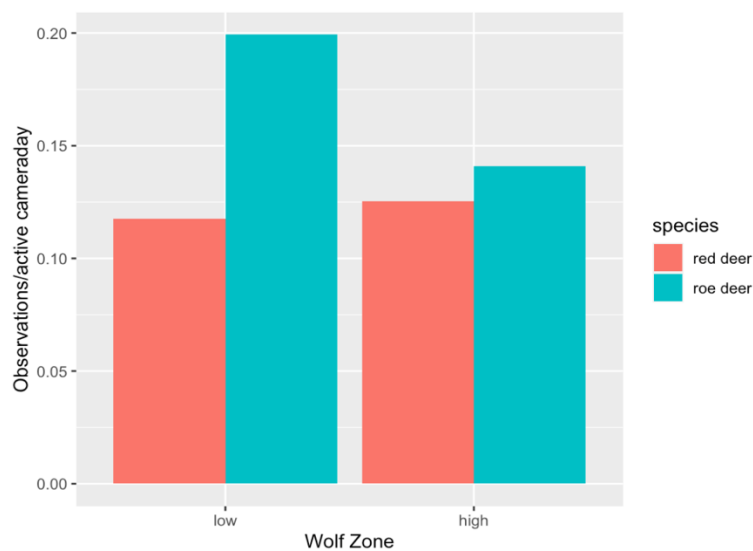


Figure 2 – frequency of red deer and roe deer observations per active camera day in zones of high and low wolf activity.

The observation frequency (observations per active camera day) of roe deer did not vary considerably with mean vegetation height, whereas the frequency of red deer observations mostly increased with vegetation height but was at its lowest in areas where vegetation height > 7 m (**fig. 3b**). Observation frequency of red deer did not vary a lot with distance to nearest house, whereas roe deer were observed twice as often in areas close to houses (0-100 m) (**fig. 3c**). Roe deer were both observed frequently close to roads (0-100 m) and far away from roads (>1000 m), whereas red deer were observed thrice as frequently far away from roads (>1000 m) as anywhere else (**fig. 3d**).

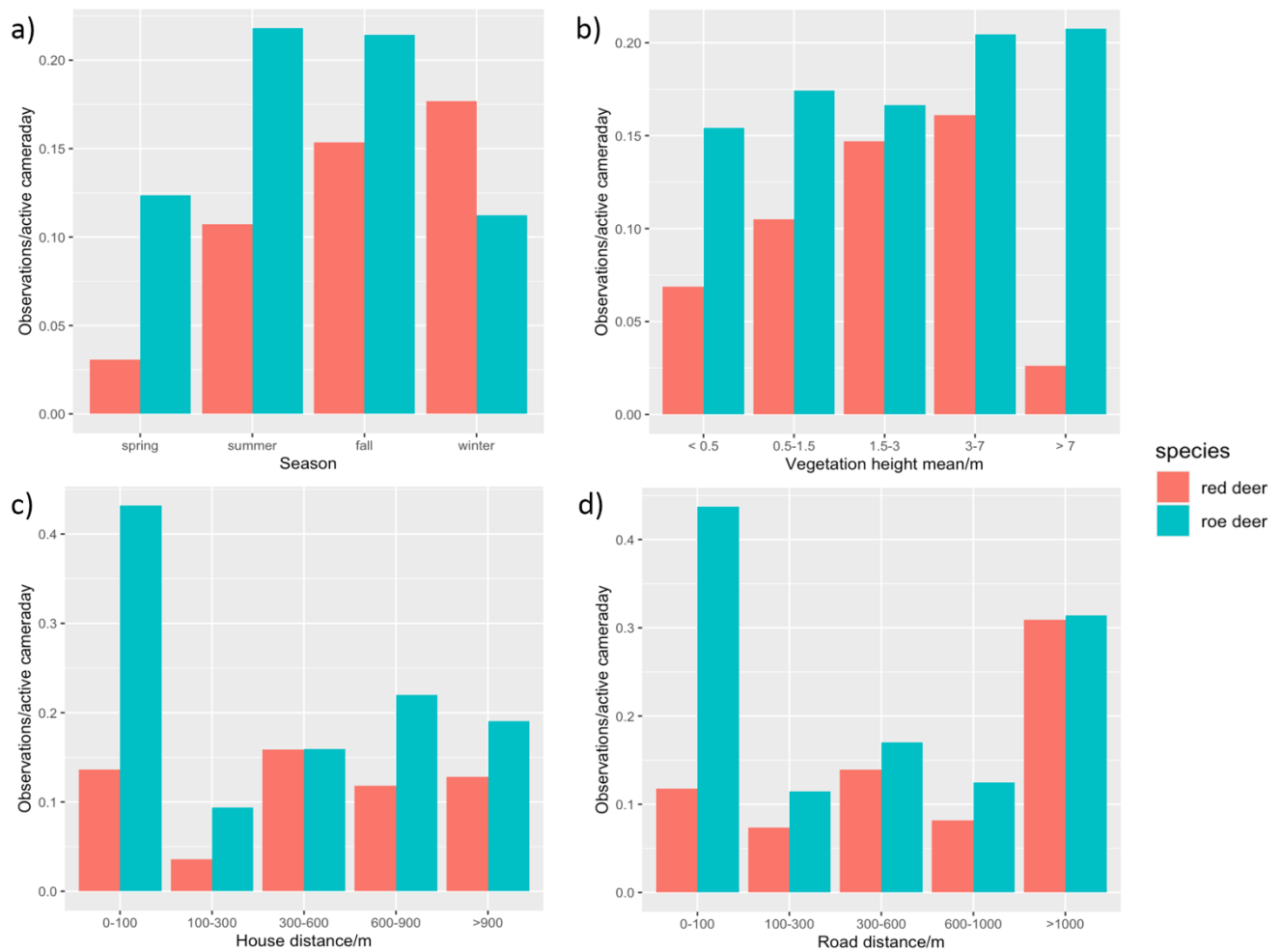


Figure 3 – frequency of roe deer and red deer observations per active camera day, **a**) in different seasons, **b**) at different vegetation heights (meters), **c**) at different house distances (meters) and **d**) at different road distances (meters).

Roe deer

The empirical support for the model including wolf activity and road distance was almost thrice that of the null model ($\Delta AIC_C = 2.14$, evidence ratio (ER) = 2.9, **table 3**). Of the two variables, wolf activity seemed to be the most important predictor as the model including wolf activity and road distance had higher support than road distance alone (ER = 2.8), whereas the addition of road distance only increased empirical support marginally compared to wolf activity alone (ER = 1.3). Models including the effect of house distance, field distance or mean vegetation height did not have more support than the null model. The final model showed slight overdispersion (7658.3 residual deviance on 7604 degrees of freedom).

Table 3 – AIC_c values and weights of different models used to predict the number of roe deer observations per active camera day. Location indicates the unique sampling point ID.

Model	AIC _c	Delta	Weight
<i>roe deer ~ season + (1 location) + wolf activity + road</i>	7676.4	0.00	0.216
<i>roe deer ~ season + (1 location) + wolf activity</i>	7676.9	0.57	0.162
<i>roe deer ~ season + (1 location) + wolf activity + road + house</i>	7678.3	1.96	0.081
<i>roe deer ~ season + (1 location) + wolf activity + road + field</i>	7678.3	1.96	0.081
<i>roe deer ~ season + (1 location) + road</i>	7678.4	2.03	0.078
<i>roe deer ~ season + (1 location)</i>	7678.5	2.14	0.074
<i>roe deer ~ season + (1 location) + wolf activity + house</i>	7678.8	2.49	0.062
<i>roe deer ~ season + (1 location) + wolf activity + field</i>	7678.9	2.56	0.060
<i>roe deer ~ season + (1 location) + road + house</i>	7679.9	2.59	0.036
<i>roe deer ~ season + mean vegetation height + (1 location)</i>	7680.0	3.67	0.034
<i>roe deer ~ season + (1 location) + wolf activity + road + house + field</i>	7680.3	3.95	0.030
<i>roe deer ~ season + (1 location) + road + field</i>	7680.3	3.97	0.030
<i>roe deer ~ season + (1 location) + wolf activity + house + field</i>	7680.8	4.40	0.024
<i>roe deer ~ season + (1 location) + field</i>	7682.0	5.66	0.013
<i>roe deer ~ season + (1 location) + house</i>	7682.0	5.67	0.013
<i>roe deer ~ season + (1 location) + house + field</i>	7684.0	7.66	0.005
<i>roe deer ~ (1 location)</i>	7724.4	48.05	0.000
<i>roe deer ~ mean vegetation height + (1 location)</i>	7724.6	48.22	0.000

From the estimates of the model with most empirical support (**table 4**), roe deer observation frequencies correlated negatively with wolf activity (**fig. 4a**), while a negative effect of the distance to roads was non-informative. On a seasonal basis, the observation frequency peaked in fall and summer and dipped in winter and spring.

Table 4 – parameter estimates for the best roe deer presence model. Bold p-values are below 0.05.

	Estimate	SD	p-value	CI
Intercept	-1.748	0.113		[-1.970, -1.525]
Wolf activity	-0.110	0.054	0.041	[-0.216, -0.005]
Road distance	-0.162	0.110	0.140	[-0.378, 0.053]
<i>Season †</i>				
Spring	-0.398	0.107	<0.001	[-0.608, -0.188]
Summer	-0.012	0.086	0.89	[-0.181, 0.158]
Winter	-0.617	0.102	<0.0001	[-0.818, -0.417]

Notes: † reference = Fall

Red deer

The best supported model included wolf activity, mean vegetation height, road distance and field distance ($\Delta AIC_C = 16.03$, $ER = > 401$, **table 5**). Including wolf activity in the final model increased empirical support significantly as the model without it had 400 times less support ($ER = 401$). The model that included distance to roads and fields had more support compared to the model only including wolf activity ($ER = 14.9$). The model that included the effect of house distance did not have higher support than the null model. The best model showed slight overdispersion (7977.4 residual deviance on 7603 degrees of freedom).

Table 5 – AIC_C values and weights of different models used to predict the number of red deer observations per active camera day. Location indicates the unique sampling point ID.

Model	AIC_C	Delta	Weight
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity + road + field</i>	6997.5	0.00	0.401
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity + road + house + field</i>	6998.8	1.36	0.203
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity + field</i>	6999.6	2.12	0.139
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity + house + field</i>	6999.7	2.24	0.131
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity + road</i>	7001.3	3.79	0.060
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity</i>	7001.8	5.37	0.027
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity + road + house</i>	7003.1	5.61	0.024
<i>red deer ~ season + mean vegetation height + (1 location) + wolf activity + house</i>	7004.8	7.36	0.010
<i>red deer ~ season + mean vegetation height + (1 location) + house + field</i>	7008.6	11.16	0.002
<i>red deer ~ season + mean vegetation height + (1 location) + road + house + field</i>	7009.5	12.03	0.001
<i>red deer ~ season + mean vegetation height + (1 location) + road + field</i>	7010.2	12.77	0.001
<i>red deer ~ season + mean vegetation height + (1 location) + field</i>	7010.6	13.15	0.001
<i>red deer ~ season + mean vegetation height + (1 location) + road</i>	7013.5	16.00	0.000
<i>red deer ~ season + mean vegetation height + (1 location)</i>	7013.5	16.03	0.000
<i>red deer ~ season + mean vegetation height + (1 location) + house</i>	7015.0	17.56	0.000
<i>red deer ~ season + mean vegetation height + (1 location) + road + house</i>	7015.4	17.88	0.000
<i>red deer ~ season + (1 location)</i>	7021.8	24.35	0.000
<i>red deer ~ mean vegetation height + (1 location)</i>	7125.0	127.51	0.000
<i>red deer ~ (1 location)</i>	7134.4	136.91	0.000

From the estimates (**table 6**) of the model with most support, red deer observation frequencies correlated negatively with wolf activity (**fig. 4b**) and mean vegetation height. Observation frequencies were positively correlated to road distance meaning that as distance to nearest road increases so does the number of red deer observations. The opposite is true for distance to fields as red deer observation frequencies decrease when distance to fields increase. The observation frequency was significantly lower in spring and summer, compared to winter and fall.

Table 6 – parameter estimates for the best red deer presence model. Bold p-values are below 0.05.

	Estimate	SD	p-value	CI
Intercept	-1.354	0.164		[-1.675, -1.033]
Wolf activity	-0.243	0.065	0.000	[-0.371, -0.116]
Mean vegetation height	-0.269	0.127	0.034	[-0.517, -0.020]
Road distance	0.324	0.157	0.039	[0.015, 0.632]
Field distance	-0.302	0.123	0.014	[-0.543, -0.061]
<i>Season †</i>				
Spring	-1.358	0.181	<0.0001	[-1.712, -1,003]
Summer	-0.509	0.127	<0.0001	[-0.760, -0.260]
Winter	0.260	0.102	0.010	[0.061, 0.459]

Notes: † reference = Fall

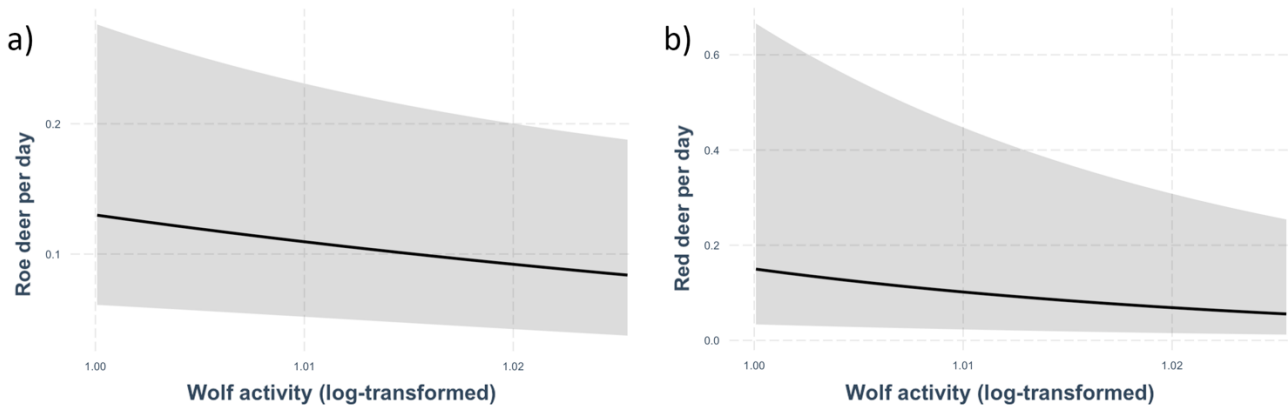


Figure 4 – effect plots showing the regression line for the number of **a)** roe deer and **b)** red deer observed per day predicted by the level of wolf activity. The shaded area indicates the 95-confidence interval.

Diel activity of deer and wolves

Roe deer, red deer and wolves all show a shift in their diel activity between summer and winter, with activity shifting closer to midnight in summer (**fig. 5**). The activity of both roe deer and red deer shows three distinct peaks in winter: one in the evening, one in the morning, and a lesser peak around midnight. In summer, the activity of the deer only has two distinct peaks: one in the late evening, and one in the early morning. The diel activity of wolves' form three peaks in winter: one in the evening, one in the morning, and a small bump in activity after midnight. In summer, wolf activity only has one large peak at around three in the morning.

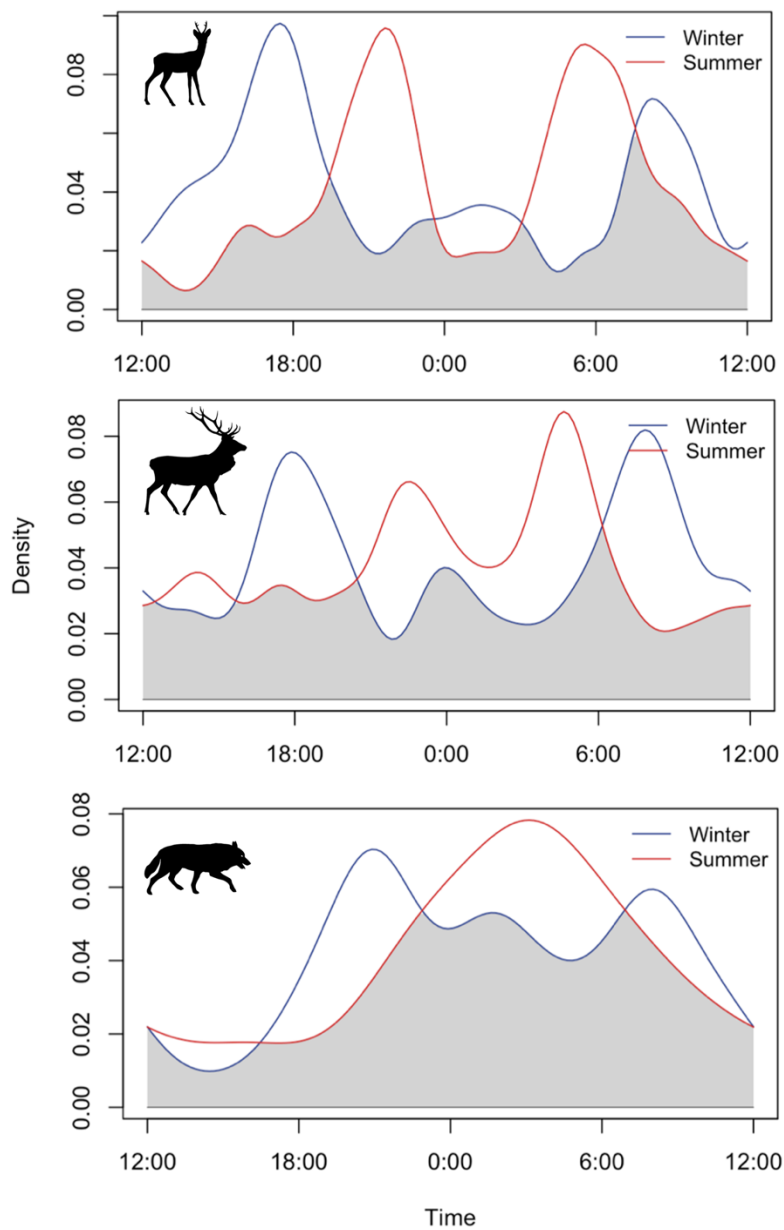


Figure 5 – density estimates of the diel activity pattern of roe deer (top), red deer (middle) and wolf (bottom) in winter (blue) and summer (red) centered around midnight.

The estimated overlap between the activity of wolves and both deer species is slightly lower in areas of high wolf activity (**fig. 6** and **7**). However, if we look at the confidence intervals, there is no significant difference between zones of high and low wolf activity in regard to the overlap in wolf and deer activity.

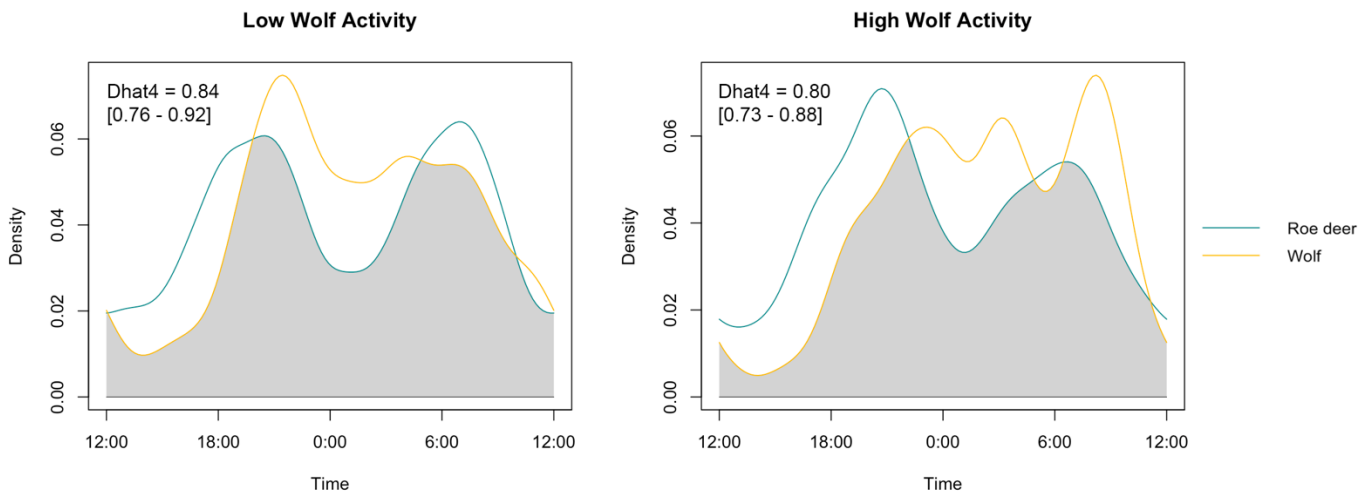


Figure 6 – density estimate of the diel activity patterns of roe deer (teal) and wolves (yellow) in areas of high and low wolf activity centered around midnight. Overlap in activity is shaded grey. Overlap in activity is 0.84 [0.76 – 0.92] in low wolf activity zones and 0.80 [0.73 – 0.88] in high wolf activity zones.

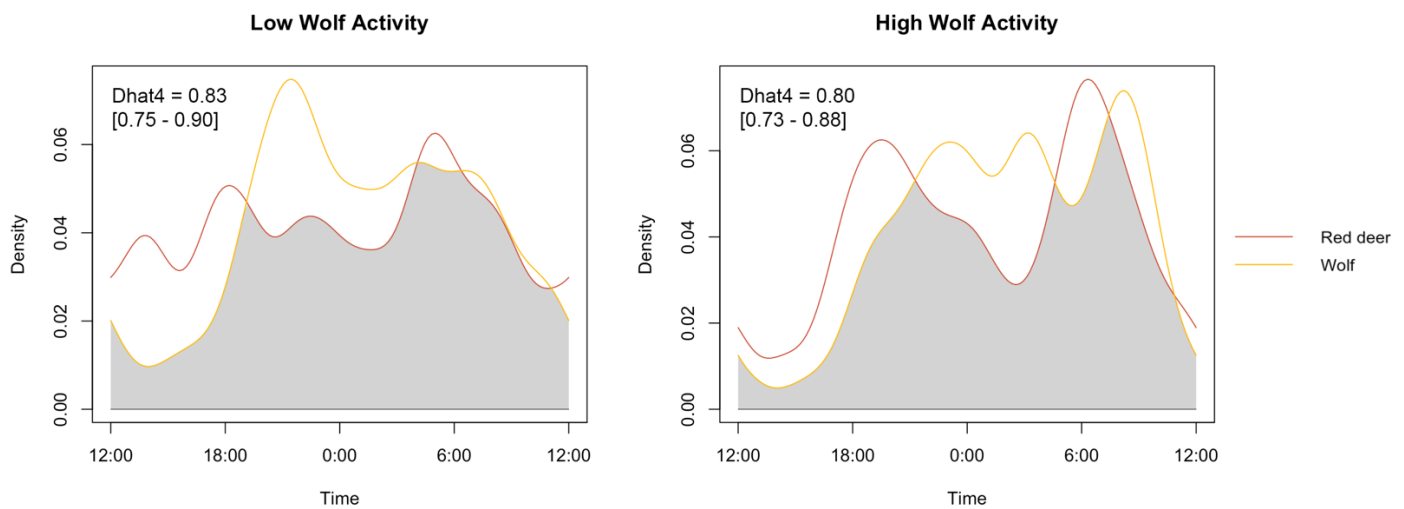


Figure 7 – density estimate of the diel activity patterns of red deer (red) and wolves (yellow) in areas of high and low wolf activity centered around midnight. Overlap in activity is shaded grey. Overlap in activity is 0.83 [0.75 – 0.90] in low wolf activity zones and 0.80 [0.73 – 0.88] in high wolf activity zones.

Vigilance behaviour of deer

When we compare the distribution of the binary vigilance scores for the two wolf zones (**fig. 8**), there is no difference in the ratio between alert and non-alert deer for either of the two species (χ^2 , roe deer: $P = 0.582$, red deer: $P = 0.297$).

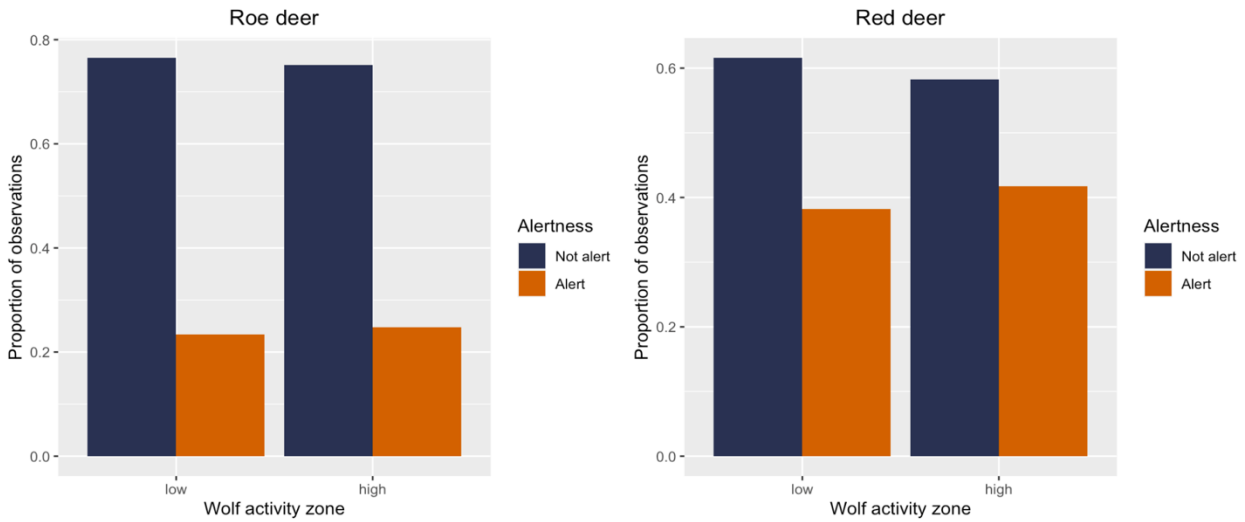


Figure 8 – the distribution of observations where deer were categorised as alert and not alert in the zones of high and low wolf activity. Roe deer (low: $n = 958$, high: $n = 395$), red deer (low: $n = 565$, high: $n = 352$).

There was a total of 35 days where roe deer and wolves were observed on the same camera and date and 39 days where red deer and wolves were observed on the same camera and date. When we look at the distribution of alert and non-alert animals between days with and without wolves, there does not seem to be a difference for roe deer (**fig. 9**, χ^2 , $P = 0.762$). However, it does look like a higher proportion of red deer exhibited alertness on days where wolves were present, although the trend is not significant (χ^2 , $P = 0.105$).

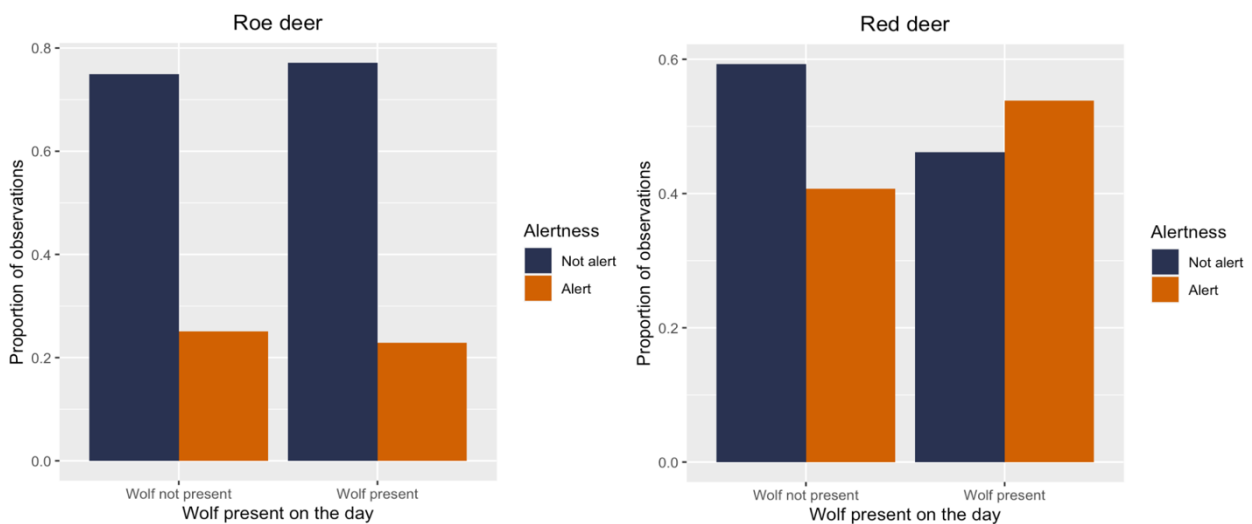


Figure 9 – the distribution of observations where deer were categorised as alert or not alert on days where wolves had been observed on the camera and days where wolves had not been observed. Roe deer (wolf not present: $n = 1095$, wolf present: $n = 35$), red deer (wolf not present: $n = 727$, wolf present: $n = 39$).

Wolf activity as a predictor of alertness

The best supported model for roe deer alertness kept wolf activity and mean vegetation height ($\Delta AIC_C = 5.03$, ER = 12.41), however the parameter estimate of mean vegetation height was not significant. Neither wolf activity nor mean vegetation height was a significant predictor for red deer alertness (**table 7**).

Table 7 – AIC_C values and weights of different models used to predict deer alertness on each observation. Light period is either daylight, twilight or nighttime. Location indicates the unique sampling point ID.

Roe deer	AIC_C	Delta	Weight
<i>alert ~ season + light period + (1 location) + wolf activity + mean vegetation height</i>	1389.8	0.00	0.534
<i>alert ~ season + light period + (1 location) + wolf activity</i>	1390.5	0.74	0.368
<i>alert ~ season + light period + (1 location) + mean vegetation height</i>	1394.3	4.56	0.055
<i>alert ~ season + light period + (1 location)</i>	1394.8	5.03	0.043
Red deer			
<i>alert ~ season + light period + (1 location)</i>	1148.3	0.00	0.357
<i>alert ~ season + light period + (1 location) + wolf activity</i>	1148.4	0.11	0.338
<i>alert ~ season + light period + (1 location) + mean vegetation height</i>	1149.8	1.56	0.164
<i>alert ~ season + light period + (1 location) + wolf activity + mean vegetation height</i>	1150.1	1.87	0.140

The parameter estimate for wolf activity was negative for the roe deer model, which means that as wolf activity increases the alertness of roe deer decreases (**table 8**). Roe deer were significantly more alert in spring compared to fall, but not significantly more or less alert in winter and summer. Roe deer were significantly more alert during twilight compared to during the day. Red deer were significantly more alert in winter compared to fall, but not significantly more or less alert in spring and summer. The light period did not affect alertness in red deer.

Table 8 – parameter estimates for the best alertness models. Bold p-values are below 0.05.

Roe deer	Estimate	SD	p-value	CI
Intercept	-1.751	0.247		[-2.236, -1.266]
Wolf activity	-0.300	0.118	0.012	[-0.531, -0.069]
Mean vegetation height	-0.222	0.135	0.101	[-0.488, 0.043]
<i>Season †</i>				
Spring	0.755	0.235	0.001	[0.295, 1.216]
Summer	-0.192	0.204	0.346	[-0.591, 0.207]
Winter	-0.080	0.233	0.732	[-0.536, 0.377]
<i>Light period *</i>				
Twilight	0.403	0.193	0.037	[0.025, 0.781]
Night	0.136	0.171	0.426	[-0.199, 0.472]

Red deer				
Intercept	-0.877	0.257		[-1.382, -0.373]
<i>Season †</i>				
Spring	0.401	0.404	0.320	[-0.390, 1.193]
Summer	-0.493	0.257	0.055	[-0.996, 0.011]
Winter	0.482	0.190	0.011	[0.109, 0.855]
<i>Light period *</i>				
Twilight	-0.031	0.234	0.895	[-0.489, 0.427]
Night	-0.229	0.183	0.212	[-0.588, 0.130]

Notes: † reference = Fall, * reference = Day

Discussion

Synopsis

This study evaluated the effect of wolf presence on two deer species in a Danish wolf territory and found that deer avoided wolves in space but not in time. Our results show that roe deer and red deer in the study area avoid wolves in space as presence of both species was negatively correlated with wolf activity. Time-wise overlap in activity between the two deer species and wolves did not change with the level of wolf activity, which means that deer did not react to increased wolf activity by avoiding them temporally. Wolf activity was a significant predictor of the vigilance behaviour of roe deer, but not red deer, with a higher wolf activity leading to roe deer being less alert.

Habitat selection including spatial wolf avoidance

The presence of both roe deer and red deer was negatively correlated with wolf activity, which supports our first hypothesis. Other studies in natural systems have also found that deer avoid areas associated with high wolf activity. For example, it has been shown that white-tailed deer *Odocoileus virginianus* visit high wolf use plots less frequently than low wolf use plots (Flagel et al., 2016) and that elk prefer to establish home ranges in areas distant from wolf territory centres (Anderson et al., 2005) and avoid areas associated with high kill densities during time periods where wolves are active (Kohl et al., 2018). Contrarily, Theuerkauf and Rouys (2008) did not find an effect of wolf density on the habitat selection of red deer and roe deer in Białowieża Primeval Forest, Poland. That deer might avoid areas based on long-term cues of wolf activity is surprising as wolves are cursorial predators which have been theorised to produce less consistent cues of risk compared to ambush predators (Preisser et al., 2007; Wikenros et al., 2020). Supporting this theory, studies simulating wolf presence using scat or urine did not find that deer reduced visitation rate or

visitation time to plots with wolf treatment (Elmeros et al., 2011; Kuijper et al., 2014; Palmer et al., 2021). However, this could just mean that the presence of olfactory cues is not perceived as an indicator of immediate risk by deer. Elk have been shown to react to more direct cues of wolf presence (e.g. short distance to wolf, wolf presence on day) by increasing movement rate and displacement (Middleton et al., 2013) and by moving into protective cover (Creel et al., 2005). That we saw a negative response to wolf activity defined for six months periods, supports our hypothesis that the deer in our study see areas associated with a higher general wolf activity as riskier.

Red deer in our study preferred lower vegetation and areas closer to fields and further away from roads. Red deer might avoid roads because of their association with human disturbance (Müller et al., 2017; Theuerkauf & Rouys, 2008). That red deer preferred low vegetation and areas closer to fields is probably due to them using these areas for foraging (Godvik et al., 2009). Roe deer presence was not significantly affected by any of the habitat variables. This is not surprising as the feeding strategy of roe deer is more versatile, and their selectiveness might make them prone to forage in many different habitat types. Furthermore, the small size and solitary lifestyle of roe deer makes it less conspicuous than the red deer who due to its size may be more limited in its habitat selection. It is important to note that we did not take time of day into account, which could have shown that roe deer preferred different habitats at different times of the diel cycle. By doing this we may also have found that red deer preferred areas with high vegetation during the day like Godvik *et al.* (2009).

Temporal overlap

Neither roe deer nor red deer decreased temporal overlap with wolves in areas with high wolf activity, which disproves our hypothesis that deer in areas of high wolf use avoid wolves in time. Whether wolves change the diel activity of their main prey have been the subject of many studies. In a similar study in the Mediterranean, fallow deer had a lower temporal overlap with wolves in areas with high wolf activity compared to areas with low wolf activity (Rossa et al., 2021). Likewise, white-tailed deer in Minnesota became less crepuscular and more diurnal on plots treated with wolf urine, which Palmer *et al.* (2021) argued was due to deer decreasing activity when wolves were hunting. However, a Swedish study did not find evidence that moose avoided wolves in a temporal sense despite being their main prey (Eriksen et al., 2011). In our study, areas were compared based on background wolf activity it is therefore possible that we failed to detect a

change in the diel activity of deer on shorter timescales. Furthermore, as wolves have been established in the area since 2016, it is possible that deer have already adapted their diel activity in the area and that this response cannot be further heightened. Another possibility is that deer in the area are keeping from becoming diurnal to limit human encounters (Bonnot et al., 2020; Gaynor et al., 2018). Our results do show that deer are crepuscular both in summer and winter as was found in other studies (Cederlund, 1989; Ikeda et al., 2016). As deer are intensively hunted, especially outside the study area, during fall and winter, human avoidance likely explains their crepuscularity.

Vigilance behaviour

Wolf activity was a significant predictor for roe deer alertness, which means that roe deer may adapt their vigilance behaviour to background wolf activity. However, they responded to increased wolf activity by decreasing vigilance, contrasting with the assumption of the *risky places hypothesis* that vigilance should increase with background risk (Moll et al., 2017). We assume that wolf activity is a proxy for predation risk, however, this may not be true as a wolf simply being present does not equal a predation event (Hebblewhite et al., 2005). Hence, we might have seen a different response if we instead of modelling wolf activity had modelled a more accurate estimate of predation risk, i.e., kill density or kill probability for each deer species. However, if we do assume that wolf activity is directly related to predation risk, our results could provide support for the *predation risk allocation hypothesis*, in which vigilance decreases in risky situations as these become more frequent (Lima & Bednekoff, 1999). In that case, roe deer would respond to an increase in risk over time by gradually allocating less time to vigilance in high-risk situations to avoid foraging costs. As this is a response to temporal variability in risk, we would have to compare the vigilance behaviour of roe deer before and after wolves settled in the area to see if they did indeed become less vigilant as time passed to properly confirm this hypothesis.

Red deer vigilance was neither affected by background wolf activity nor any of the habitat variables, which disproves the risky places hypothesis (Moll et al., 2017). Other studies similarly find that habitat characteristics do not affect the vigilance behaviour of red deer (Kuijper et al., 2014) and elk (Winnie & Creel, 2007). However, another study on elk found that vigilance did increase with distance to cover, though herd composition and immediate threat were the main drivers of vigilance (Liley & Creel, 2008). Red deer in our study were generally more alert than roe deer, regardless of wolf activity, time of day, habitat or season (with the exception of being more

alert in winter). As cameras were placed in order to maximize wolf sightings, it is possible that red deer perceived all camera locations as being risky and that the apparent lack of response is actually due to red deer being perpetually vigilant on camera locations. To test this, our data would have to be compared to control cameras in the area or to data from red deer outside of the wolf territory. Red deer in our study showed a tendency to be more vigilant on days where wolves were present, although the response was not significant. That red deer vigilance is affected by direct cues of wolf presence was seen in a study by Kuijper *et al.* (2014) where red deer were more vigilant on plots with wolf scat. Similarly, white-tailed deer were more vigilant on plots treated with wolf urine but only in the “risky” dawn hours emphasizing the temporal aspect of predation risk assessment (Palmer *et al.*, 2021). Elk have been shown to increase vigilance on days where wolves are present in an area (Creel *et al.*, 2008) and when wolves are within 1 km (Middleton *et al.*, 2013). That we did not find a significant effect of wolf presence on the day could be due to instances of wolves and red deer having occurred on different sides of midnight not counting as days where both wolves and red deer occurred. Furthermore, herd size and composition, which is known to affect elk vigilance (Childress & Lung, 2003; Creel *et al.*, 2008; Laundré *et al.*, 2001; Liley & Creel, 2008; Lung & Childress, 2006) was not accounted for in our analysis.

Ecological Implications

When predicting the possible ecological effects of returning predators, Ritchie *et al.* (2012) emphasize the need to evaluate the function of predators in the context of the environment they are returning to. Denmark has a large human population density, and natural areas are small and largely exploited for human interests, e.g., forestry, hunting and recreational activities. Even though our study area is relatively remote, commercial forestry occurs and human activity is not negligible. Thus, our results show that wolves can affect the behaviour of their prey even in areas with a moderate human presence. This could have implications for deer, who in some areas may face risk imposed by both humans and wolves, especially if the two types of predation create contrasting LOFs (Kuijper *et al.*, 2016). Studies on roe deer found that the contrasting predation pressures of humans and lynx *Lynx lynx* resulted in a strong reduction in low risk refuges (Lone *et al.*, 2014) and in both diurnal and nocturnal activity being associated with risk (Bonnot *et al.*, 2020). Based on this, Danish wolves could have a large effect on prey populations in areas where prey is already limited by human activity. Additionally, wolves may affect prey behaviour in ways that human activity does not. For instance, Raynor *et al.* (2021) found that the return of wolves in an area

reduced the number of deer-vehicle collisions by affecting deer behaviour in ways that human hunters did not.

The observed change in deer space use in response to wolf activity in our study could have ecological consequences if the change causes a permanent shift in browsing pressure in the area. Other studies have found that the negative impact of deer browsing on tree recruitment and species richness was reduced in core wolf areas (Flagel et al., 2016; Kuijper et al., 2013). However, the effect might be reduced in Denmark as natural areas are small and deer therefore might be forced to stay in areas with high wolf activity. During the first breeding period in the Ulfborg territory 95% of all observations were found within an area less than 50 km² in size (Sunde & Olsen, 2018). This is roughly the same size as the protected study area (~ 40 km²), which means that if deer wanted to avoid the core wolf area entirely, they would have to forage on the surrounding privately owned land where they are intensively hunted. Based on this, it is unlikely that the presence of wolves will cause a trophic cascade in the study area. However, vegetation structure could still be affected on a smaller scale if deer avoid foraging near escape-impeding obstructions, e.g. fallen trees or dense shrubbery, inside the core wolf area as was seen in Poland (Kuijper et al., 2013). Looking at possible changes in vegetation structure and species diversity inside core wolf areas in Denmark is an interesting topic for future studies.

Conclusion

Our study indicates that even in anthropogenic areas natural predators are able to create a landscape of fear that affects the habitat choice of their prey. However, human disturbance in these systems should not be disregarded and may have kept deer in our study from becoming diurnal in response to wolf presence. The association between wolf activity and deer vigilance was not unambiguous, which emphasizes that vigilance behaviour is complex and likely responds to risk on a finer temporal scale. As predators continue to recolonize Europe it could result in predation-mediated behavioural changes in prey populations. However, realistically speaking, prey response will be complex and depend on the interaction between human disturbance and natural predation.

Acknowledgements

I would like to thank Jens-Christian Svenning, Peter Sunde and Kent Olsen for supervising this project and providing valuable insight and guidance throughout the study period. I also wish to thank Sofie Amund Kjeldgaard for invaluable discussions and companionship, as well as excellent teamwork during our shared fieldwork. I give my sincerest thanks to Thomas Boesdal and Søren Krabbe, who spent many hours with us in the field teaching us how to monitor wolves and navigate the area. I want to thank Fonden Frands Christian Frantsens Legat for giving us the opportunity to conduct our own field work by financially supporting this project and Lars Haugaard for assisting with the procurement of trail cameras. Additionally, I wish to thank Martin Mayer for assisting with statistics in R and Emilie Pi Goll for critiquing this manuscript. Finally, I want to thank the Western Jutland department of the Danish Nature Agency for granting us access to their areas during our field work.

References

- Anderson, D. P., Turner, M. G., Forester, J. D., Zhu, J. U. N., Boyce, M. S., Beyer, H., . . . Green. (2005). Scale-Dependent Summer Resource Selection by Reintroduced Elk in Wisconsin, USA. *Journal of Wildlife Management*, 69(1), 298-310. doi:10.2193/0022-541x(2005)069<0298:Srsbr>2.0.Co;2
- Asferg, T., & Madsen, A. B. (2012). *Kronstyr* i *Dansk Pattedyratlas*. Retrieved from <https://pattedyratlas.lex.dk/Kronstyr> on 30/04/21
- Asferg, T., & Madsen, A. B. (2013). *Rådyr* i *Dansk Pattedyrsatlas*. Retrieved from <https://pattedyratlas.lex.dk/Rådyr> on 30/04/21
- Barja, I., & Rosellini, S. (2008). Does habitat type modify group size in roe deer and red deer under predation risk by Iberian wolves? *Canadian Journal of Zoology*, 86(3), 170-176. doi:10.1139/z07-129
- Beschta, R. L., Ripple, W. J., Kauffman, J. B., & Painter, L. E. (2020). Bison limit ecosystem recovery in northern Yellowstone. *Food Webs*, 23. doi:10.1016/j.fooweb.2020.e00142
- Bleicher, S. S. (2017). The landscape of fear conceptual framework: definition and review of current applications and misuses. *PeerJ*, 5, e3772. doi:10.7717/peerj.3772
- Bonnot, N. C., Couriot, O., Berger, A., Cagnacci, F., Ciuti, S., De Groeve, J. E., . . . Hewison, A. J. M. (2020). Fear of the dark? Contrasting impacts of humans versus lynx on diel activity of roe deer across Europe. *J Anim Ecol*, 89(1), 132-145. doi:10.1111/1365-2656.13161
- Brown, J. S., & Kotler, B. P. (2004). Hazardous duty pay and the foraging cost of predation. *Ecology Letters*, 7(10), 999-1014. doi:10.1111/j.1461-0248.2004.00661.x
- Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2010). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, 65(1), 23-35. doi:10.1007/s00265-010-1029-6

- Cederlund, G. (1989). Activity patterns in moose and roe deer in a north boreal forest. *Ecography*, *12*(1), 39-45.
doi:10.1111/j.1600-0587.1989.tb00820.x
- Chapron, G., Kaczensky, P., Linnell, J. D., von Arx, M., Huber, D., Andren, H., . . . Boitani, L. (2014). Recovery of large carnivores in Europe's modern human-dominated landscapes. *Science*, *346*(6216), 1517-1519.
doi:10.1126/science.1257553
- Childress, M. J., & Lung, M. A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour*, *66*(2), 389-398. doi:10.1006/anbe.2003.2217
- Christianson, D., & Creel, S. (2010). A nutritionally mediated risk effect of wolves on elk. *Ecology*, *91*(4), 1184-1191.
doi:10.1890/09-0221.1
- Churski, M., Spitzer, R., Coissac, E., Taberlet, P., Lescinskaite, J., van Ginkel, H. A. L., . . . Cromsigt, J. P. G. M. (2021). How do forest management and wolf space-use affect diet composition of the wolf's main prey, the red deer versus a non-prey species, the European bison? *Forest Ecology and Management*, *479*.
doi:10.1016/j.foreco.2020.118620
- Ciuti, S., Northrup, J. M., Muhly, T. B., Simi, S., Musiani, M., Pitt, J. A., & Boyce, M. S. (2012). Effects of humans on behaviour of wildlife exceed those of natural predators in a landscape of fear. *PLoS One*, *7*(11), e50611.
doi:10.1371/journal.pone.0050611
- Creel, S., Christianson, D., Liley, S., & Winnie, J. A., Jr. (2007). Predation risk affects reproductive physiology and demography of elk. *Science*, *315*(5814), 960. doi:10.1126/science.1135918
- Creel, S., Winnie, J., Maxwell, B., Hamlin, K., & Creel, M. (2005). Elk Alter Habitat Selection as an Antipredator Response to Wolves. *Ecology*, *86*(12), 3387-3397. doi:10.1890/05-0032
- Creel, S., Winnie, J. A., Christianson, D., & Liley, S. (2008). Time and space in general models of antipredator response: tests with wolves and elk. *Animal Behaviour*, *76*(4), 1139-1146. doi:10.1016/j.anbehav.2008.07.006
- Duncan, P. (1998). Feeding Strategies and the Physiology of Digestion in Roe Deer. In *The European Roe Deer: The Biology of Success*: Scandinavian University Press.
- Elmeros, M., Winbladh, J. K., Andersen, P. N., Madsen, A. B., & Christensen, J. T. (2011). Effectiveness of odour repellents on red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*): a field test. *European Journal of Wildlife Research*, *57*(6), 1223-1226. doi:10.1007/s10344-011-0517-y
- Eriksen, A., Wabakken, P., Zimmermann, B., Andreassen, H. P., Arnemo, J. M., Gundersen, H., . . . Storaas, T. (2011). Activity patterns of predator and prey: a simultaneous study of GPS-collared wolves and moose. *Animal Behaviour*, *81*(2), 423-431. doi:10.1016/j.anbehav.2010.11.011
- Flagel, D. G., Belovsky, G. E., & Beyer, D. E., Jr. (2016). Natural and experimental tests of trophic cascades: gray wolves and white-tailed deer in a Great Lakes forest. *Oecologia*, *180*(4), 1183-1194. doi:10.1007/s00442-015-3515-z
- Fortin, D., Beyer, H. L., Boyce, M. S., Smith, D. W., Duchesne, T., & Mao, J. S. (2005). Wolves Influence Elk Movements: Behavior Shapes a Trophic Cascade in Yellowstone National Park. *Ecology*, *86*(5), 1320-1330.
doi:10.1890/04-0953
- Gaynor, K. M., Hojnowski, C. E., Carter, N. H., & Brashares, J. S. (2018). The influence of human disturbance on wildlife nocturnality. *Science*, *360*(6394), 1232-1235. doi:10.1126/science.aar7121
- Gill, R. M. A. (1990). *Monitoring the status of European and North American cervids*: The Global Environment Monitoring System Information Series 8. United Nations Environment Programme, Nairobi, Kenya.

- Godvik, I. M., Loe, L. E., Vik, J. O., Veiberg, V., Langvatn, R., & Mysterud, A. (2009). Temporal scales, trade-offs, and functional responses in red deer habitat selection. *Ecology*, *90*(3), 699-710. doi:10.1890/08-0576.1
- Hebblewhite, M., Merrill, E. H., & McDonald, T. L. (2005). Spatial decomposition of predation risk using resource selection functions: an example in a wolf-elk predator-prey system. *Oikos*, *111*(1), 101-111. doi:10.1111/j.0030-1299.2005.13858.x
- Ikedo, T., Uchida, K., Matsuura, Y., Takahashi, H., Yoshida, T., Kaji, K., & Koizumi, I. (2016). Seasonal and Diel Activity Patterns of Eight Sympatric Mammals in Northern Japan Revealed by an Intensive Camera-Trap Survey. *PLoS One*, *11*(10), e0163602. doi:10.1371/journal.pone.0163602
- Jędrzejewski, W., Schmidt, K., Theuerkauf, J., Jędrzejewska, B., Selva, N., Zub, K., & Szymura, L. (2002). Kill Rates and Predation by Wolves on Ungulate Populations in BiaŁowieża Primeval Forest (Poland). *Ecology*, *83*(5), 1341-1356. doi:10.1890/0012-9658(2002)083[1341:Krapbw]2.0.Co;2
- Kauffman, M. J., Brodie, J. F., & Jules, E. S. (2010). Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade. *Ecology*, *91*(9), 2742-2755. doi:10.1890/09-1949.1
- Kelley, D., Richards, C., & Layton, C. (2021). Package 'oce'. Analysis of Oceanographic Data. Version 1.4-0. Retrieved from <https://cran.r-project.org/web/packages/oce/oce.pdf> on 03/04/21
- Kohl, M. T., Stahler, D. R., Metz, M. C., Forester, J. D., Kauffman, M. J., Varley, N., . . . MacNulty, D. R. (2018). Diel predator activity drives a dynamic landscape of fear. *Ecological Monographs*, *88*(4), 638-652. doi:10.1002/ecm.1313
- Kuijper, D. P., Sahlen, E., Elmhagen, B., Chamaille-Jammes, S., Sand, H., Lone, K., & Cromsigt, J. P. (2016). Paws without claws? Ecological effects of large carnivores in anthropogenic landscapes. *Proc Biol Sci*, *283*(1841). doi:10.1098/rspb.2016.1625
- Kuijper, D. P., Verwijmeren, M., Churski, M., Zbyryt, A., Schmidt, K., Jędrzejewska, B., & Smit, C. (2014). What cues do ungulates use to assess predation risk in dense temperate forests? *PLoS One*, *9*(1), e84607. doi:10.1371/journal.pone.0084607
- Kuijper, D. P. J., de Kleine, C., Churski, M., van Hooft, P., Bubnicki, J., & Jędrzejewska, B. (2013). Landscape of fear in Europe: wolves affect spatial patterns of ungulate browsing in BiaŁowieża Primeval Forest, Poland. *Ecography*, *36*(12), 1263-1275. doi:10.1111/j.1600-0587.2013.00266.x
- Laundré, J. W., Hernández, L., & Altendorf, K. B. (2001). Wolves, elk, and bison: reestablishing the "landscape of fear" in Yellowstone National Park, U.S.A. *Canadian Journal of Zoology*, *79*(8), 1401-1409. doi:10.1139/cjz-79-8-1401
- Liley, S., & Creel, S. (2008). What best explains vigilance in elk: characteristics of prey, predators, or the environment? *Behavioral Ecology*, *19*(2), 245-254. doi:10.1093/beheco/arm116
- Lima, S. L. (1998). Nonlethal Effects in the Ecology of Predator-Prey Interactions. *BioScience*, *48*(1), 25-34. doi:10.2307/1313225
- Lima, S. L., & Bednekoff, P. A. (1999). Temporal Variation in Danger Drives Antipredator Behavior: The Predation Risk Allocation Hypothesis. *Am Nat*, *153*(6), 649-659. doi:10.1086/303202
- Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, *68*(4), 619-640. doi:10.1139/z90-092

- Lone, K., Loe, L. E., Gobakken, T., Linnell, J. D. C., Odden, J., Remmen, J., & Mysterud, A. (2014). Living and dying in a multi-predator landscape of fear: roe deer are squeezed by contrasting pattern of predation risk imposed by lynx and humans. *Oikos*, *123*(6), 641-651. doi:10.1111/j.1600-0706.2013.00938.x
- Lung, M. A., & Childress, M. J. (2006). The influence of conspecifics and predation risk on the vigilance of elk (*Cervus elaphus*) in Yellowstone National Park. *Behavioral Ecology*, *18*(1), 12-20. doi:10.1093/beheco/arl066
- Magnusson, A., Skaug, H., Nielsen, A., Berg, C., & Kristensen, K. (2020). Package 'glmmTMB'. Generalized Linear Mixed Models using Template Model Builder. Version 1.0.2.1. Retrieved from <https://cran.r-project.org/web/packages/glmmTMB/glmmTMB.pdf> on 03/04/21
- Mao, J. S., Boyce, M. S., Smith, D. W., Singer, F. J., Vales, D. J., Vore, J. M., . . . Hudson. (2005). Habitat Selection by Elk before and after Wolf Reintroduction in Yellowstone National Park. *Journal of Wildlife Management*, *69*(4), 1691-1707. doi:10.2193/0022-541x(2005)69[1691:Hsbeba]2.0.Co;2
- Marshall, K. N., Hobbs, N. T., & Cooper, D. J. (2013). Stream hydrology limits recovery of riparian ecosystems after wolf reintroduction. *Proc Biol Sci*, *280*(1756), 20122977. doi:10.1098/rspb.2012.2977
- Marucco, F., Pletscher, D. H., & Boitani, L. (2008). Accuracy of scat sampling for carnivore diet analysis: wolves in the Alps as a case study. *Journal of Mammalogy*, *89*(3), 665-673. doi:10.1644/07-mamm-a-005r3.1
- Mech, D. L. (2012). Is science in danger of sanctifying the wolf? *Biological Conservation*, *150*(1), 143-149. doi:10.1016/j.biocon.2012.03.003
- Mech, L. D., & Boitani, L. (2003). *Wolves: Behavior, Ecology and Conservation*. Chicago IL: The University of Chicago Press.
- Meredith, M., & Ridout, M. (2020). Package 'overlap'. Estimates of Coefficient of Overlapping for Animal Activity Patterns. Version 0.3.3. Retrieved from <https://cran.r-project.org/web/packages/overlap/overlap.pdf> on 03/04/21
- Middleton, A. D., Kauffman, M. J., McWhirter, D. E., Jimenez, M. D., Cook, R. C., Cook, J. G., . . . White, P. J. (2013). Linking anti-predator behaviour to prey demography reveals limited risk effects of an actively hunting large carnivore. *Ecol Lett*, *16*(8), 1023-1030. doi:10.1111/ele.12133
- Moll, R. J., Redilla, K. M., Mudumba, T., Muneza, A. B., Gray, S. M., Abade, L., . . . Montgomery, R. A. (2017). The many faces of fear: a synthesis of the methodological variation in characterizing predation risk. *J Anim Ecol*, *86*(4), 749-765. doi:10.1111/1365-2656.12680
- Müller, A., Dahm, M., Bocher, P. K., Root-Bernstein, M., & Svenning, J. C. (2017). Large herbivores in novel ecosystems - Habitat selection by red deer (*Cervus elaphus*) in a former brown-coal mining area. *PLoS One*, *12*(5), e0177431. doi:10.1371/journal.pone.0177431
- Naturstyrelsen. Dagjagter - Naturstyrelsen Vestjylland. Retrieved from <https://naturstyrelsen.dk/naturoplevelser/jagt/jagt-i-statsskovene/koeb-dagjagter/betalingsjagter-vestjylland/> on 30/05/21
- Noer, H., Asferg, T., Clausen, P., Olesen, C. R., Bregnballe, T., Lauersen, K., . . . Haugaard, L. (2009). Vildtbestande og jagttider i Danmark: Det biologiske grundlag for jagttidsrevisionen 2010. *Danmarks Miljøundersøgelser, Aarhus Universitet, Faglig rapport fra DMU nr. 742.* , 288 s.
- Nowak, S., Mysłajek, R. W., Kłosińska, A., & Gabryś, G. (2011). Diet and prey selection of wolves (*Canis lupus*) recolonising Western and Central Poland. *Mammalian Biology*, *76*(6), 709-715. doi:10.1016/j.mambio.2011.06.007

- Olesen, C. R., Asferg, T., & Forchhammer, M. C. (2002). Rådyret - fra fåtallig til almindelig. *Danmarks Miljøundersøgelser, Aarhus Universitet. TEMA-rapport fra DMU, 39/2002.*
- Palmer, M. S., Portales-Reyes, C., Potter, C., Mech, L. D., & Isbell, F. (2021). Behaviorally-mediated trophic cascade attenuated by prey use of risky places at safe times. *Oecologia, 195*(1), 235-248. doi:10.1007/s00442-020-04816-4
- Preisser, E. L., Bolnick, D. I., & Benard, M. F. (2005). Scared to Death? The Effects of Intimidation and Consumption in Predator–Prey Interactions. *Ecology, 86*(2), 501-509. doi:10.1890/04-0719
- Preisser, E. L., Orrock, J. L., & Schmitz, O. J. (2007). Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. *Ecology, 88*(11), 2744-2751. doi:10.1890/07-0260.1
- Randon, M., Bonenfant, C., Michallet, J., Chevrier, T., Toïgo, C., Gaillard, J. M., & Valeix, M. (2020). Population responses of roe deer to the recolonization of the French Vercors by wolves. *Population Ecology, 62*(2), 244-257. doi:10.1002/1438-390x.12043
- Raynor, J. L., Grainger, C. A., & Parker, D. P. (2021). Wolves make roadways safer, generating large economic returns to predator conservation. *Proc Natl Acad Sci U S A, 118*(22). doi:10.1073/pnas.2023251118
- Ripple, W. J., & Beschta, R. L. (2004). Wolves and the Ecology of Fear: Can Predation Risk Structure Ecosystems? *BioScience, 54*(8). doi:10.1641/0006-3568(2004)054[0755:Wateof]2.0.Co;2
- Ritchie, E. G., Elmhagen, B., Glen, A. S., Letnic, M., Ludwig, G., & McDonald, R. A. (2012). Ecosystem restoration with teeth: what role for predators? *Trends Ecol Evol, 27*(5), 265-271. doi:10.1016/j.tree.2012.01.001
- Rossa, M., Lovari, S., & Ferretti, F. (2021). Spatiotemporal patterns of wolf, mesocarnivores and prey in a Mediterranean area. *Behavioral Ecology and Sociobiology, 75*(2). doi:10.1007/s00265-020-02956-4
- Sahlen, E., Noell, S., DePerno, C. S., Kindberg, J., Spong, G., & Cromsigt, J. P. (2016). Phantoms of the forest: legacy risk effects of a regionally extinct large carnivore. *Ecol Evol, 6*(3), 791-799. doi:10.1002/ece3.1866
- Say-Sallaz, E., Chamailé-Jammes, S., Fritz, H., & Valeix, M. (2019). Non-consumptive effects of predation in large terrestrial mammals: Mapping our knowledge and revealing the tip of the iceberg. *Biological Conservation, 235*, 36-52. doi:10.1016/j.biocon.2019.03.044
- Smith, F. A., Lyons, S. K., Ernest, S. K. M., Jones, K. E., Kaufman, D. M., Dayan, T., . . . Haskell, J. P. (2003). Body Mass of Late Quaternary Mammals. *Ecology, 84*(12), 3403-3403. doi:10.1890/02-9003
- Sunde, P., & Olsen, K. (2018). Wolves (*Canis lupus*) in Denmark 2012-2017 - Review and analysis of available population information. *Videnskabelig rapport fra DCE-Nationalt Center for Miljø og Energi. Institut for Bioscience. Aarhus University, Aarhus, Denmark.*
- Theuerkauf, J., & Rouys, S. (2008). Habitat selection by ungulates in relation to predation risk by wolves and humans in the Białowieża Forest, Poland. *Forest Ecology and Management, 256*(6), 1325-1332. doi:10.1016/j.foreco.2008.06.030
- Thomsen, P. F., Hansen, M. M., Olsen, K., & Sunde, P. (2020). Genetiske analysemetoder i den nationale overvågning af ulv (*Canis lupus*) i Danmark. *Notat fra DCE-Nationalt Center for Miljø og Energi og Naturhistorisk Museum Aarhus.*
- Thorsen, H., Olsen, K., & Sunde, P. (2019). Danske ulves (*Canis lupus lupus*) døgnaktivitets- mønster studeret med brug af vildtkameraer. *Flora og Fauna, 125*(4), 1-11.

- Wagner, C., Holzapfel, M., Kluth, G., Reinhardt, I., & Ansorge, H. (2012). Wolf (*Canis lupus*) feeding habits during the first eight years of its occurrence in Germany. *Mammalian Biology*, *77*(3), 196-203.
doi:10.1016/j.mambio.2011.12.004
- Wikenros, C., Sand, H., Bergstrom, R., Liberg, O., & Chapron, G. (2015). Response of moose hunters to predation following wolf return in Sweden. *PLoS One*, *10*(4), e0119957. doi:10.1371/journal.pone.0119957
- Wikenros, C., Sand, H., Mansson, J., Maartmann, E., Eriksen, A., Wabakken, P., & Zimmermann, B. (2020). Impact of a recolonizing, cross-border carnivore population on ungulate harvest in Scandinavia. *Sci Rep*, *10*(1), 21670.
doi:10.1038/s41598-020-78585-8
- Winnie, J., & Creel, S. (2007). Sex-specific behavioural responses of elk to spatial and temporal variation in the threat of wolf predation. *Animal Behaviour*, *73*(1), 215-225. doi:10.1016/j.anbehav.2006.07.007
- Worm, B., & Paine, R. T. (2016). Humans as a Hyperkeystone Species. *Trends Ecol Evol*, *31*(8), 600-607.
doi:10.1016/j.tree.2016.05.008

Appendix 1 - Wolf activity heatmaps

1.1 Background for data

To characterise the level of wolf activity at each camera trap location we would ideally have estimated the passage rate (wolf observations per active camera day) but that limited us to only include the 259 wolf observations entered in our database. However, all wolf observations (including sightings, genetic samples from scat and prey and camera traps) are entered in a separate database. Via this database, we had access to 990 wolf observations from camera trap photos and 381 wolf observations from scat finds from the study area across the study period (1/11-16 to 30/4-20). To check whether the two methods (camera trapping and scat searches) were comparable we generated separate heatmaps (see description in the next part) for the two methods for each 6-month period, extracted the wolf activity values for our camera trap locations and then compared the value from the two different heatmaps using Pearson’s correlation coefficient (**table 1**). To do the comparison NA values were substituted with zeros. As the correlation coefficient for the two methods varied between 0.46 and 0.97, we decided to include observations from both methods in the final heatmaps to increase sample size. By doing this we were able to divide the observations into periods of 6 months, thereby increasing the detail of our wolf activity index. Unfortunately, there were too few observations in 2016 and the beginning of 2017 to make this comparison, so in that case we assumed that they were still comparable based on the correlation coefficient for the other periods.

Table 1 – Pearson’s correlation matrix comparing the wolf activity index values for the different locations. The bold numbers are the comparison between the two methods for each period. Summer = 1/5-31/10 for the given year, winter = 1/11 in the given year to 30/4 the following year.

	Summer 17 CT	Winter 17 CT	Summer 18 CT	Winter 18 CT	Summer 19 CT	Winter 19 CT	Summer 17 Scat	Winter 17 Scat	Summer 18 Scat	Winter 18 Scat	Summer 19 Scat	Winter 19 Scat
Summer 17 CT	1	0.355	0.769	0.692	0.089	0.237	0.973	0.876	0.815	0.260	-0.151	0.278
Winter 17 CT	0.355	1	0.493	0.226	-0.673	-0.264	0.257	0.684	0.493	-0.137	-0.664	-0.420
Summer 18 CT	0.7685	0.4929	1	0.526	-0.316	0.015	0.651	0.710	0.463	-0.211	-0.498	-0.1837
Winter 18 CT	0.692	0.226	0.526	1	0.274	0.724	0.722	0.646	0.695	0.598	0.002	0.547
Summer 19 CT	0.089	-0.673	-0.316	0.274	1	0.600	0.211	-0.166	0.109	0.614	0.906	0.810
Winter 19 CT	0.237	-0.264	0.015	0.724	0.600	1	0.346	0.039	0.431	0.816	0.401	0.863
Summer 17 Scat	0.973	0.257	0.651	0.722	0.211	0.345	1	0.825	0.840	0.421	-0.057	0.413
Winter 17 Scat	0.876	0.684	0.710	0.646	-0.166	0.039	0.825	1	0.777	0.162	-0.343	0.010
Summer 18 Scat	0.815	0.493	0.463	0.695	0.109	0.431	0.840	0.777	1	0.606	-0.107	0.486
Winter 18 Scat	0.260	-0.137	-0.211	0.598	0.614	0.816	0.421	0.162	0.606	1	0.411	0.886
Summer 19 Scat	-0.151	-0.664	-0.498	0.002	0.906	0.401	-0.057	-0.343	-0.109	0.411	1	0.622
Winter 19 Scat	0.278	-0.420	-0.184	0.547	0.810	0.863	0.413	0.010	0.486	0.886	0.622	1

1.2 Heatmap

We created heatmaps representing the continuous spatial distribution of wolf activity in the study area for periods of 6 months. To do this we used a kernel density estimator in QGIS (v. 3.16.3), using georeferenced points of wolf observations as input and a smoothing factor of 1000 meters. The resulting raster map had a resolution of 10x10 meters, where the value of each pixel was the estimated number of wolf observations at that point. By setting the smoothing factor to 1000 meters the calculator took account of all wolf observations within a 1000-meter radius when estimating the number of wolf observations at each point. The final raster was visualised as a heatmap where warmer colours meant a higher number of wolf observations and cooler colours a lower number of wolf observations. Afterwards, we could project our camera trap locations onto the heatmap and extract the underlying value. The value was later divided by the total number of wolf observations in the given period to make comparison between the different periods possible. Two examples of the final heatmaps are seen below (**fig. 1** and **2**).

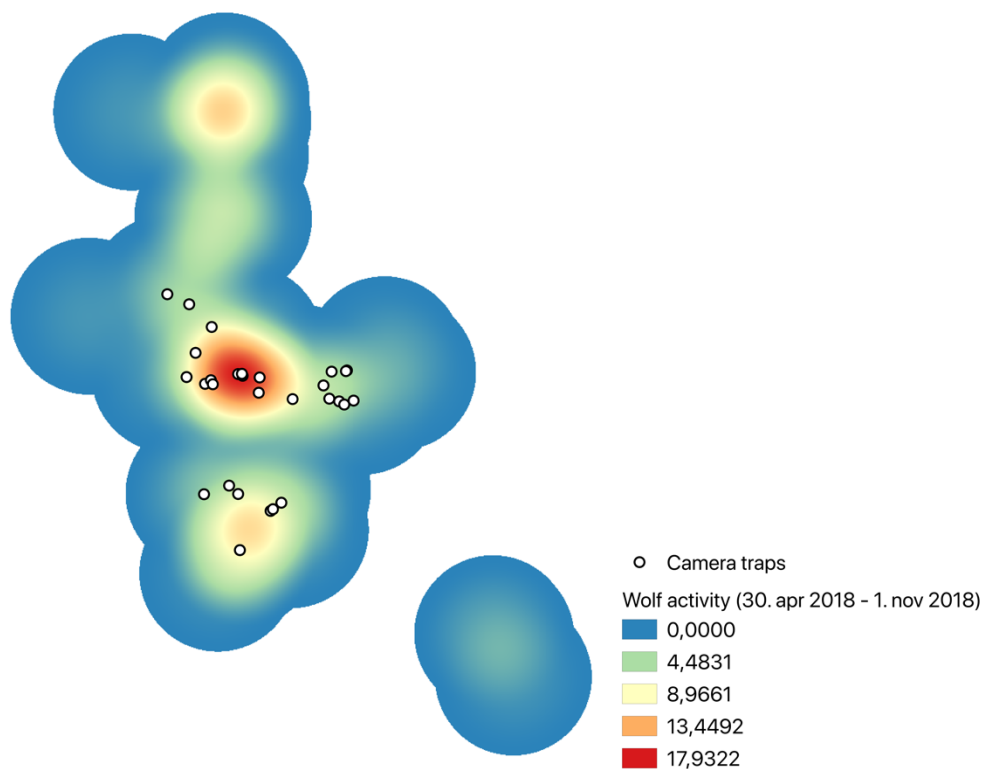


Figure 1 – visualisation (heatmap) of wolf activity in the study area between 30. April 2018 and 1. November 2018. The colours correlate with estimated number of wolf observations in the period with warmer colours indicating a higher and cooler colours a lower estimated number of observations. White dots indicate the location of camera traps in the period. The heatmap is based on 77 confirmed wolf observations from camera traps and wolf scats.

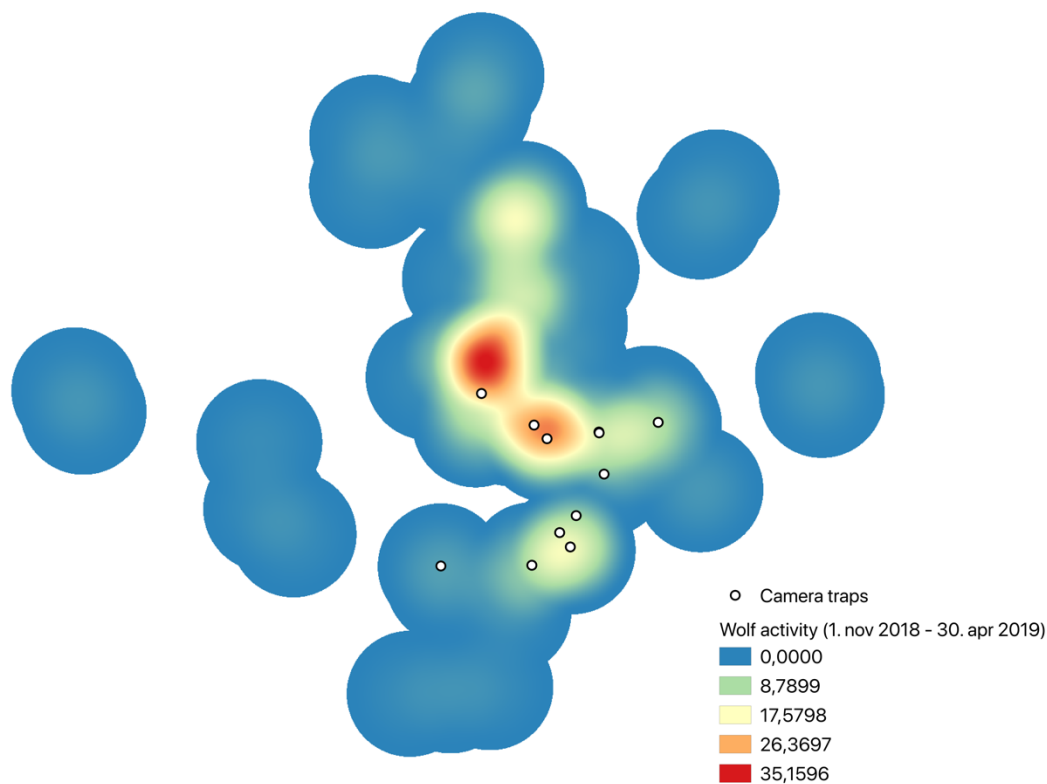


Figure 2 – visualisation (heatmap) of wolf activity in the study area between 1. November 2018 and 30. April 2019. The colours correlate with estimated number of wolf observations in the period with warmer colours indicating a higher and cooler colours a lower estimated number of observations. White dots indicate the location of camera traps in the period. The heatmap is based on 190 confirmed wolf observations from camera traps and wolf scats.

Appendix 2 - Control camera project

The purpose of this project was to determine if camera trap data from the cameras used in the National Danish Wolf Monitoring Project is representative of the general fauna activity. Determining this is necessary if the data is used to infer about the activity of other species than wolves, in which case it will be assumed that the detectability of species is not affected by the wolf strategic placement. Furthermore, wolf strategic placement of cameras is time consuming as it is based on knowledge of the movement patterns of local wolves, which requires active searches for wolf scat and tracks. Consequently, if we find that the amount of wolf observations does not differ between control cameras placed randomly and cameras placed using the wolf strategic method inside wolf territories, there is no reason why cameras cannot simply be placed in random locations.

This means that the time spent putting up cameras can be minimised substantially. Additionally, the cameras used for monitoring wolves are often moved around in an effort to match current wolf movement patterns, which hinders the potential for acquiring long data series on general animal activity. Another aim of the control camera project was therefore to place stationary cameras in the area for future studies.

For this project, we used 14 cameras funded by the Fonden Frands Christian Frantsens Legat (16 was received but unfortunately one had technical issues and another was destroyed early in the project). All cameras were of the model Bushnell Core DS (trigger speed: 0.2 seconds, recovery rate: 0.6 seconds) and were placed in a metal security box. Using an online “random location generator” random points were generated in the study area in two “batches” using the same radius but with different centre points. The two centre points were chosen to cover as much of the study area as possible. After removing points located outside the study area, the first seven locations of each “point batch” were chosen as the locations for the control cameras. When arriving at the locations the nearest tree was selected if the point was in an open area.

As we had to compare our control data to the data from the wolf monitoring cameras, we used the same method when putting up the cameras at the random locations as is used when putting up the cameras used in the wolf monitoring project. The method used is described below.

Wolf strategic camera placement:

- Cameras are placed 80-120 cm above the ground
- Cameras are placed along natural or man-made paths in the landscape (animal tracks, gravel roads etc.)
- Cameras are placed at an angle to paths in the landscape and angled slightly downwards to increase likelihood of animals crossing the sensor fields and triggering the camera
- Cameras are hidden in the landscape in an effort to avoid animals being aware of the cameras
- Cameras are placed in a way so animals are unlikely to approach the camera directly from the front (as this will often lead them to notice the cameras)

All cameras were set to take three pictures followed by a 30 sec video. The time between activations was set to “0.6 seconds”, sensitivity was set to “automatic” (depends on temperature), and range to “long”. A week after having installed the cameras we returned to check on them. If the cameras had had a large number of misfires, we increased the time between activations to “5 minutes” and removed vegetation that might have caused the camera to misfire. Subsequently, we returned to check up on and change SD cards once a month at approximately the same time as the wolf monitoring cameras were checked.

Regarding future analysis, we want to compare data obtained from the control cameras with data from the wolf monitoring cameras that have been operating in the same period. Specifically, we want to test if the observation frequency of the different species in the area is the same for the two types of cameras.

Appendix 3 – R code

The R code and data used in this project is available as a zip-file.