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LUTRA

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When leaving: about the past, the present and the future of Lutra

When I joined Lutra's editorial team as editor-in-chief in 2002, the journal was in a state of transition. I was following in the footsteps of two outstanding predecessors. A. Scheygrond (1953-1976) and Chris Smeenk (1981-1998) had determined Lutra's content for many years, and each was very successful in their own way, but it was not now clear which direction the journal should take. Scheygrond had worked hard to increase knowledge about the presence of indigenous mammals, and had also made a huge contribution to popularising mammal science in the Netherlands through Lutra and other channels. One of his many achievements was the publication in Lutra of the first Atlas of Dutch Mammals (Lutra 13-1/3; 1971). The special issues on badgers (Lutra 6-1/2; 1964) and otters (Lutra 12-1/2; 1970) were also important events. Until then, little was known about these species in the Netherlands, other than the fact that their numbers were so low that they were probably endangered. The first author of these three publications was Anne van Wijngaarden who, together with his co-authors, conducted pioneering research in the Netherlands during this period.

Smeenk had focussed on improving the scientific quality of the manuscripts submitted while also paying a great deal of attention to correct use of language and syntax. During his 'reign' in the 1980s, Lutra regularly published hefty single-theme issues, including an Otter Special too (Lutra 27-1; 1984), the Predator Special (Lutra 29-1; 1986) and the Whale Special (Lutra 30-2; 1987).

Both editors also made their mark on Lutra's appearance: during Scheygrond's period as editor-in-chief, issues were published as quires of varying thickness (to be collected and bound). Smeenk lobbied the board of the Society for the Study and Conservation of Mammals ('de Vereniging voor Zoogdierkunde en Zoogdierbescherming'- VZZ, as the Dutch Mammal Society was called before 2010), which publishes Lutra, to have the issues published with a light blue cover. This better reflected the fact that Lutra was a journal, and made issues better able to withstand rough and inexpert handling. The emblematic drawing of an otter, which had been the VZZ's logo for years, adorned the front cover.

When Smeenk retired from his post in the mid-1990s, there was no immediate modus in place for continuing the journal. Like Scheygrond, Smeenk had always done a tremendous amount of work for Lutra, but this had meant that other editors had been less involved and made it difficult to build a functional team that was not dependent on the input of one person. A somewhat precarious situation developed in the years that followed, resulting in a relatively high turnover of editorial staff; Lutra was in danger of extinction.

In around 2000, a number of young and enthusiastic researchers who not only wanted to take Lutra forward, but also wanted to give it a new lease of life, joined the editorial board. They asked me to become editor-in-chief and after giving the matter some consideration, I said 'Yes'.

I started just in time to work on a subject which, up to that point, had been a regular topic for Lutra: zoology in Zoo's. Lutra had planned a single-theme issue, the 'Zoo Special', for December 2001, but this proved to be an edition that was published only with great difficulty and a major delay in the planned publication date. These problems brought all normal editorial tasks more or less to a standstill. After this experience, the editorial team decided to set and maintain clearer boundaries with regard to the sub-disciplines we would cover. From now on, we would focus on *ecology, biogeography, behaviour and morphology of native (and wild) mammals in Europe*. Zoos did not fit within this plan. Another area that had previously received a great deal of attention in Lutra, but was scarcely dealt with by the 21st century editorial board, was the mammals of the former parts of the Kingdom of the Netherlands.

A new look was introduced in 2001, when the blue cover was replaced by a predominantly yellow cover sporting a black and white photo (replaced by colour photos in 2007). Another change was that each issue now opened with an editorial. Up until 2010, each editorial was signed by the editorial board, after which it was signed by the member of the editorial team who had written it. To mark the celebration of '50 volumes of Lutra', the editorials of three consecutive issues (Lutra 53-1, 2007; Lutra 53-2, 2007; and Lutra 54-1, 2008) took a closer look at Lutra's past and future.

Looking back, I can say that Lutra is still mainly perceived as a journal offering a wide range of more or less 'in-depth' articles. In recent years, we have paid a good deal of attention to a very contemporary research area, namely the reappearance in the Netherlands and Flanders of wild mammal species such as the raccoon dog (*Nyctereutes procyonoides*), the wildcat (*Felis sylvestris*), the wolf (*Canis lupus*) and the golden jackal (*Canis aureus*). Connections (proven and unproven) are often drawn between these reappearances and large-scale environmental changes (such as climate change, maturing forests, and the increased tolerance of humans towards indigenous mammals). As always, we regularly published articles about marine mammals. Nowadays, Lutra now and then even explores the significance of molecular and genetic research into wild mammals, especially research to determine origin and kinship.

I do not want to discuss individual contributions here, but I would like to mention some of the specials that have appeared since 2002: 1) the Rodent Special (Lutra 45-2; 2002) with contributions presented at the symposium 'Rodents – threatened or a threat?' (7 and 8 September 2002 in Antwerp, Belgium, on the occasion of the 50th anniversary of the VZZ); and 2) the Beaver Special (Lutra 46-2; 2003) with contributions presented at the Third International Beaver Symposium (13-15 October 2003 in Arnhem).

I also want to make a separate and special mention of the comprehensive North Sea Cetacean Special (Lutra 61-1; 2018), which provided an up-to-date overview of the state of research on whales in the North Sea. This special themed issue, dedicated to Chris Smeenk (2016†), came about through the benevolent and persistent efforts of a guest editorial team consisting of specialist whale researchers Peter J.H. Evans (Wales), Carl Chr. Kinze (Denmark) and Graham Pierce (Scotland). For Lutra, and we hope also for our readers, this was a substantively valuable and memorable experience, which brought together a significant body of current knowledge about the occurrence, (displacement) behaviour, ecology and taxonomy of cetaceans and dolphins in the North Sea.

In the meantime, the editors were trying to make publishing in Lutra a more attractive proposition for professional researchers by applying, in around 2010, for inclusion in the Web of Science, something which would also have conferred a Journal Impact Factor. The editorial board felt that Lutra met the criteria required for a successful application, however, the organisation (then Thomson Reuters) considered that not only were there already quite a few journals on mammals, but that the number of self-citations in Lutra was relatively high, and our application was rejected on those grounds.

The editors had terminated Lutra's bilingual policy and switched entirely to English in the years leading up to the submission, partly in order to strengthen our case. Unfortunately this was to no avail, and so Lutra resumed publication of articles in Dutch in 2017. This decision was partly to encourage a particular segment of our target group – young, amateur researchers with little or no previous experience – to publish scientific articles.

Lutra faces other structural challenges, such as a tight budget, the equally tight supply of copy – even from non-professional researchers, and the limited availability of those members of the editorial board who have a full-time job. In addition to all this, technological developments in the field of publishing mean that we must now decide whether to publish a digital version of Lutra in addition to print, or to switch to this channel entirely. We are currently considering this matter and discussing how to deal with it in the future.

Other external factors are also exerting an influence on Lutra's continued existence. New journals have been launched that are, to some extent, fishing in the same pond, including Zoogdier (the popular scientific journal of the Dutch Mammalian Society), and Cranium (the journal of the Working Group on Pleistocene Mammals).

Given my rather limited understanding of 'the digital world', I felt that it was time for me to resign. It will of course also be necessary, if possible, to find a solution for the other points I have raised here.

I don't think that it is possible to predict what is going to happen to Lutra – I am certainly unable to do so – but I do think that the Dutch Mammal Society should have its own channel where it can publish research results in a scientifically sound manner. This means that its members will continue to have access to research and developments in this area – or at least they will if they have a subscription to Lutra! In my opinion, this is not only interesting, but also necessary for its objective functioning.

This does not mean, however, that subjectivity should be forbidden: on the contrary. In addition to curiosity, an emotional connection with mammals and other animals, and more broadly with nature and the environment, is a very important reason for people to engage with nature.

It only remains for me to thank all the co-editors, authors and numerous specialist reviewers for their collaboration over the years. It was thanks to them that we managed to produce many 'fine issues', as I referred to them in the corridors. I would also like to take this opportunity to thank the board of the Dutch Mammal Society for the trust it placed in me. It has been an honour and a great pleasure to do this work, in the course of which I have been surprised time and time again by mammals and their weird and wonderful ways.

Kees J. Canters

Genetic structure of badger populations in a fragmented landscape: how do barriers affect populations on a genetic level?

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Abstract: The expansion of urban area, accompanied by the development of infrastructure, leads to more fragmented natural living space and isolation of wild populations of plant and animal species, including the badger (*Meles meles*). Fragmentation enhances the influence of genetic drift in these isolated populations. In order to reconnect fragmented populations, corridors have been implemented as mitigation measures. It is unclear, however, whether these measures fully mitigate the effects of fragmentation. The aim of this study was to measure genetic differentiation between fragmented populations of badgers by establishing the population structure of badgers in a fragmented area and examining the influence of barriers on genetic differentiation. This study was carried out in the Utrechtse Heuvelrug, Province of Utrecht, the Netherlands. Tissue samples were collected from road killed and trapped badgers, and faecal samples were collected for a selected group of clans. Microsatellites were used to study population structure of badgers. Population structure of badgers on either side of a major road, the A27 motorway, differed substantially. The degree of heterozygosity between both populations appeared to be similar, allelic diversity however was much lower for the population west of the A27 motorway. This barrier may therefore limit gene flow despite implementation of multiple corridors. Long-term studies may reveal to what extent corridors mitigate the barrier effect by major roads.

Keywords: badger, *Meles meles*, population structure, fragmentation, barrier, corridor, gene flow.

Introduction

Terrestrial species continue to live under pressure for living space, whilst land use changes due to human population growth and land becomes fragmented as roads cut through natural landscapes (Meyer & Turner 1992). Fragmentation also leads to reduction of suitable natural living space. Habitat loss has large, consistently negative effects on biodiversity (Fahrig 2003). Fragmentation of habitat amplifies the negative impact on biodiversity as it sets off time delayed biodiversity loss (Krauss et al. 2010). Species on high tropic levels have smaller population sizes and greater mobility and are, therefore, more susceptible to habitat fragmentation (Gilbert et al. 1998). By affecting different trophic levels, fragmentation may disrupt whole communities and ecosystems might collapse. Furthermore, fragmentation exacerbates edge effects and negatively affects population size of many species (Debinski & Holt 2000, van Strien et al. 2007).

The indirect effects of fragmentation might be underestimated, since populations may become isolated from each other as infrastructure and urban development limit migration chances, making populations vulnerable to the effects of inbreeding and genetic drift. The influence of habitat fragmentation on genetic variability of meta-populations has therefore been of recent scientific interest. Significant differences in genetic differentiation and in allelic diversity as a result of inbreeding were found in fragmentened populations of flightless ground beetle (*Carabus violaceus*), European tree frog (*Hyla arborea*), dice snake (*Natrix tesselata*), and desert bighorn sheep (*Ovis canadensis nelsoni*) and have led to genetically distinct populations in, for instance, the Florida black bear (*Ursus americanus floridanus*) (Gautschi et al. 2002, Keller & Largiader 2003, Andersen et al. 2004, Epps et al. 2005, Dixon et al. 2007). As fragmentation can lead to isolation, gene flow may become limited and the chance of genetic drift increased.

In order to reconnect fragmented populations, corridors have been implemented as mitigation measures. It is unclear however whether these measures fully mitigate the effect of fragmentation. Multiple methods have been applied to determine which species make use of corridors (van der Grift 2010). So far, most studies have demonstrated that corridors are frequently used to cross barriers throughout the year, during day and night, by multiple species and thus mitigate fragmentation effects as they provide safe passage and significantly reduce road mortality (Veenbaas et al. 2003, van der Grift 2005, Dekker & Bekker 2010, Rytwinski et al. 2016). Corridors prove to be especially effective for mustelids, for instance, by increasing individual life expectancy (Vink et al. 2008, van den Akker 2014). Most methods used in studies on reconnecting populations through corridors, however, lack the ability to identify specific individuals, and with that: actual dispersal or migration and gene flow facilitated by corridors. Sawaya et al. (2014) found bidirectional gene flow and genetic admixture in grizzly bears (*Ursus arctos*) but did not find this in black bears (*Ursus americanus*) within the same, with corridors reconnected, fragmented study area. Corridors can thus be useful to reconnect populations but their effectiveness to restore or maintain gene flow may depend on the species. Since corridors are intended to facilitate several species, it is difficult to determine whether the use of corridors is a fully effective measure to mitigate the effect of fragmentation.

We aimed to establish the population structure of badgers (*Meles meles*) in a fragmented area and to examine the influence of barriers on genetic differentiation. Dispersal of badgers is often to one of the neighbouring clans and rarely takes place over longer distances (Christian 1994, Rogers et al. 1998). Settling into another clan is a gradual process that may take up to nine months (Roper et al. 2003). The dispersal strategy of badgers, where clans act as stepping-stones for gene flow, makes the badger a suitable species to study genetic differentiation. Since roads function as an obstacle and as such territory bounderies, roads might directly affect gene flow. Corridors may mitigate this effect as they facilitate safe passage (Gilbert et al. 1998).

The study was conducted in a heavily fragmented area: the Utrechtse Heuvelrug. Motorways around Utrecht were found to function as territoria boundaries for badgers, adding weight to the assertion that elements such as motorways act as barriers and fragments habitat (Mulder 2016). The badger population in Utrecht originates from a small population located at Einde Gooi in the 1980s but has since spread across the Utrechtse Heuvelrug

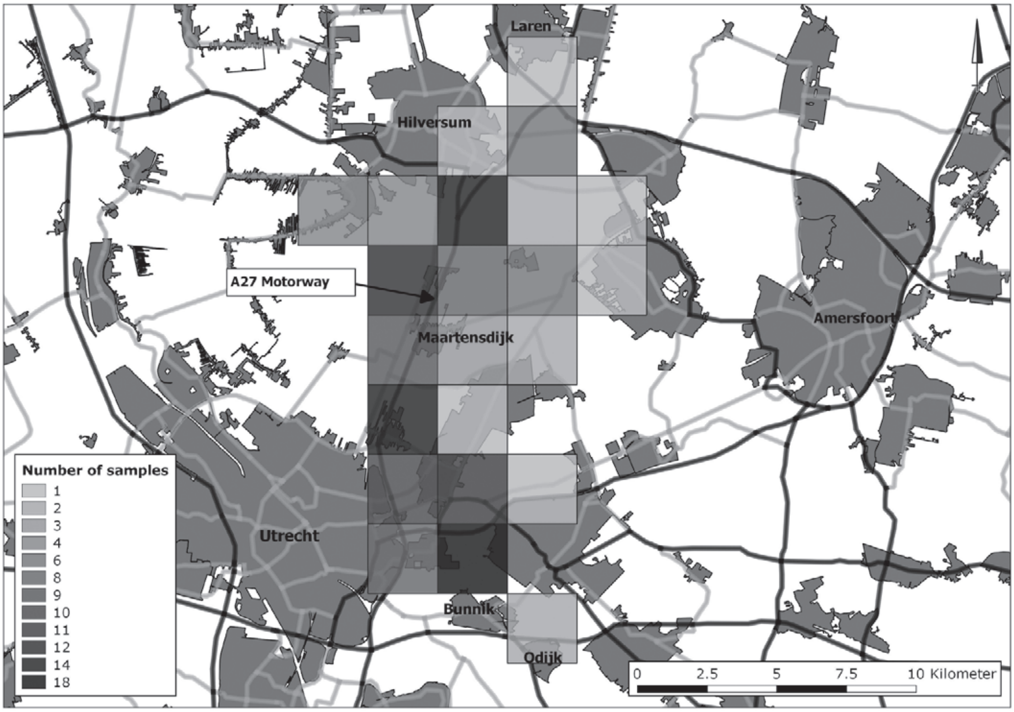

Figure 1. Area where samples originated from illustrated using a grid of 2.5 x 2.5 kilometre. Light squares illustrate areas with few samples, dark squares illustrate areas where most of the samples were collected.

area and surroundings (van Apeldoorn et al. 2006). Badgers are now expanding onto previously thought unsuitable habitat (Zekhuis & Gerrits 2015). As a result of more land becoming inhabited by an increasing badger population, the role of corridors may have shifted from a measure enabling individuals to colonise new territoria, to a measure which connects established territoria among each other.

Measuring genetic differentiation between populations of badgers in the fragmented region of Utrecht was the scope of this study. For this, we used a molecular approach. Tissue from road kills, trapped badgers and faecal samples were used to understand population structure and genetic diversity. Samples were filtered for sample quality and microsatellite analyses of seven different loci were conducted on the selected, good quality samples. Genetic differentiation was tested for populations on either sides of landscape elements that may affect gene flow. Measures in order to reconnect populations, such as corridors, are widely implemented in the study area. The origin of the population and re-connecting measures in place, led to the hypothesis that badger population structure would resemble one connected population.

Methods

Study area

This study was carried out in the Utrechtse Heuvelrug area, Province of Utrecht, the Netherlands. The Utrechtse Heuvelrug is a push moraine formed in the Saale glaciation. The moraine is characterised by sandy soils. These sandy soils change into more clay and peat rich soil as the high land transitions into low land to the east. This transition area of high sandy soils to low clay rich soils formed the main study area. Tissue samples of badgers were collected between the town of Laren and the town of Odijk, covering ± 20 kilometres. The area between Maartensdijk and Bunnik comprised the study area for collection of faecal samples of badgers in the autumn of 2018 (covering ± 7 kilometres), completely overlapped by the area where tissue samples were collected. Composition of the origin of the samples is illustrated in figure 1, exact locations of road kill and faecal samples are not displayed in order to conceal the exact locations of important badger habitat.

Sampling DNA

Tissue samples were collected from road killed (n=60) and trapped badgers (n=21) between September 2011 and August 2018. Tissue samples consisted of tongue tissue or skin biopsies. Location and date were recorded for all road kills and trapped badgers. Sex and age were recorded for trapped badgers. Faecal samples (n=113) were collected from nine clans between 1 August 2018 and 10 October 2018. During the months of September and October, badgers show an increase in the use of latrines. This increase in activity is more profound in February – March and is related to the mating season (Middleton & Paget 1974, Roper et al. 1986). Collection of faecal samples is therefore performed during these periods as much as possible, as more indiviudals from each clan use latrines more frequently.

Sampling faecal matter is a non-invasive method to collect genetic material from badgers. Faecal samples were collected during the morning. The chance of disturbing badgers in their natural habitat is low during this time of day and human scent has the time to fade before badgers become active again. Faecal matter is also most fresh during this time, as the warm sunlight has not had the time yet to warm up the faeces and speed up the process of breaking down DNA. Badgers place their faeces carefully into small pits, or latrines. Multiple faeces from different individuals could sometimes be found in the same latrine. Tissue samples and faecal samples were collected in tubes containing 96% ethanol. Tissue samples were stored at room temperature, faecal samples were stored at -18 °C. A sample of the inner matter of the faeces was collected in order to avoid DNA contamination from other faeces in the same latrine. As territoria boundaries were not exactly known for each clan, latrines were tracked down from the main sett. Latrines close to the main sett were assumed to be primairly used by the corresponding clan. Faecal samples were collected for clans who are connected by natural habitat, connected by corridors or disconnected by roads and towns.

DNA extraction

DNA was extracted using the Qiagen DNaesy Blood & Tissue kit (tissue samples) and Qiagen QIAamp PowerFecal DNA Kit (faeces). Microsatellites were used to study population structure of badgers. Microsatellites are mainly non-coding parts of the genome and are non-susceptible to natural selection, making microsatellites suited for a study of population structure. A total of eight polymorphic badger microsatellite sequences were included in the analysis; Mel102, Mel105, Mel106, Mel109, Mel111, Mel113, Mel117 and Zinc_y_ri (Carpenter et al. 2003, Frantz et al. 2003). Loci were selected for allele sizes (bp) < 250 bp to increase the chance of detection on possible degraded samples. In a first test it appeared that amplification success from faecal samples was lowest for Mel113 locus. Therefore, Mel113 was used to make a first shifting between good and bad quality samples. If Mel113 was not amplified consistently using two PCR replica's, the sample was excluded from further tests. All amplifications were performed using 12,5 μL environmental mastermix 2.0 (life technologies),

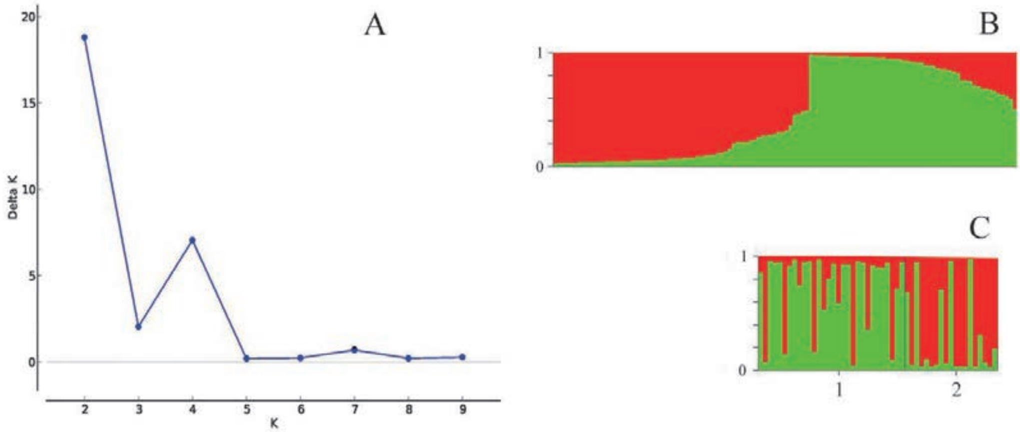

Figure 2. A. To determine population structure, Structure was used to calculate ΔK. Following Evanno et al. (2005) the highest value for ΔK: 2, represented the most accurate estimation for the number of clusters. B. Bars represent population structure for every individual. The red and green colour do not show an abrupt boundary, both populations are therefore not entirely isolated. C. Most distinct difference in population structure was found for both populations on either side of the A27 motoway (1, west of A27 motorway; 2, east of A27 motorway, north of the city of Utrecht).

1 µL of both primers (10 µM), 3 µL template DNA and 7,5 µL DNase free water. Primers were 5'-end labelled with a 6-FAM, Atto550, ATTO532 or ATTO565 dye. PCR was performed for 5 min at 50 °C, 10 min at 95 °C, 55 cycles at 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, followed by a final step at 72 °C for 3 min. PCR was performed in duplo, each marker in a separate reaction. Both PCR reactions of four micro-satellites loci were pooled and length of fragment was evaluated using an ABI 3730 DNA Sequencer.

Data analysis

Faecal samples were checked for multiple samples of the same individual per clan, this was later checked among clans. Samples with results for less than four loci were excluded from further analysis. These samples could not accurately be assigned to an individual.

Peaks were called using Geneious 9.1. Geneious enables microsatellite genotyping because it can determine the length of alleles in number of basepairs. Geneious also provides the possibilty to distinguish homozygotes from heterozygotes (Arif et al. 2010). Individual badgers were recognised from multiple samples per clan on the basis of these results. Once the individuals were identified, this same process was repeated among clans. Structure 2.3.4 was used to determine population structure (Pritchard et al. 2000). All individuals from Utrecht were included in this analysis. The total badger population of Utrecht was assumed to be one fully connected population (K=1), a heavily fragmented population (Kmax=9) and all values in between. Length of Burnin Period was set at 5,000, followed by 50,000 replicates. An admixture model with correlated allele frequency among populations was applied for structure analysis. Following Evanno et al. (2005), ΔK was taken as a measure to detect the value of K. Genepop 4.2 was used to calculate population parameters, such as observed- (H_O) and expected heterozygosity (H_E). Weir & Cockerham's (1984) version of *F*-statistics were used in this study. Here, badgers fallen victim on A27 motorway were excluded as it was unclear which side of the motorway they originated from.

Table 1. Seven loci were included in the genetic analysis. The number of successful analyses (%), total amount of alleles (N), expected heterozygosity (H_E) and observed heterozygosity (H_O) are displayed per locus and per population.

Locus	Total Utrecht population				Utrecht West population				Utrecht East population			
	%	N	H_E	H_O	%	N	H_E	H_O	%	N	H_E	H_O
Mel102	94%	6	0.78	0.67	100%	5	0.68	0.63	91%	6	0.79	0.69
Mel105	97%	6	0.56	0.55	100%	4	0.56	0.58	95%	6	0.56	0.54
Mel106	95%	5	0.52	0.42	100%	3	0.52	0.37	93%	5	0.52	0.45
Mel109	100%	3	0.14	0.14	100%	2	0.07	0.07	99%	3	0.17	0.16
Mel111	90%	5	0.40	0.40	97%	2	0.45	0.55	87%	5	0.37	0.34
Mel113	97%	5	0.56	0.52	97%	3	0.54	0.62	96%	5	0.57	0.48
Mel117	99%	4	0.37	0.38	97%	2	0.24	0.28	99%	4	0.41	0.42
AVG	96%	4.9	0.45	0.42	99%	3.0	0.43	0.44	94%	4.9	0.45	0.41

Results

DNA extration

Extraction of badger DNA, consistent amplification of Mel113, was successful for 98% of the tissue samples (*n*=81). Analysis was fully successful for 96% of these samples. Tissue samples resulted in 59% males and 41% females. Faecal samples reacted in a different way: 76% of badger faecal samples resulted in the successful extraction of badger DNA. A total of 44 different individuals were successfully identified from the faecal samples, 21 of which were found more than once. Sex ratio was less skewed in faecal samples, 51% males and 49% females.

An extensive search for badger faeces was conducted for five known clans. These territoria are located consecutively, separated by roads. Some of these clans are connected by corridors to neighbouring clans. Between five and thirteen distinct individuals were found for these five known clans. Samples were taken from four other neighbouring clans. Sample size per clan was too small to determine clan size by a mark and recapture analysis (Frantz et al. 2003).

Population structure

Following Evanno et al. (2005) ΔK was taken as a measure to detect the true value of K. The true value of K was found at K=2 (figure 2a). No absolute isolated populations were found, yet clusters of individuals resemble different population structures (figure 2b). The most profound difference in population structure was found between the badger population west of the A27 motorway and east of the A27 motorway, north of Utrecht (figure 2c). A few individuals for both east and west of the A27 motorway match the population structure for the other side of the A27 motorway rather than the side they were found at.

Genetic diversity

The frequency and distribution of alleles and the heterozygosity were used to calculate genetic diversity of the two subpopulations, west of the A27 (hereafter referred to as 'western population') and east of the A27, north of Utrecht (hereafter 'eastern population'). Expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated for the total population and for each subpopulation (table 1). The eastern badger population

Table 2. *F*-values for all loci between the two subpopulations, F_{is}: measure of deviation from Hardy-Weinberg within subpopulations, F_{st}: measure of genetic differentiation among subpopulations, F_{it}: measure of deviation from Hardy-Weinberg in total population (also called 'inbreeding coefficient').

Locus	F_{is}	F_{st}	F_{it}
Mel102	0.1145	0.0595	0.1672
Mel105	0.0298	0.0035	0.0332
Mel106	0.1816	-0.0046	0.1778
Mel109	0.0494	0.0103	0.0592
Mel111	-0.0184	0.0361	0.0183
Mel113	0.0791	0.0059	0.0736
Mel117	-0.0402	0.0365	-0.0022
Total	0.0692	0.0219	0.0896

showed a greater diversity in alleles than the western badger population.

All alleles found in the western population were also present in the eastern population. For the total Utrecht badger population H_O was found to be lower than H_E in four of the alleles. In two alleles, H_E and H_O were found to be equal and H_O was found to be higher than H_E. The average heterozygosity for the total Utrecht population was 0.420. This pattern is different between the two subpopulations. The average H_O in the western population (0.437) was found to be slightly higher than the average H_O of the eastern population (0.413), despite a lower number of alleles per loci in the western population (3.0 and 4.9 respectively).

For the total population *F* values were positive, meaning a deficiency of heterozygotes results in a devation from Hardy-Weinberg equilibrium (Scharloo & van Delden 1987). The measure of deviation from Hardy-Weinberg within subpopulations (F_{is}) overall also shows positive values indicating a deficiency of heterozygotes within the subpopulations. The eastern population showed significant ($P<0.05$) heterozygote deficit for Mel102, Mel105, Mel106 and Mel113. The western population showed significant ($P<0.05$) heterozygote deficit for Mel105 and Mel106. For these alleles more homozygotes were found than expected. More homozygotes for these alleles were also found for the total population (F_{it}). Heterozygote deficits were greater for the total population than within subpopulations.

Following Hartl & Clark (1997) F_{st} values ranging 0–0.05 indicate little differentiation, 0.05–0.15 moderate differentiation, 0.15–0.25 great differentiation and >0.25 very large differentiation. These guidelines were previously also applied in Baird's tapirs (*Tapiris bairdii*) (Norton & Ashley 2004). The F_{st} value for the badger subpopulations and the total badger population in Utrecht could indicate little differentiation (table 2). The F_{st} value in locus Mel102 indicates moderate genetic differentitation.

Discussion

The badger population in the study area in the Province of Utrecht can be considered as a population consisting of two subpopulations on either side of the A27 motorway. However, neither of the subpopulations were found to be completely isolated populations. The A27 motorway may act as an underlying factor limiting gene flow between two subpopulations. This is in contrast with our initial hypothesis that the population structure of badgers would resemble one connected population.

The Utrecht badger population shows no clear similarity in allele composition to foreign populations such as found in badger populations in England and Switzerland by Frantz et al. (2010b) or Ireland by Guerrero et al. (2018). Average heterozygosity and average number of alleles per locus in the Utrecht population measured over all loci included in this study (0.420; 4.9) appeared to be lower than in the Swiss (0.614; 5.9), English (0.557; 5.4) or Irish study populations (0.461; 6.0). Heterozygosity and allelic diversity are both indicators for genetic diversity of populations which in turn contributes to fitness and evolutionary potential. High heterozygosity, which is often accompanied by rich allelic diversity, indicates genetically diverse populations with little to no effect of inbreeding on genetic diversity. Low heterozygosity, in fragmented populations, might be caused by genetic drift and limited gene flow. The Utrecht badger population, if compared to study populations in Switzerland, England and Ireland, might therefore have lower fitness and might be more susceptible to loss of genetic diversity as a result of factors such as inbreeding.

Given the limited genetic pool from a small founder population, limited introduction of badgers and supposed little connection between the badger population on the Utrechtse Heuvelrug and Veluwe Nationaal Park, the result of two subpopulations with genetically different structures is consequential. Especially since Van de Zande et al. (2006), who studied badger populations from different regions in the Netherlands and using different loci, found coherence among badger populations. The coherence was in part explained by the relative 'connectiveness' of habitat by corridors. The population west of the A27 motorway contains fewer alleles for every locus in this study and consequently holds a lower gene diversity. The additional alleles in the eastern population might indicate gene flow between the badger population of the Utrechtse Heuvelrug and Veluwe Nationaal Park. However, genetic drift in the western population accompanied with limited gene flow from the eastern population might also be an underlying cause of the difference in alleles between subpopulations. If the latter is part of the driving factor in the genetic differentiation found in this study, then the A27 motorway can be considered a barrier dividing populations, despite efforts to reconnect the fragmented populations.

How corridors mitigate restricted gene flow in the fragmented habitat of badgers appears to be difficult to derive from this study. Factors such as the age of corridors, genetic diversity between generations and the length and complexity of badger dispersal (Roper et al. 2003) for example were not taken into account. Territorial behaviour and disperal behaviour of badgers in relation to corridors might further negatively affect gene flow. Corridors are often completely confined within one territory (Mulder 2016). With dispersing badgers often moving to a neighbouring territory (Roper et al. 2003), gene flow would in most cases only occur via the clan living near a corridor and possibly restraining gene flow.

Several individuals found on one side of the A27 motorway showed a genetic structure resembling that of the individuals on the opposite side of the A27 motorway. Successful colonisations may explain some of these exceptions as one of these concerns a badger who was caught on its sett just southeast of the village of Hollandsche Rading and the A27 motorway. This badger may successfully have migrated to the opposite end of the barrier. Most of the exceptions however (figure 2b) were badgers killed in close proximity to the A27 motorway. It is therefore likely that these badgers crossed the A27 motorway but were killed shortly after. These badgers may therefore unjustly be asigned to a subpopulation.

Collecting faecal samples for genetic population studies on badgers has proven to be a promising method. This method is non-invasive and linking individuals to main setts is found to be possible once accompanied with an elaborate field study for latrines. Faecal samples would be collected from established clans and the link to the area is therefore strong. As most badgers are killed in traffic during periods of high sexual activity, the samples collected from road kills may very well be from dispersing badgers, making it less reliable to link them to a particular area. Badger DNA was successfully extracted out of 76% of the faecal samples. Lammertsma et al. (2006) successfully extracted DNA from the faeces of otter (*Lutra lutra*) in 18% of the samples. Wilson et al. (2003) had a 89% success rate in badgers. Contrary to Wilson et al. (2003), not all faecal samples were collected for a consecutive period of time. A higher succes rate is therefore possible once adapting a method wherein latrines are carefully mapped prior to collecting samples and samples are collected when fresh.

As shown by Mulder (2016), roads act as barriers to badgers and consequently, major roads act as territory boundaries. Frantz et al. (2010a) found evidence that a motorway may also restrict badger movement. Corridors therefore do not only provide safe passage, but may also be of importance for communication possibilities between clans in a fragmented habitat. In this study several latrines were often found near corridors. Latrines undisputedly play a major social role in the life of badgers (Roper et al. 1986). They have been described as ways of communication between clans, as ways to attract mates and as ways to defend territories, setts or main foraging grounds. Latrines are assumed to play major roles in establishing territory boundaries. Male badgers were therefore found to show increased use of latrines (Kilshaw et al. 2009). Latrine use in autumn is assumed to be considerably male biased (Roper et al. 1993). The results of this study however show that use of latrines between sexes is nearly equal. This might indicate that latrines play a socially more important role during autumn, when these samples were taken, than previously assumed or that females take as much part in establishing territory boundaries as males. A more comprehensive molecular study, including more seasons, could shed more light on the actual difference in use of latrines between male and female badgers and how this changes throughout the year.

In this study we found two subpopulations on either side of the A27 motorway that differed in population structure and are slightly genetically differentiated, despite efforts to connect badger habitat by several corridors. In order to effectively conserve badger populations, the amount of possibilities for badgers to disperse and therefore opportunities for gene flow to occur may need to increase. Safe passage across barriers in the form of a corridor or 'traffic-calmed areas' (Jaarsma 1997) may be required for every territory alongside significant barriers. Obstacles such as major motorways significantly negatively affect safe passage. The effect of minor roads however should not be underestimated (van Langevelde et al. 2009). Further, we encourage efforts to collect tissue and faecal samples from badgers from different areas and years. Such samples could prove to be crucial to study fluctuations in genetic diversity and thus gene flow between subpopulations, and to be of vital importance to study the mitigating effects of corridors on population structure in the long run.

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Samenvatting

De invloed van barrières op de genetische structuur van dassenpopulaties in een versnipperd landschap

De toename van stedelijk gebied met de daarbij behorende infrastructuur zet flora en fauna verder onder druk. Dit geldt ook voor de das (*Meles meles*). Door fragmentatie van natuurlijk gebied wordt het totale leefgebied verkleind en raken leefgebieden geïsoleerd. De fragmentatie versterkt de effecten van genetische drift. Migratie, en daarmee de uitwisseling van genen (*gene flow*), wordt bemoeilijkt, waardoor steeds kleiner wordende populaties op zichzelf worden aangewezen. Faunapassages zijn toegepast om geïsoleerde leefgebieden weer te verbinden. Deze faunapassages zijn bewezen, effectieve middelen om verschillende soorten veilig barrières over te laten steken. Het is echter onbekend of faunapassages populaties ook zodanig verbinden dat de uitwisseling van genen weer hersteld wordt. Om de genetische variatie binnen een gefragmenteerde, met faunapassages verbonden, populatie vast te stellen is de genetische structuur van de dassenpopulatie ten noordoosten van de stad Utrecht onderzocht. Aan de hand van genetisch materiaal, afkomstig van dierlijk weefsel en uitwerpselen, is een studie uitgevoerd waarbij microsatellieten zijn gebruikt om individuen te herkennen en genetische variatie te meten. Uit de studie rond de Rijksweg A27 komt naar voren dat de populatiestructuur van dassen aan beide zijden van deze significante barrière opvallend blijkt te verschillen. De populatie ten westen van de A27 wordt ten opzichte van de oostelijke populatie gekenmerkt door enerzijds een vergelijkbare mate van heterozygositeit, maar anderzijds door een lagere diversiteit aan allelen. Ondanks de implementatie van verschillende faunapassages, lijkt de A27 meer dan eerder verondersteld mogelijke migratie te bemoeilijken, en daarmee de uitwisseling van genen, gezien de bestaande verschillen in de populatiestructuur aan weerszijden van de rijksweg.

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Skeleton of an otter (*Lutra lutra*) with some regular and remarkable alterations

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Abstract: On 24 April 2019 the remains of an otter (*Lutra lutra*) were found on the verge of a road on the island of Sula (Norway). About 95% of skeletal elements were collected. Inspection revealed several recently broken ribs and vertebrae, that may have led to the death of this individual. This paper describes the previously existing skeletal lesions, discusses the most probable cause of death, and attempts to estimate the age of the specimen. A comparison of skull measurements to those of otters from elsewhere in Europe (*n*=74) shows that this specimen is well within the 25th and 75th percentiles. Measurements of the baculum length / weight, shows that this otter was in the top 15% for European otters (*n*=65). Based on these comparisons, the specimen can be described as an adult male. Evidence of strong grinding of dentals and (pre)molars corroborates this estimation. The presence of a narrow strip of bone at the ventral part of the ischium increased the accuracy of the age estimation, which is set at four to five years. Besides a healed longitudinal fracture of the shaft of the left fibula, there were signs of severe arthrosis deformans on the femoral-tibia joints and erosion and enthesopathies were also noticed on the left tibia, all more extreme than on the right side. Three thoracal vertebrae showed erosion of the endplates and osteophytes to the stages of interlocking. The length of the osteophytes on each rim were quite regular. The breadth of the osteophytes, however, differed considerably. By describing these alterations, which can be described as 'normal' phenomena, this paper aims to contribute to increasing our knowledge of otters.

Keywords: *Lutra lutra*, otter, condylobasal length, postorbital constriction, baculum length, baculum weight, ventral bone-strip ischium, fibula fracture, arthrosis, osteophyte.

Introduction

On 24 April 2019 the remains of an otter (*Lutra lutra*) were found on the inland side on the verge of Road 606 on the island of Sula, on the north-western side of the Sognefjord, Norway (61°07'37"N 004°54'46"E, 12 m above sea level) (figure 1).

All of the recognisable skeletal elements were subsequently collected, resulting in the recovery of around 95% of the skeleton. Although speculative, it is estimated that more than nine months had elapsed between the individual's death and the skeleton being found.

After cleaning the remaining hairs from the skeleton, inspection revealed the characteristics of an adult specimen with several bone fractures of recent origin that may have led to its death, together with some lesions, including a healed fracture, arthrotic changes of different joints and bony outgrows (spurs or osteophytes) near the discal margins in several vertebrae.

Francois et al. (1995) define any bone out-

Figure 1. The location where the remains of an otter (*Lutra lutra*) were found on Sula (indicated by a red star), on the north-western side of the Sognefjord, Norway (from: Google Maps).

growth, but especially spurs around an osteoarthrotic joint, as an osteophyte. Macnab (1971) differentiates two types of osteophytes around vertebrae: the traction spur, with a shelf-like appearance, arising two millimetres or more from the discal margin, and the common marginal osteophyte. The latter is a claw-type spur, which has a broad base located at the discal margin and a claw-like configuration as it arches over the disc to eventually interlock with an opposing spur arising from an adjacent vertebra. While the marginal osteophyte seems to be related more to the collapse and loss of the width of the disc space, the traction spur appears to be primarily the result of abnormal motion. Both types of osteophytes, the claw-like as well as the shelf-like spur, are ossifications of the outermost annular fibers, some millimetres from the discal border (Macnab 1971). This paper describes the stages of development of the osteophytes on the vertebral margins, in accordance with the definitions by Morgan (1967), who studied the stages of osteophytes of the vertebrae in dogs (*Canis familiaris*). Stage one represented nodules (not ossified) located over intervertebral spaces and adjacent vertebral rims. Stage two consisted of small osteophytes on the vertebral margins. Stage three was characterised by larger bony projections with a cup shape but which did not extend beyond the vertebral end plate. Stage four was identified by the tip of the osteophytes extending beyond the edge of the vertebral body, without union between opposing osteophytes. In stage five bony fusion occurred between opposing osteophytes.

This paper attempts to estimate the age of the discovered specimen; describes the non-recent skeletal lesions and discusses its most probable cause of death.

The collected remains were added to the collection of the Dutch Natural History Museum in Rotterdam (Natuurhistorisch Museum Rotterdam) (NMR999000004336).

Material and methods

Heggberget (1984), observing histological sections of otter teeth, concluded that the number of dark-staining incremental cementum lines is a likely indicator of an otter's approximate age (in years). Hauer et al. (2000) presented an age pyramid of otters from eastern Germany based on the analysis of cementum annuli of canines (*n*=1027, males 58%) and concluded that only eight percent of the male otters were older than nine years, with the eldest specimen being over 15 years. However sectioning the teeth, requires the removal of a tooth and lessens the value of a skull for taxonomic purposes (Morris 1972). As a result, this destructive method was not applied in this investigation.

Dental wear has been used as a relative age indicator: the positive criterion for the oldest category (more than two - three years) is the exposure of the dentine of the upper first molar (Zeiler 1988). Another method of age determination is to examine the fusion of the epiphyseal line (*Cartilago physialis*[1]) of the postcranial bones. According to Zeiler (1988), the epiphyseal fusion in elderly otters can best be judged at the "narrow strip of bone at the ventral side of the ischium"; this line "remained unfused for up to 4-5 years in some cases. Only in animals older than 5 years was the fusion complete."

Van Bree et al. (1966) derived a set of parameters to determine the relative age of male otters: the condylobasal length and the postorbital constriction of the skull, and the length and weight of the baculum *(Os penis)*. They claimed to get reliable growth curves that can be used for relative age determinations, using a scatter diagram of the skull-index (postorbital constriction * condylobasal length^{-1}) and the baculum index (weight * length^{-1}). For the postorbital constriction lower values point to higher age categories, while for the other parameters higher values indicate older specimens.

Other parameters that can be helpful to distinguish otters within their first year of life from older ones, such as obliteration of cranial sutures, the size of the sagittal cranial crest and bone deposition around canine alveoles, are mentioned by Hauer et al. (2000).

To compare the present specimen, which was registered as NMR999000004336, with a larger dataset, besides the specimens described by Van Bree et al. (1966), some measurements of the skulls and bacula of other specimens are also included. These specimens came from the collections of the Natuurhistorisch Museum Rotterdam (NMR) (*n*=5) and one (skull only) from the collection of the Koninklijk Zeeuwsch Genootschap der Wetenschappen, Middelburg (KZGW). Of the specimens appended, one (NMR999000002556) came from Portugal, the others came from the Netherlands (table 1).

Following Hansen (1951, 1952) in this study each intervertebral space is identified by the serial number of its intervertebral disc, counted from the cranial to the caudal region. Between the skull and atlas (first cervical) there are two regular joints (without discs) and the atlas and the axis (the second cervical or epistropheus) cervical are connected by two regular joints. Therefore, the first intervertebral space with a disc (no. 1) is situated between the second and the third cervi-

[1] See note 1 of the *Nomina Anatomica Veterinaria*: "The Cartilago physialis is the plate of growing and calcifying cartilage between the Epiphysis and the Metaphysis during growth." (International Committee on Veterinary Gross Anatomical Nomenclature 2005).

Table 1. Characteristics of skulls and bacula of otter specimens from the collections of the NMR and KZGW. Abbreviations: CBL: condylobasal length, POC: postorbital constriction, LBAC: length of baculum, WBAC: weight of baculum, HCC: height of cranial crest, n.c.: not collected.*: in this specimen epiphyseal fusion was absent in all long bones, except for the proximal epiphyses of the fibulae.

Collection no.	CBL	POC	LBAC	WBAC	HCC
NMR999000001741	109.0 mm	13.0 mm	68.9 mm	2.8 g	1.4 mm
NMR999000002001	115.4 mm	18.1 mm	n.c.	n.c.	1.0 mm
NMR999000002556	114.7 mm	14.6 mm	n.c.	n.c.	1.3 mm
NMR999000003543*	120.2 mm	14.3 mm	> 40 mm	> 0.4 g	1.0 mm
NMR999000003675	119.4 mm	15.5 mm	62.0 mm	1.8	1.8 mm
NHG26170	119.6 mm	14.5 mm	n.c.	n.c.	2.0 mm

cal vertebra, and the following discs are numbered consecutively.

The perimeter of the vertebral margin was divided into four sectors that were designated as dorsal, ventral, right lateral, and left lateral (Morgan 1967). The distribution of the position of spurs on the vertebral margin was determined along its perimeter. In this study, bony outgrowths along the rim of the vertebral body were considered as one spur when they were separated by each other by clefts deeper than half of the least length of one of the neighbouring spurs.

The official terms used in international veterinary nomenclature follow the *Nomina Anatomica Veterinaria* (International Committee on Veterinary Gross Anatomical Nomenclature 2005) which has been followed in this paper. In order to avoid confusion by using terms with unclear meanings this article uses the glossary for rheumatic spinal diseases developed by François et al. (2005) for describing physiological conditions or pathological phenomena.

Results

Age estimation

The condylobasal length and the postorbital constriction of the skull of specimen NMR999000004336 measured 113.7 mm and 13.8 mm, respectively while the baculum measured 68.9 mm and weighed 3.9 g.

Figure 2 compares the parameters for the condylobasal length and postorbital constriction of this specimen with 80 male specimens from Denmark, the Netherlands and France. In these boxplots specimen NMR999000004336 is between the 25th and 75th percentile (both within the box), indicating that this animal had full-grown status.

Figure 3 shows box plots that rank the length and weight of the baculum of this specimen in comparison with 67 male specimens from Denmark, the Netherlands and France. In these boxplots specimen NMR999000004336 was ranked in the uppermost echelons, indicating a full-grown and a sexually mature status.

The maximal dimensions of the cranial crest of the recovered specimen (NMR999000004336) measured 3.6 mm, which when compared with the cranial crest dimensions of six other male otters (table 1), corroborates the impression the specimen was a fully-grown specimen. There were no visible cranial sutures (figure 5a).

The upper incisors and most of the upper premolars were evenly ground off. Both upper canines were also ground off at the tip of the dental crown exposing the dentin texture, while the attrition of the right one had almost

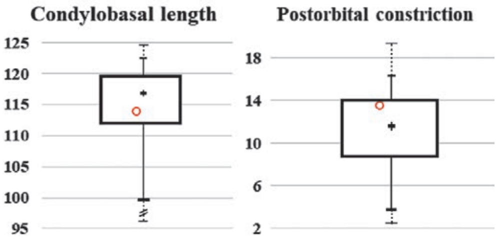

Figure 2. Boxplot of the condylobasal length (left) and the postorbital constriction (right), both based on 74 males, derived from Van Bree et al. (1966) supplemented with six additional specimens; the minimum condylobasal length is 61 mm. The position of specimen NMR999000004336 is marked with a red circle.

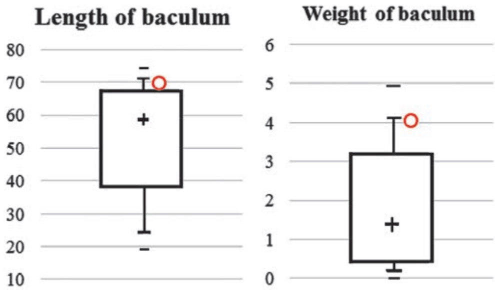

Figure 3. Boxplot of the length of the baculum based on 65 male specimens, derived from Van Bree et al. (1966) supplemented with two additional specimens. The position of specimen NMR999000004336 is marked with a red circle.

reached the pulp cavity (figure 5b). The left as well as the right fourth upper premolars showed a grinded ridge over the edge of the total length of the coronal cusp exposing the dentin texture. Both of the upper molars were heavily grinded, and the left one was partially fractured, exposing a cavity with grinded edges. Both third lower incisors were grinded: the central and middle lower incisors on the right side were fractured (and worn) and those on the left side were evenly worn down. Both lower canines were also heavily grinded at the tips, the right one even exposing a pinhole connection to the pulp cavity. The lower unicuspid premolars all were grinded, the right one more than the left, while the crown of the left second premolar was fractured and the remaining posterior root showed a ground surface. The fourth lower premolars had ground ridges over the whole length and the lower molars were ground to a lesser extent.

A comparison of this pattern of dental wear with the mandibular tooth-wear pattern used by Zeiler (1988), suggests that this otter specimen was at least three years old.

All the epiphyseal lines, were completely fused, except the strip at the ventral side of the ischium, indicating an age of 4-5 years (figure 4).

Fractures

Fractures, without any signs of healing were present on two left ribs (C5 & C13) and thirteen of the right ribs (C2C14). The tips of the spinal processes of the 4th to 7th cervical vertebrae and the 1st to 3rd and 5th thoracic vertebrae were fractured. The spinal processes of the 5th and 6th lumbar vertebrae were also fractured, with the distal fragments still attached and clearly bent towards the left side. The left scapula was fractured in seven parts, while the right one had a longitudinal fissure in the caudal part of the spine of the scapula. The outline of the shaft of the left fibula showed a fusiform thickening and, on close observation, a healed longitudinal fracture was detected (figure 6).

Arthrosis

The right femur showed a slight proliferation of the edge of the medial and lateral condyles, with the articular surface left intact. The distal part of the left femur exhibited proliferation at the rim of the anterolateral and anteromedial

Figure 4. Part of the left pelvis of the recovered otter (ventral view). The unfused strip was attached to the ischium at the Ramus ossis ischii (see also note 56 of the N.A.V.[2]).

condyles. The medial plateau of the right tibia had several circular pits, varying in diameter. On the left tibia an almost circular osseous proliferation was attached to the rim of the articular surface of the plateau. The lesion of the articular surface could be described as severe arthrosis deformans (figure 7). Large calcifications of tendon attachments (enthesophytes) were found on the dorsal side of the proximal end of the left tibia, and the proximal side of the left fibula was completely fixed with proliferations of the tibia. The right fibula showed a slight proliferation at the connection with the tibia on the proximal side.

Both of the humeri were without arthrotic signs, however the left ulna and each caput radii showed minimal arthrotic reactions around the rims of the proximal radioulnar joints.

Osteophytes

Several of the thoracic vertebrae showed extended new bulging at the rims, which were most prominent in the caudal thoracic vertebrae. There was erosion of the epiphysis on the thoracic vertebrae, bordering intervertebral space 19 (figure 8). Several thoracic vertebrae had spurs on the rims of the intervertebral spaces: light, stage II (intervertebral space 9), intermediate with kissing spines, stage III (intervertebral space 16) and severe, with interlocked spines, stage IV (intervertebral space 19) (stages after Morgan 1967) (figure 8).

The vertebrae affected with these types of spurs were positioned well before and after the transition zone of the anticlinal vertebra (*Vertebra anticlinalis*)[3], while the centre of this zone was free from these types of spurs (figure 9).

The length of the osteophytes on each rim were quite regular: the spurs along the same rim had similar measurements. The breadth of the osteophytes, however, differed considerably. The length of the spurs on the vertebral margin along the perimeter of the 9th, 16th and 19th intervertebral spaces was most prominent

[2] Note 56 of the *Nomina Anatomica Veterinaria*: "*Corpus ossis ischii, Ramus ossis ischii*. According to the N.A., Ramus ossis ischii denotes the part that was previously called Ramus symphysialis by veterinary anatomists. The qualifying "symphysialis" is now unneccesary because there is only one Ramus; the former Ramus acetabularis is included in the Corpus ossis ischii" (International Committee on Veterinary Gross Anatomical Nomenclature 2005).

[3] See note 38 of the *Nomina Anatomica Veterinaria*: *Vertebra anticlinalis*. This is the first vertebra in the caudal thoracic or lumbar region that has its Processus spinosus perpendicular to the body of the vertebra (International Committee on Veterinary Gross Anatomical Nomenclature 2005).

Figure 5. Skull of the recovered otter. A. Dorsal view. Note the absence of cranial sutures and the bone deposition around canine alveoles. B. Ventral view. Note the abrasion of the tip of the dental crowns exposing the dentin texture.

Figure 6. The left tibia and fibula complex of the recovered otter (*Lutra lutra*) with the remains of a longitudinal fracture surrounded by callus leading to a fusiform thickening of the fibula.

in the lateral sector and less so in the ventral sector; there were no spurs in the dorsal sector of the rims.

The margins of the caudal and cranial vertebral extremities facing the 17th, 18th and 20th intervertebral spaces presented the onset of shelf-like osteophytes which were irregular in length (measuring at most 1.3 mm).

At the cranial side of the sacral-caudal transition (intervertebral space 29) two continuous, shelf-like osteophytes were present on the left and right lateral sides. At the caudal side of this intervertebral space, one continuous shelf-like osteophyte was visible at the lateral and ventral sectors, with only the dorsal sector remaining free of osteophytes.

There were no signs of arthrosis of the costovertebral joints on the thoracic vertebrae, whereas on the superior parts of the zygapophyseal joints of the 2nd to 5th and 8th to 13th thoracic vertebrae only minor erosions were present. Minimal, symmetrical, osteal reactions were present at the caudal rims of the sacroiliac joints.

Discussion

Reading the values of the scatter diagrams from figures 1 and 2 in Van Bree et al. (1966) casts doubts on the relevance of using skull measurements (condylobasal length and postorbital constriction) for age estimations: the correlations of these measurements for male otter skulls could be calculated as – 0.57 for specimens from Denmark (n=41) and – 0.61 for specimens from the Netherlands (n=33). The negative value of these coefficients is due to postorbital constriction lessening in higher age categories. Correlations of the scatter dia-

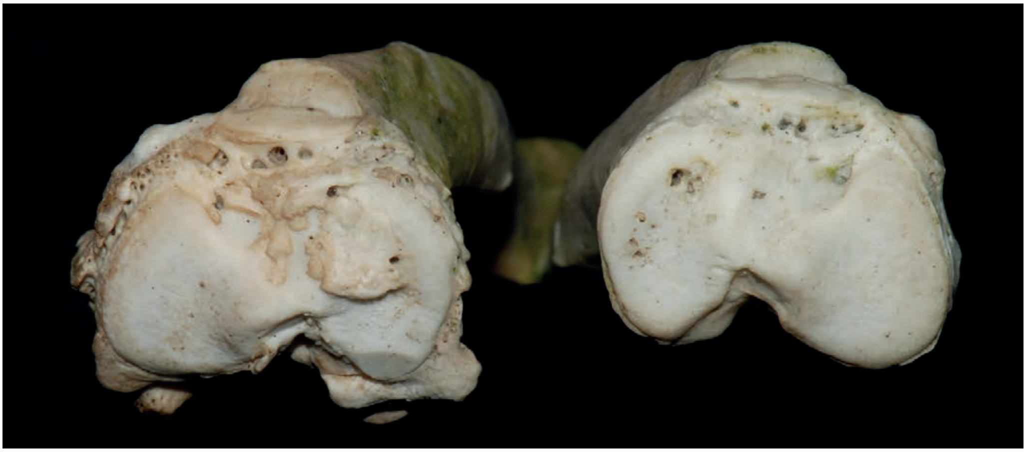

Figure 7. Proximal view of the left and right tibia plateau of the recovered otter (*Lutra lutra*). Note the severe arthrosis deformans around the left plateau (described in the text).

gram in figure 3 in Van Bree et al. (1966) of the length and the weight of bacula are much stronger for the combined specimens from Denmark (n=43), the Netherlands (n=10) and France (n=12), this correlation was calculated at 0.93. High values of condylobasal length do not seem to be an excellent predictor for old age in specimen NMR999000003543* (see table 1), although the condylobasal length equalled 120.2 mm, the epiphysis was not fused with the diaphysis in the long bones, except proximally in the fibulae. According to Zeiler (1988) epiphyseal fusion status of specimen NMR999000003543 would give it an age of barely two years.

The age of the described otter is not precisely known, however comparing the measurements of all age-related characteristics, apart from the condylobasal length and postorbital constriction with measurements of large groups (n=81, n=67), clearly indicates this specimen to be fully mature. The other skull parameter, the height of cranial crest, reaches the highest value of a rather limited number (see table 1). The wearing of the teeth, especially of canines and molars, suggests that the specimen was at least three years old. Based on the epiphyseal lines, which were all completely fused, except the strip on the ventral side of the ischium, the specimen's age can be estimated as between 4 to 5 years.

Heggberget (1984) mentions that there have been no studies done which have managed to establish the true age classes over the whole age spectre of a wild population of otter. Later on, Hauer et al. (2000) presented an age pyramid in their study of otters in eastern Germany over the period 1957 to 1998, however this was biased, as it was predominantly based (69%) on traffic victims. Kruuk et al. (1997) found a change of mean age in Scottish otters based on an increasing proportion of immature otters. Since the reintroduction of otters in the Netherlands in 2002 the population has steadily increased (Lammertsma & Niewold 2016), an increase which should be reflected in higher age categories. Recognition of elderly specimens is therefore important, as these could indicate a stable population (Hauer et al. 2000).

The recent fractures of ribs, scapulae and vertebrae observed in the retrieved otter specimen are most likely the results of a traffic accident that lead to death of the specimen. Also the location of the remains on the road verge supports this supposition. Jancke & Giere (2011) found most otter road accidents in Uckermark County (Brandenburg, north-east Germany) were located close to one or more lakes. In this case here, the road

Figure 8. Caudal (left) and cranial (right) views at the 9th, 16th and 19th intervertebral spaces. Note the bony protuberances (stages III and IV, after Morgan) at the margins of the caudal and cranial sides of the vertebral bodies. The epiphyseal surfaces themselves are rather smooth, only the vertebrae bordering intervertebral space no. 19 showed signs of erosion.

Figure 9. Left lateral view of the spinal column of the recovered otter (*Lutra lutra*) around the transition zone (t) of the diaphragmatic (left of 't') and anticlinal vertebra (right of 't') which have backward and forward sloping spinous processes respectively. Arrows indicate the osteophytes at the 16th and 19th intervertebral space (left and right respectively).

verge where the specimen was found was 21m away from the shoreline of the fjord. Jancke & Giere (2011) also found an almost linear relationship between the maximum permitted speed for motor vehicles (between 30 km/h and 100 km/h.) and the number of otters killed. Although the physical characteristics of road 606 at the spot of specimen retrieval (an unclear bend, near the top of the hill) would limit the speed of motor vehicles to 40-50 km/h, the limited vegetative cover along the road suggest either a direct hit or a return of the disturbed animal to the road, ending up with the fatal collision.

The extra formation of bone (callus) on the left fibula is the typical end stage of a healed fracture, suggesting that this fracture occurred at least several months before the animal's death. Healed fractures in feral mammals are quite regularly encountered: living in the wild can be risky. In otters traumatic injuries, principally caused by road traffic accidents and intra-specific aggression, also are common (Simpson 1997). The cause of the fibula fracture in this case is not likely to be the consequence of a road accident: the impact of a motor vehicle on a hind leg would be too massive to be restricted just a fracture of the fibula and not of the tibia. Intra-specific bite wounds are usually to the face, feet and anus/genitals (Simpson & Coxon 2000). Based on these finds and observations, it is quite possible the fibula fracture originated from such an intra-specific aggression encounter which subsequently healed.

The asymmetrical affection, predominant on the left side of the arthrosis of the tibiae (and, on a more modest level of the femora), hints to its origin: the fibular fracture would have caused a limping movement for a longer time, perhaps permanently. This condition ended up in severe arthrosis deformans.

The degenerative process at the thoracic vertebrae – the erosion of the endplates, osteophytes till the stages of interlocking and shelf formation – also illustrates the condition of this specimen. In other mammal species (e.g. dogs) the prevalence of spondylarthrosis (spondylosis deformans) accompanied by osteophytes is common among elderly specimens (Morgan 1967). It is plausible, but not certain, that there is a causative relation between the fibula fracture and the arthrotic knee on the left side. However, claiming a relation between the impaired movements, caused by the left hindquarter and the development of vertebral alterations in this otter specimen, is still speculative.

The prevalence of different appearances of arthrosis deformans in otters is unknown. Until now no cases of arthrotic malformations in otters have been described in the literature (see e.g. Gulland et al. 2018). Alterations, eventually accompanied by arthrosis deformans, seem to be considered as just a 'normal' phenomenon and perhaps for that reason have not previously been described (Anonymous 2004). This case gives a good opportunity to fill up part of this lacuna.

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Samenvatting

Skelet van een otter (*Lutra lutra*) met enkele gewone en opmerkelijke veranderingen

Op 24 april 2019 werden op het eiland Sula, aan de uiterste noordwestkant van het Sognefjord (Noorwegen), de overblijfselen van een otter (*Lutra lutra*) gevonden langs de kant

van de weg. Ongeveer 95% van de skeletelementen werd verzameld. Inspectie bracht verschillende gebroken ribben en wervels van recente oorsprong aan het licht, die ongetwijfeld de dood van dit exemplaar veroorzaakten. Op basis van schedelmetingen en lengte- en gewichtsmetingen van het penisbot kon het gevonden specimen worden beschreven als een volwassen mannetje. Slijtage van de tanden en kiezen wezen op een geschatte leeftijd van meer dan drie jaar. Aan de hand van een nog losliggende smalle strip bot, dat nog niet was vastgegroeid aan de onderzijde van het bekken, was het mogelijk de leeftijd nauwkeuriger in te schatten op vier tot vijf jaar.

Naast een genezen botbreuk van de schacht van het linker kuitbeen, werd bij het linker kniegewricht een ernstige artrose gevonden. Zo vertoonde het linker scheenbeen uitgebreide botwoekeringen op het gewrichtsplateau met daarnaast ook erosies ten gevolge van slijtage. Verder waren de peesaanhechtingen aan de achterzijde gedeeltelijk verkalkt. Beide dijbeenderen onthulden aan de gewrichtsknobbels botwoekeringen, in het bijzonder aan de linkerkant. De borstwervels vertoonden erosies ten gevolge van slijtage van de eindplaten en slijtage verschijnselen van andere borstwervelgewrichten. Aan de randen van de tussenwervelruimten 9, 16 en 19 werden diverse stadia van haakvormige uitgroeiingen vastgesteld, in de laatste met in elkaar grijpende vergroeiingen. De lengte van de uitgroeiingen op elke rand was vrij regelmatig, de breedte verschilde echter aanzienlijk. De uitgroeiingen op de wervelkolom langs de omtrek bij alle drie tussenwervelruimten waren het meest duidelijk in de zijwaarts gerichte sectoren, minder in de ventrale delen en afwezig in het rugwaartse deel van de randen. Het beschrijven van de gevonden afwijkingen, die als ‘gewone’ vondsten afgedaan zouden kunnen worden, is van belang omdat deze veel voorkomende afwijkingen kunnen bijdragen aan het correct duiden van de leeftijd van individuele dieren. Deze vaardigheid is noodzakelijk voor het juist beoordelen van de leeftijdsopbouw van populaties otters.

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A nine month small mustelid survey across four research sites in the Netherlands

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Abstract: There is a general concern amongst ecologists about the apparent decline of small mustelids in The Netherlands over the past decades. However, little is known about their actual historic and present numbers. To better understand their ecology and the cause of the decline more research into these small predators is a necessity. Between October 2016 and August 2017 four different research sites in the vicinity of Deventer, the Netherlands, were investigated by surveying 64 locations with camera-trap boxes and nest boxes fitted with tracking tunnels with the intention to gather distributional data of small mustelids common weasel (*Mustela nivalis vulgaris*) and stoat (*Mustela erminea*). Three locations were chosen for their small-scale agricultural landscapes, regarded as prime habitat for the target species. One location concerned an open large-scale intensively managed agricultural landscape, regarded as degraded habitat. Weasels were found with both camera-trap boxes and nest boxes but the footprints of weasel and stoat from tracking tunnels could not always be identified to species. Zero stoats were found during this study. Stoats are either very rare or not present at the time of research. Research materials proved not to be suitable for Western polecat (*Mustela putorius*) and other larger mustelids as the limited diameter of the entrance tubes used in the research materials averts entry. There was no clear difference in number of weasel recordings between large-scale agricultural landscape (two recordings) and small-scale agricultural landscapes (one, five and zero recordings) that were included in this study. Striking is the fact that most observations occurred between March and October. The outcome of this study concludes that small mustelid studies require innovative research materials, time consuming methods and harvest meagre results. Based on historical data and recent research results, including this study, it is not possible to provide a better foundation for the status and trend of weasel and stoat. The available data is too minimal. In order to get a better understanding of current status and population trends large-scale, long-term monitoring studies with camera-trap boxes are recommended.

Keywords: common weasel, stoat, Western polecat, stone marten, camera-trap box, nest box, large-scale agricultural landscapes, small-scale agricultural landscapes.

Introduction

Intensification of land use and large-scale agriculture has increased considerably in the Netherlands in recent years, which is the main cause of habitat loss for common weasel (*Mustela nivalis vulgaris* - hereafter called weasel), stoat (*Mustela erminea*) and Western polecat (*Mustela putorius* – hereafter called polecat) and the main reason why small mustelid populations have probably been in decline (Criel

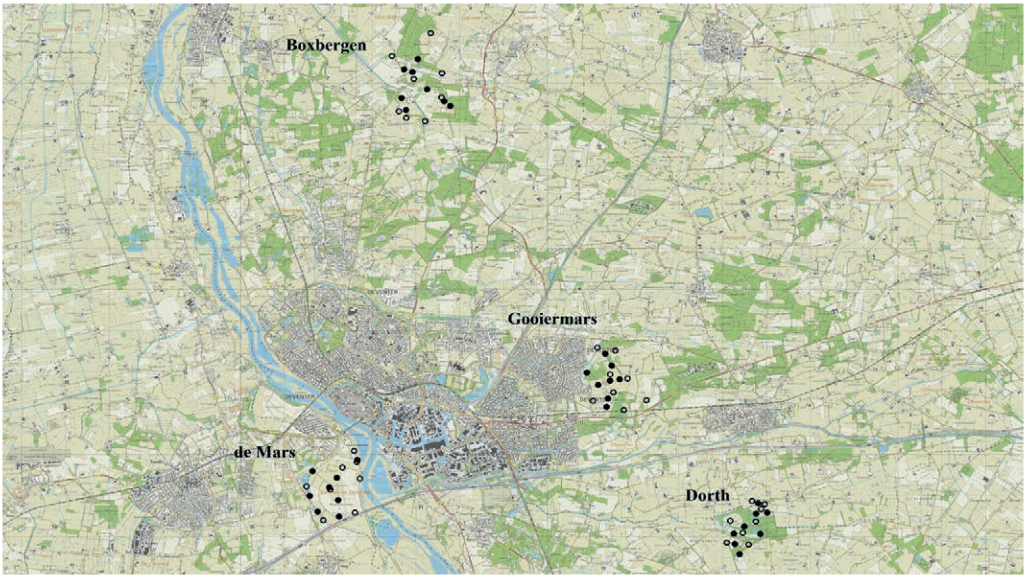

Figure 1. The location of the four field sites around the city of Deventer, the Netherlands. The open dots are locations that were surveyed in period one and two, the closed dots were surveyed in period three and four.

1990, Lange et al. 1994, Twisk et al. 2010, van Maanen et al. 2013, Bouwens 2017, Jonker 2017, Verschoor & Rozema 2017). There is a structural lack of information about the occurrence of small mustelids in the Netherlands (Jonker 2016, Bouwens 2017, Veldman & Troost 2019). To better understand their distribution and habitat use intensive innovative research is a necessity. This study was initiated by the Dutch Mammal Society to do just that and share knowledge and experiences (Westra 2017). In the past fifteen years new research methods are being developed for the notoriously difficult-to-study species group of the small mustelids: Scentinel (King et al. 2007), Camera-trap box or 'Mostela' (van Maanen et al. 2015a), Nest box (Smaal & van Maanen 2017), Audio lures (Schep 2018), Struikrover (van Uchelen & Smaal 2019). This study aims at carrying out a standardised survey, with two of these methods: the camera-trap box and the nest box. The camera-trap box is a box fitted with a camera-trap with short focus lens and open with tubing on one side luring explorative small mustelids in. The nest box is designed as a suitable nesting shelter for small mustelids and is fitted with a tracking tunnel to monitor its use.

This paper presents the results obtained in three sites within small-scale agricultural landscapes regarded as prime habit at for small mustelids and in one site within large-scale agricultural landscapes regarded as degraded or unsuitable habitat. The hypothesis is that, based on landscape features and habitat suitability, small mustelids are present at the first three sites, and not present at the fourth site. If so this may lead to a better understanding and support base for landscape management measures to improve habitat suitability for small mustelids. The main goals were to use camera-trap boxes and nest boxes as research methods for surveying weasel and stoat and to confirm that the research methods are unsuitable for surveying other mustelids. Also a comparison of the obtained results between small-scale agricultural landscape and large open agricultural landscape was desired to test the above hypothesis. Furthermore, this study could provide details about the time of year and in which microhabitat small mustelid surveys are most successful.

Figure 2. Examples of small-scale extensively managed agricultural landscapes at Dorth estate (A) and Gooiermars (B), and an example of large-scale intensively managed agricultural landscape at de Mars (C).

Materials and methods

During a nine month period between October 2016 and August 2017 four different research sites were investigated by surveying 64 locations, 16 within each of the research sites. The research sites were located in the vicinity of the city of Deventer in the province of Overijssel in the east of the Netherlands (figure 1).

Research sites

I selected research sites based on terrain characteristics, after checking aerial photographs, consulting with site managers and extensive field checks. Three sites - Boxbergen, Gooiermars and Dorth - consist of small-scale extensively managed agricultural landscapes with a pattern of linear shaped bushes, ditches, hedgerows and woodlands (figure 2A and 2B). The fourth site - de Mars - is a large-scale intensively managed agricultural landscape with mainly open grassland and some hedges (figure 2C).

Research site 1, Dorth (52.226693 N; 6.290673 E), falls within the category of small-scale agricultural landscape. It is an estate managed by Natuurmonumenten (Dutch Society for Nature Conservation) with a size of roughly 180 ha and situated along the stream Dortherbeek in the province of Gelderland. It contains woodland with historical open

Figure 3. One of the field locations at research site Gooiermars. A hedgerow as an example microsite as used in this study.

waters like moats, ponds, and streams and sandy soil with mixed deciduous and pine forest and extensively managed grasslands. The landscape can be characterised as confined and natural with intensively managed agriculture in the surrounding areas.

Research site 2, Gooiermars (52.256527 N; 6.244048 E), falls within the category of small-scale agricultural landscape (figure 3). It consists of wetland, woodland and grazelands with a size of 200 ha, managed by IJssellandschap (a local foundation for landscape management) and Bannink (local estate). The low lying area is known as the origin of the Zandwetering, a local waterway running westwards towards the river IJssel. The terrain is not accessible for recreational use. Management is aimed at nature and watershed conservation.

Research site 3, Boxbergen (52.320099 N; 6.184706 E), falls within the category of small-scale agricultural landscape. It is a century old estate with an area of roughly 220 ha and is managed by IJssellandschap. Forestry and cattle farming dominate land use in Boxbergen. Slightly sloping sandy soils in the north are overgrown with old growth deciduous forests. The southern part is a mosaic of grassland, pastures and forest. Most agricultural activity is managed intensively and borders between forest and open landscape are sharp.

Research site 4, de Mars (52.235523 N; 6.153145 E), falls within the category of open large-scale agricultural landscape. It is a flood plain of the river IJssel. It has an open typography with grassland and sugar beet farming. De Mars has an area of 200 ha and is managed by IJssellandschap. The soil consists mainly of fluvial river clay that is exploited by intensive large-scale agriculture. Linear shaped landscape features are confined to a few narrow hedges and ditches of which most are situated in the west. The management is aimed at agriculture and wet grassland birds.

Research materials

Camera-trap box

The so called 'Mostela' camera-trap box used in this survey is an instrument which allows camera-trap surveys in thick undergrowth, where otherwise camera trapping is very difficult or impossible. Small mustelids are shy

Figure 4. A camera-trap box designed for surveying small mustelids.

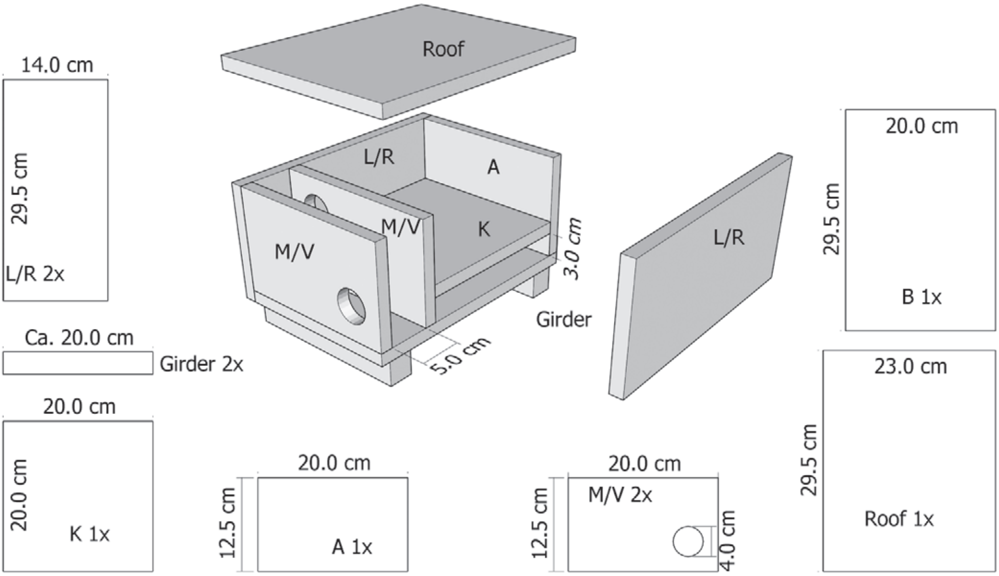

Figure 5. Schematic drawing of a nest box designed for surveying stoats (drawing reconstructed from Criel 1986).

and swift creatures that live most of their lives in dense cover where they are hard to find. This species group is rarely encountered with regular camera-trap surveys and therefore the camera-trap box was developed by the small mustelid workgroup of the Dutch Mammal Society (van Maanen et al. 2013).

The camera-trap boxes consisted of a wooden box measuring 60 cm in length, 30 cm in width and 20 cm in height (figure 4). On one short side of the interior a camera-trap (type Bushnell Trophycam 2016) was placed fitted with a short focus lens apprehended from +2.5 reading glasses. Across from the camera, on the opposite short side there was an open tube with a diameter of 8 cm running through the box where animals can enter or exit the box on both sides. In the middle of the box fish oil was applied as an attractant. The short focus lens allows the camera-trap to capture sharp images of animals that enter the box. Most of the infrared flash LED bulbs of the camera had to be obscured by duct tape to prevent overexposure of the images. A sticker with location, camera number and coordinates was put inside the camera-trap boxes so that this information was captured on footage each time the camera was triggered.

Nest box

The nest box used in this survey was first developed by the Belgian National Campaign on Protection of Predators in the 1980s (Criel 1986). At the time the aim of the development of this nest box was to be able to quickly create more refuges in an area that is prone to ecological restoration. In recent years the concept was revised to be able to employ the nest box for ecological research (Smaal & van Manen 2017).

The nest box measures 30 cm in length, 20 cm in width, 12.5 cm in height and stands 5 cm off the ground by two small girders (figure 5). Its interior has double flooring and a separate nesting compartment (20x20 cm) to further increase insulation. The entrance consists of a round opening with a diameter of 4.5 cm onto which a separate plastic pipe with a diameter of 8 cm and a length of 40 cm is connected. The measurements of this nest box were customised for stoat but since weasels are similar or smaller in size they also fit inside. The plastic pipe fitted to the front of the nesting box was used as a tracking tunnel by inserting in a small plank on the bottom of the tube with an ink pad situated in the middle. The pad was filled with a mixture of paraffin oil and graphite powder functioning as the 'ink'. Animals entering the nest box walked over the pad leaving footprints on the plank. The plank was painted with blue coloured primer to increase visibility of footprints of animals entering and to be able to easily wipe the plank clean after each check.

Sampling procedures

The exact field locations were chosen on the basis of suitable microhabitats that are important for small mustelids (see figure 3). Suitability of the microhabitats was based on tracks and signs of mustelids that were encountered at the research sites as well as habitat requirements for small mustelids as stated in literature (Lange et al. 1994, Boshi et al. 2015, Twisk et al. 2016, van Tongeren 2017, Westra & Kuiters 2018). Within each research site 16 different locations were sampled randomly, eight with camera-trap boxes and eight with nest boxes fitted with a tracking tunnel. The camera-trap box and nest box locations were often placed in densely overgrown linear structures, such as wooded banks, wood girdles, hedges, forest edges or overgrown ditches. Heaps of pruned branches, cairns and messy farmyards were also chosen as field locations. If there was no sufficient vegetation coverage the research materials were covered with branches and leaves. The research materials were deployed approximately equidistant from each other (between 200 and 300 metres as the crow flies), to enable a regular distribution of sampling units over the research site.

Each of the four research sites were surveyed identically. Research materials were deployed on a total of 64 locations regarded as potential habitat for small mustelids. A total of 16 camera-trap boxes and 16 nest boxes were used simultaneously. In October 2016 four camera-trap boxes and four nest boxes were placed in each of the four research sites. They remained at the first location for roughly 20 weeks until March 2017. An interim check was done halfway, after roughly ten weeks, in January 2017 replacing memory cards in the camera-trap boxes and photographing footprints obtained

Figure 6. Footprints of weasel and / or stoat as obtained by using the plastic pipe at the front of the nesting boxes as a tracking tunnel with a small plank and an ink pad. Male weasel prints and female stoat prints overlap in size, making them impossible to identify at species level.

from tracking tunnels attached to the nest boxes. In March 2017 all 16 camera-trap boxes and 16 nest boxes were moved within each of the four research sites to new locations. At the second location, again, they remained in place for roughly 20 weeks until the end of July / beginning of August when they were removed from the field and the study was terminated. An interim check at the second location was done half way, after roughly ten weeks, at the end of April replacing memory cards and photographing footprints. The ink pads were checked one extra time during the second sampling period in the first half of June. At each check batteries in the camera-traps were replaced if the indicator showed less than two out of four bars. Salmon oil was also refreshed in the camera-trap boxes during each of the two interim checks. The tracking tunnel attached to the nest boxes was wiped clean and the mixture of paraffin oil and graphite was re-applied on the ink pad. Animals were not trapped or handled in any way for this survey so no Dutch laws or regulations on animal welfare apply.

Data analysis

Footprints from the tracking tunnels attached to the nest boxes were analysed in the field and photographed for later species identification in the lab by consulting reference material and literature (van Diepenbeek 2003, 2013, Agnew 2009, Vercayie 2013). However, the shape and size of large male weasel prints and small female stoat prints overlap. This makes it sometimes impossible to identify these prints to species level. In that case I recorded these prints as "weasel/stoat" (figure 6). Photographs of the footprints collected in this study were presented to tracking experts Annemarie van Diepenbeek and Jeroen Kloppenburg for quality control.

Data on memory cards of the camera-trap boxes were inspected on a computer and all information was inserted into a single spreadsheet. Location and date was noted for every species the camera-trap recorded. This study concentrated on gathering distributional data and therefore multiple detections in camera-trap boxes were not archived.

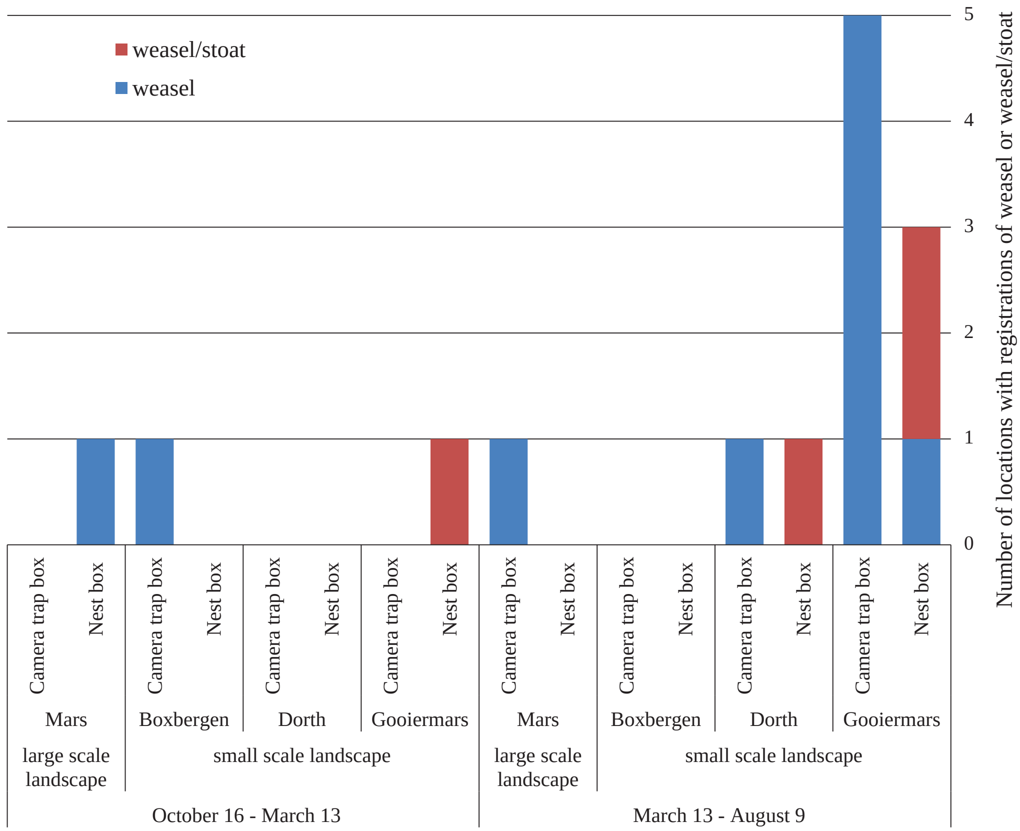

Figure 7. Overview of results at the four research sites. Note that the number of days the devices were active were not exactly the same.

Table 1. Overview of results per research site.

Research site	Category	Results
1. Dorth	Small-scale agricultural landscape	One weasel was registered in a camera-trap box and prints of one weasel/stoat were found in the tracking tunnel of a nest box. Additionally a young stone marten showed itself in one of the Camera-trap boxes.
2. Gooiermars	Small-scale agricultural landscape	Weasel was detected at five locations and weasel/stoat was detected at three locations (figure 9). A polecat showed itself in one of the camera-trap boxes.
3. Boxbergen	Small-scale agricultural landscape	Weasel was detected at one location with a camera-trap box. The camera-trap boxes registered a total of 160,000 photos at Boxbergen, of which only one single photo was taken of a weasel. No results were achieved with nest boxes.
4. de Mars	large-scale open agricultural landscape	Weasel was detected at two locations, one in a camera-trap box and one in a nest box.

Table 2. The average number of days until the first registration of weasel with Camera-trap boxes.

Research site	Species	Research instrument	Start	First registration	Number of days
Boxbergen	weasel	Camera trap box	24-01-17	06-03-17	41
Gooiermars	weasel	Camera trap box	09-03-17	11-03-17	2
Dorth	weasel	Camera trap box	10-03-17	04-04-17	25
Gooiermars	weasel	Camera trap box	20-04-17	06-06-17	47
Gooiermars	weasel	Camera trap box	20-04-17	14-05-17	24
Gooiermars	weasel	Camera trap box	20-04-17	28-04-17	8
Gooiermars	weasel	Camera trap box	20-04-17	26-07-17	97
De Mars	weasel	Camera trap box	23-04-17	19-07-17	87
Average number of days until first weasel registration					41

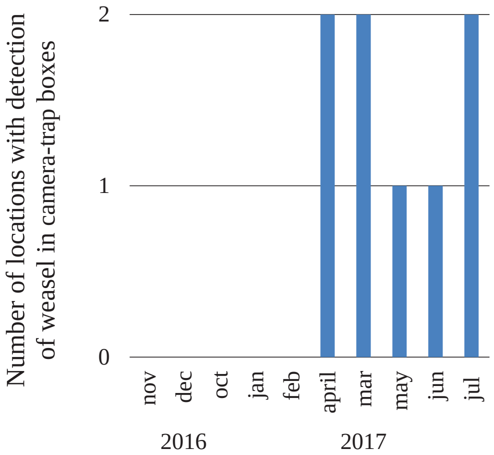

Figure 8. Overview of the number of camera-trap box registrations of weasel per calendar month

Results

During the study one camera-trap box disappeared at a location in research site Boxbergen. Therefore, from a total of not 32 but 31 locations camera-trap deployments (photo series) were collected. Once an SD memory card was found to be defective at a location in research site in Dorth, causing the sample size of the deployment to be halved. In total I collected approximately 490,000 images of 21 species in 3597 sampling days. The 32 nest box locations were interim checked once but the 16 nesting boxes were checked one extra time at the second locations. So a total of five sampling periods can be distinguished: period 1 and period 2 at the first research locations and period 3, period 4a and period 4b at the second research locations. Two nest boxes were flooded during one of the sampling periods in the study, making the tracking tunnel unusable. In a total of 4367 sampling days with 16 nest boxes times five sampling periods minus two flooded nest boxes results in photographs of 78 tracking tunnels. The footprints were studied and identified to species level if possible. The total sampling nights is 7964 for camera-trap boxes and nest boxes combined. An overview of the results at the four research sites is given in table 1 and figures 7 and 8.

Number of locations with observations and observations per research period

Results were achieved at eight out of 31 camera-trap box locations registering weasel (7x), polecat (1x) and stone marten (1x). In addition, results were obtained with five out of 32 nesting box locations, in which weasel prints (2x) and weasel/stoat prints (4x) were registered. As a combined total weasel was registered at nine separate locations (figure 9), weasel/stoat at four separate locations, stoat at zero locations, both polecat and stone marten once. By far the most results can be seen in the last six weeks of survey (figures 10 and 11).

Calculating the average number of days until first detection shows that it takes an

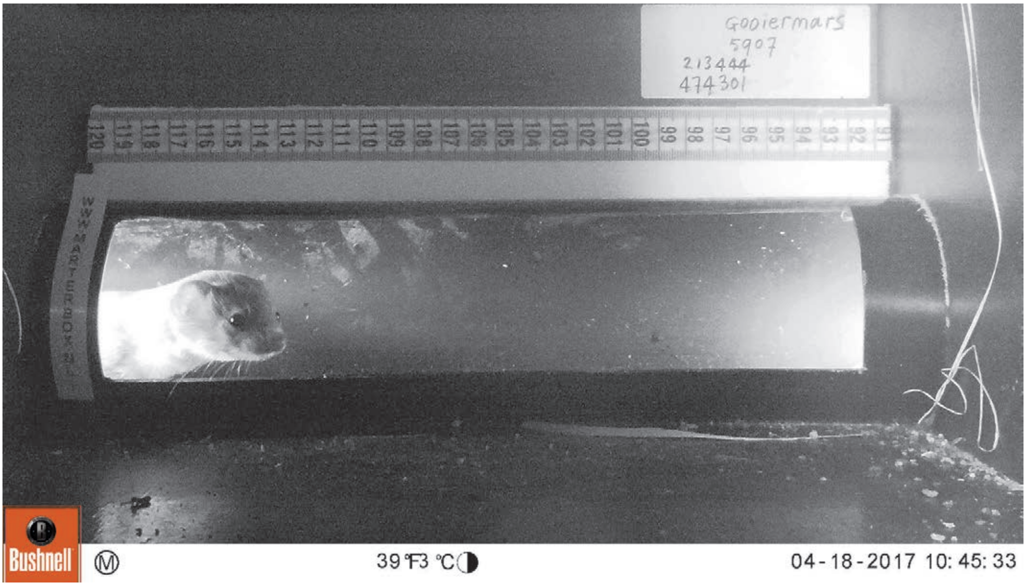

Figure 9. A weasel in a camera-trap box at one of the locations at research site Gooiermars.

average of 41 days for weasels to be registered in this study with a camera-trap box (table 2). The single recorded stone marten and polecat in this study were registered after respectively 31 and 89 camera-trap days.

Other animals also showed themselves in the camera-trap boxes. Not only wood mice and bank voles were registered but also hedgehogs, squirrels, brown rats, a domestic cat, amphibians and a variety of ground foraging birds (table 3).

Discussion

As already stated in the Introduction the intensification of land use, large-scale agriculture and urbanisation has increased considerably in the Netherlands in recent years. This increasement is the main cause of habitat loss for small mustelids and most probably the main reason small mustelid populations have been in decline. Furthermore, the absence of peak years of vole populations, the increase of other predatory mammal populations, like red fox (*Vulpes vulpes*) and stone marten (*Martes foina*), and accumulation of rodenticides from prey animals may have contributed to the decrease of small mustelids in the Netherlands (Douma et al. 2011, Broekhuizen et al. 2016). Stoats prefer low-lying wetland areas, whereas the weasel avoids wetlands (Criel 1990, Lange et al. 1994, Bouwens 2017). As a result, the weasel is likely to be able to maintain itself better in areas with large-scale agriculture (Criel 1990). The stoat appears to be much less numerous than weasel and even seems to have disappeared in parts of our country (van Maanen et al. 2015a).

Weasels were found at nine of the 64 research locations. In addition, footprints of weasel/stoat were collected at four locations. In contradiction to the hypothesis weasels were also found in open large-scale agricultural landscapes regarded as unsuitable habitat. Both camera-trap boxes and nest boxes proved successful in demonstrating the presence of weasels of weasel/stoat. The consultation of the Dutch National Database Flora and Fauna (NDFF) produced no historical data on the presence of stoats within our research locations at the time of this writing. Several road casualties and sporadic field observations in the NDFF indicate that stoats do occur in the east of the

Table 3. The species detected (mustelids in bold) and the number of locations (from a total of 64) where the species was detected during this study.

	Number of locations where detected		
Species	Camera-trap box	Nest box	Total
Mammals			
Wood mouse (*Apodemus sylvaticus*)	28	7	35
Bank vole (*Myodes glareolus*)	26	0	26
Common / Millet's shrew (*Sorex araneus/coronatus*)	19	0	19
Brown rat (*Rattus norvegicus*)	11	1	12
Weasel (*Mustela nivalis vulgaris*)	8	2	10
Greater white-toothed shrew (*Crocidura russula*)	4	0	4
Red squirrel (*Sciurus vulgaris*)	2	0	2
Western hedgehog (*Erinaceus europaeus*)	2	0	2
Soricidae spp.	2	0	2
Feral cat (*Felis catus*)	1	0	1
Pygmy shrew (*Sorex minutus*)	1	0	1
Western pole cat (*Mustela putorius*)	1	0	1
Field vole (*Microtus agrestis*)	1	0	1
Stone marten (*Martes foina*)	1	0	1
Stoat (*Mustela erminea*)	0	0	0
Muridae spp.	0	26	26
Weasel / Stoat (*Mustela nivalis / Mustela erminea*)	0	4	4
Birds			
Eurasian wren (*Troglodytes troglodytes*)	3	0	3
European robin (*Erithacus rubecula*)	4	0	4
Aves spp.	2	0	2
Common firecrest (*Regulus ignicapilla*)	2	0	2
Amphibians			
Common toad (*Bufo bufo*)	8	0	8
No data, camera trap box stolen	1		
No data, memory card error	1		
No data, no clear footprints		6	
No data, nest box drowned		2	

Netherlands. Stoats do show up in camera-trap boxes in other studies (van Maanen et al. 2013, 2015a, 2015b, Soininen et al. 2015, Hollander & Overman 2017). However it is hard to state their absence if target species are not detected. As described above, stoats often occur in lower densities than weasels. The number of detections of weasels in this study is low and therefore it is likely that stoats were present in even lower densities in the research sites at the time of this research and went undetected.

No results for polecat in this study were to be expected as the research materials used were not specifically built for surveying polecats. This is due to the limited diameter of the entrance tubes used in the research materials averts entry by larger mustelids. However, this study yielded the first known record of a polecat documented with a camera-trap box. Also a young stone marten showed itself on camera-trap footage gathered from Dorth estate. This is very surprising and probably due to the explorative nature of the young stone marten in question. The smaller size of the young stone marten made it easier to enter the camera-trap box. However, entry of camera-trap

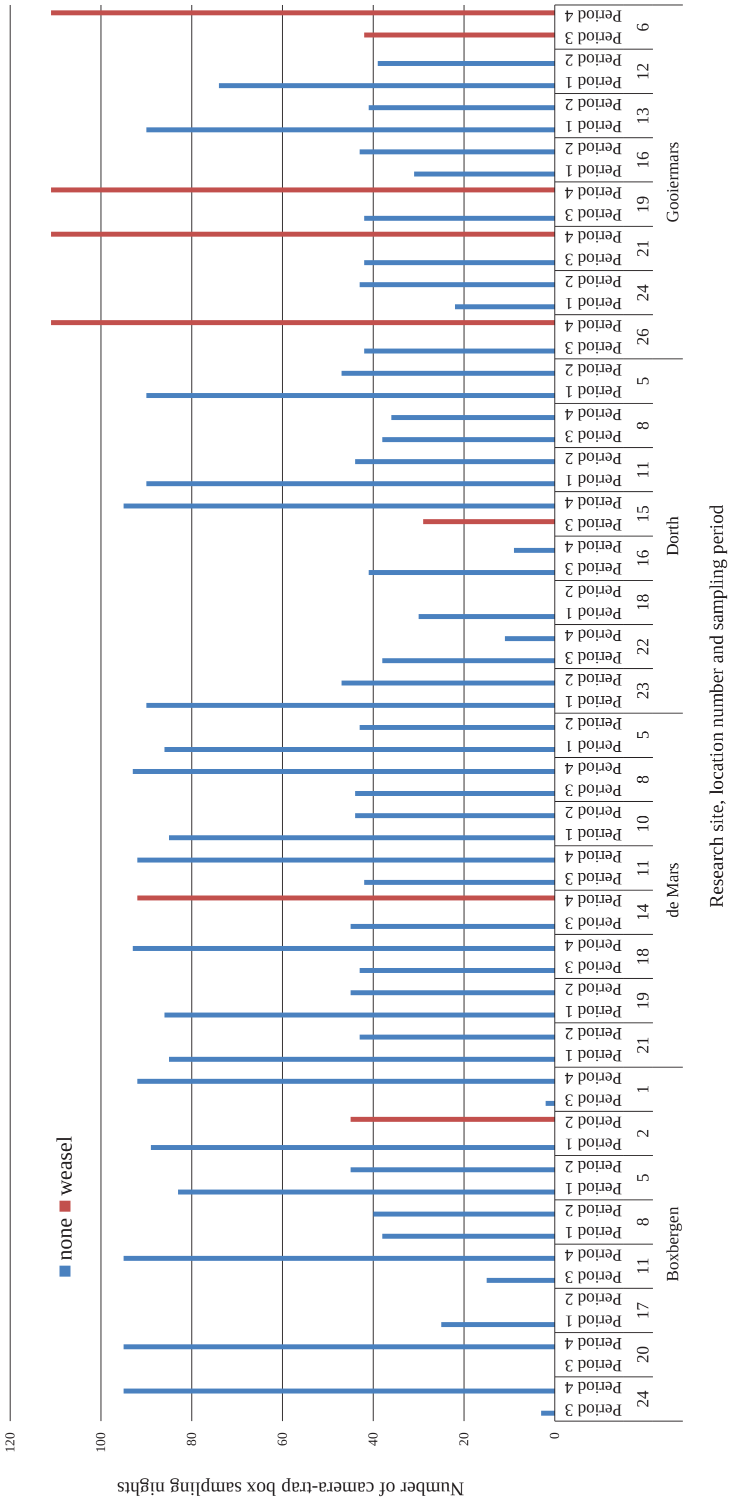

Figure 10. Number of camera-trap box sampling nights ; the locations where one or more weasels had been detected during a certain period are indicated by a red bar.

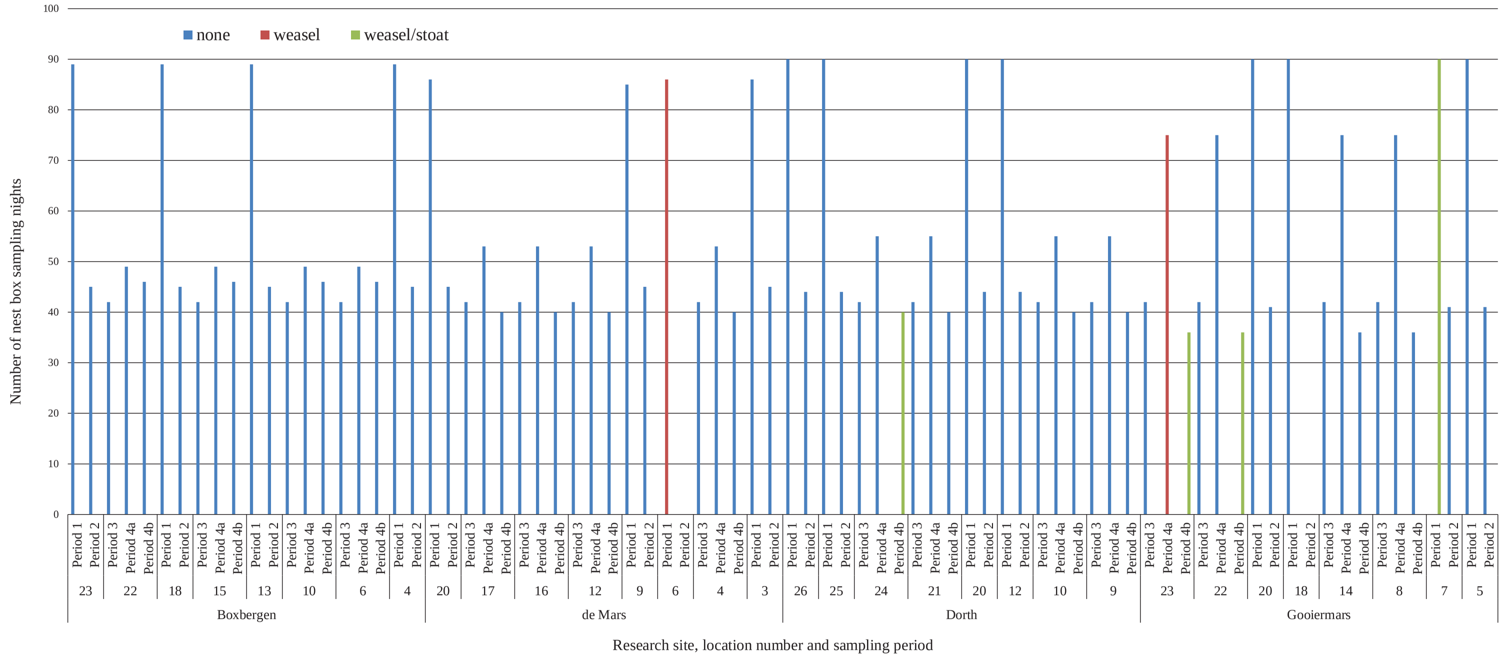

Figure 11. Number of nest box sampling nights ; the locations where one or more weasels had been detected during a certain period are indicated by a red bar; locations with weasel or stoat are indicated by a green bar.

boxes by adult martens is not impossible since a stretched out pine marten (*Martes martes*) managed to enter the camera-trap box of a local volunteer studying mustelids in Dorth estate in April 2018 (Oosterman 2018, unpublished data). Other studies showed that polecats, stone martens and pine martens do not show up in camera-trap boxes (van Maanen et al. 2015a, Hollander & Overman 2017). These observations should therefore be considered as by-catch. However, by-catch of larger mustelids and mammals is desirable in future studies to gather more information about hard to study species. Camera-trap boxes designed for small mustelid surveys have only been in use since 2012 in the Netherlands and there is still a lot to be learned about their effectiveness.

The first 30 of the 40 research weeks yielded little data, whereas the last ten weeks resulted in the bulk of the research results. Even though the extra interim check of the nest boxes during this period may have had an influence, it can be concluded that November through March appears to be a less successful survey period for small mustelids. The results indicate that April, May, June and July seem to be the best period for surveying weasels. Most likely this is weather related since small mustelids are less active during the colder months of the year (Smaal & van Manen 2017, Veldman & Troost 2019). Therefore they travel less distance in these months and the chance of being detected decreases.

Camera-trap boxes do yield the best results with regard to small mustelid surveys compared to tracking tunnels from nest boxes (figures 10 and 11). Tracking tunnels are easily contaminated with large amounts of footprints from small rodents making tracking of small mustelids impossible and short term interval interim checks a necessity and therefore labour intensive. Also, size and shape of small mustelids prints found in tracking tunnels often overlap making identification to species level impossible which is highly undesirable in most ecological surveys. In future studies tracking tunnels are only to be used as secondary method or if distinction between weasel and stoat is not a necessity.

At the beginning of this study intervals of ten weeks between field checks turned out to be too long for the tracking tunnels causing the footprints to be washed away by rain or the ink pads eaten by slugs and mice. Tracking tunnels should ideally be checked at least once every few weeks. Lastly, the sticker with information that was put in the interior of the camera-trap boxes was repeatedly eaten by snails. It is advisable to write down the required information on a piece of paper and to stick it in a plastic sleeve before adhering it in the camera-trap box. This way it cannot as easily be eaten by snails or mice.

Sometimes identification based on limited quality footage from camera-trap boxes proves difficult. Furthermore, small mustelids are swift and generally do not spend more than a few seconds inside a camera-trap box. It is recommended that camera-trap boxes used in any study should utilise the highest quality camera-trap available, with regard to image quality and trigger speed, as it is essential to increase the chance of detection and identification of the species. As an example, 164,000 photos (138 GB) were collected at Boxbergen research site, of which only one single photo captured weasel. Quickest trigger time and dynamic infrared flash intensity being most important features in a camera-trap to be used in a camera-trap box.

The analysis of camera-trap footage is very time consuming and labour intensive. A camera-trap box deployment (series of photos) takes on average about two hours to process manually. Manual processing was the quickest option at the time of this survey. In future studies it is recommended to use camera-trap footage processing software, of which several good options are currently available (Hendry & Mann 2018, Liefting & Jansen 2019).

Fish or fish oil is generally accepted as the most effective lure for small mustelids. It is, however, unclear whether lures are effective

Weasel. *Photo: Paul van Hoof.*

at all (Koenders 2018). There is currently very little literature on the subject in a Western European setting. Therefore, the effectiveness of the use of different types of scent lures and or baits is a subject that needs to be investigated more thoroughly. A pilot study in 2017 using soundtracks of prey animals in distress as a lure did not yield desired results (Schep 2018). Subjects like these need follow up studies because it is likely they can increase the efficiency of the use of camera-trap boxes in small mustelid surveys. Gloves or any other measures against human odour on camera-trap boxes and nest boxes have not been used in this study. This may have had an adverse impact on attraction to small mustelids and thus possibly influenced the results. Some studies imply that human scent masking does not improve capture rates (Muñoz et al. 2014), others imply that human scent might have an negative effect on capture rates (King 1994). Therefore it is advised to take adequate actions in future studies to reduce human scent on research materials and to report results.

Wildlife surveys with use of camera-trap boxes and nest boxes are time consuming. With large quantities of research instruments in the field the risks of defects, battery failure and theft influencing results are high. This can be kept to a minimum by interim checking at least monthly.

The average number of days that a camera-trap box was in the field for a weasel to be detected was 41 days. Also, no detections were made between October and February since all eight weasels were recorded between March and July. It is therefore recommended, in future studies to place the boxes effectively in the field for at least 56 days (eight weeks) outside of the winter season and to not move them within this period but to achieve at least one interim check after four weeks. Furthermore, in future studies the number of observations should be registered to be able to evaluate effectiveness of research materials and approximate density of mustelids.

eDNA is a promising new method for detecting species unique DNA sequences in samples of water, soil, tissue or faeces. Faeces of small mustelids are not easily encountered in the field but if they are they can be easily collected and analysed. This method has currently been tested (S.A. Westra & K. van Bochove, unpublished data). The outcome could be an additional survey method for detection of presence of small mustelids that can easily be combined with placing and checking research materials in the field.

Seven of the 13 successful research locations in this study were located at the head of linear landscape elements. In future studies, it is recommended that priority be given to research locations at the head of linear elements.

General concern amongst Dutch ecologists about the status of small mustelids has encouraged improvement of legal protection in the past years. In at least five of twelve Dutch provinces the three small mustelid species now have a protective status (Jonker 2016, Bouwens 2017, Veldman & Troost 2019). Many questions did arise about their status and conservation issues. Based on historical data and recent research results, including this study, it is not possible to provide a better foundation for the status and trend of weasel and stoat. The available data are too minimal. In order to get a better understanding of current status and population trends, a large-scale, long-term monitoring study with camera-trap boxes is recommended. This monitoring could have a similar design to this project but the research duration should be at least a few years to increase detection and decrease the missing of weasels/stoats when they are actually present.

Conclusions

Weasel is known to be present in the area and the results of this study confirm this. Weasel numbers demonstrated are very low. It is very difficult and in most cases impossible to distinguish individuals from the research data, and therefore it is impossible to say anything about densities. Comparisons are made between the four research areas based on difference in land use and distributional data of weasel and stoat gathered here.

Stoats could not be detected during this study. The total of one recording of polecat (and one stone marten) during this study can be seen as a lucky shot and is disregarded in this conclusion. The results obtained in this study supports the outcomes of previous small mustelid studies in the Benelux (Criel 1990, van Maanen et al. 2015a): limited results are to be expected when surveying small illusive predatory mammals with camera-trap boxes and nest boxes.

In the Dorth research site - regarded as suitable habitat for weasel – I detected a weasel in one location which was found in the central, heavily forested part of the estate. However, the species was expected more along the extensive pasture areas and the pools that lie therein. It is possible that weasels are influenced by human and pet activity and scent.

In the Boxbergen research site - regarded as suitable habitat for weasel - a total of only one weasel was found in a hedge row, however more results were expected. The sharp transitions from closed to open landscape types at Boxbergen estate means that there are relatively few refuges in comparison with the Gooiermars and Dorth. The intensively managed agricultural lands that are found here makes the site a less suitable habitat compared to Dorth and Gooiermars. A camera-trap box disappeared at one of the locations at Boxbergen estate, so the research effort and the chance of success has been slightly lower than in the other research sites.

The research site of de Mars – regarded as marginal habitat for weasel - is characterised by a very open landscape with a limited number of narrow linear landscape structures. In contradiction to what was expected two weasels were found here. This is possibly due to the natural agricultural practices of field border management that is carried out here since 2016 in combination with the presence of refuges in the western part of the research area adjacent to the winter dike. This is beneficial for small mustelids. It is also known that weasels can adapt to living in open landscapes that are similar to de Mars (Twisk et al. 2016). Furthermore, the above is possibly caused by a sampling issue. If a smaller part of the landscape is suitable for weasels, the chance of detecting them increases in the parts of the landscape that are suitable. So higher detection might not come from higher weasel presence but from a higher detectability.

In the research site of Gooiermars – regarded as suitable habitat for weasel – weasels were found at five locations, by far the most numerous of the four research sites. What has become clear is that in the Gooiermars much more data have been obtained from the presence of weasel than at other research sites. In contrast to the other research sites only in the Gooiermars does the presence of a large area of apparent suitable habitat actually result in higher densities of weasels. The Gooiermars is characterised by poor soils with wet natural grassland, extensive grazing, and linear landscape elements with sufficient coverage. In addition, a large part of the area is closed to recreational users, which makes the chance of disturbance by people and pets very small. This type of landscape has disappeared in large parts of the Netherlands and with that possibly the weasel and stoat. Despite efforts in this study we know little more about this subject than before and further study to confirm the above is a necessity.

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Samenvatting

Een onderzoek van negen maanden naar het voorkomen van kleine marterachtigen in vier onderzoeksgebieden in Oost-Nederland

Onder ecologen bestaat bezorgdheid over de (al dan niet vermeende) achteruitgang van kleine marterachtigen in Nederland. Er is weinig bekend over hun aantallen in verleden

en heden. Om hun ecologie en de oorzaak van de achteruitgang beter te begrijpen is meer onderzoek naar deze soortgroep noodzakelijk. Om zo mogelijk in deze kennislacune te voorzien heeft de Zoogdiervereniging een ecologisch onderzoek geïnitieerd dat is uitgevoerd door Silvavir ecologisch advies. Tussen oktober 2016 en augustus 2017 werden vier verschillende gebieden in de omgeving van Deventer onderzocht. Daarbij werden 64 locaties met behulp van Nestkasten en Marterboxen geïnventariseerd op het voorkomen van kleine marters. Beide technieken betreffen nieuw ontwikkelde onderzoeksmaterialen om het voorkomen van de kleine marterachtigen wezel (*Mustela nivalis vulgaris*) en hermelijn (*Mustela erminea*) vast te stellen. Aangezien bekend is dat kleine marters een voorkeur hebben voor kleinschalig agrarisch cultuurlandschap werden drie van de vier onderzoeksgebieden in dat type landschap gesitueerd. Eén locatie betrof een grootschalig open agrarisch cultuurlandschap - beoordeeld als aangetast leefgebied. De aanwezigheid van wezels werd zowel met cameravallen als met nestkasten vastgesteld. Voetafdrukken in sporenbuizen waren niet altijd met zekerheid te herleiden tot wezel of hermelijn. Er werden geen hermelijnen geregistreerd. Hermelijnen zijn in de betreffende onderzoeksmaanden óf zeer zeldzaam óf niet aanwezig in de onderzochte gebieden. De gebruikte onderzoeksmaterialen bleken niet geschikt te zijn om het (veronderstelde) voorkomen van bunzing (*Mustela putorius*) vast te stellen omdat de beperkte diameter van de opening van de inloopbuis van de onderzoeksmaterialen de toegang bemoeilijkt. Er is geen duidelijk verschil te zien in de gegevens tussen grootschalig open agrarisch cultuurlandschap (twee registraties) en kleinschalig agrarisch cultuurlandschap (één, vijf en nul registraties). Opvallend is het feit dat de meeste waarnemingen werden gedaan in de periode tussen maart en oktober. De conclusies die uit dit onderzoek getrokken kunnen worden zijn dat: 1. Kleine marterachtigen een onderzoeksuitdaging vormen. 2. Ondanks de inzet van innovatieve onderzoeksmaterialen - waarvan het gebruik veel tijd vergt -, er weinig (positieve) gegevens konden worden verzameld. Op basis van historische data en recente onderzoeksresultaten, waaronder deze studie, is het nog steeds niet goed mogelijk om verantwoorde uitspraken te doen over de status en trend van wezel en hermelijn. Om hier beter grip op te krijgen is grootschalig, langetermijnonderzoek met wildcamera's nodig.

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Observations on a red deer (*Cervus elaphus*) in the province of Drenthe, the Netherlands, during the winter of 2018-2019

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Abstract: Red deer (*Cervus elaphus*) are rare in the Dutch province of Drenthe. Most observations in the past decades were found to be linked to escapes or illegal introductions. In all of those cases, the animals were shot, in accordance with the policy of the province. This article describes a red deer of wild origin, wintering south of Assen in the period December 2018 - May 2019. Intensive surveillance of tracks, combined with camera trapping and analysis of DNA from droppings was conducted to study its home range, behaviour and origin. Within less than half a year, over 1300 observations of this individual were collected. Pictures from camera traps revealed a 3-4 year old male deer. Based on regularly visited locations, its home range was estimated to be over 2000 hectares. This specimen was almost strictly nocturnal. In agricultural areas it was found foraging occasionally on grassland and crop remains of potatoes, flower bulbs and sugar beets. Basic data of Dutch red deer track measurements were never published before, this article provides the first documented set. DNA analysis showed a clear similarity with the red deer population from the Kroondomeinen area at the Veluwe (province of Gelderland), 85 km from the wintering range in Drenthe.

Keywords: red deer, *Cervus elaphus*, home range, dispersal, behaviour, DNA analysis, Drenthe, Veluwe, Netherlands.

Introduction

On 26 November 2018, I happened to notice footprints of a large ungulate on some sandy roads on the 'De Haar' military training grounds near Assen, Province of Drenthe, The Netherlands. On that particular day I ignored the unfamiliar prints, but within a week, on 2 December 2018, I found the same large footprints, less than 200 m from the previous spot and decided this time to take some measurements. To my surprise it became clear that a red deer (*Cervus elaphus*) was the only animal that fitted the size of the prints.

Red deer are rare in the province of Drenthe. Most observations in the past decades were found to be linked to escapes or illegal introductions (table 1). These animals were all shot, in accordance with the policy of the province to discourage illegal introductions (Provincie Drenthe 2014). According to this policy, migration and temporary presence of wild red deer in Drenthe is permitted and the presence of roaming wild deer will be assessed case by case with regard to agricultural damage and the risk of traffic accidents. After a brief check with the authorities of the province of Drenthe it became clear that there were no reports of a recent escape or introduction and it appeared likely that the red deer was wild. I decided to start a surveillance in order to get more information about its behaviour. The behaviour of red deer outside their Dutch range in the province of Gelderland and the Oostvaardersplassen and outside enclosures such as Weerterbos and Groene Woud has never been described before. From a genetic perspective, populations of red deer in the Netherlands and neighbouring Germany are investigated quite well (de Groot et al. 2016). They are genetically clearly separate, so movements of red deer from these populations can be reconstructed if genetic material is obtained. The goal of the study in Drenthe was to better understand the behaviour of a red deer in a fragmented landscape that was previously thought to be unsuitable for such a large animal. Filling this knowledge gap, at least partly, may contribute to more fact-based discussions on introduction projects or natural colonisation of red deer in the Netherlands.

Methods

The study area is located roughly between Assen, Smilde and Beilen, bordered by the highway A28 and the canals Drentsche Hoofdvaart and Oranjekanaal.

Field work was conducted between 2 December 2018 and 14 July 2019. First of all, I searched on average three days per week for red deer hoof prints on sandy roads and walked transects across arable fields in a wide area around De Haar. Once a track was found, I tried to follow the trail for as far as possible. Approximately half of the tracks found were logged in detail with a GPS, with additional waypoints taken every 50-60 m. For the other half of the tracks, only the waypoints every 50-60 m were taken. Documented tracks were removed or marked in order to prevent multiple counts of the same track. Red deer trails were found relatively easily on arable fields and on sandy roads but were difficult to find on meadowland and almost impossible to spot in woods and heathland (only during snow cover). The main goal of mapping tracks was to investigate the activity area of this deer. However, following this animal step by step, kilometre after kilometre, also provided a wonderful insight in its behaviour. From the type of tracks, indicating for instance a walk or gallop, it's possible to deduce whether the animal was searching for food (walking slowly in different directions), just passing by from one place to another (walking in a straight line) or running from danger. I started to follow the tracks from the beginning of December 2018. Since most arable fields were prepared for new crops in the last week of March, I henceforth restricted the survey to the edges of these fields. Additionally, by random sampling (only fresh tracks and samples distributed widely over the area and research period), measurements were taken of the hoof prints and parts of the tracks. Sizes were registered in the field with an accuracy of 0.5 cm for hoof prints and track width and 1 cm for stride length.

Secondly, eight camera traps were placed in locations where the red deer could be expected based on tracks found previously. Camera traps were used to identify the animal and confirm that there was only one animal roaming the area (appendix 1). They were also useful for improving estimates of the age of the tracks (by providing the exact time the deer passed by) and provided data on the nocturnal activity of this deer. Two cameras were located at De Haar, the others a few kilometres further to the south within the Hijkerveld nature reserve. In order to avoid additional disturbance, camera traps were mounted within 100 m from the nearest public path (average distance 66 m) or at the edge of arable fields. The first camera was placed on 12 December 2018, the last cameras were removed from the field on 14 July 2019. Altogether, the camera traps provided 24,681 observation hours.

The home range of the deer was estimated

Figure 1. Red deer caught on camera trap. Assen, De Haar, 2 February 2019.

based on locations (tracks or camera trap observations) that were visited on at least two separate occasions during winter, following the definition of Powell and Mitchell (2012): "the best concept of a home range is that part of an animal's cognitive map of its environment that it chooses to keep updated." From these locations, a minimum convex polygon (MCP) was calculated to outline the home range of the deer.

Finally, samples of fresh droppings were collected on 12, 17 and 24 February 2019 and stored frozen in 96% alcohol. DNA from these samples was analysed by Wageningen Environmental Research for genetic identification of this red deer (de Groot 2019). Results were compared with populations as described by De Groot et al. (2016).

Results

Pictures from camera traps revealed a 3-4 year old stag (figure 1). Its age was estimated based on the antlers[1] and general body shape as described by Worm (2010a).

In less than half a year, over 1300 records of this red deer were collected, covering (parts of) 34 kilometre grid squares (figure 2). Out of these records, 35 were made by camera trap, all the others were the result of the search for tracks. The deer was recorded at every location where a camera trap was mounted. All camera trap records were obtained during the twilight and at night, never in broad daylight. Observations with the camera traps were on average 170 minutes after civil dusk or before civil dawn and on average 209 minutes after sunset or before sunrise. Only one record was made during civil twilight, 11 minutes

[1] Brow tines in December-March relatively small, indicating the second set of antlers (animal three years old). After casting the brow tines of the new antlers were considerably larger (four years old, see appendix 1). Pedicles were visible (excluding stags from eight years or older).

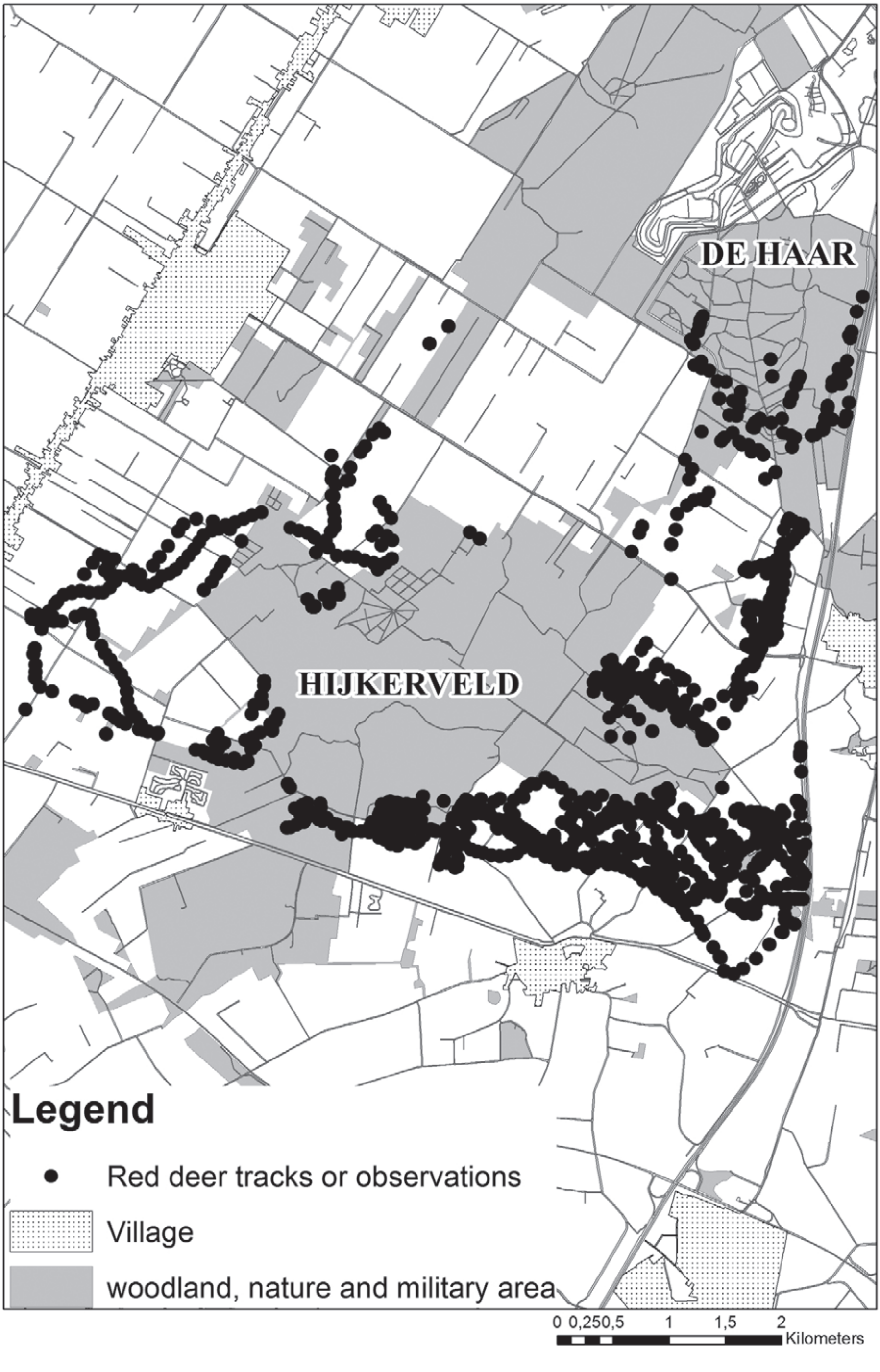

Figure 2. Locations of tracks or camera trap observations of the red deer stag in 2018-2019. Note that vegetation cover (heathland, woods or pastures) prohibited search for tracks in the central part of the Hijkerveld and parts of the agricultural area around as well.

Table 1. Reported red deer in Drenthe in the past decades.

Date	Details
6 October 2003	1 adult female: Gasterse duinen : escape/illegal introduction: animal was shot later on
12 April 2015	1 young male, 2 years old : Assen, A28 : traffic killed : DNA analysis pointed out the populations of Dämmerwald and Üfter Mark in Germany as most probable origin.
7-25 March 2017	2 adult females : Veenhuizen-Norg: illegal introduction : both animals were shot at 6 April 2017
8 June 2018 – 17 June 2019	1 female : Drents-Friese Wold : escape from a red deer farm in Smilde: animal was shot 17 June 2019. A few other red deer that were involved in the same escape were shot already within the day of their escape close to the farm.

before sunrise. Most activities (54%) of this deer were observed within two hours of civil dusk and dawn, therefore within nautical and astronomical twilight and at night. The camera traps from De Haar and Hijkerveld revealed an activity pattern in which the stag visited De Haar at night but returned to the Hijkerveld to rest during daytime.

According to GPS data, a total of 33.2 kilometres of tracks were logged in detail. The shortest trail was a mere 58 metres but the longest one stretched out for over 5.5 kilometres. On average, it was possible to follow the tracks over a distance of 722 metres (n=46). It became clear that although De Haar was regularly visited, the Hijkerveld was the main area of residence for this deer. From there the deer explored the surrounding landscape, resulting in an estimated home range of over 2000 hectares. Although the centre of the Hijkerveld could not be investigated, based on the locations where the red deer entered or left the area it is likely that the central part was used as well. This is illustrated by an observation by the shepherd of the Hijkerveld who encountered the stag by chance on 4 December 2018 in the middle of the heathland, more than 500 metres from the nearest observations in this study (B. Zoer, personal communication). From the tracks that were mapped, combined with data from the camera traps, it is clear that the red deer easily travelled 5-10 kilometres at night.

After the first observations in 2018 the red deer remained present in the study area until 18 May 2019. After this date, no new records were collected with camera traps and despite an intensive search, no more tracks were found. Clearly the animal had left its wintering grounds.

The tracks showed that this deer mostly walked in a steady direct register gait. Stride length was on average 119.7 cm and showed little variation (table 2). The trail width was also very consistent, on average 20.8 cm, illustrating that this deer walked mostly in a very straight line. Size measurements of the hind hoof were on average 8.1 cm long and 5.5 cm wide.

Gallop tracks were relatively rare (1.3% of 1293 track waypoints) and typically found close to roads. It seems likely that these tracks indicate disturbance. In several cases the deer approached a road in a normal walking gait at first and then backed off in a gallop before crossing. Stride length in gallop was impressive compared to the normal walking gait: on average 296.3 cm and a maximum of 435 cm. At gallop speed, trail width increased to an average of 27.2 cm. The same hind hooves that were measured in direct register gait clearly left larger prints in gallop (on average 8.4 cm long and 6.6 cm wide).

Among the tracks, signs of feeding behaviour were found at only 17 waypoints. This was mostly on grassland (8x) and furthermore on remnants of harvested flower bulbs (4x), sugar beets (3x) and potatoes (2x). There is one camera trap observation of foraging on young

Table 2. Measurements of red deer tracks. Measurements of hooves all without dew claws. Stride length = distance between one print and the corresponding following one. Measurements of trail width all from the outsides of the prints. Direct register print = print of the hind (rear) hoof overlapping with the front hoof. L=length, W=width, *n*=sample size.

Type	Average (cm)	Sd (cm)	Median (cm)	Min (cm)	Max (cm)	*n*
Walk (direct register)						
Stride length	119.7	2.5	120	115	127	115
Trail width	20.8	3.0	21	14	32	106
Direct register print (L/W)	9.8 / 8.1	1.0 / 1.0	10 / 8	8 / 6.5	13 / 11	65
Hind hoof (L/W)	8.1 / 5.5	0.3 / 0.4	8 / 5.5	7.5 / 4.5	9 / 6	34
Gallop						
Stride length	296.3	85.0	258.5	214	435	32
Trail width	27.2	4.4	27.5	20	38	52
Front hoof (L/W)	9.9 / 7.7	0.6 / 0.5	10 / 7.5	8.5 / 7	11 / 9	20
Hind hoof (L/W)	8.4 / 6.6	0.5 / 0.6	8.5 / 6.5	7.5 / 6	9 / 8	19

beech leaves (*Fagus sylvatica*, appendix 1).

Discussion

The three faecal samples showed identical DNA profiles, based on eight microsatellites markers (de Groot 2019). Cluster analysis in STRUCTURE (de Groot et al. 2016) placed the red deer for 65% into the cluster 'red' and smaller percentages in the clusters 'purple', 'orange' and 'green' (see figure 6 and 7 in De Groot et al. 2016). The cluster 'red' is typical for red deer from the Kroondomeinen and National Park Hoge Veluwe. Based on the exact alleles found at each microsatellite, all are known from the Kroondomeinen reference set but two of them are absent in the Hoge Veluwe reference dataset. This indicates the Kroondomeinen as most the likely origin of this red deer. It should be mentioned that the reference dataset of Wageningen Environmental Research does not include animals from red deer farms in the Netherlands. So, even though there is a sound genetic match with the Kroondomeinen, other options, although unlikely, cannot be ruled out with certainty.

Red deer inhabit a limited number of areas in the Netherlands. The largest populations live in the provinces of Gelderland (Veluwe 1900 animals) and Flevoland (Oostvaardersplassen, 490 animals[2]) (Spek 2014, Provincie Flevoland 2018). Furthermore, they are kept in enclosures in the province of Limburg (Weerterbos, 150 ha, introduction in 2005, 30 animals in October 2018) and Noord-Brabant (Groene Woud, 300 ha, introduction in 2017, 26 animals in October 2018) (Middeljans 2008, Tielemans 2017, Dekker & Houben 2018, Gabriëls 2018; B. Houben, personal communication). From the perspective of Drenthe, the nearest populations are in the north-eastern part of the Veluwe (70 km from Assen) and Forst Bentheim in Germany, approximately 80 km from Assen. However, in April 2015 a young stag was hit by a car near Assen and DNA analysis pointed to Dämmerwald and Üfter Mark (both Germany) as its most probable origin (de Groot & Jansman 2016). Red deer from the German Dämmerwald and Üfter Mark would have to walk 140 km to Assen, measured in a straight line. Other observations in the past decades were found to be linked to escapes or illegal intro-

[2] 490 red deer is the population size that the province of Flevoland finds acceptable. The actual numbers are much higher: 1525 were counted in October 2019 (Cornelissen et al. 2019).

ductions (table 1). In the eastern parts of the provinces of Overijssel (Twente) and Gelderland (Achterhoek), roaming red deer occur more often (Worm 2010b). Unfortunately, the observations from Twente and the Achterhoek are mostly poorly documented incidental encounters. These red deer were supposedly all from Forst Bentheim, Dämmerwald and Üfter Mark. This seems likely, due to relatively short distances (10-25 km) between the Dutch observation sites and the German areas of origin. The stag that was observed in Drenthe in the winter of 2018-2019 is the first connection between the Veluwe (Kroondomeinen) and Drenthe. It is clear that red deer are able to cover fairly large distances and can cope with infrastructural barriers such as motorways and canals. The common assumption that red deer in Drenthe are probably escapes seems no longer valid. The introduction of red deer in Drenthe has been discussed in the past (Spek & Worm 2014), with a focus on the Drents-Friese Wold. The animals from 2015 (Dämmerwald and Üfter Mark) and 2018-2019 (Kroondomeinen) show that an introduced population of red deer in Drenthe could occasionally receive fresh blood from other populations. This would reduce the risk of inbreeding.

Red deer are the largest terrestrial mammals in the Netherlands. It is remarkable that such a large animal is able to travel almost unnoticed for months through the landscape of Drenthe. I expected that the presence of this deer would soon become public knowledge and farmers, hunters, nature conservation employees and others who spend a lot of time in the field would either observe the animal or find tracks. But it appears that either no one else found the tracks or that anyone who did, did not realise that red deer could occur in the area. It therefore seems likely that chance observations lead to an underestimation of the occurrence of red deer outside their regular distribution areas as described by Worm (2010b).

Both the evidence from the camera traps and the lack of regular observations during the daytime show that this deer was strictly nocturnal. The edges of civil twilight are much better suited than sunrise and sunset for the human eye to spot a red deer. Since there was only one camera trap observation during civil twilight and none during daylight, it makes sense that hardly anybody has actually seen the animal while it was in the region. Apart from the shepherd of the Hijkerveld, who saw the deer once, only a single resident of the area mentioned to me that he had seen the stag. It is possible that these observations during daylight were caused by disturbance of the red deer at its resting place. The nocturnal rhythm of red deer is a well-known response to human disturbance (Hoogeveen 1991, Dekker & Groot Bruinderink 2010). From this perspective it is noteworthy that the Hijkerveld, when compared to De Haar, is less accessible for the public and dogs are not allowed due to the presence of the sheep. It seems that this red deer adapted well to the area in Drenthe by using the quietest parts of the terrain (Hijkerveld) for rest during daytime and exploring the wider region only at night.

Since the red deer tracks were easily found on arable fields and sandy roads but were hard to find in pastures and almost impossible to spot in woods and heathland it is clear that the data do not reflect the complete use of the home range. Almost no data was obtained from the core area in the Hijkerveld. Nevertheless, it is evident that the animal frequented a large area. This knowledge is relevant with regards to new introductions of red deer in the Netherlands. Spek and Worm (2014) estimated the home range needed for red deer in the Drents-Friese Wold in areas influenced by human activity at 100-500 hectares. This seems questionable based on the results of the 2018-2019 study in Drenthe. A radio telemetry study of 20 male red deer in the Slovakian Carpathians found a home range of 564-1762 ha for resident deer and 1250-6393 ha for migrants (Kropil et al. 2014), depending on the type of modelling used. In a Hungarian lowland area, radio-tracked red deer were found to have much larger home ranges: the mean home range of twelve stags was 6697 ha (min: 1774 ha, max: 17,748 ha) (Szemethy et al. 1998). A telemetry study in the fragmented landscape of Schleswig-Holstein in Germany found a wide variety of home ranges with the largest ones, for stags, at 721-6300 ha, (Reinecke et al. 2013). Based on these studies, it appears that a home range of over 2000 ha is not exceptionally large for a red deer stag. It also makes clear that red deer in small enclosures like Weerterbos and Groene Woud are unable to display complete natural behaviour patterns.

Though tracks were followed over long distances, the agricultural landscape seemed to be of little importance as a foraging area. More time was spent on grassland, but feeding on crop remains was incidental. This is strikingly different to the behaviour of roe deer (*Capreolus capreolus*) in the same area, which systematically dig up remnants of flower bulbs, sugar beets or potatoes.

Basic data of Dutch red deer track measurements have not been published until now, this article provides the first documented set. Animal track guides (Bang & Dahlstrøm 1980, Nauta & Pot 2019) all lack essential information and do not distinguish between track sizes of male or female deer, front or hind hooves, or direct register walk or gallop. The hoof measurements from Drenthe fit within the range described in the guide books, but the maximum stride length in gallop (435 cm) is much greater than the maximum (350 cm) mentioned by Bang & Dahlstrøm (1980).

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Samenvatting

Een edelhert (*Cervus elaphus*) in Drenthe in de winter van 2018-2019

In de provincie Drenthe leeft geen populatie edelherten. Waarnemingen vielen in het nabije verleden vrijwel steeds te herleiden tot bekende ontsnappingen of illegale uitzettingen. Conform het beleid van de provincie, dat is gericht op het voorkomen van illegale uitzettingen, zijn dergelijke dieren steeds geschoten. Van tenminste 2 december 2018 tot 18 mei 2019 overwinterde een edelhert van wilde herkomst in het gebied tussen de plaatsen Assen, Smilde en Beilen. Door intensief sporenonderzoek, gebruik van cameravallen en DNA-analyse van gevonden keutels zijn homerange, gedrag en herkomst van dit dier onderzocht. Er zijn in minder dan een half jaar tijd meer dan 1300 waarnemingen van dit individuele dier vastgelegd. Het hert is op basis van gewei en lichaamsbouw geschat op een mannelijk dier van 3-4 jaar oud. De plaatsen die dit hert met enige regelmaat bezocht wijzen op een homerange van meer dan 2000 ha. Het dier was nagenoeg strikt nachtactief. In agrarisch gebied werd incidenteel gefoerageerd op grasland en oogstresten van aardappel, bloembollen en suikerbiet. DNA-onderzoek van drie keutelmonsters wijst op de populatie van de Kroondomeinen op de Veluwe als meest waarschijnlijke herkomstgebied. Dat is hemelsbreed 85 km van het overwinteringsgebied in Drenthe. De kennis die is opgedaan met het onderzoeken van dit dier kan benut worden in discussies over herintroductie en spontane kolonisatie van edelherten in Nederland. Belangrijke vragen daarbij zijn de grootte van een territorium dat nodig is voor volledig natuurlijk gedrag en de mate waarin genetische uitwisseling tussen populaties mogelijk is. Twee gevallen uit Drenthe maken nu duidelijk dat zwervende edelherten over een afstand van hemelsbreed 85 tot 140 km de provincie kunnen bereiken en daarmee een eventueel geïntroduceerde populatie in theorie van vers bloed kunnen voorzien. Aanvullend leverde het onderzoek een dataset op van afmetingen van sporen hetgeen voor Nederland zo nog niet eerder was vastgelegd.

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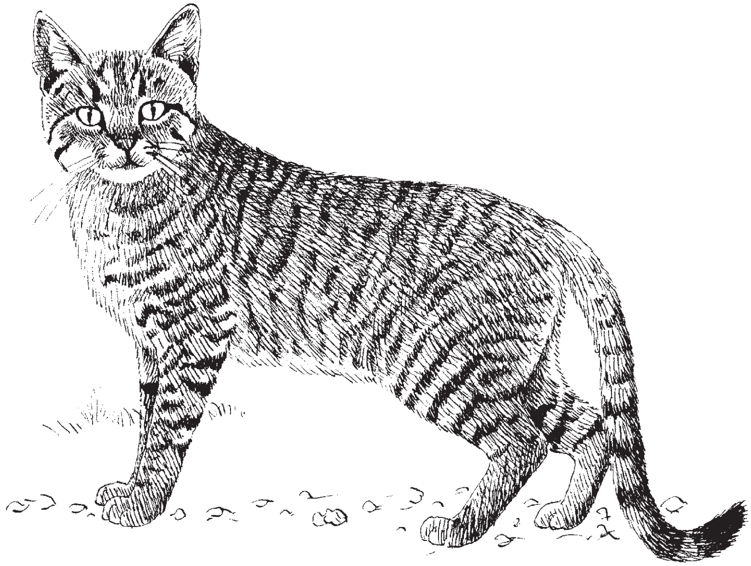
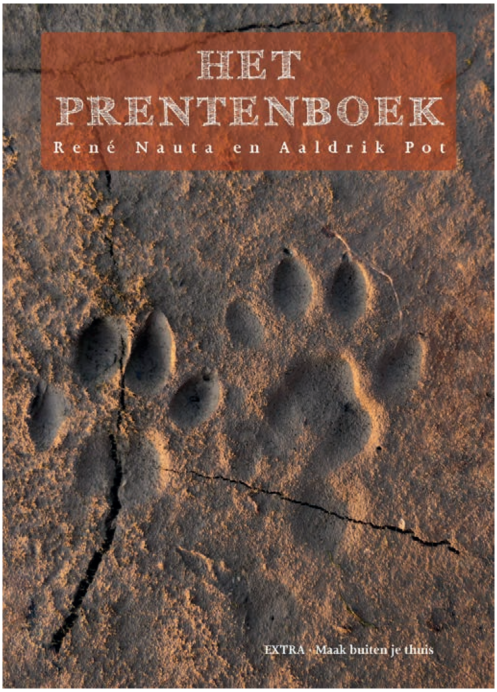
Appendix 1. Links to a few camera trap videos of the red deer described this article.
https://www.youtube.com/watch?v=gyx74sQ0cPs
https://www.youtube.com/watch?v=Vdih2LQrhOM
https://www.youtube.com/watch?v=MB-K4-v611w



Het Prentenboek

René Nauta & Aaldrik Pot 2019. Extra, Vledder, Nederland. 448 blz. ISBN 9789082453812.



Het Prentenboek, een nieuw boek over dierprenten, is met veel enthousiasme en liefde gemaakt. Dat blijkt al uit de speelse dierentekeningen op de binnenkant van de kaft, het opdragen van het boek aan de dieren en niet aan mensen, de aparte kaderteksten met persoonlijke belevenissen, die onder andere eerlijk het leerproces bij de auteurs zelf verhalen en, achterin, aan de manier van het bedanken van alle mensen die aan het boek hebben meegewerkt. René Nauta beschikt bovendien over de gave om mooie, exacte en educatieve illustraties met potlood, pen en waterverf te maken. Leuk is ook dat de auteurs Annemarie van Diepenbeek, de sporenpionier van Nederland, terecht op een voetstuk plaatsen. Ze doen dit door haar zowel het voorwoord te laten schrijven als haar het eerste exemplaar plechtig te overhandigen.

Geen sporen maar prenten

Het prentenboek gaat, zoals de titel aangeeft, niet over sporen in het algemeen, maar bijna uitsluitend over prenten. Beschrijvingen van krabsporen van boommarters (*Martes martes*) of eekhoorns (*Sciurus vulgaris*) aan een beuk of die van zeul- of ligplekken van hoefdieren zul je niet aantreffen. Voor deze en andere sporen blijven de Veldgids Diersporen (2013) en de recentelijk verschenen en geheel herziene Veldgids Diersporen Europa (2019) van Annemarie van Diepenbeek vooralsnog de beste bronnen. De manier van lopen van de beschreven diersoorten worden overigens wel behandeld, maar pas helemaal achterin in het boek.

Dat de focus zo sterk op geïsoleerde, losse prenten ligt is niet verwonderlijk. Beide auteurs wonen en werken in Nederland en daar kunnen sporenreeksen in de sneeuw slechts een beperkt aantal dagen in het jaar worden waargenomen. De rest van het jaar ben je in Nederland veelal aangewezen op sporen zoeken in stukjes slijk of in los zand. Soms moet je het doen met een individuele prent, andere keren met een korte reeks prenten van variabele kwaliteit. Het is onder die omstandigheden van groot belang om goed te kijken en alle details goed in je op te nemen. De auteurs laten waarschijnlijk mede om die reden de maten van prenten geheel uit de beschrijvingen weg. Alleen in de bijlages worden deze voor zoogdieren en vogels tabellarisch weergegeven. (Helaas zijn ze onhandig alfabetisch gerangschikt waardoor verwante soorten niet makkelijk met elkaar vergeleken kunnen worden.)

Introductie

De inleidende teksten gaan vooral over het hoe, wat en waarom van sporen zoeken. Het begrip

’CyberTracker’ wordt hier uitgelegd. Alhoewel je dan al snel denkt aan de cyberspace of het internet heeft de term een andere oorsprong en betekenis. Het komt er op neer dat het een soort internationale leerschool is voor het lezen van diersporen met gedefinieerde criteria en vaste toetsen voor het behalen van bepaalde kennis- en vaardigheidsniveaus. Beide auteurs gaan hier helemaal in op en verhalen over de niveaus die ze tot nu bereikt hebben.

De beschrijving van de mensen die een grote inspiratie waren en zijn voor de ontwikkeling van het CyberTracken komt - ongetwijfeld onbedoeld - nogal stereotypisch over. De term ’Bosjesman’ wordt meermaals gebruikt, maar er wordt niet verklaard waarom deze koloniale term in plaats van de term ’San’ nog steeds gebruikt wordt. Verder vind ik anonimisering (de drie mensen met pijl en boog worden in tegenstelling tot andere mensen in het boek niet met naam genoemd) en veralgemenisering (de auteurs hebben het over ’De bosjesmannen’, en «omdat ‘ze’ niet kunnen lezen en schrijven») niet op zijn plaats. In een apart kader wordt één persoon (#Ui!) daarentegen wel met naam genoemd.

Wat je eerst moet weten

In het hoofdstuk ‘Kennis vooraf’ gaat het over de opbouw van zoogdier- en vogelvoeten, hoe prenten ontstaan en waar je in het bijzonder op moet letten. Het stukje over de evolutie van voeten en poten bij zoogdieren geeft de lezer inzicht in het hoe en waarom van het ontstaan van verschillen tussen de poten van soorten. Ik mis de kijk van een taxonoom die een paar foutjes had kunnen voorkomen. Zo hebben niet álle dieren zich verder ontwikkeld uit zoolgangers. De oude orde insecteneters bestaat niet meer. Zoolgangers kunnen best snel lopen (denk aan een beer die een eland aanvalt), maar niet aanhoudend. De duim is bijzonder nuttig voor zoogdieren die veel klimmen. En bij vleermuizen hebben de handen en niet de voeten zich fascinerend ontwikkeld. De illustratie met een vleermuis (p. 24) schetst bovendien de achtervoet verkeerd. De benen zijn bij vleermuizen namelijk 180 graden gedraaid waardoor de zool van de voeten als het dier op zijn rug ligt naar de kijker is toegekeerd. (Deze draaiing heeft grote voordelen bij het hangen en bij het vangen van prooidieren.) Dit is overigens de enige fout die ik bij de illustraties heb kunnen ontdekken.

Leuk en ook met educatieve waarde is de vergelijking van de voeten van zoogdieren met een menselijke hand. Die heb je immers altijd bij je en is een goed geheugensteuntje bij het duiden van prenten. Enigszins verwarrend is dat de auteurs voor de voeten het begrip ’hand’ en voor de tenen de namen van de verschillende vingers gebruiken en dus niet zoals te doen gebruikelijk de tenen nummeren.

Een apart stukje verklaart duidelijk met behulp van tekst en illustraties de in het boek veel gebruikte begrippen. Onduidelijk blijft echter waarom de auteurs aan het woord ‘hak’ boven ‘hiel’ de voorkeur geven. Het laatstgenoemde begrip is bij mensen en andere zoogdieren toch de algemeen gebruikte term voor het achterste deel van de voet? Verderop in het boek worden ook Engelse (Amerikaanse) termen zoals ‘understep’, ‘indirect register’ en ‘bound’ gebruikt. Deze begrippen zijn waarschijnlijk aan het CyberTracker-universum gekoppeld, maar zijn niet meteen begrijpelijk en daarmee onduidelijk. Meer consensus tussen auteurs van Nederlandstalige sporenboeken over dit soort begrippen is wenselijk.

Het hoofdstuk vervolgt met uiteenlopende tips die je op het goede spoor (!) zetten, zoals van het bewust waarnemen van je omgeving, over plekken waar je vaak prenten kunt aantreffen en de rol van de stand van de zon bij het bekijken van prenten. Als de auteurs het hebben over natuurlijke trechters in het landschap en bijgevolg allerlei door mensen geschapen structuren zoals een dam over een sloot, een trekkerspoor in een akker en een duiker, blijkt weer eens indirect hoe het Nederlandse landschap door mensenhand gevormd is. De boodschap is echter duidelijk:

probeer je in een dier te verplaatsen en voor te stellen hoe en waar dat dier zich in het landschap bevindt en beweegt.

Het stappenplan voor het kijken naar prenten van zoogdieren is onderverdeeld in elf stappen en deze geven de lezer duidelijk houvast bij het 'lezen' van individuele zoogdierprenten. Daarbij is het ook belangrijk te weten dat er een hoop variatie is en dat er vaak verschillen zijn tussen voor- en achtervoet, tussen man en vrouw en tussen jong en oud. Ook de invloed van de ondergrond is van groot belang. Het laatste wordt duidelijk geïllustreerd met informatieve foto's. Bij prenten in de sneeuw wordt benadrukt hoe goed deze te lezen zijn als je een 'perfecte', dunne laag sneeuw hebt op een donkere achtergrond. Hier had aanvullend vermeld kunnen worden dat je dit bij uitstek aantreft op ijs en dat het bij dikkere pakken sneeuw de moeite loont om te speuren naar plekken onder bomen of andere overhangende structuren waar de sneeuwlaag vaak dunner is.

Ook voor vogelprenten is er een apart voorbereidend stukje. Wederom is de vergelijking met de mensenhand bijzonder educatief. Het stappenplan voor het kijken naar prenten van vogels bestaat uit zeven stappen.

Prentbeschrijvingen

Het hoofdstuk met de prentbeschrijvingen per diersoort neemt grofweg driekwart van het boek in beslag. De volgende soortgroepen worden behandeld: zoogdieren (170 blz), vogels (50 blz), amfibieën en reptielen (20 blz) en ongewervelden (20 blz). Ik ken geen enkel Europees sporenboek waar de prenten van de laatste drie groepen zoveel aandacht krijgen. Alleen dat al is een pluspunt!

De pogingen om prenten van veel op elkaar lijkende soorten, zoals rode en grijze eekhoorn (*Sciuris vulgaris* <-> *Sciuris carolinensis*), bruine en zwarte rat (*Rattus norvegicus* <-> *Rattus rattus*), tamme en wilde kat (*Felis catus* <-> *Felis silvestris*) en hond en wolf (*Canis lupus familiaris* <-> *Canis lupus*), toch uit elkaar te houden verdienen waardering. Jammer dat de goudjakhals (*Canis aureus*), die nu volop het studiegebied verovert, niet apart wordt behandeld. Gelukkig krijgt de goudjakhals wel aandacht bij de behandeling van de hond.

Volgens het kaartje in de inleiding zou het studiegebied ook het zuiden van Noorwegen en Zweden omvatten. Een bespreking van de prenten van lemming (*Lemmus lemmus*), boslemming (*Myopus schisticolor*), poolvos (*Vulpes lagopus*) en veelvraat (*Gulo gulo*) ontbreekt echter. De soorten worden overigens systematisch besproken. Een soortgroep, handig gekenmerkt door kleurvlaggetjes onderaan de zijkant van de pagina's, wordt veelal eerst met een inleidend hoofdstuk behandeld.

De prentbeschrijvingen per soort (bij de niet-zoogdieren gaat het vaak om soortengroepen in plaats van soorten) volgen een vast stramien: een inleidend stukje geeft informatie over de plaatsing in het dierenrijk, de uiterlijke beschrijving van de soort, de verspreiding, het voedsel, het leefritme, de leefwijze, de manier van lopen en te verwarren soorten. Dit stukje wordt geïllustreerd met een foto van de betreffende soort. Vervolgens wordt uitgelegd waar je de sporen (prenten!) het beste kunt aantreffen. Dan worden de kenmerken van de voor- en achtervoet uitgelegd, geïllustreerd door foto's van de voetzolen (veelal van dode dieren) en van de prenten en een illustratie van de prenten. Bij het laatste staan nummertjes die verwijzen naar de beschreven kenmerken. Vervolgens worden praktijkvoorbeelden van prenten met foto's en bijbehorende tekst gegeven. Tenslotte volgt een stuk over prenten van soorten die voor verwarring zorgen. Een prentillustratie van de besproken soort wordt voorafgaand aan illustraties van de te verwarren prenten gepresenteerd. Stapsgewijs wordt, uitgaande van de te verwarren soorten, verklaard wat de verschillen zijn. Iedere soortbeschrijving neemt drie tot vier bladzijden in beslag.

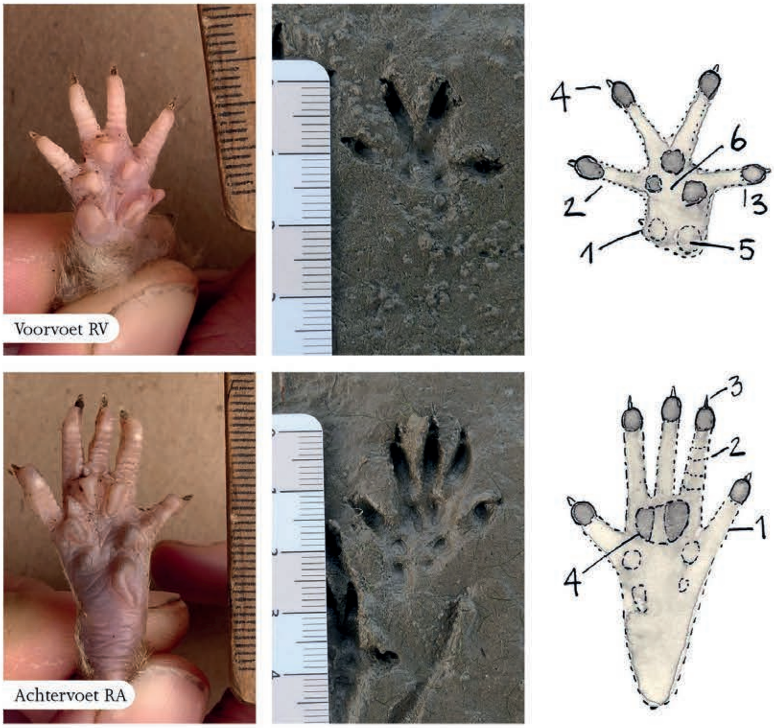
De opzet van de soortbeschrijvingen ervaar ik als uitermate pedagogisch. De lezer wordt van begin tot het eind bij de hand genomen.

Het inleidend stukje over de herkenning en de biologie van de zoogdiersoorten is informatief en geeft de prent een 'gezicht'. Het is - zonder dat het expliciet vermeld wordt - een beknopte versie van de soortbesprekingen van de Veldgids Europese Zoogdieren (Twisk et al. 2010). Jammer dat de auteurs veelal geen gebiedsinformatie van net over de Nederlandse grens hebben meegenomen. Zo zou het boek in België, met name in Vlaanderen, een nog groter bereik hebben gekregen. Bij het meelezen van de inleidende tekst heeft de uit zes personen bestaande leesgroep wel een paar onvolkomenheden over het hoofd gezien. Zo gebruiken de auteurs het begrip Scandinavië verkeerd. Dit omvat namelijk Noorwegen, Zweden én Denemarken, maar niet Finland. De steenmarter (*Martes foina*) komt ook in Denemarken voor. De wezel (*Mustela nivalis*) heeft een grotere sprongafstand dan 20 cm, hij kan wel sprongen van 1 m maken. De huismuis (*Mus musculus*) komt in praktisch geheel geografisch gescheiden gebieden voor, waarbij de oostelijke vorm niet egaal grijs is, maar een duidelijke demarcatielijn naar een witte buik heeft. De herintroductie van de wisent (*Bison bonasus*), met name in Duitsland had gerust genoemd kunnen worden, evenals dat je schapen in Noorwegen praktisch overal in de natuur kunt aantreffen. De manier van voortbewegen wordt bij de slaapmuizen niet vermeld. Ook wordt verzuimd te vermelden dat met name de hazelmuis (*Muscardinus avellanarius*) in een winternest op de grond overwintert en daarbij dus wel degelijk op de grond komt. Vreemd vind ik dat beweerd wordt dat je prenten van andere soorten muizen, zoals bosmuis (*Apodemus sylvaticus*) en rosse woelmuis (*Myodes glareolus*), nauwelijks in het veld tegenkomt. Zo komen bijvoorbeeld veldmuis (*Microtus arvalis*) en aardmuis (*Microtus agrestis*) toch wijdverspreid voor en moeten prenten van deze soorten nabij akkers en ruigten wel degelijk te vinden zijn.

Het Prentenboek zou meer compleet zijn als ook de afmetingen van prenten en van spoorgroepen zouden zijn vermeld. Deze maten kunnen namelijk wel degelijk nuttig zijn voor een juiste identificatie. Voor deze informatie en voor de schat aan informatie van andere sporen blijven we aangewezen op andere sporenboeken.

De foto's van de zolen van de behandelde diersoorten zijn zeer nuttig. Dat de zolen niet met een standaardachtergrond of met eenzelfde meetlat gefotografeerd zijn is, verassend genoeg, niet storend. De kwaliteit van de foto's is goed. Zie ter illustratie de beschrijvingen van voor- en achtervoet van bruine rat (p. 116 uit het boek) op p. 123. Helaas dekken sommige van de door de opmaak over de foto's heen gemonteerde, toelichtende etiketten (zoals over voor- of achtervoeten) details van de voeten af (zoals bij de nevenhoeven van de hoefdieren). Bij de voetzolen van de prentbesprekingen van spitsmuis, haas (*Lepus europaeus*) / sneeuwhaas (*Lepus timidus*) en woelmuis is niet aangegeven om welke soorten het precies gaat. Hoewel het om een al bekend fenomeen gaat, wordt mooi gedocumenteerd dat boommarters soms weinig haar en steenmarter soms veel haar onder de voeten hebben en dat je bijgevolg het afdrukken van wel of geen beharing in een voetprent niet als sluitend argument voor het determineren van marterprenten kunt gebruiken. Ik heb bij de prenten- en of sporenfoto's geen duidelijke fouten ontdekt. Dat het spoor over een besneeuwd meer in Lapland van een bosmuis afkomstig zou zijn, lijkt me echter onwaarschijnlijk aangezien zowel bosmuis als grote bosmuis (*Apodemus flavicollis*) praktisch niet in Lapland voorkomen en in Noord-Scandinavië sterk aan menselijke bebouwing gebonden zijn. Maar zoals de auteurs terecht vermelden: zeg nooit nooit!

Bij het verklaren van de verschillen tussen gelijkende prenten hebben de auteurs er voor gekozen om het hele soortenspectrum telkens opnieuw te behandelen. Bij de prenten van de otter vind je bijvoorbeeld vergelijkingen met de prenten van de das (*Meles meles*), een middelgrote hond, de pardellynx (*Lynx pardinus*) en de wasbeer (*Procyon lotor*). Bij de das worden vervolgens de prenten van otter (*Lutra lutra*), de lynx (*Lynx lynx*), de wasbeer,



Kenmerken

Voorvoet

Zoolganger. Voorvoet kleiner dan achtervoet. Omtrekvorm rond. Asymmetrisch. Vijf tenen. Duim nauwelijks aanwezig. Laat soms nog kleine afdruk achter in perfecte ondergrond. Is dan gekoppeld aan duimkussen (1). Overige tenen staan gespreid afgedrukt. Middelvinger en ringvinger wijzen schuin naar voren. Wijsvinger (2) en pink (3) wijzen vaak ver naar buiten, soms zelfs iets naar achteren. Vaak is de hoek aan de buitenkant (tussen pink en ringvinger) groter dan de hoek aan de binnenkant (wijs- en middelvinger) van de voet. Nagels klein en scherp, kunnen afdrukken (4). In totaal vijf voetkussens waarvan één polskussen (5). Polskussen zit schuin naast kleiner duimkussen, die het hoogst staat van de twee. Overige drie voetkussens dicht bij elkaar, drukken vaak als 'samengesmolten' driehoek af. Geen negatieve ruimte tenzij alleen teentoppen afdrukken. Geen haar onder voetzool.

Achtervoet

Zoolganger. Achtervoet groter, langer dan voorvoet. Omtrekvorm staand langwerpig. Hak drukt heel vaak niet af. Licht asymmetrisch. Vijf tenen. Vergelijk eigen hand: duim en pink wijzen opzij, andere drie tenen wijzen (redelijk parallel) vooruit. Pink (1) langer dan duim. Duim vaak op gelijke hoogte met pink. Tenen vrij lang en dun. Soms drukken plooien zichtbaar af (2). Nagels dun en scherp, drukken vaak af (3). In totaal zes losse voetkussens in drie tweetallen verdeeld over voet. Twee grootste, met kenmerkende driehoekige vorm, staan dicht onder drie voorste tenen (4). In perfecte afdruk zie je die driehoekige vorm. De andere vier zijn het grootst aan binnenkant (duimzijde) van voet. Geen negatieve ruimte tenzij alleen teentoppen afdrukken. Geen haar onder voetzool.

Knaagdieren 116 Prenten

Pagina 116 uit het Prentenboek: beschrijvingen van voor- en achtervoet van bruine rat.

de grote marters en een middelgrote hond behandeld. Voor de gebruiker van het boek is dit bijzonder aangenaam omdat je door deze opzet niet veel heen en weer hoeft te bladeren en altijd kunt uitgaan van de door jou gevonden prent. Het nadeel hiervan is wel dat informatie veelvuldig voorkomt waardoor de omvang van het boek toeneemt.

Een etiketaanduiding van wat voorpoot en wat achterpoot is, zou nuttig geweest zijn. Bij het vergelijkingsoverzicht van de muizen (spitsmuizen zijn geen muizen!) kan de lezer snel op het verkeerde been gezet worden door het aantal tenen van de voorvoet (vijf). Pas bij de beschrijving van de duim wordt duidelijk dat deze bij de meeste knaagdieren praktisch nooit afdrukt en dat je daardoor in de prent maar vier tenen telt. Wanneer prenten van lynx en sneeuwhaas vergeleken worden had vermeld moeten worden dat bij de sneeuwhaas het verschil tussen voor- en achterpoten bijzonder groot is.

Manier van lopen

De manier van lopen, bij de meeste sporenboeken als inleidend hoofdstuk, wordt zoals bovenstaand al opgemerkt, na de prentbesprekingen uitgelegd. Mogelijk omdat de auteurs willen dat de gebruiker van het boek het kijken naar individuele prenten eerst en bovenal moet leren. Zoals de auteurs aangeven komt de uitleg over de manier van lopen voor veel mensen vaak technisch en abstract over. Ze doen zelf een moedige poging om het anders uit te leggen. De lezer krijgt houvast door de uitleg van een set van kenmerken (groeplengte, spooras, spreiding etc.). Verder helpen de beschrijving van het loopritme (1,2,3,4,1,2,3,4,… bij de stap; 1,2,1,2,1,2,.. bij de draf), het geluid van een paard of van je eigen hond bij het lopen en natuurlijk de vele foto's. Toch blijft het voor mij abstract.

De grootste handicap is natuurlijk dat wijzelf op twee voeten lopen en ons daardoor moeilijk kunnen verplaatsen in het lopen met vier voeten. Misschien is het allemaal makkelijker te snappen door zelf op handen en voeten te lopen en de verschillende manieren van lopen - voor zover mogelijk - te ervaren? Bewegende beelden helpen uiteraard ook voor het begrijpen van de verschillende loopwijzen. Ik ben ervan overtuigd dat de auteurs het in het veld veel levendiger kunnen uitleggen. Toch zou, zoals in andere sporenboeken gebruikelijk, een schets van de prentenreeks bij iedere loopvorm een goede steun geweest zijn. De auteurs presenteren zulke reeksen pas - gebundeld - aan het eind van het hoofdstuk. Verwarrend is dat twee van de drie illustratiefoto's bij de stap, draf tonen. Ook verwarrend zijn de verschillende begrippen. Terwijl sprong en hop inleidend als twee van de zes verschillende loopvormen worden genoemd, worden deze gebundeld onder het begrip sprong gepresenteerd. Bij de illustratie van de loopvormen zijn dit vervolgens drie begrippen: sprong, 'bound' en hop.

Dat de sprongengalop veel energie kost staat haaks op de uitspraak dat dit voor marters een zeer karakteristieke manier van bewegen is. Geen dier beweegt zich langdurig op een manier die veel energie kost. Dat de lichaamsbouw bij marters de sprongengalop energetisch efficiënt maakt had uitgelegd kunnen worden.

Materialen, onderzoek en 'trailing'

Eén van de afsluitende hoofdstukken, 'Materialen en middelen', gaat over hulpmiddelen die handig zijn om mee te nemen als je op stap gaat, hoe je prenten het beste kunt vastleggen (fotograferen, natekenen, gips- en kleiafdrukken, roet-, olie- en inktplaten), het gebruik van cameravallen en marterkisten. Bij het erop volgend hoofdstuk 'Toepassingen en onderzoek' gaat het onder andere over belangrijke thema's als 'trailing', het inschatten van de leeftijd van een prent en de waarde van prenten voor onderzoek. Over trailing schrijven de auteurs: "Trailing is het volgen van een diersoort tot het moment dat je de maker van het spoor gevonden hebt". Dit spreekt natuurlijk vele natuurgeïnteresseerden aan. Tenslotte hebben ontelbare generaties voor ons dit dagelijks gedaan om, bewust of onbewust, in hun behoefte aan dierlijke proteïnen te voorzien. Het zit als het ware in onze genen. Ook vergt dit het uiterste van al je zintuigen. En dan uiteindelijk de beloning: een dier in het wild zien. De auteurs vermelden terecht dat hier ook een ethische kant aan vastzit: het komt er in de praktijk vaak op neer dat je een dier opjaagt en wil je dat wel? Dit dilemma hadden de auteurs best wat verder mogen uitwerken. Als je geïnteresseerd bent in het natuurlijk gedrag van een dier, dan probeer je je eigen invloed op het dier zo veel mogelijk uit te sluiten en in ieder geval tot het minimum te beperken. Het langere tijd volgen van een spoor in de looprichting van het dier is daarbij taboe. Dit wordt duidelijk bij sporen in de sneeuw. Sneeuw documenteert immers zowel je eigen bewegingen als de bewegingen van het te volgen dier genadeloos. De consistentie van sneeuw verandert veelal in de loop van de dag. Daardoor vormt een spoor in de sneeuw ook een tijdlijn. Bij het volgen van bruine beren (*Ursus arctos*) en lynxen blijkt zo vaak dat de roofdieren als ze merken dat ze gevolgd worden posities in het terrein innemen waarvandaan ze jou ongezien kunnen gadeslaan. De geur van de mens verraadt immers onze aanwezigheid lang voordat enig zichtcontact mogelijk is. Daarom wordt in Zweden en Noorwegen aanbevolen om sporen van onder andere grote roofdieren tegen de looprichting in te volgen. Deze methode wordt gelukkig ook door de auteurs vermeld. Het effect van trailen op dieren die zich niet in een hol kunnen verstoppen is natuurlijk het grootst. Dat trailing in Nederland verboden is en elders niet, is echter geen vrijbrief om het op deze wijze speuren naar dieren in het buitenland ongeremd wel te doen.

Cursussen, nog meer tips, dank- en nawoord

Het boek eindigt met een hoofdstuk over educatie en nuttige internetadressen voor meer informatie en cursussen die de auteurs geven. Tot slot worden alle fotografen die aan het boek hebben bijgedragen vermeld en bedankt, evenals alle anderen die hebben bijgedragen aan het prentenboek.

Een lovend en wijs nawoord van, waarschijnlijk, één van de grote mannen in het cybertracken en een presentatie van wie de auteurs zijn en waar ze heen willen sluiten het boek af.

Opmaak

De auteurs hebben er voor gekozen om het boek in eigen regie uit te geven. Dit geeft een grote vrijheid bij onder meer de keuze van de opmaak en de hoeveelheid tekst en informatie die je kunt en wilt opnemen. Deze vrijheid hebben de auteurs volop benut, zoals bij het geven van tips en uitleg, het gebruik van foto's en het bieden van afwisseling zoals in de vorm van persoonlijke anekdotes.

De opmaak is over het algemeen goed geslaagd en de foto's en illustraties zijn groot genoeg, al wens je altijd dat met name de sporenfoto's nog groter afgebeeld zouden zijn. Er is vrij veel wit op de bladzijdes, waardoor er ruimte is voor aantekeningen. Die vrije regie heeft wel tot een relatief dik boek geleid. Die omvang en daardoor ook het gewicht maken het boek minder geschikt voor in het veld.

De anekdotische teksten zijn op een bruinrode achtergrond gedrukt, wat ze goed herkenbaar maar slechter leesbaar maken. Ook zijn de teksten die over de foto's heen zijn gedrukt moeilijk leesbaar. Her en der wordt vergeten te vermelden welke soort de pootafdruk op een foto heeft gemaakt. Ik heb hier geen structuur in kunnen ontdekken: waarom wordt bij sommige foto's wel en bij de vele andere geen vindplaats vermeld?

Conclusie

De kracht van het Prentenboek is volgens mij hierin gelegen dat het mensen enthousiast maakt voor het zoeken naar sporen en benadrukt dat daarbij het nauwlettend bekijken van prenten van groot belang is. Ook de wat meer ervaren spoorzoeker heeft er met dit boek een bron met nieuwe en bovenal op educatief aantrekkelijke wijze gepresenteerde informatie bij. Door het lezen van het boek ben ik gestimuleerd om niet alleen de naam van een soort aan een prent te koppelen, maar ook om beter te kijken naar voor- en achterpoot, naar links en naar rechts. Prenten waar ik vroeger aan voorbijliep, omdat ze bijvoorbeeld van huisdieren waren of moeilijk herkenbaar, zijn nu ook interessant geworden.

Ik hoop oprecht dat dit boek zal bijdragen aan de erkenning van het spoorzoeken als een betrouwbare en in het veld ook zeer goed bruikbare inventarisatie- en onderzoeksmethode.

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