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RESEARCH PAPER

How do seasonal changes in adult wolf defecation patterns affect scat detection probabilities?

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Abstract. Wolves are currently recolonising their historic range in France. The collection of scats is a widely used a non-invasive survey method to monitor wolf population size. However, seasonal changes in wolf faecal deposition patterns might affect the results of surveys. We used a detection dog and camera trapping (CT) to compare wolf scat detectability during winter and the nursing season. We collected 113 scats deposited by adult wolves at 29 marking sites on forest roads in the Sainte-Baume Regional Park, Provence, France. After parturition, the mean number of adult wolf scats increased by 160% inside the nursing territory and decreased by 80% outside of it. Around the time the pups are born, changes in faecal deposition patterns of adults make it easier to find scats around the wolf den (87% probability per wolf marking site) and harder to find scats outside the nursing territory (11% probability). During winter, the chance to find scats is equal (38 to 40% probability per wolf marking site) inside *vs.* outside the nursing territory. The combined use of a detection dog and camera traps allowed us to gather data on wolf defecation patterns non-invasively. Detectability of adult wolf scats during the nursing season is highly variable compared to winter due to seasonal behavioural changes affecting scat location. We conclude that surveys to collect samples and estimate wolf population size should be conducted exclusively during winter to avoid sampling biases.

Key words: detection dog, faecal samples, camera trapping, nursing season, large carnivores, non-invasive sampling, breeding

Introduction

Wolves are currently recolonising parts of their historic range in France, following the first official confirmation of the species' presence in the early 1990s in the French Alps (Peillon & Carbone 1993, Valière et al. 2003, Ciucci et al. 2009, Louvrier et al. 2018a). Monitoring large carnivores such as wolves is demanding because they are nocturnal, elusive, highly mobile, and occur in low densities over wide territories (Long et al. 2012, Ausband et al. 2014). Between 2009 and 2012, four she-wolves were radio-collared in south-eastern France. None survived more than five months after human intervention (Anceau

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et al. 2015). Due to these failures, there are currently no monitoring programs for radio-collared wolves, and the French government is reluctant to authorise the capture and radio-collaring of wolves. Currently, a "Capture-Mark-Recapture" (CMR) model based on genetic sample collection from scats is used by French authorities to estimate population size and survival rates (Duchamp et al. 2012). Non-invasive survey methods for carnivores are commonly used (Long et al. 2007a, b, 2012, MacKay et al. 2008), and the combination of both camera trap (CT) and genetic sampling of scats often offers greater efficiency for carnivore monitoring than one survey technique employed alone (Karanth et al. 2006, Long et al. 2007a, Mattioli et al. 2018); in addition to these non-invasive methods, a new a promising field of research is based on wolf howling structure characteristics (Hennelly et al. 2017). Wolf scats are thus opportunistically collected in the French Alps by a network of field observers supervised by the French Office of Biodiversity and are the primary source of genetic samples (600-700 each year), along with carcasses of legally culled wolves. The collection of wolf scats occurs mainly during winter since snow cover in the French Alps facilitates the detection of signs of wolf presence. However, scat collection is more difficult in newly colonised areas outside the Alpine mountains because snow events may be rare. As a result, genetic samples from newly colonised French territories are limited.

French authorities have recently addressed this challenge using a dog team to find wolf scats and provide information on the distribution and relative abundance of wolves in newly colonised areas; the use of a detection dog significantly improved wolf genetic monitoring and delivered up to a 99.6% time saving relative to monitoring by human trained observers (Roda et al. 2020). Over the last two decades, conservationists have used the extraordinary sense of smell of domestic dogs to locate various samples in a variety of habitats and from numerous species; trained dogs find more scat than do human observers and are less prone to detection sampling biases (Engeman et al. 2002, Smith et al. 2003, Wasser et al. 2004, Browne et al. 2006, Cablk et al. 2006, DeMatteo et al. 2014, Orkin et al. 2016, Richards et al. 2018). One must remember that differences in faecal deposition patterns related to age, sex, social, or reproductive status of wolves might also lead to detection heterogeneity and errors in analyses based on faecal DNA genotypes (Cubaynes et al. 2010). Detection heterogeneity could be a direct consequence of species biology or result from heterogeneities in the sampling effort (Devineau et al. 2006, Louvrier et al. 2018b). Previous studies showed that seasonal changes in the defecation patterns of kit foxes might alter scat detection probabilities by dogs and lead to an underestimated population size (Ralls et al. 2010).

The defecation patterns and scent marking of wolves have been the subject of many studies. Scent marking is considered a mechanism for territory defence in wolves (Zub et al. 2003, Mech & Boitani 2010). Wolves mark their territory with visual (scratching and faecal drops) and olfactory marks (urine, faecal drops and secretions from the anal sacs and interdigital glands; Mech & Boitani 2010). Pack members accumulate these marks on the edge of their territories or surrounding *rendezvous* sites (Zub et al. 2003, Barja et al. 2005, Mech & Boitani 2010, Stenglein et al. 2011). Wolf activity is highly flexible and may be adapted to various environmental conditions with a peak of activity during summer, which corresponds to the nursing period (Fancy & Ballard 1995, Eggermann et al. 2008); however, breeding females decrease their mobility during summer (Eggermann et al. 2008). The reasons for lower mobility during summer are denning and nursing, when wolf activity concentrates on the den site and the breeding female does not move far from the den (Eggermann et al. 2008, Tsunoda et al. 2009). The intensity of territorial marking predicts wolf reproduction (Llaneza et al. 2014). The accumulation of scats left by adults at specific points of the den area seems to be the consequence of repeated visits during the reproductive period; scat deposits may be far higher in the den area than in the outer zones (Barja et al. 2005).

In newly colonised territories in France, the locations of wolf den areas are unknown. During the nursing period, the chosen sampling transect may pass near a wolf den or in the outer zones of the territory. If so, surveys based on scat collection during the nursing season could give a misleading impression of wolf distribution, space use and relative abundance. Therefore, we sought to evaluate how the seasonal changes in wolf defecation patterns may affect the detection probabilities of predetermined sampling surveys. We speculated that defecation patterns during the nursing period might lead to significant heterogeneities in detection. To evaluate this hypothesis, we collected wolf scats using a dog each month between December 2019 and August 2020 in the known nursing territory and outer zones. The use of CT complemented the wolf monitoring based on wolf scat collection.

Material and Methods

Dog training

The dog team comprised one dog and the handler (first author). The dog (a female Belgian shepherd selected by a professional dog breeder) had been living with its handler from the age of two months and has been trained to discriminate wolf scats from non-target species (Roda et al. 2020). Based on positive reinforcement, the training protocol was adapted from previous conservation detection work (Smith et al. 2003, Wasser et al. 2004, Sentilles et al. 2016, DeMatteo et al. 2019) and remains fundamentally the same. The dog progressed to an interim training stage similar to that described in Statham et al. (2020), graduating from controlled settings with artificial scat provision into known, occupied wolf territories to encounter naturally occurring wolf scats. The handler initially trained the dog with samples from 60 genetically confirmed wolf scats from the French Alps provided by the French Office of Biodiversity (Roda et al. 2020). The selection of scats for training included genetically confirmed samples from target species (scats from male and female wolf haplotype group w22 (*sensu* Pilot et al. 2010), i.e. characteristic haplotype from grey wolves originating from the Apennine Peninsula and French Alps). Scats known to be from non-target species (genetically identified red fox *Vulpes vulpes* scats and scats from breeding domestic dogs fed with wild ungulate meat) were also provided during training (as per DeMatteo et al. 2019). The training protocol consisted of searching off-leash, with the dog indicating having found a wolf scat by freezing, lying down, and barking. For each find, the dog was rewarded by playing with a ball. Once the dog showed consistent recognition and detection of naturally occurring wolf scat, the handler deemed it ready to deploy on surveys. In a previous study (Roda et al. 2020), the dog team was deployed in 12 wolf-pack territories to collect wolf scat samples. All samples of this previous study were genetically analysed and demonstrated that the dog was consistently accurate (96%) at finding only wolf scats (and ignoring those of non-target species) in natural field conditions. In another study realized in October 2021 with the same experienced detection dog, scats were collected in the adjacent wolf-pack (called "Sirius Black", Fig. 1). All collected scats were genotyped, and the accuracy of the detection dog was confirmed to be 95% (F. Roda & J.N. Philibert, unpublished data). As the accuracy of the detection dog was consistent over time and within the same region (adjacent wolf-pack), we considered that the same detection rate (95-96%) of the dog could be

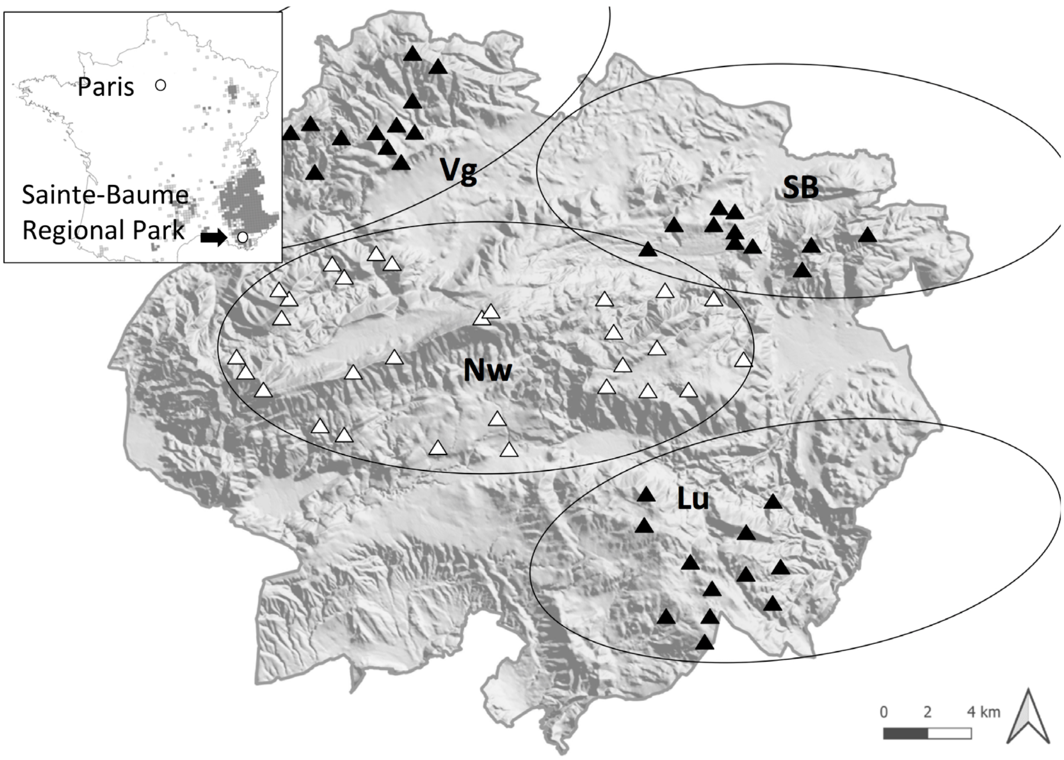

Fig. 1. Study area, showing the territory of the four wolf packs present in the Sainte-Baume Regional Park. The administrative boundaries of the Park are shown in light grey. White triangles indicate the marking sites of the "Neowise" wolf pack; black triangles indicate the marking sites of three adjacent wolf packs. Nw: Neowise wolf-pack; Lu: Lupi wolf-pack; SB: Sirius Black wolf-pack; Vg: Véga wolf-pack. Marking sites of the Lu, SB and Vg packs outside park boundaries are not shown.

extrapolated in the present study (see data modelling for further details). We used the same dog in our study to reduce sampling bias. The dog was four years old during the study and had three years of field detection experience.

Study area

The study area is situated in South-eastern France in the boundaries of the Sainte-Baume Regional Park (43°32 N; 5°83 E) and covers approximately 810 km^2. The Sainte-Baume Regional Park map is available at https://inpn.mnhn.fr/espace/protege/FR8000053. Elevations range from 286 to 1,148 m. The climate of the Sainte-Baume Regional Park is Mediterranean, with hot and dry summers, mild winters and moderately rainy autumns and springs (mean maximal temperature in July 27.6 °C; mean minimum temperature in July 19.4 °C; mean precipitation in July 5.6 mm; annual mean 613.4 mm). Forest covers 70% of the area and displays various profiles of vegetation according to forest management stages, mostly downy oak (*Quercus pubescens*), evergreen oak (*Quercus ilex*), Scots pine (*Pinus sylvestris*), Aleppo pine (*Pinus halepensis*) with patches of European beech (*Fagus sylvatica*). Numerous stands contain a mix of pine and oak.

Sport hunting is mainly focused on big game species, especially wild boar (*Sus scrofa*). Wild boar abundance is high throughout the forest terrain, with high numbers harvested (from 3.0 up to 6.3 wild boars killed/km^2/year). In contrast, the harvest of roe deer (*Capreolus capreolus*) is scarcer (from 0.4 up to 1.0 animal killed/km^2/year). In addition, small clusters of red deer (*Cervus elaphus*), chamois (*Rupicapra rupicapra*) and fallow deer (*Dama dama*) have been introduced by game managers (Office Français de la Biodiversité 2021a). Livestock animals are scarce or absent in the prospected areas. Our camera traps failed to capture free-ranging dogs during the two years preceding this study or during the current study (nine months), so we assumed that there were no free-ranging dogs in the Park.

The Sainte-Baume Regional Park is home to four referenced wolf packs (Fig. 1). We decided to study the wolf pack named "Neowise" because it occupies a central territory in the Sainte-Baume Regional Park that is entirely comprised within the Park boundaries. The Neowise wolf-pack was constituted of a pair of breeding wolves raising the pups without the aid of young wolves from previous reproductive events. Both breeding wolves were already known and genotyped as s58-02 (the male) and s69-09 (the female), respectively (Office Français de la Biodiversité 2021b). The marking sites of the other wolf packs are well-known, and individual wolves are genetically identified (F. Roda & J.N. Philibert, unpublished data). During the study, no incursions of adult wolves from the adjacent wolf packs were recorded by CT in the Neowise territory.

The density of forest roads is almost uniformly 4 km/km^2 throughout the wolf territory. No paved roads run through the territory of the Neowise wolf-pack. The public is not allowed to use motor vehicles on forest roads. The human traffic on roads is important throughout the year due to mushroom picking, big game hunting, and leisure hiking on sunny days.

Data collection

The team surveyed predetermined transects that were preferably circular for logistical reasons (return to the vehicle); in some cases, a member of the author's team (Poulard F.) transported the dog and handler, thus allowing a line-transect along forest roads. The dog team surveyed trails previously covered by other observers (Poulard F., D'Antuoni C. or Nasi N.) during previous years or where camera trapping captured at least one wolf. The transects were not randomly selected but were chosen to cover the whole area of the wolf-pack territory and replicate sampling used by previous observers or CT-based detections. All the transects were conducted on forest roads; no scats were searched or collected off-road. We equipped the dog and the handler with GPS devices (Dogtra pathfinder) to record survey tracks and distance covered and to enable mapping of all scats indicated by the dog. In a preliminary work (data not published), we censused 29 marking sites (Fig. 1) with the detection dog. We considered that a "marking site" was a 50 m radius location where at least one faecal dropping was left at two different dates. Each marking site was surveyed monthly from 1st December 2020 to 1st September 2021. The scat collection protocol was standardized with an observation effort similar between each marking site, using the same detection dog. All scats marked by the dog were collected (from adults + pups). For this study, only scats deposited by adult wolves were counted (scats from adults are much larger than scats produced by young pups; during the nursing period, no scat left by young pups was detected beyond 600 m from the den). The faeces collected in the present study will be analysed as part of the genetic monitoring program of the French Office of Biodiversity (Duchamp et al. 2012). The genetic results provided by the French Office of Biodiversity primarily concern areas of new wolf colonisation (not the case of the Sainte-Baume Regional Park), so we do not know when these results will be available. As genetic data were unavailable, and to consider that dog detection errors on non-target species may lead to an overestimation of wolf scats counts, we modelled and applied a correction on data (see data modelling).

Camera trapping was conducted in the Neowise wolf-pack territory between 1st December 2020 to 1st September 2021, using five cameras left in the nursing territory for 990 trap days. One camera was placed near the den area (at a distance of 600 m). In addition to these five cameras, five other cameras were used outside the nursing territory as "supplemental CT". These five supplemental cameras were often displaced and were never left in the same place for more than three weeks. These supplemental cameras have been used to better track wolves' movements outside the nursing territory. Remote motion-activated cameras (Browning spec obs BTC8A) were placed on forest roads used by wolves. Each camera trap was active 24 h per day, seven days each week, and was visited by observers (Poulard F., Ayache G., Nasi N., D'Antuoni C.) at variable intervals (from 2 to 20 days) to change batteries and SD cards. The male of the breeding pair of the studied wolf-pack was easily recognizable thanks to a scar on the hind leg. All cameras were provided with a passive infrared sensor and LED flash.

For this study, we considered the period from December to April as "Winter" and from May to August as "Summer". The date of parturition was estimated between the 1st and the 6th of May (thanks to CT), so the period of "Summer" is also the "nursing period". The precise boundaries of the "nursing" and "outside" territories were defined *a posteriori* (see Data modelling and analysis).

Data modelling and analysis

We performed the spatial analysis of geo-referenced wolf scats with "Magrit", an interoperable thematic cartography software (http://magrit.cnrs.fr/; Commenges 2017). The Magrit (0.8.14 version) smoothing tool was used, computing an inverse distance model of the probability of occurrence, or "potentials of population"; the function is exponential. Magrit computes the same algorithm as the R "Potential" package (https://riatelab.github.io/potential/), written by the same team who edits Magrit. With a grid of 1 km, we used a beta of 2 and a span of 0.8. The maps were designed on GIS software QGIS.

To model the probability of detection by the dog team, we used a single-season site-occupancy model to account for what we termed imperfect detection (*sensu* Kéry 2010; i.e. missing a scat when present) and to estimate the power of the dog survey method (MacKenzie 2006, MacKenzie et al. 2009). The survey was carried out over 29 marking sites (Fig. 1) each month, and it lasted less than three half-days in sample unit "i" (i.e. the Neowise wolf-pack territory). Within the sample unit "i", the protocol ensured that the dog team passed through 29 marking sites where wolf presence signs were previously detected each month.

The model (adapted from Hines et al. 2010, Roda et al. 2020; Appendix S1) was implemented in WinBugs (Kéry 2010) with 30,000 iterations and three chains. Each marking site was designed as "j" in the occupancy analysis. The data were subdivided into temporal replicates (each month) for the survey at each marking site "j" (Appendix S2). We checked convergence visually by inspecting the chains and checking that the Rhat statistic was below 1.1 (Kéry 2010, Brooks & Gelman 2012).

We defined from *a posteriori* analysis the "nursing territory" as the territory comprised in a 4 km radius from the den (corresponding roughly to a 50 km^2 area). We chose this distance because a previous study by Tsunoda et al. (2009) showed that the mean travel distance from the den for radio-tracked wolves of both sexes during the nursing period rarely exceeded a total distance (round trip) of 8 km/night or day, with a mean daily range of 11.3 ± 3.6 km^2 centred around the den. Consequently, seven marking sites were included in the nursing territory.

We used a t-test to compare mean detection probabilities per marking site during winter *vs.* summer (i.e. the nursing period); to compare mean detection probabilities per marking site inside *vs.* outside the nursing territory; and to compare the probabilities to photo-capture wolves during winter *vs.* during summer, both inside and outside the nursing territory.

As the use of the same dog in previous studies showed 95-96% successful detection of wolf scats *vs.* non-target species (Roda et al. 2020, F. Roda & J.N. Philibert, unpublished data), the 4-5% error could lead to an over-estimation of wolf scats counts and potentially bias the results. After collecting data, we tested four

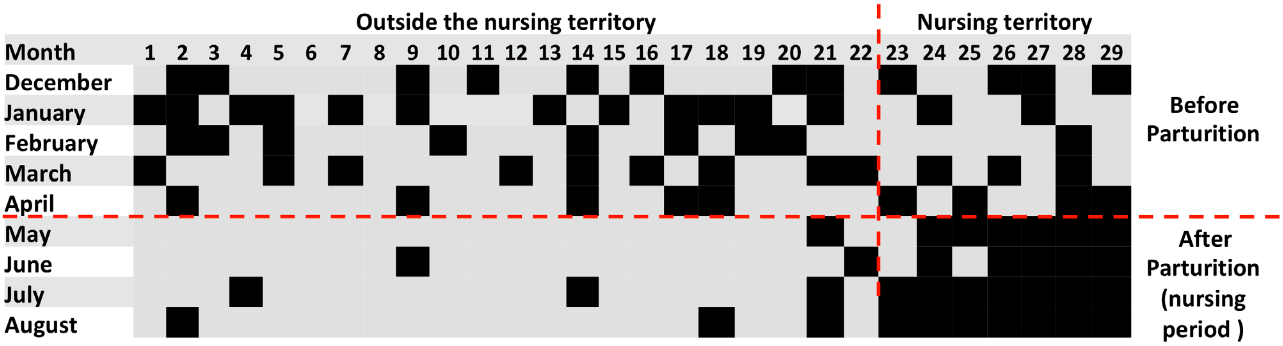

Fig. 2. Monthly differences in faeces deposition at each marking site (numbered 1 to 29) outside and inside the nursing territory; black rectangles show the detection of one or more droppings found at each location, light grey absence (non-detection) of faeces.

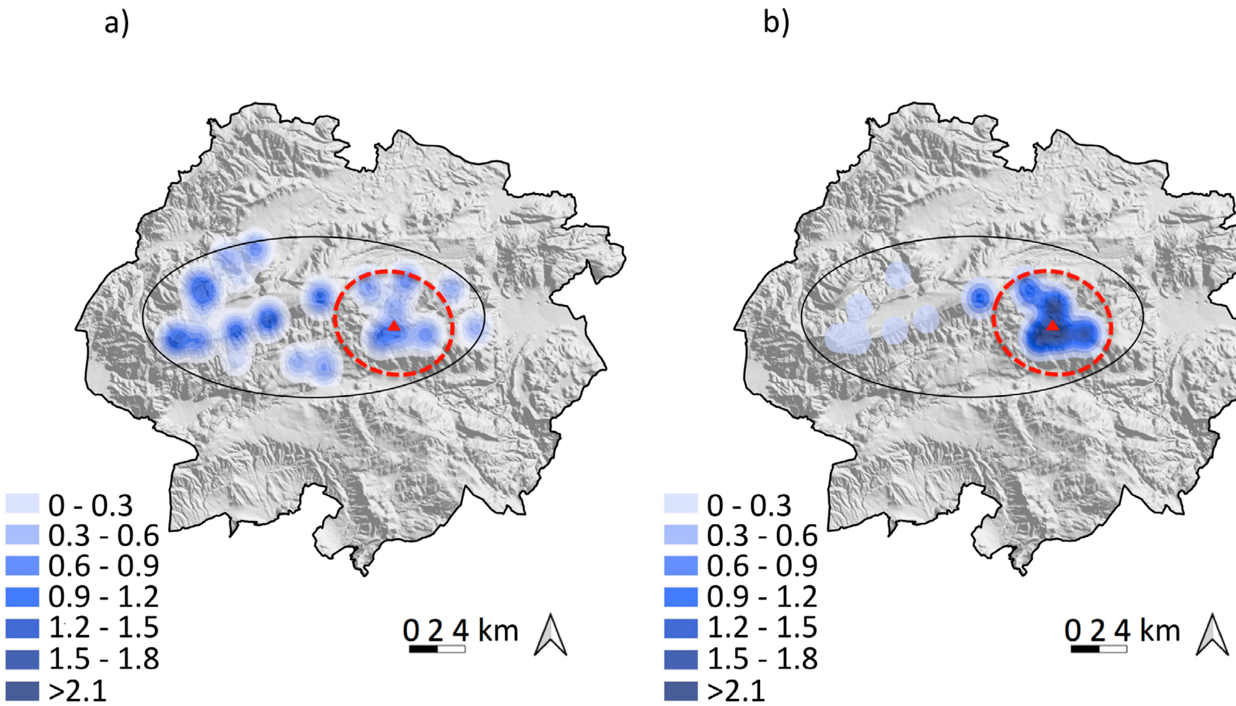

Fig. 3. Seasonal differences in faeces marking abundances of the Neowise wolf-pack. The graduation in shades of blue shows the mean number of faeces/per site/per month; a) winter and b) nursing period. The boundaries of the wolf-pack are shown in black; the limits of the nursing territory are shown with a dashed red line. The red triangle shows the location of the den.

likely scenarios: two scenarios in which the detection error rate of the dog was constant throughout the year; and two scenarios supposing that all the errors were made during summer. We simulated data and applied a correction factor supposing a 5% error spread throughout the year and across the whole territory (Model 1); a 20% error spread throughout the year and across the whole territory (Model 2); 5% errors spread only during summer and across the whole territory (Model 3); 20% errors spread only during summer and across the whole territory (Model 4). All simulated models were checked, and results were compared to the real data obtained in the field (Appendix S3).

All statistical analyses (other than occupancy with WinBugs) were performed using R-software.

Results

From December 2019 to August 2020, the dog team collected 113 wolf scats (Fig. 2). Once pups emerged from the den, we found both pups and adult scats near the den (< 200 m). No scats from pups were found at the sampling marking sites. For this study, only scats from adults were counted. During the study, only the two adult wolves of the breeding pair were recorded on CT; no adults of the adjacent wolf packs or free-ranging dogs were photographed.

We found a comparable number of scats every month (9 to 15; mean 12.5) with a constant sampling effort of two half days per month on the forest roads of the Park; the mean number of scats found each month did not differ between winter and the nursing period (t = 0.07, df = 6.96, P = 0.94). During winter, the mean number of scats found at marking sites was similar inside and outside the nursing territory (0.44 and 0.54, respectively; the difference was not statistically significant; t = 0.14, df = 61.66, P = 0.89). Based on our occupancy model, the per month chance for successful detection of wolf scats at each marking site was similar inside and outside the wolf-pack nursing territory (38% and 40%, respectively). Therefore, we calculated that during winter months, it was necessary to sample ten marking sites to reach a 99% probability of detecting wolf presence, regardless of the location of the marking site outside or inside the wolf-pack nursing territory.

Faecal deposition patterns changed between winter and the nursing period (Fig. 3). After parturition (estimated between the 1^{st} to 6^{th} of May), the mean number of wolf scats found outside of the nursing territory decreased by 80% and increased by 160% in the nursing territory as compared to winter season (0.09 and 1.43, respectively; difference statistically significant; t = –7.72, df = 29.82, P < 0.001; Fig. 4). We calculated that after parturition the per month probability of scat-marking at each marking site decreased threefold outside of the nursing territory (dropping from 38% to 11%). In contrast, it increased twofold in the nursing territory (increasing from 40% to 87%). We calculated that during the nursing period, the sampling of 20 marking sites was necessary to obtain a 90% probability of detecting wolf presence outside of the nursing territory; in the nursing territory, the sampling of only three marking sites was sufficient to obtain a 99,7% probability of detecting wolf presence. When taking into account the potential overestimation of scat counts due to non-target species detection by the dog, the overall results were similar, and the findings and conclusions of the study did not change (see details in Appendix S3).

Using results from camera trap videos, the probability of capturing wolves of the breeding pair in the nursing territory decreased after parturition (from 76% to 63% per month and per camera). During winter, the breeding pair used main forest carriage roads allowing fast travel (6.5 sights per month/CT). During the nursing season, wolves avoided the main forest roads (0.2 sights per month/CT); the difference was statistically significant (t = 7.51, df = 12.96, P < 0.001). Instead, wolves preferentially used secondary forest roads and quiet paths during the nursing season (5.6 sights per month/CT). From May to August, only the male was photographed far (up to 17 km) from the nursing territory. Secondary forest roads were avoided during winter (0.2 sights per month/CT); the difference was statistically significant (t = –3.52, df = 5.17, P = 0.016). The den was far from any anthropogenic disturbance in dense cover and along steep slopes.

Discussion

This study is the first to quantify and map how the seasonal changes in adult wolf defecation patterns

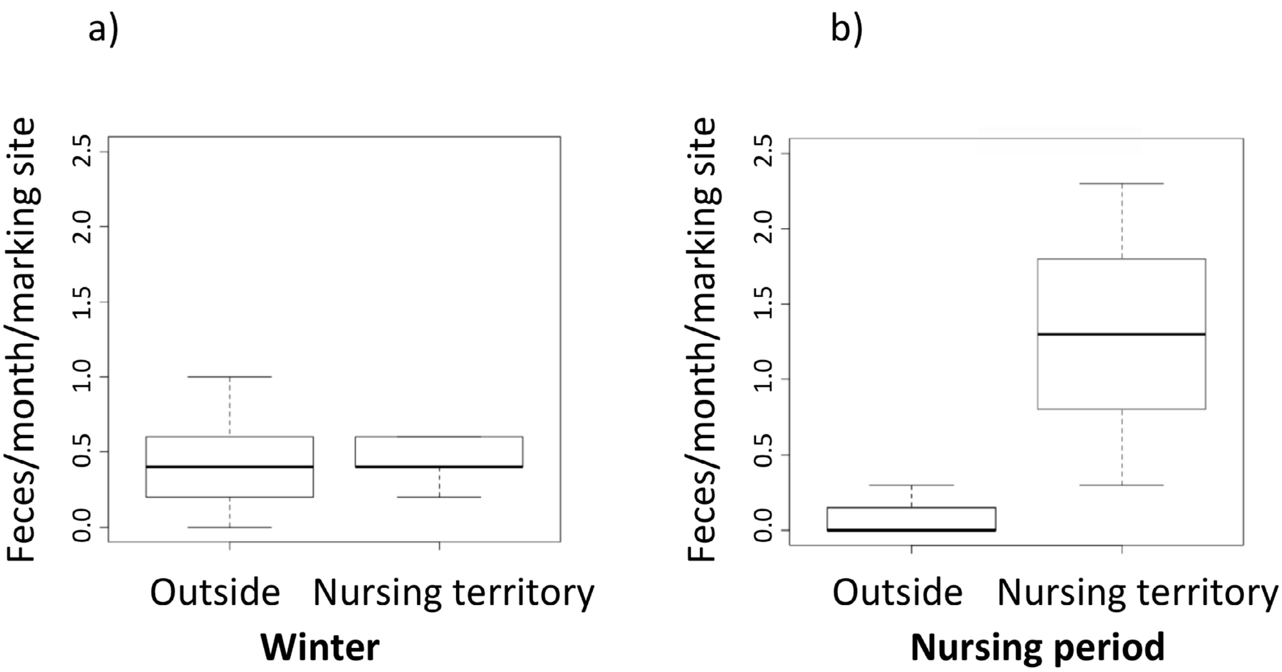

Fig. 4. Seasonal differences in mean abundances of faeces collected monthly at each marking site outside and inside the nursing territory: a) during winter and b) during the nursing period.

may affect detection probabilities. During the nursing period (i.e. summer), the probability of finding scats inside the nursing territory increases due to a change in the defecation pattern of the breeding pair; these changes affect scat location and, thus, scat detectability. On the other hand, the chance to find scats in winter is the same inside or outside the nursing territory. These results are of scientific importance because in non-invasive surveys for wolves in the French Alps (with human-only teams), ignoring detection heterogeneity may lead to an underestimated population size by an average of 27% (Cubaynes et al. 2010). Outside the French Alps (where snow events are scarce), surveys to estimate abundance are conducted with humans only and often during the reproductive season. Moreover, the underestimation of wolf population size may be even greater in newly colonised areas outside the Alpine mountains. For example, in a previous study, we found that human teams missed 75% of wolf genetic samples compared to dog teams (Roda et al. 2020). Capture heterogeneity (i.e. differences in probabilities of finding scats from different individuals) and its causes is an important consideration because it can lead to an underestimate of population size in capture-recapture models (Marescot et al. 2011, Louvrier et al. 2018b). Following our initial hypothesis, ignoring seasonal wolf scat marking changes could give misleading interpretations of wolf population size. They could result in an underestimate of population size unless the seasonal heterogeneity in scat detectability is considered.

Although the number of scats outside the nursing territory declined during the summer months, it was not zero. Because systematic genetic analyses of scats were not performed, we do not know if both sexes showed changes in faecal deposition patterns associated with the pup's birth. Changes in male and female defecation patterns during the nursing season are known in other canids. Kit foxes (*Vulpes macrotis*) (Ralls et al. 2010) also exhibit such changes. Other carnivore species, such as female mountain lions (*Puma concolor*) (who rear their young without male help), bury their faeces when they have young kittens (Seidensticker et al. 1973). We suspect that the studied male wolf deposited fewer scats near the den than the female during the nursing period since only the male was photographed far from the nursing territory during the summer, but this hypothesis needs further investigation. The male wolf exhibited a typical and visible scar on the hind leg, and no other male wolf (that could have biased the scats deposits) was captured on CT. We are confident that no other adult wolf roamed the area and could have biased the scats deposits, but we cannot exclude this hypothesis. In contrast to males, female wolves with young pups may remain near the den, which probably affects their defecation behaviour. Male and female wolves have different roles in the bi-parental care of pups (Packard 2003); the male contributes by feeding the female and therefore spends most of the time hunting away from the den (Mech & Boitani 2010). However, one must remember that young wolves did not help the Neowise wolf-pack to provide food to the female as other wolf packs do; hence, the defecation patterns may be different if one or more young wolves from a previous reproduction help the breeding couple parenting the pups. This hypothesis seems likely, as a previous study from Tsunoda et al. (2009) showed that a radio-collared male (of a breeding pair raising the pups without aid, as in our study) was mostly away from the den and his activity and movements were accordingly greater than those of the female during the nursing season. In the following years, when the male's pack consisted of seven wolves, his activity pattern and movements matched those of the female. The faeces collected in the present study will be analysed in a further study as part of the genetic monitoring program of the French Office of Biodiversity (Duchamp et al. 2012), and the response to these questions will be known in the future. However, as these data are not yet available, we acknowledge that this is a limitation of the study and its implications. Indeed, the detection dog may have marked non-target species, which could have resulted in an overestimation of scat counts. To overcome this issue, we simulated data and took into account that the ability of the detection dog to discriminate wolf scats from non-target species was evaluated in previous studies; the dog was found to be 95-96% accurate (Roda et al. 2020, F. Roda & J.N. Philibert, unpublished data). The differences in defecation patterns found in this study were so significant that the overall results were not changed, even when an exceptionally high error rate (20%) was considered. One must remember that free-ranging dogs were never recorded on CT, so we are confident that there are no free-ranging dogs in the Park that could bias the results.

Wolves in our study used forest roads and crossroads for scent-marking (but no surveys were conducted off-road, as in Bojarska et al. 2020). During winter, they used main forest roads to travel fast and far across their home range at night. As revealed by CT, wolves of the breeding pair strongly avoided the main forest roads during the nursing period and selected secondary trails in forested areas. Wolves of the Neowise wolf-pack used main forest roads during winter to travel and connect distant parts of the territory; during the nursing season, the journeys were more limited (but not non-existent). One must keep in mind that our sampling design should have taken into account off-road scats deposits; thus, it is evident that only a fraction of all the available faeces has been collected, which could be a source of bias. However, we are confident that our results represent the reality of seasonal changes, as our sampling points are spread over the entire territory. All in all, wolves in the Neowise territory likely avoided humans and selected roads at times of no human activity. We believe that both legal culling (Grente 2021) and illegal wolf shooting, which is regularly reported in France (Mathieu et al. 2021), contribute to maintaining this behaviour. Although forest roads in the Sainte-Baume Regional Park usually have too little traffic to pose a risk of wildlife-vehicle collision, they may increase wildlife mortality by easing access to legal hunting and poaching (Person & Russel 2008). Our results are in accordance with recent findings showing that wolves may take advantage of forest road infrastructure for travel and scent marking while minimizing human encounters by spatio-temporal avoidance of roads (Bojarska et al. 2020). In addition, wolf response to roads with low traffic may vary according to wolf behavioural and social status (pack members *vs.* floaters), time of day and season (Mancinelli et al. 2019). In summer, the avoidance of the main roads and human activity during the day is common to many wolf-packs in different European countries (our study, Eggerman et al. 2008, Mancinelli et al. 2019, Bojarska et al. 2020).

In conclusion, the combined and complementary use of a detection dog and camera traps give a good understanding of wolf seasonal space use, human avoidance and marking behaviour. The limited quantity of data provided by this kind of survey differs from that provided by radio-collared wolves, but this is balanced by the fact that wolves are not disturbed by this technique. Because using radio collars is essentially prohibited in France, this original method is an excellent non-invasive alternative that avoids wolf capture, anaesthesia and disturbance. Behaviour and adult wolf scat deposits are highly variable during the nursing season compared to winter, affecting detectability. We conclude that dog surveys to collect samples should be conducted exclusively during winter to avoid sampling biases. This conclusion can probably be extrapolated to other species as numerous carnivores exhibit such seasonal changes in defecation patterns.

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Author Contributions

F. Roda raised and trained the dog, conducted fieldwork, collected wolf scat data, carried out statistical analyses, performed interpretation of results, wrote the original draft, reviewed and edited the final draft. F. Poulard conducted fieldwork and data collection from camera traps. G. Ayache designed and provided GIS mapping. N. Nasi and C. D'Antuoni assisted with fieldwork. R. Mathieu provided advice in the early stage of manuscript redaction. G. Cheylan supervised the work. All authors reviewed the original draft.

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Supplementary online material

Appendix S1. WinBugs code for the model.

Appendix S2. Data.

Appendix S3. Model of error due to non-target marking by the dog.

(https://www.ivb.cz/wp-content/uploads/JVB-vol.-71-2022-Roda-et-al.-Appendix-S1-S2S3-1.pdf)