War and wildlife

The continuing war in Ukraine, and especially the disastrous destruction of the Kachovka Dam about one month ago, made me think about the collateral damage of war to wildlife and international scientific collaboration. The breach of the Kachovka dam in the early hours of the 6th of June unleashed a torrent of water that flooded villages and towns, led to people and livestock drowning and caused the evacuation of tens of thousands of people from both sides of the Dnipro River in Ukraine's southern Kherson region. It also caused what many fear will be one of Europe's biggest environmental disasters in decades. Mud and toxic substances, including pesticides, buried in the sediments of the Kachovka Reservoir have spread over large areas downstream of the dam. The inundated area is far larger than the former flood plains that were previously inundated by natural floods on the Dnipro river, before the construction of the Kachovka Dam. For example, the fluvioglacial sand dune landscape of the Oleshky Sands National Nature Park is now inundated. Until this event, this site contained about half of the world's population of the sandy blind mole-rat (*Spalax arenarius*), a species endemic to Ukraine, with a very restricted range along the lower Dnipro River. Another species, Nordmann's birch mouse (*Sicista loriger*), while not endemic to Ukraine, has its main strongholds in remnant steppe habitats along the lower Dnipro river. According to the Ukrainian Nature Conservation Group, 70% of its world population has been affected by the flooding.[1]

Dr Pavlo Goldin, of the Smalhausen Institute in Kyiv, gave an interview with the popular science media outlet Kunsht, that not only gives a sound commentary on the consequences for wildlife, but also compares the breach of the Kachovka Dam to deliberate inundations in the Netherlands in the 16th century during the Eighty Years War. Examples include the sieges (and reliefs) of Alkmaar (1573) and Leiden (1574). While the Dutch have a long standing tradition of using inundation to prevent enemy armies invading the western part of the country (particularly through the water-based defences of the Old and New Dutch Waterlines) it seems to me that this is not ecologically comparable to the destruction of the Kachovka Dam.

One (unintended) side-effect of the New Dutch Waterline is that its fortifications have now become a stronghold of hibernating bats (see Buys et al. 2022 in last year's Lutra Special on hibernating bats). But in Ukraine, bats continue to suffer from the raging war. For example, in the town of Kharkiv in 2022 an estimated 7,000 noctules (*Nyctalus noctula*) have died as a consequence of war damage to buildings (Vlaschenko et al. 2023).

[1] https://uncg.org.ua/en/the-consequences-of-the-russian-terrorist-attack-on-the-kakhovka-hydroelectric-power-station-hps-for-wildlife/

International scientific collaboration in joint projects with Russia is another victim of the war in Ukraine. The European Mammal Foundation is currently in the process of producing a Second Atlas of European Mammals, this time including Belarus, Moldova, Russia and Ukraine, which were not included in the first atlas. However, as the war continues unabated and, despite the best efforts of the European Mammal Foundation over a considerable period, finding an acceptable way to engage in scientific cooperation with Russia has proved inappropriate, if not impossible. With great regret, the European Mammal Foundation has decided to abandon the original scientific objective of mapping mammal distributions across the whole of geographic Europe and will now exclude the Russian part of that area from the project. Following on from this decision Russia has announced that it will produce its own mammal atlas.

The editors of Lutra sincerely thank our dear colleague Dr Jan Piet Bekker who in the editorial of December last year announced his resignation from the editorial board. Jan Piet has been a dedicated editor of Lutra for 19 years and was also a very active contributor to the journal. He wrote or co-authored ten editorials and 28 or so articles for Lutra, many on morphology, such as the article on the frequency of the *radnensis* form of a molar of the root vole in this issue of Lutra. The origin of the name *radnensis* derives from the description by Éhik in 1942 of a supposed new species of snow vole, described by the author as *Microtus* (*Chionomys*) *radnensis*, first collected in the Rodna mountains (German: Radnaer Gebirge) in the north of Romania. However, later *radnensis* turned out to be just a tooth anomaly, which also occurs in other mouse species, such as Savi's pine vole (*Microtus savii*) and the root vole.

This issue of Lutra also contains papers dealing with: non-lethal, but disruptive and aversive, stimuli to protect livestock from wolves (Van Dessel & Snijders); seals that died from asphyxiation after eating Dover sole (Haelters et al.); offshore windfarms as a possible threat to noctules (Lagerveld & Mostert), and; the recently discovered presence of hibernating Leisler's bats in the Low Countries (Janssen et al.). The authors of the latter paper suggest that a part of the Leisler's bat population migrates to southwestern regions, while others stay and hibernate in their summer range, which may be due to climate change (editor's interpretation). In ecological terms the conclusions of these papers carry mixed messages. Van Dessel & Snijders' paper argues for the need for a more evidence-based approach to human-wolf conflict as being essential in building viable cohabitation arrangements between wolves, livestock farmers and pastoral activities. Lagerveld & Mostert's findings are somewhat more comforting, and suggest that offshore wind farms probably pose little risk to coastal noctule populations, at least for most of the year. The finds of Haelters et al. may be surprising to some readers: in spite of Dover sole being dangerous to the seals that eat them, it remains one of their favoured preys.

I thank Dr Lena Godlevska from the Schmalhausen Institute of the Ukrainian Academy of Sciences, who has provided excellent commentaries on the consequences of the war in Ukraine for wildlife.

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Johan Thissen

The form *radnensis* in the upper second molar in isolated subpopulations of the root vole (*Alexandromys oeconomus arenicola*) in the Netherlands

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Abstract: During a preliminary study of unsorted barn owl pellets from Texel, the Netherlands, twelve out of 140 upper second molars (M^2) of the root vole (*Alexandromys* (*Microtus*) *oeconomus arenicola*) were identified as the form *radnensis*. Besides the regular or normal form (N), four different stages of *radnensis* are recognized, which show a progressive incision of the lingual field: V_1, V_2, V_3, and V_4. The aim of this study is to determine the frequencies of the prevalence of the form *radnensis* of M^2 in the known subpopulations of the root vole in the Netherlands, to establish whether or not these frequencies differ from each other and, if so, whether this can be explained by the age structure of the root vole subpopulation. We analysed the skulls of root voles from owl pellets from all the known subpopulations in the Netherlands. The subpopulation of Texel shows 12.5% *radnensis,* significantly higher than all the other subpopulations. No link was found with age. It is concluded that the prevalence of the form *radnensis* is unevenly distributed over the subpopulations of root voles in the Netherlands.

Keywords: *Alexandromys oeconomus arenicola*, form *radnensis* M^2, genetic drift, *Microtus*, Netherlands, root vole, Texel, upper second molar.

Introduction

The root vole (*Alexandromys oeconomus arenicola*) is an endemic subspecies in the Netherlands and is considered an ice age relict (van Laar 2018). Under the criteria mentioned in the proposal for a revised Red List for Mammals of the Netherlands, the root vole's status remains 'vulnerable' (van Norren et al. 2020). In the past many areas became unsuitable habitats for the root vole, leading to the development of isolated subpopulations. Small populations are more prone to genetic drift, a greater chance of minor physical alterations and, potentially, of local extinctions. As such it is important to monitor seemingly minor alterations as these changes to the phenotype could indicate a genetic impoverishment of this vulnerable endemic subspecies.

During a preliminary study of unsorted barn owl pellets from Texel, the Netherlands, the presence of a number of variants that can be described as an inverted omega (Ω) at the lingual side of the upper second molar (M^2) in the root vole (*Alexandromys* (*Microtus*) *oeconomus arenicola*) was immediately obvious (Figure 1). Twelve out of 140 M^2s appeared to be of this specific form, also known as the form *radnensis* (Ruprecht 1967). Within this subset, eight were on the left side and four on the right side.

Angermann (1984) reported the occurrence of different stages of the form *radnensis* of M^2 of the root vole from 30 localities from all over its circumpolar distribution in Central and Northern Europe, Central Asia and Beringean. The highest prevalence of *radnensis*

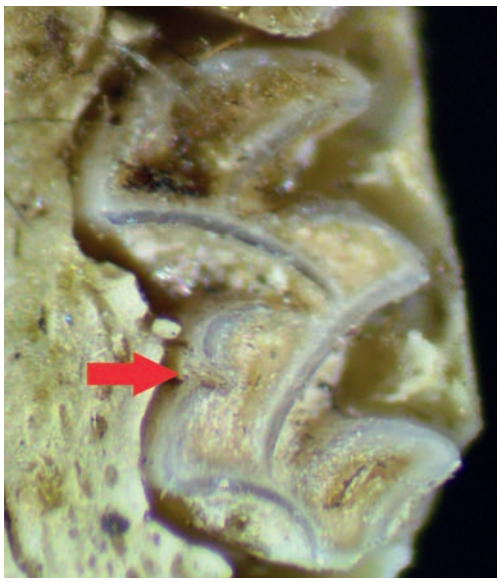

Figure 1. The omega shaped indentation of the left second upper molar (M^2) of the root vole (*Alexandromys oeconomus arenicola*) described as the form *radnensis* (V_2). *Photo: J.P. Bekker*.

(10.5%) was found in a sample of 124 M^2s from specimens from St. Lawrence Island, USA and from 145 M^2s from the Netherlands (locations not specified), which was the second highest percentage (7.6%).

Ruprecht (1967) studied 610 skulls of the root vole from the Kujawy area, Poland, and discovered, for the first time, a specimen with a remarkable feature: the triangle in the second field of M^2 was completely divided (the form *radnensis*). Ten other specimens, also from this area, had M^2s with a partial division. Besides the regular or normal form (N), Ruprecht (1967) recognized four stages in the form *radnensis* : V_1, V_2, V_3, and V_4, representing progressive incision of the lingual field (Figure 2).

Van Laar's study (2018) on the origin and distribution history of the root vole in the Netherlands, identifies at least five subpopulations. These historically-based subpopulations are: 1. the island of Texel; 2. Friesland and the adjacent IJsseldelta; 3. the moorlands of Noord-Holland; 4. the moorlands of Zuid-Holland and adjacent parts of Utrecht; 5. the estuaries of the rivers Rhine and Meuse (almost always referred to as the 'Delta') and (maybe) 5a. the Biesbosch. Van den Brink et al. (2011) made a study of differences of skull shape (geometric morphometrics) and slightly altered this classification; recognizing the same subpopulations for the island of Texel, Friesland and the IJsseldelta, unifying the moorlands of Noord-Holland, Zuid-Holland and adjacent Utrecht. In addition to the Delta these authors identified the area around the Vlaardingse Vlietlanden, north of the Nieuwe Waterweg, as having a separate relict population. Based on the distribution of the root vole in the Netherlands and its recent discontinuities, this led to reclassification of the five subpopulations: 1. Texel; 2. Friesland; 3. the moorlands of Noord-Holland; 4. the moorlands of Zuid-Holland; 5. the Delta (Koelman & Bekker 2016, especially the map on page 128).

The origin of the term *radnensis* derives from the description by Éhik (1942) of a new species of snow vole, described by the author as *Microtus* (*Chionomys*) *radnensis*, collected in the Rodna mountains (German: Radnaer Gebirge) in the north of Romania. In the described specimen the second field of the M^2 was partly divided and could be classified as stage V_2 under Ruprecht's classification (1967) (see photograph opposite to page 30 in Éhik (1942)). In this photograph it is also clear that the form *radnensis* is present at both sides. Tast (1982) regarded this snow vole, *M. nivalis radnensis*, as a subspecies, although this subspecies is no longer mentioned in the Handbook of the Mammals of the World (Wilson et al. 2017).

In addition to the snow vole, *Chionomys nivalis radnensis*, and the root vole, the form *radnensis* is also described in recent specimens of Savi's pine vole (*M. savii*) (Contoli 1992). Malez & Rabeder (1984) described special shapes of occlusal views of different *Microtus* specimens from an Early Pleistocene fissure filling of Podumci 1 in Croatia. In their

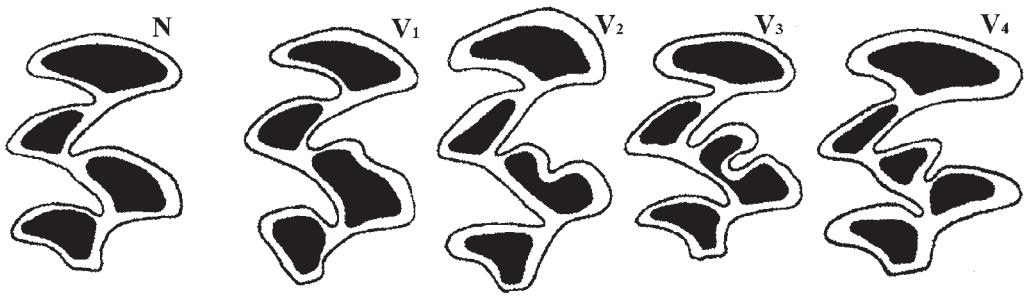

Figure 2. The regular right second upper molar (N) and the different stages of M^2 in the form *radnensis* (V_1, V_2, V_3, and V_4) of the root vole (adapted from Ruprecht 1967).

study another form of a left M^2 is depicted and described as *praeradnensis*, without further comments on frequencies. Malez & Rabeder (1984) also describe a left M^1, with a "*radnensis*"-wrinkle at the mesial side of the lobus anterior.

The aim of this study is to determine the frequencies of the prevalence of *radnensis* of M^2 in the known subpopulations of the root vole in the Netherlands and to establish whether the frequencies differ significantly from each other. In the samples studies by Ruprecht (1967) and Jorga (1973), there was not an even distribution of the different stages (V_1-V_4) of the form *radnensis* which was higher among the lower stages than the higher ones (Table 1). However the limited number of unsorted skulls from Texel showed equal numbers of both V_1 and V_4, raising the question of whether this skewed distribution of the form *radnensis* would be found in a larger set of observations from the total population in the Netherlands. In voles the molar patterns of opposing molar pairs suggest a transient role for the second upper and lower molars. While the early developmental architecture of such traits is masked by later stages of growth, it may still be deciphered from the adult phenotype, if careful attention is paid to the relevant features (Labonne et al. 2014). Assessing ontogenetic changes in molar complexity during the process of tooth wear in the narrow-headed vole (*Microtis gregalis*), closely related to the root vole, suggested that morphotype dental patterns could not be clearly established in half-month old animals due to presence of juvenile characteristics and for animals of one month and older, age differences in morphotype dental patterns were insignificant (Markova et al. 2013). If an inverse relation (i.e. decreasing numbers from V_1 to V_4) could be demonstrated, this raises the question as to whether the age structure of the root vole population could be the explanation of this skewed distribution.

Table 1. Frequencies of the stages of the form *radnensis* in M^2 of root voles in a preliminary and two regular studies.

Author / form of *radnensis*	N	V_1	V_2	V_3	V_4
Unsorted skulls Texel	140	6			6
Ruprecht 1967	610	11	6	2	1
Jorga 1973	251	21		1	1
Total	861	38	6	3	8

Material and methods

This study analyses owl pellets that were collected from known localities on the island of Texel, between 2015 and 2020, together with the available skulls of root voles from owl pellets, collected between 1994-2020 from all over the Netherlands, which were provided by the Dutch Mammal Society (Zoogdiervereniging). Additional root vole skulls from owl pellets from Schouwen-Duiveland, mainly

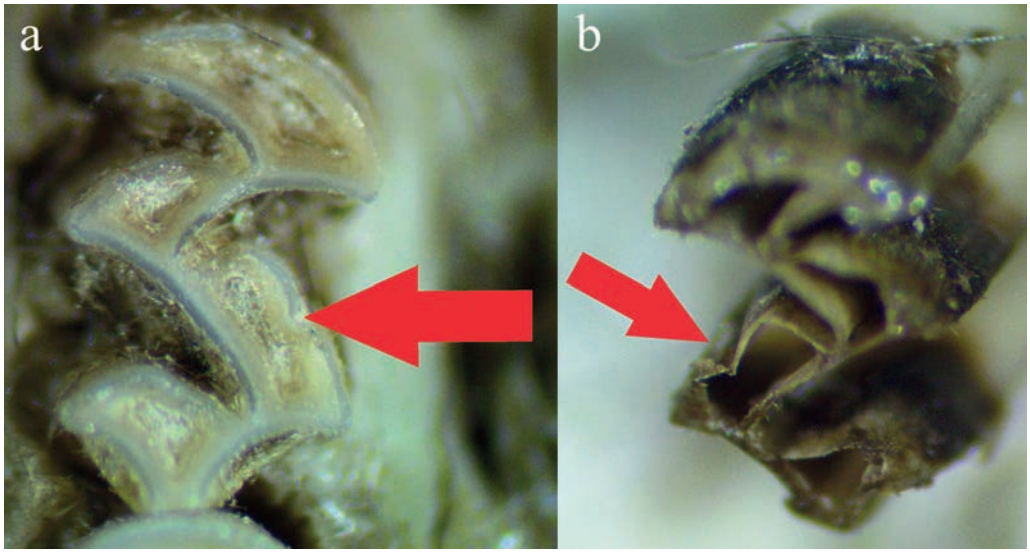

Figure 3. Occlusal view (a) and root rim view (b) of M^2 with form *radnensis* V_1; the indentation of the occlusal view is hardly visible, while the indentation of the root rim view is obvious. *Photos: J.P. Bekker*.

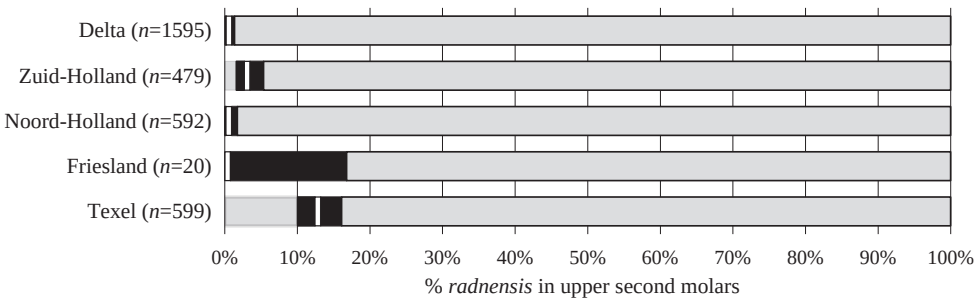

Figure 4. Percentages of the form *radnensis* in upper second molars, with limits of confidence (black), of root voles in subpopulations in the Netherlands.

collected during the years 1998-2003, completed the sample. The skulls with M^2s were sorted by the collection site and used to determine the frequencies of the form *radnensis* in the subpopulations. The following numbers of available skulls, with M^2s present on one or two sides were counted: 303 from Texel, 315 from the moorlands of Noord Holland, 253 from the moorlands of Zuid Holland and 853 from the Delta, and 10 from Friesland.

Based on the drawings and detailed descriptions of Ruprecht (1967) and Jorga (1973) the definitions of the regular form (N), and the stages V_1, V_2, V_3, and V_4 of the form *radnensis* can be described as follows. The regular form N can be characterized as the lingual line of the second field of the upper second molar (M^2) being slightly convex till plane and showing no concavity. It may show a slight concavity outside of the centre of the lingual enamel ridge; a form that is also assigned to the regular form N. The form V_1 shows an indentation rather than a concavity in the middle of the oral line, which is less than the thickness of the enamel ridge. The concavity in the form V_2 is deeper than the enamel ridge oral line, but does not reach the middle of the breadth of the triangle of the second field. The concavity in the form V_3 reaches deeper than half of the breadth of the triangle, but not to

the enamel ridge of the opposing side of the triangle. In the form V_4 the concavity is even deeper, so that both opposing enamel ridges touch or coalesce. In some cases it may be difficult to assign a specific molar into the right category, e.g. the difference between a form N with a slight concavity, outside the centre of the lingual enamel ridge or form V_1 (Figure 3a); in such cases it is helpful to observe the root side of this molar (Figure 3b).

Two types of analysis were conducted in order to determine whether the distribution of the stages of the form *radnensis* is determined by the age structure of the investigated subpopulations of root voles or by another cause: age dependent factors and a comparison of the left and right M^2s from the same skull (which by definition were of the same age).

Markowski (1980) proposed ten skull measurements for root voles but in this study we only use the upper molar row length, measured from the anterior margin of the alveolar of M^1 to the posterior margin of the alveolar of M^3. This is because this measurement has the fewest drop-outs as vole skulls in owl pellets are often broken. The values of the age categories in root voles are derived from Markowski (1980) and presented in boxplots using the mean values and twice the standard error. The upper molar row lengths of the four stages V_1, V_2, V_3 and V_4 *radnensis*, are also presented in boxplots for comparison.

M^2s from the same individuals were compared by determining the correlation of the presence of the stages V_1, V_2, V_3 and V_4 between the left and the right sides. Frequencies (including 95% confidence intervals) of the form *radnensis* are presented as percentages of the total numbers of the observed upper second molars (M^2). The 95% confidence interval is calculated around the measured difference in percentages (Wilson's formula, RIVM 2020; see also Bekker 2020) to determine whether the two percentages differ. The probability of the true value of the difference falling within the interval is 95% (Rothman & Greenland 1998). In the case of zero observations, the upper limit of the 95% confidence interval is calculated using the Byar formula (RIVM 2020).

The chi-square test was used to determine whether or not there is a significant association between two categorical variables. The pairwise differences in left/right presence were tested with confidence interval tables (Diem & Lentner 1968).

Results

The percentages of the form *radnensis* in the second upper molars of root vole skull material in the different subpopulations in the Netherlands is presented in Figure 4. The subpopulation of Texel shows 12.5% *radnensis*, which is higher than in the other subpopulations and, with the 5% confidence limit, is well above the 95% limits of confidence, except the percentage from Friesland (=0%), which is based upon a very limited number of M^2s (n=20). The subpopulation of the moorlands of Zuid-Holland shows 2.7% *radnensis,* much lower than the percentage from Texel, but higher than the percentages of subpopulations of the moorlands of Noord-Holland (0.17%) and the Delta (0.19%). There was no discernible difference between the percentages of the subpopulations of the Delta and the moorlands of Noord-Holland. The sample size of M^2s from Friesland was low (n=20) and because of this it is difficult to draw any conclusions regarding this subpopulation.

Table 2 shows the total numbers of the forms *radnensis* stages V_1, V_2, V_3, and V_4 in the second upper molars of root vole skull material in all the subpopulations in the Netherlands. The totals are not evenly distributed, but there are fewer cases of the higher forms.

The five coloured boxes in Figure 5 represent different age categories. The mean values ('x') of the upper molar row length indicate an upward pattern for the five age categories (coloured boxes, age classes accord-

Table 2. Upper second molars of root voles in the Netherlands, rated according to the form *radnensis* V_1, V_2, V_3, and V_4 (chi square test: $P<<0.01$).

	V_1	V_2	V_3	V_4
Expected M^2s with *radnensis*	23	23	23	23
Established M^2s with *radnensis*	36	31	18	7

Table 3. The presence of N and f. *radnensis* stages V_1-V_4 in left and right M^2s, with symmetrical presence (including unsorted skulls from Texel; skulls with missing M^2s on one side were excluded).

	Left	Right
N	1503	1504
V_1	16	20
V_2	19	11
V_3	8	10
V_4	3	4
Total	1549	1549

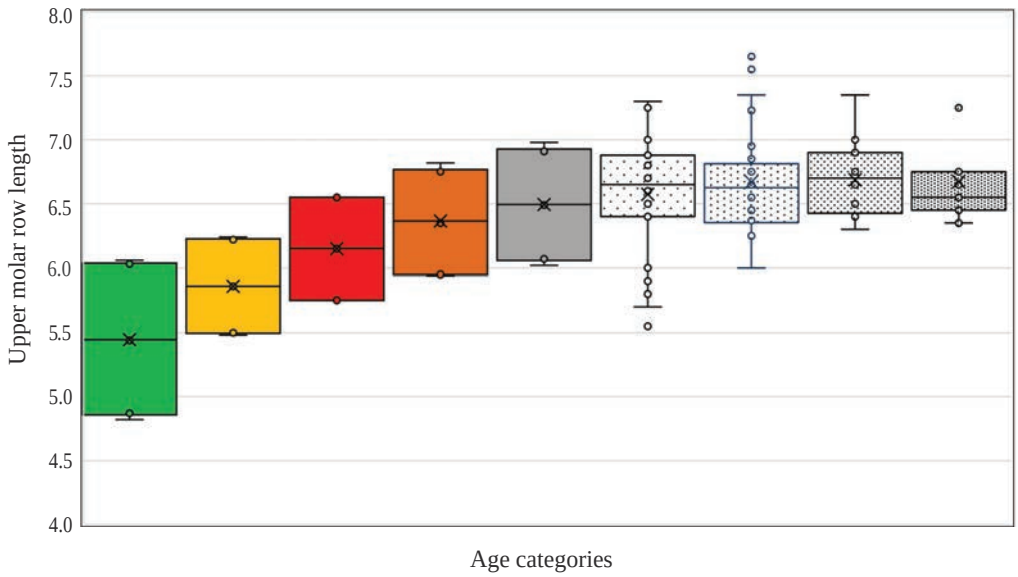

Figure 5. Boxplots of upper molar row lengths in mm (left axis) by age categories I (< 1 month: green), II (1 month - 6 weeks: orange), III (6 weeks - 3 months: red), IV (4-7 months: brown) and V (> 7 months: grey) (age classes derived from Markowski (1980) and the stages of the form *radnensis* (dotted boxes V_1, V_2, V_3 and V_4, from left to right).

ing to Markowski 1980). The upper molar row lengths of all the available skulls (including 'unsorted' material from Texel) were determined for the presence of *radnensis* V_1-V_4 M^2. A comparison of the four dotted boxes (V_1, V_2, V_3 and V_4) with the coloured boxes in Figure 5, shows an upward pattern, suggesting that there is no relationship between the form *radnensis* (stage V_1-V_4) and age and that the presence of the different types of M^2s with *radnensis* is not linked to age.

The distribution of the four stages of *radnensis* from specimens with M^2s present on both left and right side (n=1549) is presented in Table 3. These results do not show a tendency for the form *radnensis* stages (in any of its stages) to appear on either the left or right M^2s among any of the subpopulations of root vole in the Netherlands (chi-square test: P=0.16).

Discussion

The prevalence of the form *radnensis* is unevenly distributed over the subpopulations of root voles in the Netherlands. The subpopulation on the island of Texel has by far the highest percentage (12.5%) followed by the moorlands of Zuid-Holland (2.7%). The occurrence of *radnensis* is very limited in the subpopulations of the Delta (0.3%) and the moorlands of Noord-Holland (0.2%). The number of M^2s from Friesland was too small (20) to draw any conclusions.

Until the 1980s the root vole was the only vole on the island of Texel. In 1985 the first remnants of a field vole (*M. agrestis*), in an owl pellet, was reported by Lange (1986), and field vole specimens were trapped close to that owl pellet four years later (van Apeldoorn et al. 1991). One possible explanation of the high prevalence of the form *radnensis* in root voles on Texel is the island's lengthy isolation (almost 5000 years) from the mainland (Fries-Drents Plateau) (van Laar 2018). During this period, genetic drift may have caused a higher prevalence of *radnensis* than is found in the other subpopulations in the Netherlands. Superficially the situation on Texel is similar to other islands (e.g. Schouwen-Duiveland, the Delta), where root voles have also expanded their ecological niche due to the absence of field voles. However Texel is more isolated from the mainland than other islands, such as Schouwen-Duiveland. So the longer and more emphatic isolation on Texel, rather than the broader ecological niche, might have been the factor influencing the prevalence of the form *radnensis* on Texel. It is also possible that this extra enamel loop provided by this form enables more efficient grinding of vegetable matter.

The percentage of M^2s in root voles with the form *radnensis* found on Texel is slightly higher (12.5%) than that (10.5%) found on St. Lawrence Island (Angermann 1984). The latter island, west of mainland Alaska in the Bering Sea, south of the Bering Strait, is thought to be one of the last exposed portions of the land bridge that once joined Asia with North America during the Pleistocene, ca. 25,000 years ago (Anonymous 1979). Although both islands are considered to be continental islands, their isolation from the nearest mainland (and thereby from neighbouring populations of root voles) probably causes the high percentages of the form *radnensis*. These figures can be compared with findings by Angermann (1984), who examined 70 M^2s from an island in Germany (Riems) and found no presence of the form *radnensis*. This is probably due to this island's close proximity to the mainland (ca. 500 metres), to which it is connected by a dam since the early 1970s.

The results of this study do not suggest any correlation between the upper molar row lengths in the ordinal ranked stages of *radnensis* forms with age. A more precise, direct comparison of upper molar row length stages of *radnensis* forms with the upper molar row lengths in different age categories is not possible as the age class specimens are retrieved from other work by Markowski (1980), conducted in East Poland (*A. o. stimmingi*), while the *radnensis* form specimens are present in a different subspecies (*A. o. arenicola*).

There was no evidence of a prevalence of the form *radnensis* V_1, V_2, V_3 and V_4 in the left or right M^2 of root voles in any of the subpopulations of root vole in the Netherlands.

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Samenvatting

Verschillen in frequentie van de vorm radnensis M^2 tussen geïsoleerde subpopulaties van de noordse woelmuis

(*Alexandromys oeconomus arenicola*) in Nederland

Bij een analyse van ongesorteerde kerkuilbraakballen uit Texel bleken twaalf van de 140 bovenste tweede kiezen (M^2) van de noordse woelmuis (*Alexandromys* (*Microtus*) *oeconomus arenicola*) de vorm *radnensis* te vertonen: deze manifesteert zich als een omgekeerde omega (Ω) aan het driehoekje aan de tongzijde. Naast de reguliere of normale vorm (N) wordt de vorm *radnensis* in vier stadia ingedeeld: V_1, V_2, V_3 en V_4, waarbij V_1 het minst en V_4 het sterkst is ingesneden. Het doel van dit onderzoek is om de frequenties van het voorkomen van de vorm *radnensis* van M^2 in de bekende subpopulaties van de noordse woelmuis in Nederland te bepalen en vast te stellen of er verschillen zijn tussen de subpopulaties. In andere studies wijzen de totalen van de gevonden vormen van de stadia V_1-V_4 op hogere totalen in de lagere stadia. Het is de vraag of de leeftijdsopbouw van de noordse woelmuizenpopulatie de verklaring zou kunnen zijn voor deze scheve verdeling. Naast materiaal van Texel zijn ook alle beschikbare schedels van noordse woelmuizen uit heel Nederland onderzocht. Het betreft schedels afkomstig van: Texel (*n*=303), Noord-Hollandse laagveengebieden (315), Zuid-Hollandse laagveengebieden (253) en de Delta (853); het aantal schedels afkomstig uit Friesland was relatief laag (10). Om de relatie met de leeftijdsopbouw te onderzoeken is van beschikbare exemplaren van de M^2 de stagering van de vorm *radnensis* bepaald en is van dezelfde schedels de lengte van de bovenste tandenrij gemeten. De subpopulatie van Texel toont het hoogste aandeel *radnensis*, 12,5%, gevolgd door de subpopulatie van de laagveengebieden van Zuid-Holland met 2,7%. De percentages *radnensis* binnen de andere subpopulaties zijn lager. Een mogelijke verklaring voor de hoge prevalentie van *radnensis* op Texel is de langdurige isolatie (>4000 jaar) van het eiland, waardoor het aandeel *radnensis* (als gevolg van genetic drift) kon toenemen. Metingen van de bovenste kiezenrijlengtes waar minimaal één *radnensis* aanwezig was, laten stijgende waarden zien bij toenemende leeftijd. Een positieve relatie tussen de bovenste kiezenrijlengte van de vormen *radnensis* V_1-V_4 met de vijf leeftijdscategorieën kon echter niet worden bevestigd. Ook een verschil in frequentie van de vorm *radnensis* V_1, V_2, V_3 en V_4 tussen linker en rechter M^2s van noordse woelmuizen in alle subpopulaties in Nederland kon niet worden aangetoond.

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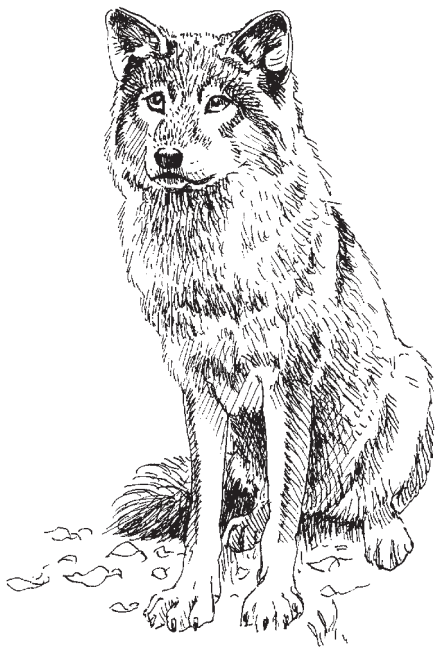

Effectiveness of behaviour-based interventions in reducing livestock depredation by wolves (*Canis lupus*)

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Abstract: Sustainable coexistence between wolves (*Canis lupus*) and humans primarily relies on the availability of effective mitigation practices to reduce livestock depredation by wolves. As a result of wolf recovery, domestic animal losses have been rising, despite the broad implementation of both lethal and nonlethal management efforts. This growing conflict of interest between livestock activities and wolf conservation requires an evidence-based insight into the effectiveness of nonlethal livestock protection interventions. Therefore, in this systematic review, we synthesised the evidence available on the effectiveness of behaviour-based interventions in reducing livestock depredation by wolves. We systematically searched Scopus and Web of Science and screened for literature in a specialised systematic map database created by Snijders et al. (2019). We retrieved 2825 publications, of which 16 articles (and their 31 corresponding studies) were included in the review after the screening process. We used relative risk ratios (RR) and standardised mean differences (SMD) as measures of the intervention effect size and subsequently performed a meta-analysis. Our study revealed a worrying lack of published empirical evidence on nonlethal behaviour-based interventions, at least in the English language, despite their broad application in practical management efforts. Nevertheless, most interventions included in the review, particularly fladry, demonstrated high effectiveness in deterring wolves from approaching- or predating livestock and bait carcasses. The limited size of the evidence base did not allow exploration of the factors that may further moderate the effect size. Knowing these so-called effect moderators could lead to more tailor-made practical recommendations on when and where to employ which type of behaviour-based intervention. Overall, our results suggest that behaviour-based nonlethal measures could be a promising mitigation tool, provided that more research supports these findings. Therefore, we strongly recommend scientists, conservation practitioners and management authorities to collaborate and to further research nonlethal interventions, especially investigating efficacy in real depredation scenarios. A more evidence-based approach to human-wolf conflict is essential in building a viable future for wolves, livestock and pastoral activities.

Keywords: wolves, *Canis lupus*, human-wildlife conflict, livestock, predation, nonlethal, systematic review, meta-analysis.

Introduction

Livestock depredation by wolves (*Canis lupus*) poses a considerable threat to human-wolf coexistence, putting both human livelihoods and wolf conservation at risk (Khan et al. 2019, Kusi et al. 2020). Since the 1990s, wolves are making a strong comeback across the European and North American mainland and are adapting to a wide range of different environments (Mech 1995, Reinhardt et al. 2019), including highly anthropogenic landscapes such as the Netherlands and Bel-

gium (Chapron et al. 2014, Van Den Berge 2019, Jansman et al. 2021). With wolves living closer to human settlements (Ronnenberg et al. 2017) and wolf populations expanding both in numbers and distribution (Andersen et al. 2015), the prevalence of conflict with livestock activities is increasing considerably (Iliopoulos et al. 2009, Hosseini-Zavarei et al. 2013, Torres et al. 2015, Bocci et al. 2017, Meuret et al. 2017, Khorozyan & Heurich 2022). For centuries, the primary method used to (attempt to) reduce depredation was culling predators, which resulted in the near extinction of wolves in large parts of their historical range (Young & Goldman 1944, Mech 2017). Recently, studies have shown that killing wolves may be ineffective in the long-term and can even lead to counter-productive effects by increased wolf reproduction efforts the subsequent year or territory colonisation by adjacent wolf packs (Wielgus & Peebles 2014, Treves et al. 2016, Lennox et al. 2018, Grente 2021). Although lethal control remains part of wolf management, livestock depredation is increasingly managed through nonlethal methods that aim to protect livestock and support wolf conservation (Shivik et al. 2003, Gehring et al. 2006, Stone et al. 2017).

Nonlethal strategies to deter wolves from livestock are predominantly based on theories regarding animal behaviour (Wilkinson et al. 2020, Blackwell et al. 2016, Miller & Schmitz 2019) and aim to manipulate wolf behaviour and distribution in such a way that it prevents wolves from attacking or approaching domestic animals (hereafter *depredation*) (Smith et al. 2000, Eklund et al. 2017). Underlying behavioural theories include the carnivore landscape of fear and the optimal foraging theory (Brown et al. 1999, Blackwell et al. 2016, Wilkinson et al. 2020). According to the landscape of fear hypothesis (LOF), prey animals change their behaviour and distribution based on the presence of predators to optimise their tradeoff strategy between food provisioning and safety (Bleicher 2017, Wilkinson et al. 2020). Similarly to how wolves can change the behaviour and distribution of prey species such as elk (Creel & Christianson 2008, Laundré et al. 2014), humans can be perceived as apex predators that influence the landscape of fear of carnivores (Smith et al. 2017). Wild wolves have a natural fear of humans and show high avoidance behaviour (Carricondo-Sanchez et al. 2020, Versluijs et al. 2022), except for rare cases of bold, habituated wolves (Gese et al. 2021). By simulating human presence around livestock (e.g. guarding dogs, lights and sounds), behaviour-based nonlethal measures can elicit avoidance behaviour resulting in top-down cascading effects that reduce damage to livestock (Frid & Dill 2002, Laundré et al. 2010). Moreover, the optimal foraging theory (OF) states that – for an individual to optimise their fitness – they must select a feeding strategy that is the lowest in cost and the highest in benefits (MacArthur & Pianka 1966). Based on OF theory, wolves are confronted with a tradeoff between the costs of hunting (spending energy, risk of injury and death) and feeding on prey with high caloric rewards. Because most livestock species have lost their anti-predator behaviour through domestication (Flörcke & Grandin 2013) and are in good physical condition, livestock depredation tends to be a high-benefit and low-cost resource strategy (Wilkinson et al. 2020). Additionally, the energy a predator requires to detect prey kept in predictable locations (i.e. livestock) is low (Sih 2005). Therefore, the cost of predating on livestock is low, at least when human retribution is also low. Nevertheless, costs for predators can be increased through protection measures. Behaviour-based intervention methods aim to artificially increase the cost of livestock depredation and shift the balance negatively in order to persuade predators to shift to a more profitable prey type (Haswell et al. 2019). Eliciting the behaviourally mediated effects predicted by LOF and OF hypotheses is done using disruptive and aversive stimuli (deterrents and repellents) designed to heighten the perceived risk and cost of approaching or predating on domestic animals.

Turbo Fladry barrier used to protect sheep from wolves in the Netherlands. This nonlethal tool combines both disruptive (fladry strips) and aversive (electrified wires) stimuli to keep wolves from trespassing into pastures and from predating on livestock. *Photo: Van Bommel Faunawerk.*

Aversive and disruptive stimuli are unpleasant to the animal and may cause pain, discomfort or fear (Shivik & Martin 2000). Typically, *disruptive stimuli* (deterrents) are used to induce fear, making use of *neophobia* (the fear of new) to prevent or change a specific behaviour (Shivik & Martin 2000), whereas aversive stimuli make use of unpleasant experiences or pain to elicit a negative association with an undesirable behaviour. The major difference is that aversive stimuli rely on negative experiences and can become more effective with learning (i.e. with repeated exposures to the negative stimulus), whereas disruptive stimuli rely solely on novelty and are only efficient when learning (or habituation) does not occur (Shivik et al. 2003). Examples of disruptive stimuli are fladry, the radio-activated guard box (RAG), fox lights and range riders. Aversive stimuli interventions can include conditioned taste aversion (CTA), turbo fladry, electric fences, shock collars and nonlethal projectiles (Shivik 2003, Appleby et al. 2017). Some interventions combine disruptive and aversive stimuli, such as livestock guarding dogs (LGD) that can elicit fear by barking (simulated human presence) and may represent a real risk of injury or death to wolves when they attack. Disruptive stimuli disrupt predatory behaviour by inducing a startle or fright response in the animal, discouraging it from approaching further (Shivik 2004) and can come in the form of chemical, visual, acoustic, or physical cues, or a combination thereof (Smith et al. 2000, Shivik 2004). The major issue related to disruptive stimuli interventions is *habituation* to the stimuli (Musiani et al. 2003). Due to the absence of negative consequences of the stimulus, the initial fear fades over time, resulting in decreased intervention effectiveness (Shivik & Martin 2000). *Aversive stimuli* can induce direct negative experiences and thereby disrupt behaviour but can also be used for aversive conditioning. By pairing a behaviour (such as approaching livestock) with an aversive stimulus (such as an electric shock from a collar) a strong learned association is created between the performed behaviour and the unpleasant stimulus by means of *positive punishment* (Rossler et al. 2012). If true conditioning is acquired, the animal shows the aversion even when the unconditioned stimulus (e.g. shock) is absent (Snijders et al. 2019), making conditioning a promising tool for mitigating predation conflicts. However,

Livestock guardian dog (LGD) guarding sheep in a mobile fence enclosure, Veluwe, the Netherlands. *Photo: Van Bommel Faunawerk.*

a recurrent issue related to aversive stimuli is the phenomenon of *extinction*, where – without frequent re-exposure to the stimulus - the learned association fades over time (Appleby et al. 2017). Another drawback is *confounding* of the (conditioned) association, resulting, for example, in an individual acquiring an aversion to humans instead of the targeted behaviour (here approaching or attacking livestock), as seen in studies on dingos (*Canis lupus dingo*) (Appleby et al. 2017). To optimise the association between actual predation behaviour (attack) and a negative (aversive or disruptive) stimulus, it is therefore important that nonlethal tools are as highly behaviourally contingent as possible and deter/repel the predator at the moment of attack initiation (Breck et al. 2002).

To conclude, through behaviour-based intervention methods, wolves are confronted with a behavioural tradeoff between the risk of illness, injury or even death and the benefits of feeding. When used correctly, nonlethal intervention methods can thus decrease the motivation for depredating livestock. But what does it mean to use them correctly? Despite the wide implementation of protection measures, livestock depredation remains a considerable issue (Meuret et al. 2017). Therefore, it is crucial to better understand how effective these interventions are and under what circumstances they are worthwhile (Bruns et al. 2020). Gaining this knowledge can contribute to more evidence-based and adaptive approaches to wolf-livestock conflicts, with the ultimate goal of a better coexistence between wolves and humans (Stone et al. 2017, van Eeden et al. 2018).

Therefore, in this systematic review, we explored the literature to investigate if and to what extent behaviour-based interventions were able to reduce livestock depredation by wolves. Moreover, we researched whether certain interventions were more effective than others at limiting depredation events. Based on the theories of optimal foraging (OF) and landscape of fear (LOF) (MacArthur & Pianka 1966, Brown et al. 1999), we predicted that behaviour-based interventions would effec-

tively reduce *depredation* events by increasing the (perceived) cost or risk of livestock predation and that interventions inducing a higher (perceived) cost or risk would be more effective. Finally, we hypothesised that a wide range of environmental factors could affect the effectiveness by shifting the cost-benefit ratio in favour of livestock depredation (e.g. low wild prey availability (Bocci et al. 2017, Janeiro-Otero et al. 2020)) (Wilkinson et al. 2020), but were unable to test this due to a limited number of articles in the evidence base (*n*=16). Ultimately, with this review, we hope to divulge evidence-based information that can support stakeholders in choosing appropriate livestock protection measures and inform future research in human-wolf coexistence.

Methods

Following systematic review methodology, we framed the primary research question '*What is the effectiveness of behaviour-based interventions in reducing livestock depredation by wolves (*Canis lupus*)?*' around a PICO structure (Livoreil et al. 2017): *Population* (wild wolves), *Intervention* (behaviour-based methods), *Comparator* (no or any non-behaviour-based intervention) and *Outcome* (livestock depredation).

Literature search

Search strategy

To identify and retrieve as much evidence as possible on the effectiveness of livestock protection measures, we searched multiple platforms, including two bibliographic databases: Web of Science (WoS Core Collection) and Scopus (both accessed through the Wageningen University and Research Library institutional subscriptions). Searches were performed on 14 September 2021 (WoS) and 1 October 2021 (Scopus); hence any relevant study published in these databases beyond this date was not considered in this review. We also accessed the specialist Zotero database of Snijders et al. (2019), comprising articles from their Systematic Map '*Effectiveness of animal conditioning in reducing human-wildlife conflicts*'. We searched the following Zotero subfolders: *Personal Communication, Google Scholar, Bibliographic databases* and *Specialist Websites* using the full-text search tool on 30 September 2021.

Search string

Within the Zotero database (Snijders et al. 2019), we searched each subfolder at the full-text level using the search terms "*wolf*", "*wolves*", and "*canis lupus*". In WoS and Scopus, we searched articles on the title and abstract level using a search string in English constructed from the PICO elements *population, intervention* and *outcome* and containing the search terms:

wolf OR wolves OR "canis lupus" AND *condition* OR aversi* OR disrupt* OR repel* OR nonlethal OR non-lethal OR learn* OR train* OR avoid* OR "negative reward" OR punish* OR reinforce* OR habituat* OR protect* OR "livestock guard* dog" OR "livestock protection dog" OR LGD OR LPD OR collar* OR herd* OR shepherd OR "human presence" OR "range rider" OR fenc* OR fladry OR CTA or "conditioned taste avers*" OR capture* AND *predat* OR depredat* OR attack* OR kill* OR conflict* OR loss* OR livestock OR domestic OR sheep OR cattle OR "farm animal" OR cow OR lamb OR approach OR movement OR calf OR calves*

To estimate the comprehensiveness of the search, we proofed the outcomes with five priorly established benchmark articles: Hawley et al. 2009, Gehring et al. 2010a, Lance et al. 2010, Rossler et al. 2012, Stone et al. 2017 and Iliopoulos et al. 2019. We tested the search string in Web of Science Core Collection on 9 September 2021, and results retrieved all five benchmark publications.

Inclusion criteria and study selection

We used CADIMA (version 2.2.3.), a free online tool for systematic reviews (Kohl et al. 2017, https://www.cadima.info/index.php) to perform our article selection and data extraction.

For an article to be included in the review, it had to conform to the key elements of the study question (PICO), which we translated into the following selection criteria: 1. *Population*: all subspecies of wolves (*Canis lupus*) that are free-ranging or captive at the time of the intervention. Studies on captive wolves were taken into consideration up until the data extraction phase as to ensure having sufficient studies in case insufficient data was available on free-ranging wolves; 2. *Intervention*: all non-lethal intervention methods that are behaviour-based and have as a goal: OR the conditioning of wolves against depredation through learned associations with intervention stimuli OR the blocking of depredation behaviour (attack and precursor behaviour) at the moment of intervention either by hindering (deterrents) or averting the behaviour (repellents) of the target (and non-target) individuals OR a combination thereof; 3. *Comparator*: no intervention OR any other intervention that is not behaviour-based and has as goal to reduce livestock depredation by wolves, this could be, for example, translocation or lethal control; 4. *Outcome*: all quantitative measures for livestock (or domestic animal) depredation OR quantitative measures for precursor behaviours that could lead to depredation (e.g. approaching livestock or trespassing a barrier).

We first screened all recovered articles on the title and abstract level before performing full-text screenings on selected publications (Figure 1). In addition to the PICO-based criteria, we excluded studies that did not present any (primary) quantitative data, such as qualitative studies (e.g. interviews), modelling studies or reviews.

Data extraction and study validity-assessment

From each selected study, we first extracted relevant meta-data. Subsequently, quantitative data on the effectiveness of intervention methods were extracted from the article's main text when available or was extracted from figures using WebPlotDigitizer version 4.5. (Rohatgi 2021, https://apps.automeris.io/wpd/). For articles where data was relevant, but no measure of variance was given, we contacted the authors requesting raw data (n=3). Studies for which the missing data could not be retrieved were excluded from the quantitative analysis but included in the narrative synthesis, and corresponding findings were discussed in a separate qualitative analysis (see results).

We performed a critical appraisal to assess the validity of the studies in the evidence base (see Figure 3 caption for appraisal criteria). The outcome of the study validity assessment was not used to exclude additional studies from the quantitative synthesis, as the sample size was already limited, but rather to give context to the evidence.

Quantitative synthesis methods

Because each study reported its intervention effectiveness using different outcome measures, we standardised the outcomes between studies by using two types of effect sizes, relative risk ratios (RR) and standardised mean differences (SMD):

Relative risk ratio (RR)

Whenever possible, we used relative risk ratios (RR) to express the effectiveness of livestock protection measures. RR was defined as the ratio between the likelihood of depredation occurring in the intervention group (livestock protection) compared to the likelihood of depredation occurring in the control group (no protection measure) (see Figure 4

caption for RR-value interpretation) and was calculated based on the following formula:

$$Relative\ risk\ ratio\ (RR) = \frac{a/(a+b)}{c/(c+b)}$$

where *a* is the number of depredated units in the treatment group, *b* is the number of non-depredated units in the treatment group ('survival' measure), *c* is the number of depredated units in the control group, and *d* is the number of non-depredated units in the control group (based on Eklund et al. 2017).

For studies that reported a measure of trespassing rather than a number of depredated animals, we calculated a survival measure as: *b = outcome measure events outside – outcome measure events inside protected area*.

Throughout the study, we defined *depredation* as both (1) animals being predated upon, resulting in injuries and/or death and (2) the performance of precursor behaviours that could lead to an attack: approaching livestock, trespassing on intervention areas.

When appropriate, we transformed the RR into a more user-friendly measure giving the percentage of risk reduction associated with a given intervention:

$$Relative\ risk\ reduction\ (RRR) = 100\% * (1 - RR)$$

Standardised Mean Difference (SMD)

Some studies did not report any measure for the number of non-depredated units (*b*) required to obtain a RR. For the studies that reported a mean and variance instead, we calculated a standardised mean difference between control and treatment groups following the formula:

$$SMD = \frac{\bar{x}_1 - \bar{x}_2}{SD_p}$$

where $\bar{x}_1$ is the mean of the intervention and $\bar{x}_2$ the mean of the control and is the pooled standard deviation (see Figure 5 caption for SMD-value interpretation).

Handling of complex data structures

To avoid mistakes with complex data structures and to stay as close as possible to true values, we decided not to transform the data with additional statistical analyses. For example, in studies with a BACI design, we only used before-after data and did not transform BACI to control-impact, in order to minimise the risk of non-intended (environmental) variables between study groups. Moreover, in studies that reported multiple time points (several years), we either counted each year as a study (when considered as independent data) or used only one year for the effect size calculation. When, for example, two before years (n-1 and n-2) and one intervention year (n) was reported, we took the before year that was closest to the intervention year (n-1) to minimise annual variations.

Statistical methods

Because we found no truly robust way of converting between standardised mean differences and risk ratios (Viechtbauer 2022), we decided to use SMD and RR studies in two separate analyses. Data analysis was performed in R version 4.1.2 using the *metafor* package (Viechtbauer 2010). Throughout the study, we used two-sided tests with a significance threshold of $P<0.05$.

Risk ratios

We used the *escalc(measure =RR)* function to calculate the log *risk ratios* (RR) of the intervention effectiveness. This *metafor* function automatically corrected zero cell errors by adding a ½ correction to all the cells, this was important because some studies did not observe any depredation events, especially under intervention. After calculating the RR for each study, we fitted a random effects model (REM) using the *rma* function to obtain the summary effect size of all behaviour-based intervention studies. Further-

more, we did a meta-regression on the moderator *intervention* to investigate differences between interventions. Finally, we performed a subgroup analysis for *fladry* and *biofence* because they differed significantly (P=0.0001) and we wanted to gain better insight into the impact of biofence on the overall effect size.

Standardised mean differences

To calculate the standardised mean difference between control and intervention groups and thus the magnitude of the intervention effect, we used the *escalc(measure =SMD)* function. This function automatically corrected small sample sizes, transforming the outcomes into corrected and more accurate *Hedges g* effect sizes. Subsequently, we fitted a random effects model to get the summary effect size of all interventions and studies. Because of the small number of studies (n=6), we did not perform any meta-regressions to investigate effect modifiers on this dataset.

Results

Narrative synthesis of the evidence base

The initial search across bibliographic databases and the Zotero database (Snijders et al. 2019) retrieved 4292 articles, 2825 after duplicate removal (Figure 1). We included fifty articles based on title and abstract, of which 47 articles were retrievable in full text. Finally, this yielded 16 articles: Breck et al. 2002, Musiani et al. 2003, Shivik et al. 2003, Schultz et al. 2005, Hawley et al. 2009, U.S. Fish and Wildlife Service 2009, Davidson-Nelson & Gehring 2010, Gehring et al. 2010a, Lance et al. 2010, Rigg et al. 2011, Rossler et al. 2012, Salvatori & Mertens 2012, Ausband et al. 2013, Stone et al. 2017, Iliopoulos et al. 2019, Samelius et al. 2021. These sixteen articles represented 31 studies (multiple studies per article) which were used in the narrative synthesis to represent the evidence base as a whole. Finally, we included 18 studies out of ten different publications in the quantitative synthesis (meta-analysis), and excluded thirteen studies from seven articles from the quantitative synthesis but included these in a qualitative synthesis (non-statistical exploration of study results). The full list of excluded studies (n=13) with corresponding criteria for exclusion from the meta-analysis can be found in the supplementary materials (Table S1[1]).

The evidence base for the effectiveness of animal behaviour-based interventions in free-ranging wolves was small (n=16 publications, n=31 studies, Figure 1). Moreover, the number of studies on interventions protecting living livestock was also limited, with more than half of the studies testing precursor and consumption behaviours rather than attacks on livestock (n=17 vs n=14, Figure 2c). The evidence base comprised twice as many studies on disruptive stimuli compared to aversive stimuli (n=16 vs n=8, Figure 2a), while seven studies investigated intervention methods that combined both aversive and disruptive stimuli, such as livestock guarding dogs (LGDs), and electrified fladry. Fladry was studied more frequently than other interventions (n=9, Figure 2b), followed by shock collars (n= 4), LGD's (n=4) and electric fences (n=4).

Finally, the geographical areas of the studies were strongly skewed towards North American countries, most importantly the U.S. (n=16, Figure 2d) followed by Canada (n=4). In Europe, ten studies were conducted on behaviour-based interventions from a total of three publications. For Asia, we found only one article, in Mongolia.

Critical appraisal

Overall, a high number of studies (n=19) from the evidence base allowed the evaluation of nonlethal tools in a real-life setting, with only one-third of studies being performed on units

[1] https://www.zoogdiervereniging.nl/publicaties/2023/lutra-66-1-2023

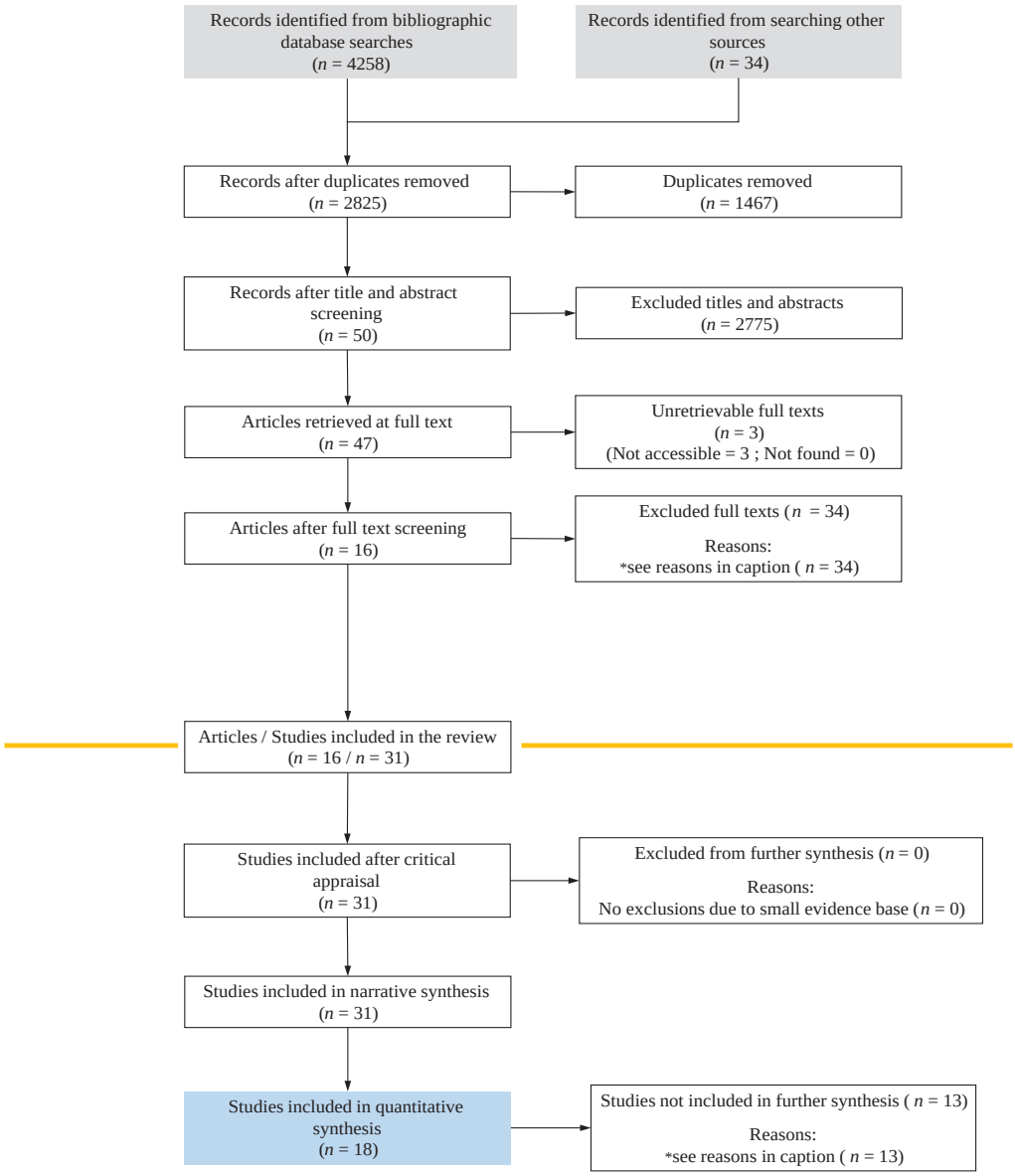


Figure 1. Flow diagram of the systematic search in WoS, Scopus and the Zotero databases from Snijders et al. (2019) with the subsequent inclusion/exclusion of articles at all stages of the review. Reasons for exclusion from the review were the following: 1. *Reasons for exclusion at full text: Population: study on captive wolves (*n*=7), Intervention: no appropriate intervention (*n*=8), Comparator: no appropriate control (*n*=17), Outcome: no appropriate outcome measure (*n*=11), Full text untranslatable (*n*=1), Full text duplicate of other study (*n*=3), note: some articles were excluded based on multiple criteria. 2. Reasons for exclusion from quantitative synthesis: lack of variance measure (*n*=7), no quantitative measure for before intervention (*n*=1), unreliable or lacking control group (*n*=4), combination of multiple interventions (*n*=1). Studies with corresponding reasons for exclusion can be found in the supplementary materials (Table S1[1]).

[1] https://www.zoogdiervereniging.nl/publicaties/2023/lutra-66-1-2023

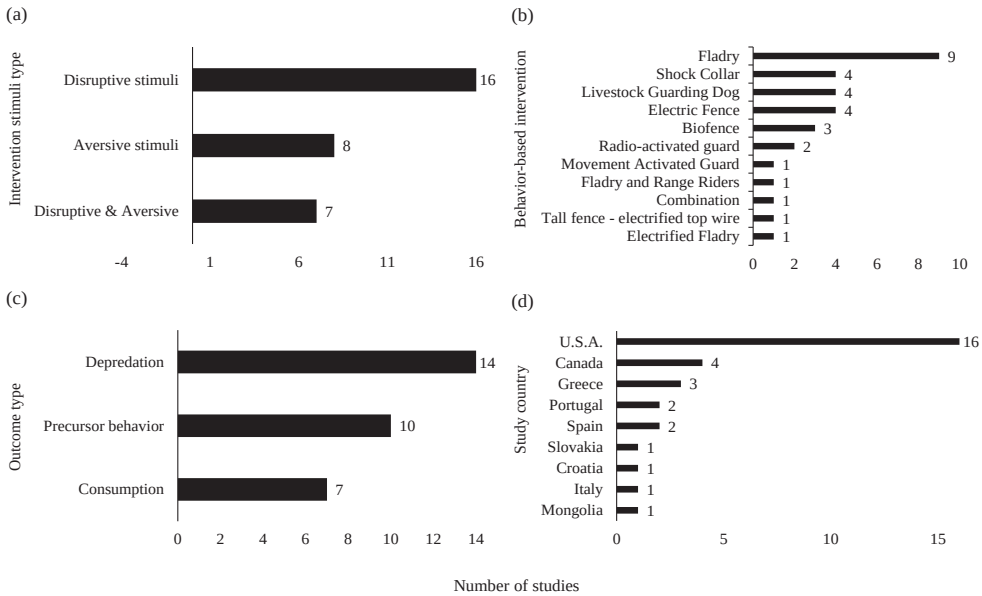


Figure 2. Number of studies in the evidence base categorised by (a) intervention stimuli type, (b) type of intervention, (c) study outcome type and (d) study country (n=31 studies from n=16 articles).

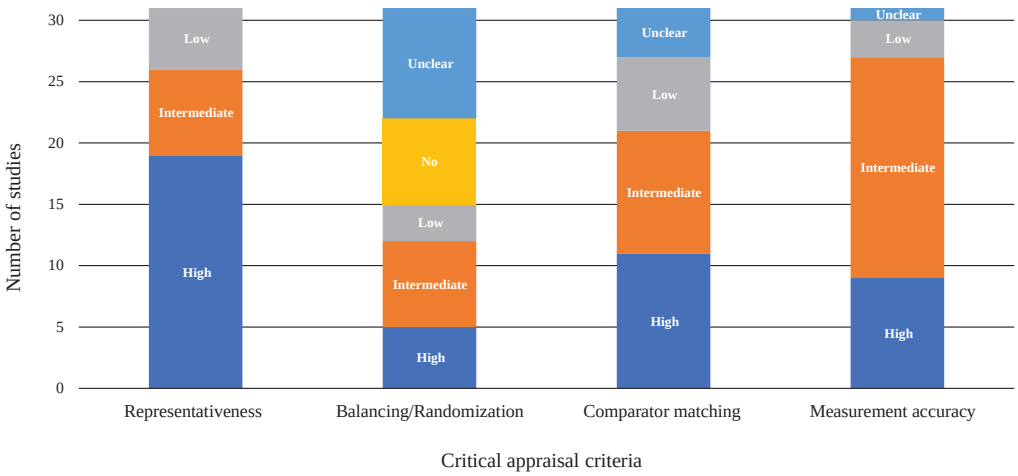


Figure 3. Study validity assessment outcomes per appraisal criteria. Each bar contains the cumulative number of studies in each category, totalling 31 studies (n=16 articles). Representativeness indicates how representative the study is in demonstrating an interventions' ability to reduce livestock depredation by wolves; Balancing/randomisation (selection bias) indicates if intervention units were balanced and/or randomised in a way that allowed minimisation of any pre-treatment differences between study groups; Comparator matching (performance bias) indicates how the comparator group/period was matched with the treatment group/period in terms of variables other than intended and whether they differed enough in intended variables; finally, measurement accuracy represents whether the study outcome was measured following a method that could truly measure the effect of the treatment. High scores represent a better study validity for the specific question, whereas Low, Unclear or No scores indicate that the studies did not perform well for the corresponding criteria.

other than living livestock (*n*=12), like bait sites. However, many studies used intermediate or low-accuracy measurement methods to quantify depredation events (n=21), such as track swaths and large-scale self-reported losses. Nevertheless, nine studies used highly robust measurement methods, such as VHF telemetry and verified depredations to measure outcomes. Although randomisation or high balancing of study groups was scarce (*n*=5), high or intermediate comparator matching was present in over two-thirds of the studies (see Figure 3 caption for the explanation of the critical appraisal criteria).

Quantitative synthesis

Across all studies included in the relative risk-ratio random effects model (*n*=12), behaviour-based nonlethal tools had high overall effectiveness (relative risk reduction= 82%) and significantly reduced depredation events (attack- and precursor behaviours) by wolves (estimate= -1.7290, df=11, *P*= 0.004, Figure 4).

Furthermore, we found a strong effect of intervention type on the effectiveness of reducing depredation, with biofence differing significantly from fladry (Fladry: estimate= -3.6341, df=2, *P*<0.0001; Biofence: estimate= 0.4851, df= 2, *P*=0.1450, Figure 4) but not from turbo fladry (estimate= -1.6891, df=2, *P*=0.222).

Subsequent subgroup REM analyses showed that fladry was significantly more effective than biofence (summary RR=0.04 vs RR=1.54, Figure 4). Fladry had high overall effectiveness (relative risk reduction= 96%) and significantly reduced livestock depredation across the eight studies included in the analysis (estimate= -3.1543, df=7, *P*<0.0001). Whereas the use of biofence increased the trespassing rate of wolves into a pack exclusion area, leading to a counter-productive effect of this particular intervention (estimate= 0.4348, df=2, *P*=0.4256). Finally, turbo fladry apparently reduced the trespassing of wolves into six cattle pastures (relative risk reduction= 70%), however, the effect was not significant (estimate= -2.2040, df=0, *P*=0.3361).

To summarise the overall effectiveness of nonlethal tools presented in studies reporting means, we fitted a random effects model on standard mean differences. In alignment with results presented in Figure 4, the nonlethal measures proved to be highly effective (Hedges g= |-1.19|> 0.8, Figure 5) in reducing depredations by free-ranging wolves, with a significantly lower average number of depredation events in intervention groups (estimate= -1.1870, df=5, *P*=0.0046).

Shock collars, livestock guarding dogs and tall fences showed a very large reduction effect on trespassing behaviour and livestock depredation (Hedges g= |-1.27 to -2.94|), and the use of Movement Activated Guard reduced carcass consumption to a smaller - but still large - extent (Hedges g= |-0.87|> 0.8).

In contrast, carcass consumption rates increased by 0.38 standard deviations on bait sites protected by fladry compared to sites without fladry, indicating a small (0.2 < 0.38 < 0.5, Figure 5) but non-significant effect.

Qualitative synthesis

Thirteen studies from seven articles (see supplementary materials, Table S1) were excluded from the quantitative synthesis, representing seven different nonlethal interventions: shock collars (*n*=2), livestock guarding dogs (*n*=3), electric fences (*n*=4), radio-activated guard boxes (RAG box, *n*=2), fladry and range riders (*n*=1 with *n*=4 cases for fladry and *n*=4 cases for range riders) as well as one study on the combination of multiple intervention methods. Overall, the findings of these studies supported our quantitative synthesis results that behaviour-based nonlethal tools effectively reduce livestock depredation by wolves. More specifically, fladry was highly effective and reduced livestock killings by 100% when used in management activities to reduce depreda-

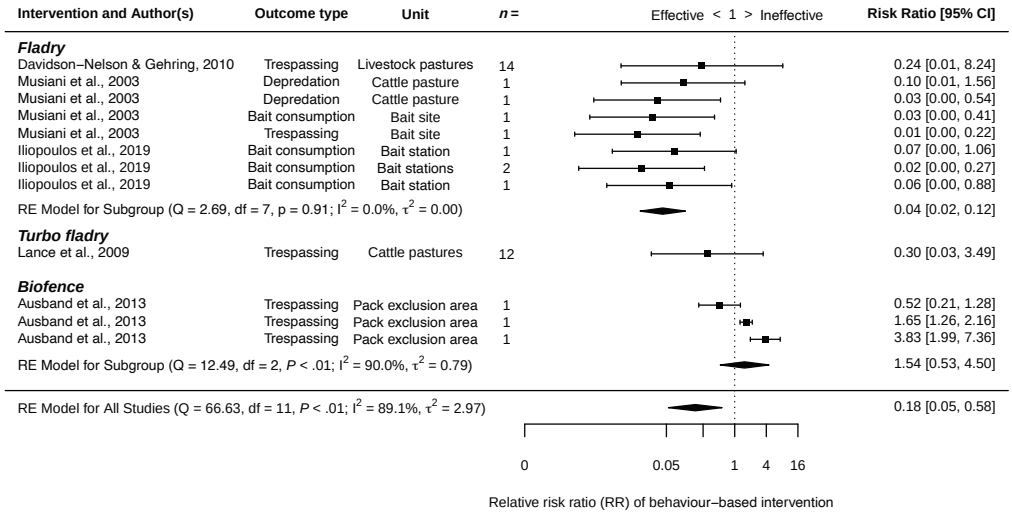


Figure 4. 95% CI interval relative risk ratio of behaviour-based intervention effectiveness to reduce livestock depredation by wolves. When RR=1, there is no difference in the risk of depredation between intervention and control groups. An RR<1 indicates that the risk of depredation is higher in the control group, with intervention effectiveness increasing as the RR gets closer to 0. Contrastingly, when R>1, the risk of depredation is higher in the intervention group, indicating a counter-productive measure.

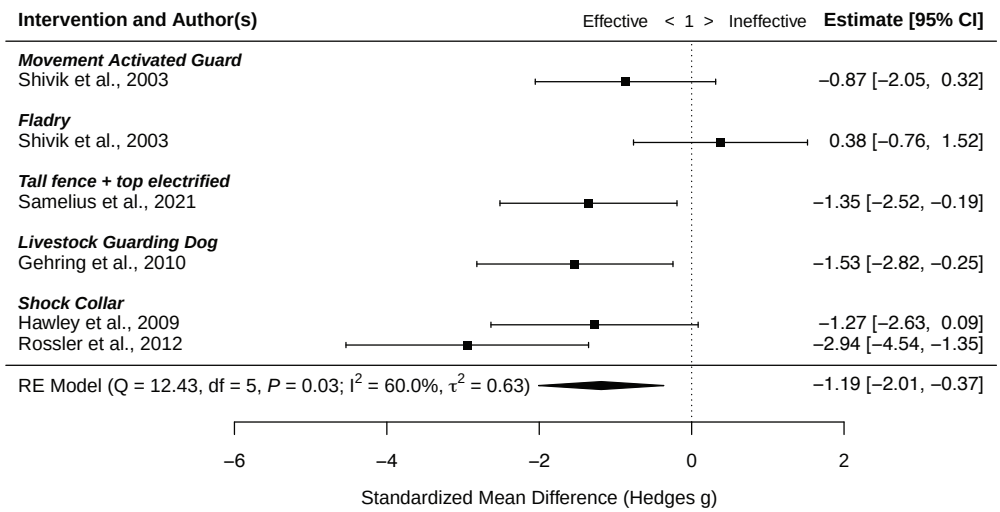


Figure 5. 95% CI interval effect size (Hedges g) of behaviour-based interventions. The sign of the effect size (g) indicates the direction of the effect, with negative values suggesting the treatment to be effective (lower mean number of depredation events in the intervention group). The amplitude of the effect can be interpreted following the rule of thumb: 0.2 = small effect, 0.5 = medium effect and >0.8 = large effect. The (absolute) value of Hedges g represents the number of standard deviations in which the intervention and control group differ.

tion in Arizona and New Mexico (*n*=4) (U.S. Fish and Wildlife Service 2009). Additionally, two studies suggested that shock collars effectively reduced wolf visitation to livestock farms that had previously suffered depredation. Moreover, in both studies, no livestock was killed by shock-collared wolves during the period of intervention (Rossler et al. 2012). Also, livestock guarding dogs were effective, reducing the number of killed livestock two- to threefold (*n*=1 and *n*=2 studies, respectively) (Salvatori & Mertens 2012), supporting the results from our meta-analysis (Figure 5).

Other protection measures that were not represented in the quantitative synthesis also suggested effective conflict mitigation. For example, electric fences used as part of the LIFE nature projects reduced depredations by 100% on Portuguese- (*n*=10) and Croatian (*n*=11) livestock holdings, compared to a 99% reduction in Spain (*n*=30) and a lower 57.80% reduction in Italy (*n*=239) (Salvatori & Mertens 2012). Moreover, RAG boxes efficiently detered wolves from depredating cattle in small pastures (Breck et al. 2002). Furthermore, management officials found that proactive use of range riders was effective in two out of four cases, with no attacks on livestock during the intervention (*n*=2), whereas two cases reported one and ten depredation incidents when range riders were active (eight months intervention), respectively (Fish and Wildlife Service 2009). Finally, a seven-year study on the adaptive use of predator deterrents and husbandry techniques in large sheep bands found that sheep depredations were 3.5 times lower in the protected area compared to the non-protected area (Stone et al. 2017).

Discussion

In this systematic review, we aimed to elucidate if – and to what extent – behaviour-based livestock protection measures are effective in discouraging wild wolves from approaching and killing livestock. The study results demonstrate that across all included studies, behaviour-based methods are highly effective at reducing livestock *depredation* by wolves, supporting our hypothesis that nonlethal tools can manipulate the (perceived) cost-benefit ratio in such a way that it deters wolves from predating on domestic livestock. Furthermore, it reinforces the application of behavioural principles in human-wildlife conflicts, as proposed by Blackwell et al. (2016). However, our study also underlines a key concern: the number of scientific publications evaluating behaviour-based protection measures against free-ranging wolves is very low (*n*=16), with even scarcer numbers of studies investigating interventions in actual conflict scenarios (on living livestock). This suggests that – although nonlethal methods are widely promoted – there is limited published empirical evidence to substantiate or guide the correct use of livestock protection measures.

Effectiveness of behaviour-based methods

The effect of intervention type: fladry, biofence and turbo fladry

The initial findings showed some fluctuation in effectiveness between studies within the same intervention and between different interventions. Although we could not test for the differences between aversive and disruptive stimuli interventions specifically, a meta-regression investigating the effect of *intervention* on the RR studies did demonstrate statistical differences in effectiveness between fladry, biofence and turbo fladry.

First, the results revealed that – across the included studies (*n*=12) – fladry was more effective than turbo (electrified) fladry at reducing depredation events. This goes against our hypothesis that interventions with a higher cost should be more effective, since electrified fladry induces both fear and pain (shock) compared to fladry which is

solely based on neophobia. Hence, here we could conclude that our hypothesis was not supported and that stimuli type does not appear to influence depredation reduction. Alternatively, certain confounding factors could explain the unexpected result. First, the outcome measurement method might not have been appropriate enough to illustrate the difference in effects between the intervention types. During electrified fladry testing, Lance et al. (2010) measured effectiveness by track surveys (number of tracks inside- and outside of pasture), which did not allow for verifying whether wolves that had trespassed the fladry barrier had also received a shock. Without electric shock administration, electrified fladry would have exposed the animal to the same (disruptive) stimulus as classic fladry, potentially leading to similar effect sizes. Furthermore, only one fladry study evaluated similar conditions to the electrified fladry: trespassing on living livestock pastures (Davidson-Nelson & Gehring 2010). When isolating these two studies, the effectiveness is comparable (risk reduction of 70% for electrified fladry and 76% for fladry).

Secondly, fladry proved to be significantly more effective than biofence in reducing depredation and trespassing by wolves. Biofence (mimicking wolf presence by deploying wolf faeces and urine) was counter-productive and increased the number of visitations (trespasses) into the protected area, suggesting this method might not be effective at keeping wolves out of livestock pastures. This is in line with previous results by Anhalt et al. (2014), where the biofence failed to significantly manipulate wolf movements. However, here we examined the outcome measure 'number of trespass locations' instead of 'average trespass distance (km)' (unusable data due to lack of variance measure). Under natural inter-pack dynamics, wolves respect territory boundaries to avoid being killed by adjacent packs (Smith & Ferguson 2012). Nevertheless, wolves may cross boundaries without entering far into the rival pack's territory, as each added distance increases intraspecific mortality risk (Mech 1994). When considering the outcome 'average distance trespassed', wolves moved less far into the treated area, suggesting the bio boundary changed wolf behaviour to some extent by mimicking adjacent pack presence. Further research could investigate whether wolves would respond better to biofence material retrieved from dominant individuals, as in this study, authors could not control for the hierarchical origin of the scat and urine. It remains to be tested whether biofence could be a useful tool to apply in real management scenarios. Although the labour-intensive maintenance of the biofence (frequent refreshing of odour cues) might limit extensive practical applications, it could be trialed as a tool for smaller-scale interventions like deterring young dispersing wolves from livestock pastures.

To conclude, more research is needed before we can make strong inferences about the differences in effectiveness between interventions and identify the additional factors that may partly explain the observed variation in effect sizes. Such confounding factors are crucial to elucidate in future studies, as this can help build stronger guidelines for using protection measures in different depredation scenarios.

Effectiveness of disruptive and aversive stimuli interventions

Disruptive methods attempt to repel predators from livestock by making use of fear-inducing stimuli that instigate a flight response. Fladry has been used for centuries in wolf hunts and was later deployed to frighten wolves away from livestock by relying on the flapping of fladry fabric strips in the wind. In this review, fladry was the most studied intervention (n=9) and showed highly promising results as a tool to reduce trespassing behaviour and livestock depredation by wolves. Nevertheless, our second meta-analysis (SMD) revealed an apparent counter-productive, yet non-significant, effect in one of

the fladry studies (Shivik et al. 2003). Here, fladry use coincided with increased carcass consumption by predators at bait sites, which unexpectedly contrasted with our hypothesis. It, however, remains unclear to what extent this study reflected the deterrence ability of fladry towards wolves, given that the study evaluated the total amount of bait consumed simultaneously by multiple predators, including raptors such as eagles. Pre-treatment food conditioning could have made bait stations an increasingly popular diversionary feeding location, leading to increased consumption during fladry treatment by flying scavengers that did not have to physically trespass the fladry barrier. This is in line with previous research findings that deterrent tests intended for one species could serve as an attractant to other species (Woodroffe et al. 2007). Although in eight out of nine studies, fladry significantly reduced depredation events, it remains a continuous visual cue with a strong risk of habituation, making treatment duration a key factor. Musiani et al. (2003) found that fladry was effective for 60 days, after which trespassing and depredation resumed, suggesting that fladry is best applied punctually during critical moments such as calving or lambing seasons. Electrified fladry proved to have the capacity to reduce trespassing (see earlier) and could provide a suitable alternative to limit habituation, provided more research is done to support this hypothesis.

Aversive stimuli such as shock collars induce discomfort or pain to prevent an individual from performing an unwanted behaviour. Shock collars emit an electric current into the neck of wolves when they trespass on a protected area, to link the act of approaching livestock (or bait) to an aversive experience. Our findings show that shock collars were highly effective in two studies included in the quantitative synthesis and proved to reduce depredation behaviour in the remaining two studies from the qualitative review. These studies were typically of high reliability thanks to the use of VHF telemetry as an outcome measure and the high balancing/randomisation between study groups. The quality of the studies and the large effect sizes represented in the SMD meta-analysis suggest that this type of aversive stimulus can strongly influence the behaviour and movement of wolves by representing an increased cost (OF) or risk to survival (LOF) as proposed by Haswell et al. (2019) and Miller & Schmitz (2019). Although shock collars have proven highly effective in these studies, its practical implementation might be limited by the financial and logistical resources required to locate and capture wolves and maintain the equipment (Rossler et al. 2012). Because of the labour-intensive aspect of collaring wolves and the relatively short-term battery life, it is doubtful that shock collars will be a cost-effective method to mitigate large-scale wolf-livestock conflicts. However, it could be a viable solution in areas where capturing- and GPS collaring are already part of management or study efforts and where depredation events are recurring in specific areas.

Electric fences are based on the same principles as shock collars and induce an aversive electric shock to wolves when they attempt to enter livestock pastures. Yet, their easy deployment might have a higher applicability in the field. Today electric fences are widely used in management efforts throughout the world. It is therefore surprising to uncover that only four studies from one single article (Salvatori & Mertens 2012) performed an empirical evaluation of the method on wild wolves, at least according to our systematic English-based search results. Overall, these studies support electric fences and suggest that this aversive and behaviourally contingent (direct consequence to an undesired behaviour) stimulus could prevent wolves from trespassing into livestock pastures. Nevertheless, in Italy, the reduction in depredation was much lower than in the other three countries (58% vs 99-100%), likely due to some livestock owners (total n=239) not keeping their entire flock inside the fences (Salvatori & Mertens

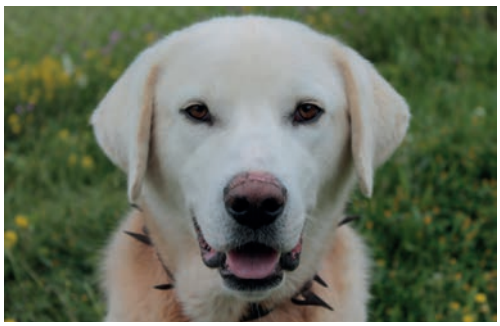
2012). It is also not excluded that wolves were able to dig under or jump over fences. Jumping was presumably the reason for Samelius et al. (2021) to set up 2-metre-high fences with an electrified top wire to protect Mongolian livestock. Their results demonstrated that tall fences can prevent further damage to livestock and that providing an initial physical barrier (energetic cost), in addition to an aversive stimulus (electrified top wire), can prevent wolves from trespassing into enclosures. Tall fences also require situation-specific use, as they are labour-intensive and cannot be used to protect large domestic flocks (Samelius et al. 2021). In addition, such tall fences could obstruct the migration routes of other wildlife. In these cases, smaller electric fences may be more applicable. But first more reported evaluations are needed.

In contrast to electric stimuli, livestock-guarding dogs (LGD) have been used for millennia to protect domestic animals from wolves and other predators (Gehring et al. 2010b). Overall, guarding dogs proved a reliable measure to reduce domestic animal losses to wolves. Livestock guard dogs limited wolves killing sheep, cattle and mixed livestock in several countries but generally did not prevent depredations completely. Use of guarding dogs combines disruptive and aversive stimuli and the dogs can serve both as a warning signal to wolves (Landry et al. 2020) and pose a considerable threat to their survival if habituation occurs. Dogs can also disrupt wolf feeding on livestock carcasses, adding an additional cost to predating on LGD protected livestock. The ability of some wolves to still predate on LGD protected livestock could be attributed to age- and breed-specific traits. Indeed, livestock guarding dogs mostly become (cost-) effective starting age two, and show variation in effectiveness between breeds (Kinka and Young 2019). Moreover, when working with living protection measures, there are other parameters to consider such as individual variation in behaviour or health as well as the inter-specific interactions that might occur with other species. Guarding dogs might, for example, wander off in pursuit of wildlife (Smith et al. 2020) or attract wolves by their presence instead of repelling them (Woodroffe et al. 2017). The latter is especially true in the mating season when LGDs were seen mating with wolves, resulting in hybridisation (Kopaliani et al. 2014, Kusak et al. 2018). In areas prone to hybridisation, it might be useful to consider sterilisation, which can also reduce wandering behaviour. The effectiveness of guarding dogs in wolf territories might also be season specific, as suggested by Stone et al. (2017). Stone et al. (2017) recommend dogs should not be deployed in core-wolf areas during denning and puppy season as it could increase wolf-dog aggression with wolves attacking and killing LGDs to protect their pups. Therefore, it is important to tailor the use of guarding dogs to the context and avoid an increase in conflict.

The success of adaptive approaches to wolf management and blended use of nonlethal tools was demonstrated in a seven-year study by Stone et al. (2017). In this management effort, large bands of 10,000 – 22,000 transhumant sheep were protected by context-dependent use of deterrents (e.g. fladry, fox lights, starter pistols, LGDs) and adaptive husbandry practices such as fencing at night, avoiding moving sheep through wolf rendezvous sites and guarding sheep with range riders. This method resulted in 3.5 fewer sheep killed in the protected area compared to the non-protected area, and no lethal control of wolves being necessary in the protected area. This study demonstrates that nonlethal tools can efficiently reduce livestock losses even on large operations in rugged and remote areas. Although this study did not allow the identification of single-intervention effectiveness, it does point to the promising potential of combining multiple methods to manage conflict scenarios. Biologically, alternating interventions can reduce habituation and combining multiple stimuli can increase the perceived intensity of human presence and/or reduce

Foxlight on a stick used as a visual deterrent to keep wolves from approaching sheep in Idaho, USA. *Photo: Wood River Wolf Project*.

the benefits initially associated with livestock predation. Human presence, however, does not systematically provoke a strong effect: the varying success of range riders seen in management practices across New-Mexico and Arizona (U.S. Fish and Wildlife Service 2009), could indicate that fear for the human "super predator" as suggested by Smith et al. (2017) can fade over time when no negative consequences are linked to their presence. In this regard, the duration of protection measure implementation might influence the outcome. In the management efforts of the U.S. Fish and Wildlife Service (2009), losses occurred solely during longer eight months range rider interventions and not during three- and four months interventions. To counter habituation, in many management efforts, range riders also deploy deterrents upon the perception of wolves or their indices of presence (tracks, howls, GPS-positions), rendering the effect of range riders more behaviourally contingent. Other behaviourally contingent measures in this review were the movement-activated guard (MAG) and radio-activated guard boxes (RAG). These boxes emit frightening sounds (cracker shells, pistol- and helicopter sounds) and strobe lights when (1) wolves pass in front of the box activating the movement sensor or (2) radio-collared wolves trespass an area protected by the RAG box monitor (invisible GPS monitored-line), respectively. The MAG-box reduced predator consumption on bait sites (Shivik et al. 2003), whereas RAG box efficiently repelled wolves from predating on calves (Breck et al. 2002). It is fairly certain the RAG boxes were able to increase the risk landscape for wolves, and manipulate their behaviour because when a RAG box malfunctioned, the wolves immediately killed a calf in the no-longer protected pasture. This was not due to habituation because after reparation of the box, no more depredations occurred. These scarcely studied behaviourally contin-



Young livestock guardian dog (LGD) with spiked collar used to protect sheep and goats in remote mountain village, Vikos Aoos National Park, Greece. *Photo: Thaana Van Dessel.*

gent tools would benefit further research to evaluate whether they could be used in larger-scale management efforts.

Behavioural principles as a basis for livestock protection measures

As seen earlier, most studies in this systematic review demonstrated that behaviour-based interventions were effective at decreasing wolf approaches and attacks, suggesting that non-lethal tools are, in fact, able to induce behaviourally-mediated effects that manipulate wolf behaviour and movement in a way that reduces depredation events. This review supports previous results and theoretical frameworks by Blackwell et al. (2016), Haswell et al. (2019), Miller & Schmitz (2019) and Wilkinson et al. (2020) proposing that behavioural principles – and, more specifically, the landscape of fear (Brown et al. 1999) and optimal foraging theory (MacArthur & Pianka 1966) – could be a valuable basis for human-carnivore conflict mitigation by using stimuli that heighten the perceived risk and cost associated with attacking domestic animals. It is unlikely that factors other than the interventions were responsible for increasing the cost/risk of predation between control and treatment units since, in most studies, before-after study designs were used, ensuring similar environmental- and farm conditions within one study (high and intermediate comparator matching). Nevertheless, we cannot exclude that higher hunting pressures between control and intervention study years could have affected the landscape of fear of wolves in such a way that they did not approach or predate on livestock anymore. Although we could not evaluate this, behaviourally-mediated effects due to hunting remain doubtful as most studies reported some measure of wolf presence in the area, both before and during the intervention through track surveys, GPS positions and camera traps. Controlling for wolf presence also excludes the confounder of intervention success due to wolves having deserted the area during the implementation of the intervention.

In contrast, variation in effectiveness between studies or intervention types, rather than within study, could have been influenced by a wide range of other factors that we were unable to statistically evaluate in this study due to the limited sample size. One of these factors – the availability of alternative prey sources – plays a pivotal role in the cost-benefit tradeoff between predating on wild- or domestic animals (Torres et al. 2015, Soofi et al. 2018, Werhahn et al. 2019) and could ultimately influence the effectiveness of protection measures. Nonlethal measures attempt to artificially increase the cost of livestock depredation by inducing fear, pain or a risk of injury (Haswell et al. 2019). However, how this cost is perceived (low or high) can be influenced by intrinsic motivation (hunger, pups to feed) and the obtainability of wild prey. Wild prey abundance and distribution, and the energy and risk associated with attacking wild animals (armaments, defence mechanisms), are all important parameters in evaluating the benefits of turning to (protected) livestock. Livestock is generally a low-cost and low-risk food source for wolves, as they are available in large numbers, usually under predictable distribution patterns, and exhibit low- to no antipredator behaviour (Flörcke & Grandin 2013). Although protection measures take away some of these ben-

efits, when wild prey populations are depleted or prey are particularly risky to attack, it can become a viable foraging strategy for wolves to be bolder towards nonlethal measures and attack livestock (Torres et al. 2015). Although we could not test this, we hypothesize that the effectiveness of behaviour-based interventions will be highly dependent on the availability of wild prey as an alternative resource.

To summarise, the results from this review strongly encourage the use and further development of behaviour-based tools in wolf management, since using behavioural principles to "educate" wolves could be a promising nonlethal alternative that supports both livestock farming and wolf conservation.

The evidence base

Sustainable mitigation of human-wolf conflicts relies strongly on using nonlethal methods that can efficiently prevent wolves from killing livestock and that support the conservation of wolf populations by avoiding lethal control. The evidence gap on the effectiveness of mitigation measures unveiled in this review has strong implications for wolf management and coexistence efforts (van Eeden et al. 2018), especially when livestock owners are already doubtful or withstanding methods that do not include (lethal) removal of the predator (Scasta et al. 2017). When strongly advocated measures are ineffective, it might heighten the mistrust towards interventions and management efforts, increase negative attitudes towards wolves and potentially limit the possibility for management agencies to recommend other methods in the future (Bogezi et al. 2021). Even with the additional studies conducted in the past five years, the results of our review strongly align with the findings by Eklund et al. (2017), Miller et al. (2016) and Treves et al. (2016), who reviewed the effectiveness of protection measures against large carnivores and Bruns et al. (2020), who studied measures against wolves specifically.

Although our review strongly supports previous findings – suggesting our search methods were indeed comprehensive – we cannot rule out that our results were to some extent limited by the scope of our literature search. Especially the restriction to the English language and the limited amount of grey literature may have led us to exclude relevant studies. These factors work in concert given that grey literature is often published in the local language. Grey literature was available and retrieved through the specialist database (Snijders et al. 2019), but only one of the studies (U.S. Fish and Wildlife Service 2009) met the selection criteria and could be included in the (qualitative) review. Nonlethal methods are often implemented in larger-scale management efforts, providing valuable insights into the effectiveness of these methods, but without necessarily being scientifically reported. Hence, not having a more comprehensive inclusion of grey literature could have caused our results to be, in some degree, affected by *publication bias*, since negative results are more likely to be reported in grey literature (if at all). When considering livestock protection measures, these negative or weak study outcomes – if they occur – are critical components to effectively adjust and adapt existing mitigation tools.

Our results may also have been affected by the duration of the intervention treatments, especially for studies on disruptive stimuli that rely on fear to be effective and are at risk for wolf habituation. Since habituation occurs over time with the repeated exposure to the (disruptive) stimuli, a longer exposure time can decrease the effectiveness of the protection measure, and vice-versa (Shivik & Martin 2000). Hence, the shorter the intervention treatment duration, the higher the probability of overestimating the effectiveness of a given protection measure. In our review, most studies evaluating the effectiveness of disruptive stimuli interventions used short treatment durations of a couple of months, ranging from 16 days to six months for fladry (Shivik et al.

2003: 16–29 days, Musiani et al. 2003: 60 days, Davidson-Nelson & Gehring 2010: 75 days, Iliopoulos et al. 2019: 23–165 days (per pack), U.S. Fish and Wildlife Service 2009: 180 days), 16–29 days for the Movement Activated Guard box (Shivik et al. 2003), 60–90 days for the Radio-Activated Guard box (Breck et al. 2002) and 90 days for biofence (Ausband et al. 2013). In future research, it would be useful to gain more insight into the longer-term effectiveness of these measures by evaluating livestock losses over multiple seasons, or years, if possible.

We acknowledge that field studies – especially on wolves – can be challenging and limit the possibility of achieving a golden standard in research. Therefore we would recommend further research to at least focus more on real conflict scenarios. With wolf-livestock conflict increasing in magnitude, the need for data on changes in depredation rates of living livestock is more urgent than ever. Moreover, it would be beneficial to study and further develop behaviourally contingent measures, to incorporate data on wild prey densities when evaluating protection measures, and to broaden the geographical distribution of studies, which are currently primarily aggregated in the United States. A wider and more robust evidence-base will allow for stronger reviews and meta-analyses that will have the power to unveil patterns in depredation events as well as the factors that influence them. This, in turn, could inform adaptive and context-specific implementation of protection measures that can more efficiently target unwanted wolf behaviour and reduce conflict situations.

Conclusions

The results of this systematic review demonstrate that across the available studies, the protection measures were highly effective and were able to manipulate wolf behaviour and distribution in such a way that it decreased depredation events. But the findings also revealed a considerable lack of evidence on the effectiveness of behaviour-based methods against free-ranging wolves. The current evidence base supports the hypothesis that nonlethal tools can increase the perceived cost or risk (OF and LOF) of predating on livestock, making it more beneficial for wolves to turn to alternative prey. This cost-benefit tradeoff could, however, strongly be influenced by environmental factors, as variation in effect size between different studies of the same intervention type was frequent. To better understand to what extent- and in which circumstances behaviour-based interventions are effective, there is a strong need for additional high-quality studies to fuel the evidence base. Reducing conflicts of interest between wolves and livestock activities is crucial for wolf conservation, livestock welfare and farmer livelihoods. Therefore, we urge the scientific community to support sustainable coexistence between humans and wolves by further investigating the use of behaviour-based nonlethal methods to protect domestic animals from wolf depredation.

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Samenvatting

De effectiviteit van gedragsmatige interventies ter vermindering van predatie van vee door wolven

Om mensen en wolven (*Canis lupus*) op een duurzame manier te laten samenleven hebben we interventies nodig die effectief de predatie door wolven van vee kunnen verminderen. Doordat wolven in hun aantallen en verspreiding weer toenemen, nemen ook de verliezen van vee en huisdieren toe, ondanks de grootschalige implementatie van zowel letale als niet-letale beheersmiddelen. Het groeiende belangenconflict tussen natuurbeschermers en veehouders vereist, onder andere, een aanpak gebaseerd op feiten en wetenschappelijk gefundeerd inzicht in de effectiviteit van niet-letale beheersmiddelen. We hebben daarom een systematische review uitgevoerd en zo de beschikbare studies omtrent de effectiviteit van (dier)gedragsmatige interventies in wilde wolvenpopulaties onderzocht en samengevat. Door de kosten/baten afweging bij het wel of niet aanvallen van vee te beïnvloeden kunnen gedragsmatige interventies namelijk wolven motiveren om voor alternatieve prooien te kiezen. We doorzochten systematisch academische databanken zoals Scopus en Web of Science en zochten naar literatuur in een gespecialiseerde literatuur databank gemaakt door Snijders et al. (2019). We hebben 2825 publicaties gevonden, waarvan 16 artikelen (en hun 31 gerapporteerde onderzoeken) na een screeningproces zijn opgenomen in deze review. Als maten voor de grootte

van het interventie-effect, gebruikten we zogenoemde 'relative risk ratios' (RR) en 'standardized mean differences' (SMD) en voerden vervolgens een meta-analyse uit. Ons onderzoek bracht een zorgwekkend gebrek aan gepubliceerd empirisch onderzoek naar niet-letale interventies aan het licht, althans in de Engelse taal. Dit, ondanks de wijdverspreide toepassing van deze interventies. Desalniettemin toonden onze synthese dat de meeste interventies, met name fladry, zeer effectief wolven weerhouden van het aanvallen of benaderen van levend vee of lokaas. Als gevolg van het beperkte aantal analyseerbare studies, was het niet mogelijk om factoren te onderzoeken die mogelijk de grootte van de effectiviteit van een gegeven interventie beïnvloeden. Dergelijk onderzoek zou in de toekomst kunnen leiden tot meer op maat gemaakte praktische aanbevelingen over wanneer en waar welk type interventie te gebruiken. Over het algemeen suggereren onze resultaten dat gedragsmatige niet-letale maatregelen een veelbelovend hulpmiddel kunnen zijn, op voorwaarde dat verder onderzoek onze bevindingen ondersteunt. Daarom raden we wetenschappers, natuurbeschermers en beheersautoriteiten sterk aan samen te werken en nader onderzoek te verrichten naar gedragsmatige interventies. Bovenal is ons advies om de werkzaamheid van deze interventies systematisch in reële predatiescenario's te onderzoeken (in plaats van met lokaas). Een meer op wetenschappelijk bewijs gebaseerde benadering van mens-wolfconflicten is een essentieel onderdeel van een duurzame toekomst voor wolven, landbouwdieren en pastorale activiteiten.

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Are offshore wind farms in the Netherlands a potential threat for coastal populations of noctule?

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Abstract: Offshore wind farms likely cause mortality amongst migratory bats. Yet it remains unknown whether resident coastal bat populations may be affected by offshore wind developments. We performed an analysis to assess the potential risk of offshore wind farms in the Dutch North Sea for local coastal populations of noctule (*Nyctalus noctula*). First, we assessed the potential overlap between their foraging range and areas with operational and planned offshore wind farms. Subsequently, we tracked 14 noctules from a coastal population during late summer and autumn and analysed their movements. In general, it seems unlikely that offshore wind farms in the Netherlands will significantly affect coastal populations of noctule since offshore wind developments take place beyond their regular foraging range. In some cases however, noctules do perform distant flights ('swarm flights'), possibly in response to migrating insects. We recorded six distant foraging trips both over land and over sea with a maximum distance of 18.5 km from their roost and 12.7 km from shore. Acoustic records confirm that noctules are occasionally present in offshore wind farms at distances of 15-25 km from shore. During such an event, noctules face the risk of a collision as virtually all their flight activity occurs at heights within the rotor swept area of offshore wind turbines.

Keywords: bat mortality, energy transition, collision, barotrauma.

Introduction

The development of the offshore wind sector plays an important role in the Dutch energy transition. In 2022 a capacity of 2.5 GWH has been realized, consisting of 260 turbines in six different offshore wind farms. The installed capacity should increase to 11 GWh in 2030, which equals to 8.5% of the total energy consumption in the Netherlands and 40% of the current electricity use (Offshore Wind Energy Roadmap 2030). Offshore wind farms will therefore enable a considerable reduction in greenhouse gas emissions in the Netherlands.

Despite this environmental gain, there are biodiversity concerns at the same time: onshore wind turbines are known to cause mortality amongst bats due to collisions (Johnson et al. 2003, Bach & Rahmel 2004, Kunz et al. 2007, Arnett et al. 2008, Rydell et al. 2010, Cryan et al. 2014, Thaxter et al. 2017) and possibly barotrauma (Grodsky et al. 2011, Rollins et al. 2012, Lawson et al. 2020). Significant numbers of fatalities have been reported on land (Hayes 2013, Voigt et al. 2015, O'Shea et al. 2018), which may cause declines in local as well as migratory populations (Lehnert et al. 2014). In temperate regions most fatalities concern migratory species and occur during late summer and autumn (Rydell et al. 2010, Voigt et al. 2015, Frick et al. 2017, Rodrigues 2018). In order to reduce fatalities curtailment

measures have been implemented in many wind farms on land which limit the production time during periods when bats are most active (Arnett et al. 2011, Peste et al. 2015, Adams et al. 2021).

Yet, there is no information on bat mortality in offshore wind farms (OWFs). As bats forage in a similar way around offshore wind turbines as they do around onshore wind turbines (Ahlén et al. 2009), it seems likely that fatalities also occur at sea. Based on the precautionary principle mitigation measures have been issued for the Dutch offshore wind farms in the Borssele area (Zeeland) to protect Nathusius' pipistrelles during their autumn migration over sea (Staatscourant 2016).

A total of 21 noctules from a coastal population near Sint Maartensvlotbrug (Noord-Holland) was tagged in October 2018 and August-September 2019. The obtained GPS data revealed that some of the nocturnal feeding trips took place over sea. This was the first evidence that at least some local noctules in the Netherlands venture out offshore during nightly foraging trips onto the North Sea.

As foraging distances up to 26 km from the roost have been reported (Kronwitter 1988), local populations of noctules along the Dutch coast may be affected by offshore wind developments. This effect may be significant when: (1) their foraging range overlaps with offshore wind farms, (2) a large proportion of the population forages offshore, and (3) they fly at heights overlapping the rotor swept area (RSA), thereby running a high risk of collision and/or barotrauma.

In this paper, we assess the potential risk of offshore wind developments in the Netherlands on local coastal populations of noctules, based on:

1. The location of maternity colonies of noctules along the Dutch coast, and the potential overlap of their foraging range with operational and planned offshore wind farms.
2. The activity pattern of noctules from a coastal colony at Sint Maartensvlotbrug (Noord-Holland), based on their foraging distance from the roost, their time budget spent over land and over sea, and their flight height.

Material & methods

Overlap of potential foraging range and offshore wind farms

First, we assessed the geographical locations of the maternity colonies in the Dutch coastal provinces, excluding the areas with Pleistocene sandy soils in the eastern and southern parts of these provinces, using a subset of the data obtained between 2010 and 2021 by Mostert (in prep.) during a national survey on the occurrence of noctules in the Netherlands. During this survey roosts were found during pre-dawn visits to forests and estates, using acoustic detectors to detect swarming individuals. Subsequently, the number of individuals leaving the roost at dusk was counted. In some cases, additional roosts were found during evening visits, as noctules are often very vocal and audible before sunset. For more details on bat inventories see Helmer et al. (1987).

Next, we assessed the geographical locations of operational OWFs and those in the preconstruction phase (National Georegister: Licensed Wind Farms). Areas in which offshore wind developments will take place in the future were also identified (National Georegister: Designated wind energy areas).

Finally, we assessed the overlap between the geographical locations of the operational and planned OWFs and the presumed maximum foraging range of noctules of 26 km from the roosts (cf Kronwitter 1988).

Activity pattern of noctules from a coastal population at Wildrijk

Wildrijk is a small forest reserve of 18 ha which is located at N 52.791 E 4.704 near Sint

Figure 1. Wildrijk (dark green) and surroundings.

Maartensvlotbrug in Noord-Holland. The area surrounding the forest is characterized by flower bulb cultivation, some small scattered villages, the nature reserve Zwanenwater and the coastline. The distance to shore is 2.2 km (Figure 1). The ground level of Wildrijk and its surroundings typically lies within 1 m below to 1 m above sea level. Zwanenwater is somewhat higher, with elevations between 5-8 m in most of the area, whilst the outer dunes reach a maximum of 20 m a.s.l. (https://ahn.arcgisonline.nl/ahnviewer/).

Tagging bats and tag retrieval

Bats were caught by hand from 'Swegler 2Fn bat boxes' during daylight hours, as well as with mist nets after dark. Before being tagged, each individual bat was measured, weighed, sexed, aged and its reproductive status was assessed using the criteria described by Haarsma (2008). All work has been executed under the Nature legislation permit 2018-057682 (Wageningen Marine Research) and the Animal Welfare protocol AVD248002016459 / VZZ-18-005 (Dutch Mammal Society).

The initial aim of this study was to obtain a dataset to validate the location estimation algorithm for the Dutch Motus network (Lagerveld et al. in prep.). Bats were therefore equipped with multiple tags:

1. A GPS tag (Pathtrack nanoFix® GEO – MINI (0.90 g) to obtain accurate location fixes, which are stored on the device.
2. A coded VHF tag (LOTEK NTQB2-1; 0.32 g) to get simultaneous detections at one or more Motus receiver stations (www.motus.org), and,
3. A VHF beeper tag (Telemetrie Service Dessau V5; 0.28 g) to ensure relocation of the tagged animal, or to find the fallen-off tag-pack in the field.

The coded VHF tag and the VHF beeper tag were glued onto the GPS tag using Superglue (Bison, Goes, the Netherlands) and the GPS tag was glued to the area between the shoul-

der blades using SAUER Hautkleber original (Manfred-Sauer-Stiftung, Lobbach Germany). This glue dissolves in a few weeks and in case tags cannot be retrieved, they will fall off automatically. Bats caught in bat boxes were placed back after being tagged, and bats caught in mist nets were released in the immediate vicinity. The total weight of the tags was never more than 5% of the weight of the animal. Because tagging disrupts normal nighttime activities (and thus potentially influences behaviour; e.g. Aldridge & Brigham 1988, Kenward 2000), the GPS tags of the animals that were caught after dark were programmed to start data collection the night after the animal had been tagged. Data collection of the animals that were tagged during the day (and placed back in their roost) started at dusk on the same day.

The capacity of the GPS battery enabled data collection during 1-4 consecutive nights, depending on the settings of the tags (Table 2). Note that GPS tags need an unobstructed view of the sky to make a location fix. While bats reside in their roost, the GPS tag produces null-fixes. Therefore, when a location fix is made, in principle the animal is in flight. However, when GPS tags fall off at a location with satellite coverage (e.g. in the open field, or in an area with limited tree cover) before the data collection has ended, they are still able to make location fixes. When this happens, long series of location fixes are made which are typically less than 20 metres apart. Therefore, this situation is easily identifiable in the data. During this study, two GPS tags fell off before the data collection ended: ID 12 from 3 September 2019 19:00 UTC onwards and ID 14 from 27 August 2019 21:30 UTC onwards, consisting of 69 location fixes in total. These location fixes were removed from the data set (9.9% of the data).

Analysis

The analysis of the activity pattern was done in R 4.0.2 (R Core Team 2022). We created a map of the flight paths using the package ggplot2 (Wickham 2016). Of each GPS fix the distance to the centre of Wildrijk as well as the distance to shore was assessed, using the package rgeos (Bivand & Rundel 2020). The time budget over land and over sea of each individual was calculated based on the number of GPS fixes in each habitat divided by the total number of GPS fixes. Subsequently, the time budget was assessed in relation to the distance from Wildrijk. We did not assess the home range given the limited amount of monitoring hours/days of each individual. Finally, we assessed the flight heights over sea and over land. As GPS altitude data refers to the altitude above mean sea level, the recorded altitude data over sea corresponds with the flight height. To assess the flight heights over land we subtracted the ground level (https://ahn.arcgisonline.nl/ahnviewer/) from the GPS altitude. Subsequently, we compared the flight heights over land and over sea, using a two-sample *t*-test. Our GPS data contained two negative altitude values (-11.3 m and -4.5 m, both recorded at Wildrijk), which we removed from the analysis.

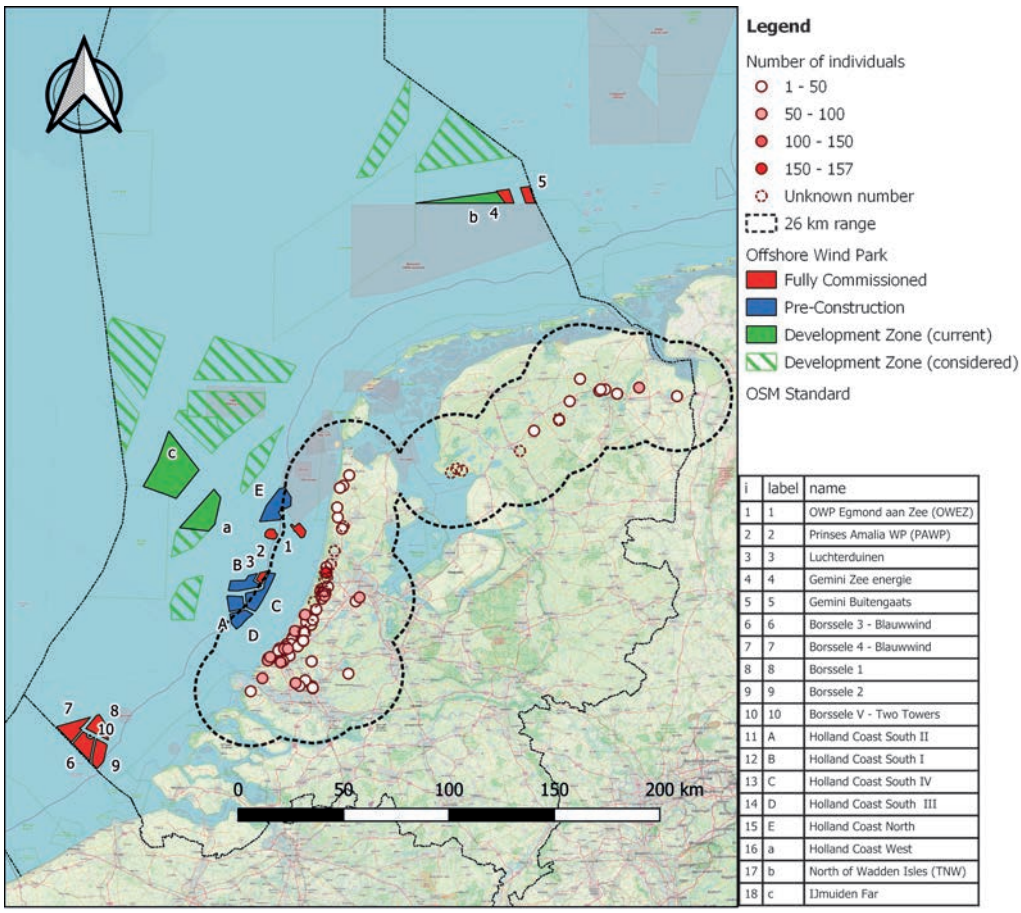
Results

Overlap of OWFs and potential foraging range

Offshore Wind Farm Egmond aan Zee (OWEZ) was the first OWF in the Netherlands and was commissioned in 2006, followed by Princess Amalia Wind Farm (PAWP) in 2008, Luchterduinen (LUD) in 2015 and Gemini (Buitengaats and Zee-energie) in 2017. The OWFs in the Borssele area (lot 1, 2, 3, 4 and 5) became operational throughout 2020–2021. Characteristics of the operational OWFs are shown in Table 1. Currently several OWFs are under development in the areas Holland Coast South and Holland Coast North. Further west and north several areas for future OWFs have been designated (Offshore Wind Energy Roadmap 2030).

Table 1. dimensions and number of wind turbines of operational OWFs. In OWFs relative close to the coast (OWEZ, PAWP and LUD) the RSA varies between 20–153 m above sea level.

Operational OWF	Nacelle heigth (m)	Rotor diameter (m)	RSA heigth (m)	Number of wind turbines
OWEZ (Offshore Wind Farm Egmond aan Zee)	70	90	35 - 125	36
PAWP (Princess Amalia Wind Farm)	60	80	20 - 100	60
LUD (Luchterduinen)	81	112	25 -153	43
Gemini (Buitengaats & Zee-energie)	89	130	34 - 164	150
Borssele 1 & 2	117	167	33 - 200	94
Borssele 3 & 4	?	164	?	97
Borssele 5	?	164	?	2



i	label	name
1	1	OWP Egmond aan Zee (OWEZ)
2	2	Prinses Amalia WP (PAWP)
3	3	Luchterduinen
4	4	Gemini Zee energie
5	5	Gemini Buitengaats
6	6	Borssele 3 - Blauwwind
7	7	Borssele 4 - Blauwwind
8	8	Borssele 1
9	9	Borssele 2
10	10	Borssele V - Two Towers
11	A	Holland Coast South II
12	B	Holland Coast South I
13	C	Holland Coast South IV
14	D	Holland Coast South III
15	E	Holland Coast North
16	a	Holland Coast West
17	b	North of Wadden Isles (TNW)
18	c	IJmuiden Far

Figure 2. Location of the maternity colonies in the coastal provinces (excluding Pleistocene areas), their presumed maximum foraging range of 26 km (Kronwitter 1988), operational OWFs as well as OWFs under construction and designated areas for wind farm development.

Table 2. Overview of tagged animals and settings of the tags.

ID	Sex	Age	Tagged			GPS			
			Latitude	Longitude	Data/Time [UTC]	Tag number	Time-on [UTC]	Time-off [UTC]	Interval [min]
1	Male	Adult	52.791	4.704	03-10-2018 17:15	20461	17:00	22:00	3
2	Male	Adult	52.791	4.704	03-10-2018 17:17	20462	17:00	22:00	3
3	Female	Adult	52.794	4.707	09-10-2018 09:00	20468	17:00	22:00	3
4	Female	Adult	52.794	4.707	09-10-2018 09:10	20469	17:00	22:00	3
5	Female	Adult	52.794	4.707	09-10-2018 09:20	20466	17:00	22:00	3
6	Male	Adult	52.789	4.703	09-10-2018 20:00	20464	17:00	22:00	3
7	Female	First year	52.789	4.701	19-10-2018 15:00	20464	15:00	20:00	3
8	Female	Adult	52.789	4.701	19-10-2018 15:10	20466	15:00	20:00	3
9	Male	Adult	52.794	4.707	19-10-2018 16:00	20462	15:00	20:00	3
10	Male	Adult	52.791	4.705	27-08-2019 09:20	21233	19:00	22:00	1
11	Male	Adult	52.794	4.708	27-08-2019 09:50	21287	19:00	22:00	1
12	Male	Adult	52.790	4.705	27-08-2019 10:30	21305	19:00	22:00	2
13	Male	Adult	52.791	4.704	27-08-2019 09:00	21490	19:00	22:00	2
14	Male	Adult	52.790	4.705	27-08-2019 10:45	21312	19:00	22:00	2
15	Male	Adult	52.791	4.704	27-08-2019 09:30	21349	19:00	22:00	1
16	Male	Adult	52.794	4.707	02-09-2019 15:00	21279/21313	19:00	22:00	2
17	Male	Adult	52.794	4.708	03-09-2019 09:00	21468	19:00	22:00	2
18	Male	Adult	52.794	4.707	20-09-2019 09:00	56321	18:00	20:00	2
19	Male	Adult	52.794	4.707	20-09-2019 09:15	21506	18:00	20:00	2
20	Male	Adult	52.794	4.707	20-09-2019 09:30	21341	18:00	20:00	1
21	Male	Adult	52.794	4.707	20-09-2019 09:45	21509	18:00	20:00	1

During the national survey on the occurrence of noctule in the Netherlands (2010-2021) a total of 97 maternity colonies was found in the Dutch coastal provinces, excluding the areas with Pleistocene sandy soils in the eastern and southern parts of these provinces. In the area concerned 56 colonies were found in Zuid-Holland, 25 in Noord-Holland, 8 in Friesland and 8 in Groningen. No colonies could be found in Zeeland. From 79 colonies, the number of individuals was assessed, resulting in an average of 31 individuals per colony (range 10–157).

Figure 2 shows the overlap between OWF areas and the potential foraging range, using a maximum distance of 26 km from the roosts (cf Kronwitter 1988). Currently one operational OWF (OWEZ) lies within the area of overlap, while PAWP and Luchterduinen are located just outside the presumed maximum foraging range. The area of overlap includes also the eastern part of the development zones Holland Coast South and Holland Coast North.

The operational OWFs in Borssele and Gemini, as well as all designated areas for future wind energy developments are located beyond the potential foraging range of coastal noctules.

Activity pattern of noctules from a coastal population at Wildrijk

A total of nine individuals was tagged in 2018 (between 3 and 19 October) and twelve individuals in 2019 (between 27 August and 20 September), including 16 adult males, one first year and four adult females. The GPS tags collected data during 2-5 hours per day and

Table 3. Retrieved tags and monitoring data.

ID	Sex	Age	Tag retrieved			GPS data		
			Latitude	Longitude	Data/Time [UTC]	Number of days	Number of location fixes	Time budget over sea
1	Male	Adult			-			
2	Male	Adult	52.793	4.707	17-10-2018 18:00	3	48	0%
3	Female	Adult			-			
4	Female	Adult			-			
5	Female	Adult	52.797	4.723	17-10-2018 18:00	3	37	3%
6	Male	Adult	52.789	4.702	17-10-2018 16:00	2	30	10%
7	Female	First year	52.790	4.703	19-10-2018 15:00	1	6	0%
8	Female	Adult	52.789	4.701	19-10-2018 15:10	1	16	0%
9	Male	Adult			-			
10	Male	Adult	52.790	4.703	10-09-2019 16:00	2	15	0%
11	Male	Adult	52.783	4.712	30-08-2019 18:00	1	83	0%
12	Male	Adult	52.790	4.705	03-09-2019 09:00	1	58	78%
13	Male	Adult	52.799	4.725	29-08-2019 16:00	1	41	20%
14	Male	Adult	52.799	4.724	29-08-2019 17:00	1	53	0%
15	Male	Adult	52.793	4.705	28-08-2019 16:00	1	58	0%
16	Male	Adult	52.794	4.707	05-09-2019 09:00	2	37	0%
17	Male	Adult			-			
18	Male	Adult	52.800	4.726	26-09-2019 16:00	4	68	7%
19	Male	Adult	52.799	4.725	26-09-2019 16:00	4	79	0%
20	Male	Adult			-			
21	Male	Adult			-			

used time intervals of 1-3 minutes between the fixes (Table 2).

We obtained data from 14 out of 21 tagged individuals (Table 3). Six GPS-tags were not retrieved and the data of one could not be extracted from the GPS due to technical problems. The dataset included eleven males (all adults) and three females (one first year and two adults).

The number of monitored nights per individual was on average two nights (range 1-4) and depended on the settings of the GPS tag (Table 3). On average 45 location fixes were obtained per individual (range 6–83 location fixes). Nine individuals were detected exclusively over land, whereas five individuals were detected both over land and over sea. On average 8% of the time budget of the animals included in this study was spent over sea. One individual (ID 12) spent most of its time (78%) over sea.

Figure 3 shows the flight tracks of all individuals combined. Note that tracks are frequently incomplete as the GPS tags were programmed to collect during a few hours per day (Table 2).

Most flight activity occurs relatively close to the roost (Figure 4). In total, we recorded 629 location fixes. Of these, 58% were made up to 3 km from the centre of Wildrijk; 72% up to 5 km and 89% up to 7 km. Within this 7 km flight range, 3% of the location fixes occurred over sea. In some cases (11% of the data) more distant flights were performed during which 71% of the location fixes occurred over sea. For example, ID 12 reached a maximum distance of 18.5 km from Wildrijk and 12.7 km from shore at 27 August 2019 20:04 UTC and ID 6 which was detected 3.3 km from shore at 10 October 2018 17:03 UTC when it flew back to the coast at a distance of 10.5 km from Wil-

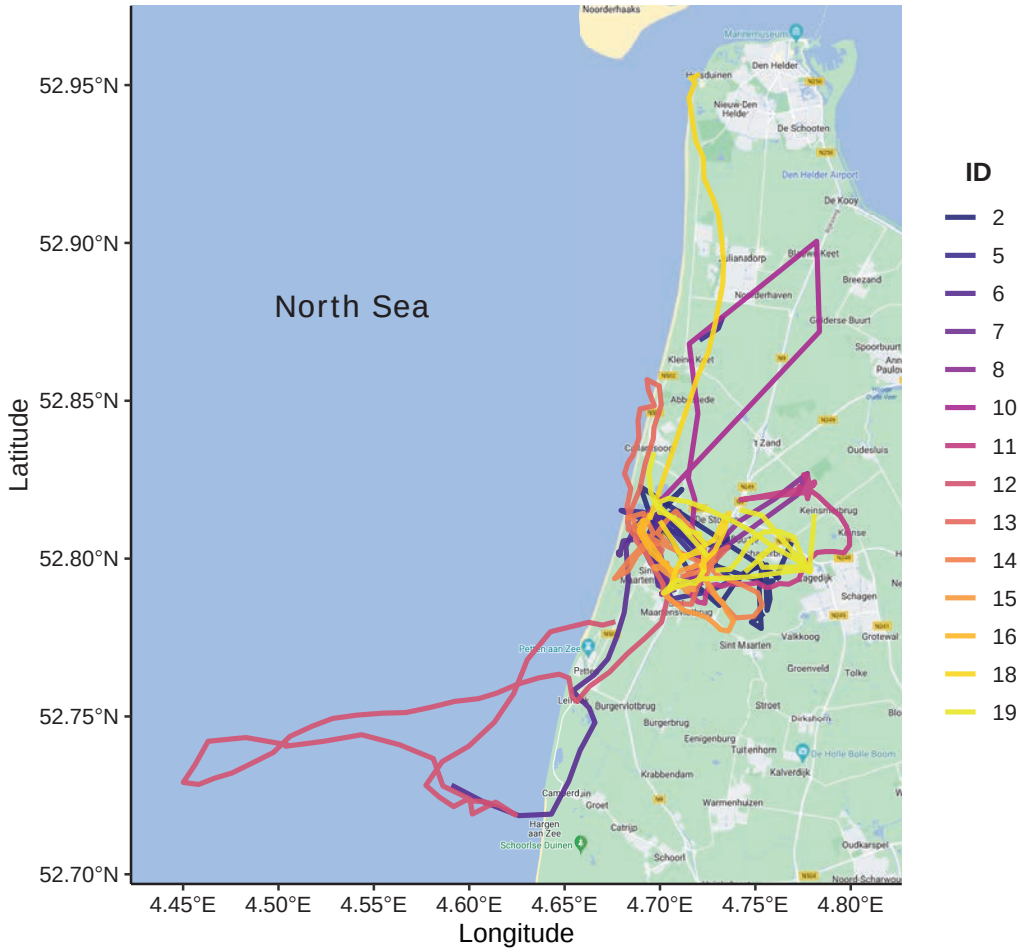


Figure 3. Flight tracks of all individuals.

Table 4. Flights over 7 km and weather conditions at de Kooij (retrieved from https://www.knmi.nl/).

ID	Date	Time (UTC)	Max distance Wildrijk (km)	Habitat	Wind direction (degrees)	Wind speed (m/s)	Temperature (°C)	Precipitation (mm/h)	Atmospheric Pressure (Hpa)
6	10-10-2018	17:03	10.5	Land & Sea	70	5	16.2	0	1013.8
5	11-10-2018	17:01	9.7	Land & Sea	130	4	18.9	0	1010.2
13	27-8-2019	19:36	7.3	Land & Sea	70	3	25.4	0	1013.3
12		20:04	18.5	Land & Sea					
10		20:37	13.2	Land					
18	22-9-2019	18:00	18.5	Land & Sea	130	3	20.7	0	1005.8

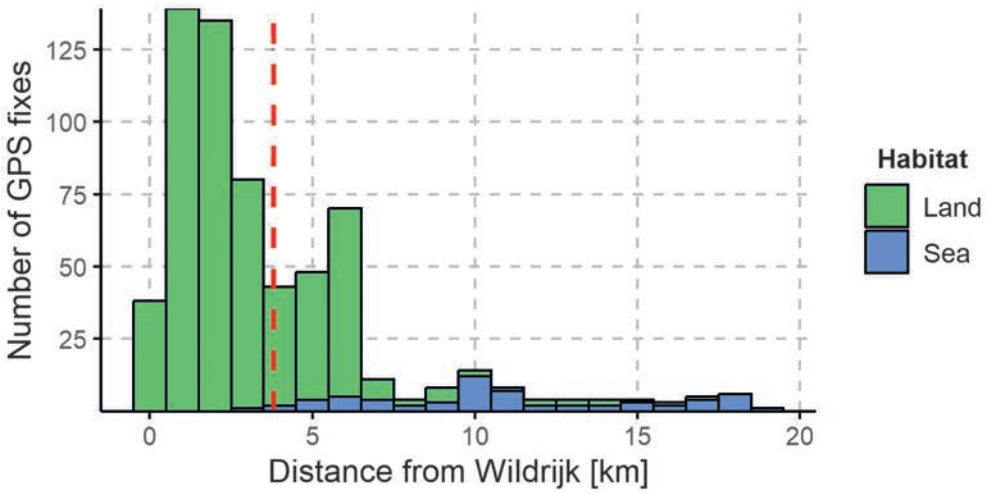


Figure 4. Histogram of the distance between the GPS location fix and Wildrijk. The vertical dashed line indicates the mean distance (3.8 km).

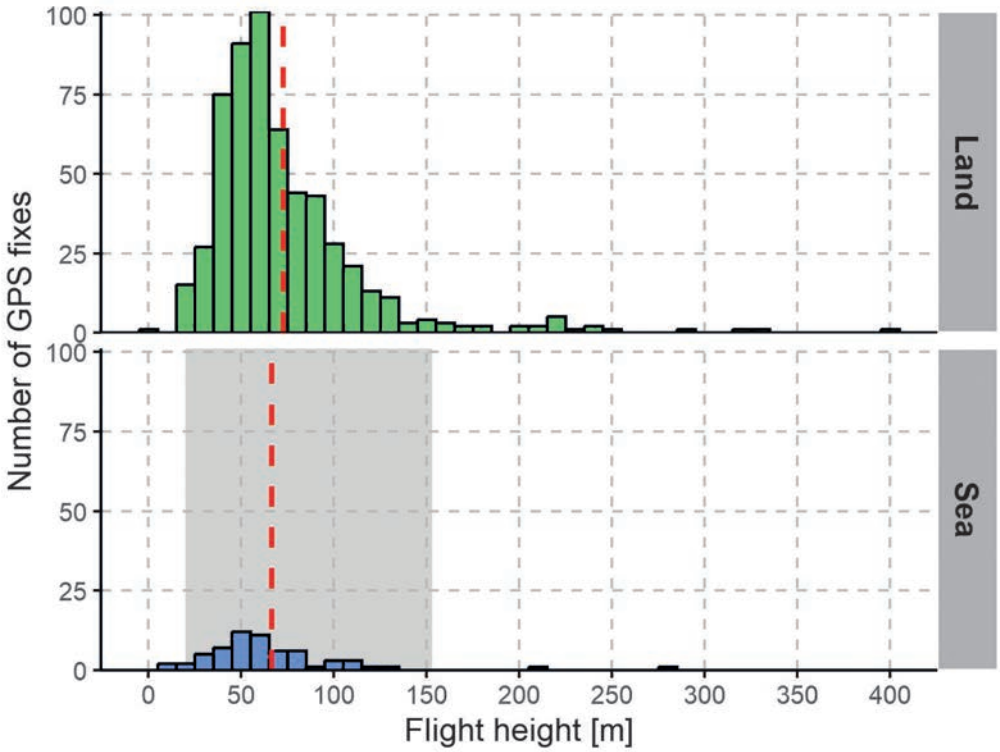


Figure 5. Histogram of the recorded flight heights over land and over sea. The dashed vertical lines indicate the mean flight heights. The shaded area represents the RSA of offshore wind turbines at OWEZ, PAWP and LUD.

drijk (Figure 3). All other individuals detected over sea (ID 5, 13 and 18) flew at distances within 0.4 km from shore.

Table 4 summarizes the flights over 7 km and corresponding weather conditions at de Kooij/Airport den Helder (retrieved from https://www.knmi.nl/). Distant flights coincided with low easterly winds, no precipitation, relatively high temperatures and relatively high atmospheric pressure. One trip occurred exclusively over terrestrial habitats while the remaining five occurred both over land and over sea.

Figure 5 shows the flight heights over land and over sea. The average flight height over land was 72.6 m (SD 42.9, range 6–400) and over sea 66.4 m (SD 42.1, range 8-280). This difference proved to be not significant (t=1.0848, df=625, P-value = 0.2784). On land 94.4% and at sea 93.5% of the flight activity occurred at heights between 20–153 m.

Discussion

We found the highest density of coastal maternity colonies in the western part of the Netherlands, in a relatively narrow strip along the inner dunes between The Hague and IJmuiden, where many estates and old forests are present. A few scattered colonies occur further north in Noord-Holland. Agricultural areas east of the dunes in Zuid-Holland and Noord-Holland are largely unsuitable for noctules, with the exception of some older forests around Amsterdam and Rotterdam. We found no colonies in Zeeland and the Wadden Islands, even though some suitable habitat is locally present. The Wadden Sea coast of Friesland and Groningen consists of an open agricultural landscape which does not harbour colonies. In southern parts of Friesland and Groningen there are some small estates and forests with a few colonies of noctule. Since males generally roost individually or in small groups in the vicinity of a maternity colony (Sluiter & van Heerdt 1966, Limpens at al. 1997), we consider the observed distribution of the maternity colonies representative for the species' range of local populations along the Dutch coast.

Our study shows that most flight activity occurs within a few km from Wildrijk; 58% of the data was recorded within a range of 3 km and 89% within 7 km. The observed main flight range corresponds well with foraging distances between 3-6 km in other areas in the Netherlands (Limpens et al. 1997), distances between 2.4-5.2 km in Germany (Kronwitter 1988, Roeleke et al. 2020) and 4.2 km in the UK (Mackie & Racey 2007). Furthermore, our study shows that the time budget spent over sea within 7 km from Wildrijk was low (3%), despite its proximity to the coast (2.2 km).

We recorded six foraging trips over 7 km with a maximum distance of 18.5 km from Wildrijk and 12.7 km from shore (Figure 2). During flights over 7 km the average time budget spent over sea was relatively high (71%). Distant flights up to 26 km are also known from Kronwitter (1988), who considers this 'swarm flights', as they are not confined to specific foraging areas and last longer in comparison to regular foraging trips. Swarm flights seem to be confined to the period between mid-August and mid-September. When they occur, the regular foraging areas are virtually abandoned (Kronwitter 1988). The distant flights we recorded occurred between late August and mid-October during nights with low easterly winds, no precipitation, relative high temperatures and relatively high atmospheric pressure. It seems plausible that foraging opportunities may trigger extensive foraging trips and thus the presence of noctules over sea. High temperatures trigger insect activity and easterly winds may drift these insects offshore. In particular during late summer/early autumn, when large numbers of migrating insects can be expected (Drake & Gatehouse 1995, Chapman et al. 2004, Drake et al. 2012), there can be increased availability of insects over sea. Offshore foraging noctules pursuing insect

swarms have also been reported from the Baltic Sea (Ahlén et al. 2009).

Assuming a 26 km maximum range of 'swarm flights' (cf Kronwitter 1988), OWEZ as well as areas along the eastern edge of the wind farm development zones Holland Coast South and Holland Coast North are within reach of coastal colonies. PAWP and Luchterduinen are located just outside the presumed maximum range. The occurrence of noctules in this area is confirmed by acoustic records from OWEZ (3 and 8 September 2012 and 4 September 2014, 15 km from shore), PAWP (4 September 2014, 9 and 12 September 2016, 25 km from shore) and LUD (23 August 2016, 23 km from shore) (Lagerveld et al. 2014, 2015 and 2017). However, the number of noctules occurring in this area is likely to be underestimated due to limitations of acoustic monitoring in general (Barataud 2020, Voigt et al. 2021) and the presence of only one acoustic detector in each OWF. In addition, only 15% of the recorded 'Nyctaloids' (including *Nyctalus*, *Vespertilio* and *Eptesicus* species) were identified to species level. To date, there are no acoustic records (with certainty) from monitoring locations in the Dutch North Sea further south, west and north (Lagerveld et al. 2014, 2015, 2017 and 2019). The only two offshore records well beyond the presumed 26 km maximum foraging range include two grounded individuals at gas production platforms at 5 September 1994 and 10 September 1996, respectively 5 km north off Ameland and 15 km north off Texel (Boshamer & Bekker 2008).

Given the timing of the occurrence, from late August until mid-September, it seems plausible that the acoustic records and visual observations at the Dutch North Sea refer to resident noctules. Seasonal migrants are likely to occur later in the season, as the main migration period in the Netherlands appears to run from late September until mid-November (https://www.trektellen.nl/species/graph/1/0/1090/0?jaar=0).

Aerial hawking bats like noctules are vulnerable to wind turbine induced mortality as their flight activity overlaps with the RSA (Rodrigues et al. 2015, Roeleke et al. 2016, Roemer et al. 2017). The operational OWF within the presumed maximum foraging range (OWEZ) as well as the ones just outside this range (PAWP and LUD) consist of wind turbines with RSAs between 20–153 m. a.s.l. Our data shows that 93.5% of the flight activity at sea occurs between 20–153 m, indicating that virtually all flight activity occurs at heights within the RSA. The observed average flight height over sea was lower in comparison to land (respectively 66.4 m and 72.3 m), but this difference proved to be not significant. A study in eastern Germany showed that 95% of noctule flight activity in open habitats occurs at heights between 0 and 144 m above ground level (Roeleke et al. 2016). This matches our observations very well, as we recorded 95.2% of the location fixes within this altitudinal range.

When interpretating the results of tracking studies one should always take the accuracy of the measurements into account (Hulbert & French 2001, Frair et al. 2010, Péron et al. 2020). The horizontal position accuracy of GPS wildlife tracking equipment ranges from less than 5 m in open habitats (Hulbert & French 2001) to 30 m at locations with high canopy cover (Frair et al. 2010). In our study we observed horizontal position errors up to 20 m. Given the extent of the movements we recorded, we can safely conclude that these errors cannot affect the observed spatial use. The vertical GPS error is generally larger than the horizontal position error, and even in good conditions vertical errors up to 20 m can be expected (Péron et al. 2020). Obvious erroneous GPS altitude data in our study included two GPS locations fixes (0.3% of the data) with negative heights of respectively -4.5 and –11.3 m, and we cannot exclude the possibility of other GPS location fixes containing vertical errors too. However, we think it is unlikely that our dataset contains many erroneous GPS altitude data as we recorded an identical altitudinal flight activity pattern as Roeleke et al. (2016). In the unlikely event our dataset is biased and the actual average flight height is for example 20 m lower or

20 m higher, virtually all flight activity will be still be at heights within the RSA of offshore wind turbines (see Figure 5).

Conclusions

In general, it seems unlikely that offshore wind farms in the Netherlands will significantly affect coastal populations of noctule since offshore wind developments take place beyond their regular foraging range. In some cases however, noctules do perform distant flights ('swarm flights'), possibly in response to migrating insects. We recorded six distant foraging trips both over land and over sea with a maximum distance of 18.5 km from Wildrijk and 12.7 km from shore. Acoustic records confirm that noctules are occasionally present in offshore wind farms at distances of 15–25 km from shore. During such an event, noctules face the risk of a collision as virtually all their flight activity occurs at heights within the rotor swept area of offshore wind turbines.

Note however, that this study cannot be considered 100% representative for all colonies along the coast and at all times of the year, as it is based on a limited number of individuals from one local population during autumn (and adult male biased). To gain more certainty about the potential risk, it is therefore recommended to conduct additional research into the habitat use of coastal noctules from different colonies, during their entire active period and for different sex and age classes, hereby specifically focusing on the extent and orientation of swarm flights, as well as the conditions in which these occur.

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Samenvatting

Zijn offshore windparken in Nederland een potentiële bedreiging voor de populaties van rosse vleermuizen langs de kust?

Offshore windparken veroorzaken waarschijnlijk sterfte onder migrerende vleermuizen tijdens hun trek over zee. Het is echter nog niet bekend of de ontwikkeling van de offshore windsector een effect kan hebben op lokale populaties vleermuizen langs de kust. We voerden een analyse uit om het potentiële risico te bepalen van offshore windparken in de Nederlandse Noordzee op populaties van rosse vleermuizen (*Nyctalus noctula*) langs de kust. We bepaalden eerst de overlap tussen hun potentiële foerageergebied en de operationele en geplande offshore windparken. Vervolgens analyseerden we de vliegbewegingen van 14 gezenderde rosse vleermuizen aan het einde van de zomer en in de herfst. In zijn algemeenheid lijkt het onwaarschijnlijk dat offshore windparken in Nederland significant negatieve effecten hebben op lokale populaties rosse vleermuizen, omdat de ontwikkeling van de offshore windsector buiten hun reguliere foerageergebied plaatsvindt. Echter, in sommige gevallen maken rosse vleermuizen langdurige en verre foerageervluchten ('zwermvluchten'), die mogelijk samenhangen met migrerende insecten. Gedurende deze studie stelden we zes verre foerageervluchten vast, zowel over land als over zee, met een maximale afstand van 18,5 km van hun verblijfplaats en 12,7 km uit de kust. Akoestische monitoring in offshore windparken op 15-25 km uit de kust bevestigt dat rosse vleermuizen hier soms aanwezig zijn. Op die momenten lopen rosse vleermuizen risico op een aanvaring, omdat vrijwel al hun vliegactiviteit plaatsvindt op rotorniveau.

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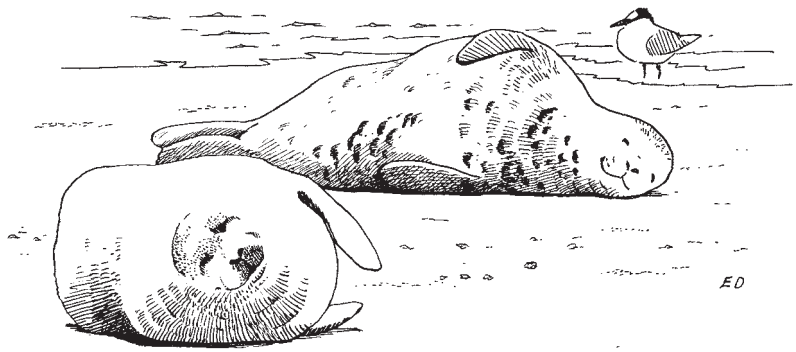
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Prey that kill: Dover soles (*Solea solea*) causing fatal asphyxiation in seals in the southern North Sea

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Abstract: Along with the increase of harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) in the southern North Sea, the number of stranded dead and dying seals has risen sharply in recent decades. A selection of animals stranded in Belgium and the Netherlands is examined, with as one of the main objectives to collect information about the cause of death. One of the causes of death is suffocation from fish that enter the trachea, or from fish that are too large to be swallowed completely. In this contribution, eleven of such cases are discussed. It appears that Dover sole (*Solea solea*) is especially dangerous for seals.

Keywords: *Phoca vitulina*, *Halichoerus grypus*, mortality, suffocation, *Solea solea*, Belgium, the Netherlands.

Introduction

Two species of seal are indigenous to the southern North Sea: the harbour seal (*Phoca vitulina*) and the grey seal (*Halichoerus grypus*). The harbour seal population crashed several times during the 20th century, while the grey seal had been virtually absent in this area for centuries (Brasseur et al. 2015, 2018). However, numbers of both species have increased dramatically during the last decades. The number of harbour seals currently present in the French North Sea and Channel, the Dutch Delta area, the Wadden Sea and south-east England is estimated to amount to more than 35,000 animals (OSPAR 2023). In Belgium, only two haul-out sites exist, holding very few seals. In the Wadden Sea area and on Helgoland 2,214 grey seal pups were counted in the winter pupping season of 2021/2022, and in April 2022 8,948 grey seals were counted in the Wadden Sea (Schop et al. 2022). Estimates of the number of grey seal births at the colonies in eastern England amounted to more than 11,000 in 2019 (SCOS 2022, OSPAR 2023). Together with the increase in numbers present, the number of stranded dead and dying seals has increased. A selection of animals stranded in Belgium and the Netherlands is investigated, with as one of the main objectives identifying the cause of death. In this contribution we focus on the pathology and the occurrence of an unexpected cause of death: asphyxia due to the ingestion of Dover sole (*Solea solea*).

Material and methods

In Belgium, the Royal Belgian Institute of Natural Sciences (RBINS) is responsible for recording strandings and incidental catches of marine mammals and organising the col-

Table 1. Data of the seals used in this study; M: male, F: female; TL: total length; DCC: decomposition code; NCC: Nutritional Condition Code; ND: not determined; B: Belgium; NL: the Netherlands

Case no.	Species	Date	Location	Rehab. tag	Weight (kg)	TL (cm)	Sex	Age	DCC	NCC	Images available
1	*Halichoerus grypus*	25/09/2014	Oostende (B)	No	148	ND	M	Adult	3	5	Yes
2	*Halichoerus grypus*	21/04/2015	Middelkerke (B)	No	Approx. 200	230	M	Adult	3	1	Yes
3	*Halichoerus grypus*	14/03/2017	Middelkerke (B)	SEALIFE (B)	40	132	M	Juvenile	2	1	Yes
4	*Halichoerus grypus*	5/04/2018	Wenduine (B)	No	85	176	M	Subadult	2	1	No
5	*Halichoerus grypus*	1/05/2018	Burghsluis (NL)	Pieterburen (NL)	29.8	124	F	Juvenile	2	1	Yes
6	*Phoca vitulina*	8/11/2012	Texel (NL)	Pieterburen (NL)	18.5	116	F	Juvenile	2-3	1	No
7	*Phoca vitulina*	25/04/2013	Julianadorp (NL)	No	43.6	147	F	Subadult	2	2	Yes
8	*Phoca vitulina*	2/05/2013	Texel (NL)	No	104	184	F	Adult	3	2	Yes
9	*Phoca vitulina*	12/10/2013	Texel (NL)	No	61	145	F	Adult	3	3	No
10	*Phoca vitulina*	5/06/2014	Den Helder (NL)	No	72,5	158	F	Adult	3	1	Yes
11	*Phoca vitulina*	12/05/2017	Sint Maartensdijk (NL)	Chip FDXB	103.5	170	F	Adult	3	1	Yes

lection of a selection of stranded and bycaught animals. Selection is mostly based on decomposition and size, with limited decomposition as the most important driver for selection. Collected animals are submitted to a necropsy performed at the University of Liège or at the University of Ghent, or on-site in case of very large seals. A standardized necropsy protocol is used (Jauniaux et al. 2002, Pugliares et al. 2007).

In the Netherlands, the University of Utrecht occasionally performs necropsies on stranded seals.

To the animals necropsied, a decomposition code (DCC) is attributed, with as DCC 2: freshly dead, intact animal; DCC 3: moderate autolysis, with organs still intact, moderate swelling due to gas, skin sloughing and discernible smell of decomposition, and DCC 4: advanced decomposition, with major bloating, skin peeling, and organs beyond recognition. For each animal also a nutritional condition code (NCC) was scored on a scale from 1 (very fat) to 6 (emaciated) (Pugliares et al. 2007).

The main objective of the necropsies was to establish the cause of death.

Results

During necropsies of seals washed ashore in Belgium and the Netherlands between 2012 and 2018, an obstruction of the airways by a fish was diagnosed in eleven cases: five grey seals and six harbour seals (Table 1). The fish was either obstructing the oral cavity, or it was partially or entirely stuck in the trachea. In all cases in which the fish was identified to species level (n=7), it turned out to be Dover sole (*Solea solea*), with an additional case in which the fish was suspected to be a Dover sole. Most of the animals in this study were found between March and June (n=8). An

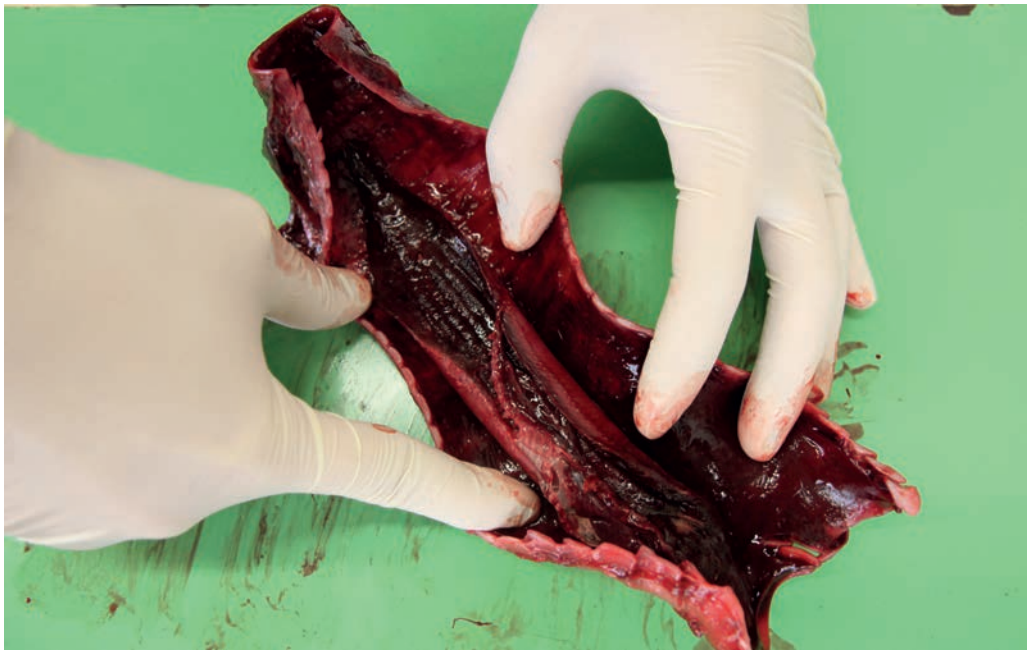

Figure 1. Dover sole present in, and blocking the trachea of a grey seal (case 1). *Photo: University of Ghent, Belgium, Faculty of Veterinary Medicine, dept. Morphology*.

additional animal (a grey seal that washed ashore at Westkapelle, the Netherlands, on 11 April 2017) that choked on a flounder (*Platichthys flesus*) is not discussed here, as it was not subjected to a necropsy.

Description of cases

The description of the cases, with a focus on the obstruction of the airways and the suspected cause of death, was extracted from the necropsy reports. Comments, not from these reports, are put in square brackets.

Case 1. The cranial part of the trachea of the animal was obstructed by an undamaged Dover sole, head down (Figure 1). According to the necropsy report, the stomach contained a lot of fish. [No stomach content analysis was performed.]

Case 2. A Dover sole was present, head down, in the cranial part of the trachea, completely blocking it (Figure 2). The stomach contained only Dover soles, 14 in total, in different stages of digestion (Figure 3).

Case 3. A 37 cm, 0.5 kg Dover sole was stuck, head down, in the oesophagus. Its head region had penetrated the oesophagus up to a depth of 7 cm; the rest of the fish's body was partly present in the oral cavity, blocking it completely, and partly sticking out of it (Figure 4). The seal stomach contained four undamaged Dover soles of 15 to 18 cm long, with a total weight of 0.45 kg. The animal had a flipper tag indicating that it had been born in fall/winter of 2016 and had been admitted for care at Sealife Blankenberge, Belgium.

Case 4. A Dover sole was stuck in the trachea, completely blocking it. The stomach contained 2 kg of Dover soles. [No further stomach content analysis was performed.]

Case 5. A Dover sole of 33 cm was stuck in the oesophagus, compressing the larynx (Figure 5). The stomach was filled with fish and with anisakid nematodes (*Contracaecum osculatum*). The animal had a tag indicating that it had been taken care of at Zeehondencentrum Pieterburen, the Netherlands. [No

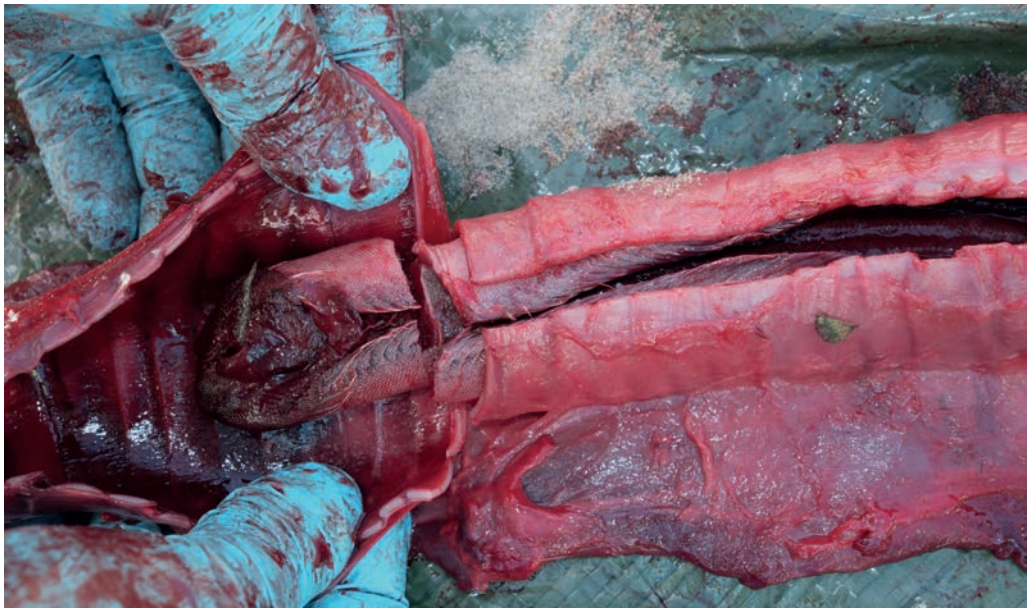

Figure 2. Dover sole present in, and blocking the trachea of a grey seal (case 2); the fish was cut in two during the necropsy. *Photo: Jan Haelters / RBINS.*

Figure 3. Stomach content of a grey seal: Dover soles in various stages of digestion (case 2). *Photo: Jan Haelters / RBINS.*

Figure 4. A large Dover sole partly present in the oral cavity of a grey seal (case 3). *Photo: Jan Haelters / RBINS*.

Figure 5. A dover sole blocking the oral cavity of a grey seal (case 5). *Photo: Jooske IJzer, Utrecht University*.

further information could be obtained about the stomach content.]

Case 6. The animal had a subcutaneous haemorrhage of 10 by 5 cm in the neck at the level of vertebrae C2–C3 and was diagnosed as a possible bycatch. A small fish, headfirst, was found in the trachea. The animal had an internal chip indicating that it had been in care at Zeehondencentrum Pieterburen, the Netherlands. [No further information could be obtained about the fish in the trachea.]

Case 7. A Dover sole of 13 cm long and 2.6 cm wide was present in the cranial part of the trachea. The stomach contained several fish. [No further information could be obtained about the stomach content.]

Case 8. The animal suffered a broken neck at the first cervical vertebra, leading to a separation between the spinal cord and the brainstem. A haemorrhage was present at the ventral side of the neck, from the mandibula to the cranial part of the scapula. According to the necropsy report, the severe neck trauma was possibly sustained during agony. In the oesophagus, a fish was present, completely blocking the nasopharynx. The animal was pregnant with an almost fully-grown foetus of 7.1 kg. [No further information could be obtained about the fish blocking the nasopharynx.]

Case 9. A 10 cm long, 4 cm wide flatfish, probably a Dover sole, was present at the bifurcation of the trachea. The stomach contained many fish, and the oesophagus contained bony remnants of fish. [No further information could be obtained about the fish in the oesophagus, given their state of digestion most probably the result of regurgitation, or stomach.]

Case 10. Postpartum, lactating animal. A 15 cm long fish was present in the nasopharynx and trachea. The oesophagus was filled with fish as well. The blubber in the neck was very red, with many 3-5 mm round haemorrhages and pooling of blood [indicative of a peri-mortem lesion]. [The species of fish in the nasopharynx and trachea remained unidentified and its size was not mentioned in the necropsy report.]

Case 11. The cranial part of the trachea was completely obstructed by a 15 cm long Dover sole; its tail was present in the oropharynx on top of the epiglottis. The stomach was filled with 5 kg of flatfish, consisting predominantly of Dover soles. The animal was pregnant with an almost fully-grown foetus of 8 kg. The animal had an internal chip indicating it had been in care at a rehabilitation facility, but no further information could be obtained. [No further information on the presence of other species than Dover soles in the stomach could be obtained from the necropsy report.]

Discussion

Suffocation due to fish in marine mammals

The respiratory system of marine mammals is well adapted to life in the water. As in other mammals, systems exist that try and prevent communication between the nasopharynx and the oropharynx (Reidenberg 2007). Seals evolved an upper aerodigestive tract valving mechanism adapted to a marine environment, with the larynx acting as a valve to assure the separation of the respiratory and digestive pathways during swallowing to prevent the aspiration of food into the trachea and lungs (Adams et al. 2020).

Suffocation due to a fish blocking the airways is well known in marine mammals. The problem may be caused by either a spiny fish that got stuck in the throat, an unusual prey, or a very agile fish. Additionally, overenthusiastic feeding on overly large prey can result in asphyxiation. In cases described earlier, the fish either blocked the cranial part of the oesophagus, concurrently leading to an obstruction of the airways, it dislocated the larynx and impeded its re-articulation, or it directly blocked the trachea.

Published cases of death of marine mammals due to spiny fish or fish that were too large to be swallowed concern bottlenose dolphins (*Tursi-*

ops truncatus) and Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Nash 1974, Byard et al. 2003, Watson & Gee 2005, Mignucci-Giannoni 2009, Byard et al. 2010, Stolen et al. 2013), harbour porpoises (*Phocoena phocoena*) (Orr 1937, Scheffer 1953, 1980, Ryan & Bolin 2014, Haelters et al. 2017, Elliser et al. 2020), a beluga whale (*Dephinapterus leucas*) (Rouse et al. 2017) and a northern elephant seal (*Mirounga angustirostris*) (Stroud & Roffe 1979).

In many other cases, asphyxiation was caused by sole (Soleidae), fish that are usually not too big nor too spiny for marine mammals and are probably a normal part of the diet. Soles are relatively narrow-bodied flatfish that are very agile compared to many other flatfish. Soles have flexible bodies: they can bend longitudinally into a circular shape, and laterally into the form of a cigar. Additionally, they have a rough skin which, together with gill covers and short fin rays, prevents fish returning once lodged. Cases of suffocation by Dover sole are known in common bottlenose dolphin (Perkins et al. 2015), long-finned pilot whales (*Globicephala melas*) (IJsseldijk et al. 2015) and in harbour porpoises (Benke et al. 1998, Siebert & Frese 1993, Siebert et al. 2001). Nine cases of fatal pharyngeal entrapment of Dover sole in harbour porpoises were described from the island of Sylt (North Sea, German waters) between April and July 2016, but in a retrospective evaluation in total 48 cases were identified between 1990 and 2019. In all but one case, the fish concerned were flatfish (Gross et al. 2020). Live sole have even been identified as having caused suffocation in humans by blocking the trachea, after they made their way into the mouth of fishermen while being handled by the teeth or while being kissed goodbye before release (Roper 2012, Pinheiro et al. 2013, Horton 2017).

Analysis of the cases described here

It is likely that in a number of the cases described here (cases 1, 2, 4, 6, 7, 9, 10 and 11), fish movement in the seal's mouth, in an attempt to escape, interfered with the system to separate the seal's digestive and respiratory tracts, and caused the fish to enter the trachea. Perhaps assisted by inhalation by the seal, the fish even entered the trachea completely, blocking the airways. It should be noted that it is possible that the fish outlived the seal and that its position observed during the necropsy could have changed since the death of the seal (Gross et al. 2020). In other cases (cases 3, 5 and 8), a relatively large ingested fish had caused a compression of the pharynx. This probably led to a laryngospasm and/or a physical blocking of the flow of air into the lungs. Either mechanism could result in a severe impairment of the ventilation to the lungs, followed by a hypoxia-induced loss of consciousness and death. It thus seems that Dover soles pose a risk to seals, both through their agility (entering the trachea) and size/roughness (irreversibly blocking the airways).

We noted haemorrhages in the cervical spine area in three cases (6, 8 and 10). In one of these (case 8), the cervical damage was immediately lethal by itself. It is likely that the stimulation of the larynx by aspirated food produced a strong cough reflex to protect the lungs. This, combined with violent head movements and strong muscle contractions before loss of consciousness (McEwan 2016), could have produced self-inflicted internal damage. Also Byard et al. (2010) suggested this for a bottlenose dolphin that choked on a fish. It should be added that in animals consciousness is maintained longer following tracheal occlusion compared to e.g. in people (McEwan 2016), and this may be the case in seals that can hold their breath for a relatively long time. It is notable that in the case of the animal with the fractured neck, the fracture occurred at the C1 vertebra, which is also the level of the pharyngeal isthmus between nasopharynx and oropharynx. During agony, vomiting could occur, which would explain the remains of partly digested fish remains in the oesophagus in several cases.

Dover sole in the diet of seals

Dover sole has been identified as prey of grey seals, and even as constituting an important part of the diet in certain areas (Prime & Hammond 1990, Hammond et al. 1997, Brasseur et al. 2004, Ridoux et al. 2007, Brown et al. 2012, Gosch et al. 2014, Smout et al. 2014, Aarts et al. 2018; own observations). Also in harbour seals, Dover sole forms, seasonally, an important part of the diet (Hall et al. 1998). Most of the animals in this study were found in spring. In this period of the year, Dover soles aggregate in shallow coastal waters to spawn. Dover sole in the diet of harbour seals in the south-western North Sea peaks in spring, probably related to availability in terms of distribution, abundance and size, and/or the (in)availability of other species (Hall et al. 1998). Dover sole is one of the most common flatfish species in the southern North Sea. It is striking that also in harbour porpoises in Germany, most cases of asphyxia due to Dover sole occurred between April and July (Gross et al. 2020).

Out of eleven of the seals investigated here, four had been, at one point in their life, taken from the beach given they were considered in need (very young, diseased, injured) and they were released after being treated at a rehabilitation facility. This is probably only illustrative of the high percentage of seals that is taken care of, mostly as pups, and any relationship between this and their fate, described in this study, remains speculative. In any case, Dover sole, or any other flatfish, is not on the menu in rehabilitation facilities.

Suction feeding vs. grip and tear feeding

The mechanism of feeding could partly explain why suffocation by sole occurs. Stomachs of grey seals often contain large quantities of undamaged or almost undamaged Dover soles (personal observations; this study). Although most pinnipeds are considered as biters (Kienle et al. 2018), the soles causing problems must have been consumed whole through suction feeding (Hocking et al. 2017). Grip and tear feeding is a strategy often used with prey that is too large to be swallowed (personal observations; Hocking et al. 2017). However, studies on feeding strategies of harbour seals suggest that suction feeding is the primary feeding strategy (Marshall et al. 2014, Kienle et al. 2018) and it appears that seals may consider soles as prey they can simply suck in.

Conclusions

Suffocation of seals due to Dover sole blocking the airways can occur due to fish entering the trachea or due to fish, too big to be swallowed, becoming stuck in the oesophagus and oral cavity. Consuming Dover sole, seasonally the most favoured prey for at least some seals, apparently comes with a risk. Given that also other flatfish species occur in the same area, and similarly sized fish are also consumed by seals, the problem for seals lies with (1) the mechanism of feeding – probably suction feeding rather than bite-and-tear feeding, and (2) the species of fish. This cause of death might have become more noticeable recently because seals became more common in the southern North Sea and nowadays more stranded seals are subjected to a necropsy.

As a mechanical obstruction of the airways is easily recognizable, even in the case of a decomposed animal and in animals that never make it to the necropsy table, we recommend even a very limited necropsy on stranded dead seals to provide more insight into the general importance of this cause of death and to assess trends.

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Samenvatting

Prooien die doden: verstikking door tong (*Solea solea*) bij zeehonden in de zuidelijke Noordzee

In de zuidelijke Noordzee komen twee soorten zeehonden voor: de gewone zeehond (*Phoca vitulina*) en de grijze zeehond (*Halichoerus grypus*). De populatie gewone zeehonden is in de 20e eeuw meermaals ingestort, terwijl de grijze zeehond eeuwenlang vrijwel afwezig was in dit gebied. De aantallen van beide soorten zijn de afgelopen decennia echter sterk toegenomen. Samen met deze toename is ook het aantal gestrande dode en stervende zeehonden sterk gestegen. Een selectie van in België en Nederland gestrande dieren wordt onderzocht, met als een van de belangrijkste doelstellingen het achterhalen van de doodsoorzaak. Een verrassende doodsoorzaak is verstikking door vissen die in de luchtpijp terechtkomen, of door vissen die te groot zijn en de mondholte volledig blokkeren. In deze bijdrage worden elf dergelijke gevallen besproken. Het blijkt dat vooral de zeer lenige tong (*Solea solea*) gevaarlijk kan zijn voor zeehonden.

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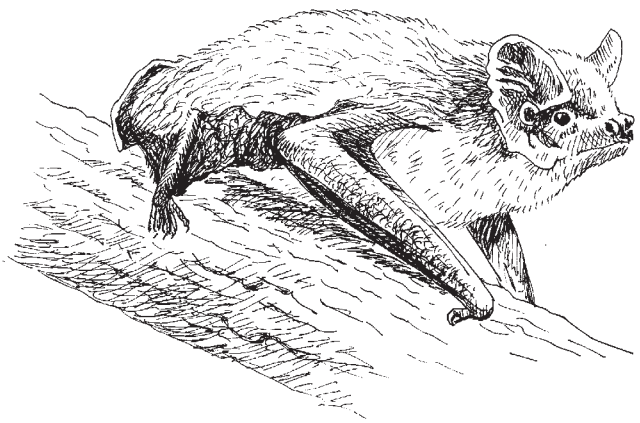

First records of hibernating Leisler's bats (*Nyctalus leisleri*) in Belgium and the Netherlands

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Introduction

Leisler's bat (*Nyctalus leisleri*) is a rare, but widespread species in Belgium and the Netherlands. The species typically roosts year-round in tree cavities, although bat boxes are also sometimes used (Boston et al. 2020). While Leisler's bat is recorded across the region, known maternity roosts are limited to larger, ancient forest fragments in the south of Flanders and the eastern part of the Netherlands. Leisler's bat is recorded from April to October (waarnemingen.be and waarneming.nl), but virtually nothing is known about hibernation of the species in Belgium and the Netherlands. In Europe the species is often considered migratory (Boston et al. 2020). Recoveries of ringed individuals (mainly in Germany and Spain) revealed that individuals from northwestern Europe migrate in a southwesterly direction, with several recoveries over more than 1500 km in the Iberian peninsula (Hutterer et al. 2005, Steffen et al. 2007). Ohlendorf et al. (2001) describe a case of a female Leisler's bat that was ringed in Germany in May 1998, recaptured in Spain in September 1999 (more than 1500 km to the southwest) and then recaptured in May 2000 at the original ringing site in Germany. Although data is sparse, many of such long distance recoveries were females (Roer 1989, Wohlgemuth et al. 2004), indicating a possible sex difference in migratory behaviour, as has been observed in noctules (*Nyctalus noctula)* (Dechmann et al. 2014, Lehnert et al. 2018). The migratory behaviour of Leisler's bat is also supported by stable isotope analyses. By examining hydrogen isotopes in fur keratin, Voigt et al. (2012) showed that wind turbine victims of this species found in Germany likely originated from the Baltic states or Belarus. A previous record also indicated migratory behaviour in the Belgian population. In autumn 2010, a ringed adult female was found in the province of Namur. This individual was ringed in autumn 2007 in Lizaso, Spain - over 960 km to the south-west of the recovery site (Alcalde et al. 2013). Winter records of Leisler's bat are also known from northwestern and Central Europe, for example from Germany and Switzerland, in tree cavities, bird and bat boxes (Kulzer et al. 1987, Roer 1989, Kretzschmar et al. 2005, Ohlendorf et al. 2010, Kohnen et al. 2020, Windeln 2023).

Results

In 2022 and 2023, Leisler's bats were recorded

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for the first time in winter in Belgium and the Netherlands. At all the sites where these observations were made, the species was also previously recorded in spring, summer and autumn.

On 26 February 2022, the third author checked 27 bat boxes in two forest plots on the Veluwe, namely Hoog Buurlo and Hoenderloo (Province of Gelderland, the Netherlands). In the boxes of plot Hoog Buurlo, two Leisler's bats were found roosting solitarily in Schwegler 1FS bat boxes (Schwegler Vogel- und Naturschutzprodukte GmbH, Schorndorf, Germany). In this batbox plot, 15 bat boxes were installed (five Schwegler 1FS, four Schwegler 2F, two Schwegler 1FF and four flat wooden boxes, model Boshamer).

The same day, four Leisler's bats were also found hibernating at bat box plot Hoenderloo. In this plot twelve Schwegler 1FS boxes were installed. Three of these bats were roosting together, while the fourth one was roosting solitary. Weather conditions preceding these observations were relatively cold (-3.0 °C the night before).

On 8 February 2023, the first two authors checked bat boxes in four forest plots in the southeast of the Province of Limburg (Belgium), namely the Nietelbroeken, Jongenbos, Bellevuebos en Kolmontbos. At these sites, 210 bat boxes (Schwegler 2FN) had been installed in 2012 (Janssen & Dekeukeleire 2012). These boxes are regularly checked in spring, summer and autumn, which often leads to observations of maternity colonies and solitary individuals of Leisler's bat. This winter check was aimed at cleaning the boxes for the next season. The weather during the preceding weeks was relatively cold, with temperatures of -4.5 °C the night before. Six Leisler's bats were found hibernating in five different bat boxes (Figure 1). Furthermore, one Nathusius' pipistrelle (*Pipistrellus nathusii*) was observed hibernating in a bat box.

As the bats were immediately awoken when the bat boxes were opened, it was decided to briefly handle the bats. Sexual state, age, and mass was determined (Table 1) following epiphyseal plates in the finger bones, secondary sexual characteristics and facial colouration (Haarsma 2008). Three solitary individuals were young of the year, namely two males and one female. One solitary adult male was also found. An adult male and an adult female were roosting together in a single bat box. Interestingly, the adult male was still sexually active (filled epididymides; Haarsma 2008).

Table 1. Mass and forearm length of the Belgian winter records. YOY: Young-of-the-Year; Ad: adult.

Age	Mass (g)	Forearm length (mm)
YOY male	14.6 |13.2	43.3 | 42.5
YOY female	11.6	45.5
Ad male	12.1 | 14.4	43.0 | 45.0
Ad female	13.6	43.8

Discussion

These observations indicate that a part of the Belgian and Dutch Leisler's bat population does not migrate, but hibernates in their summer roost areas. Conversely, the Belgian recovery of an individual ringed in Spain (Alcalde et al. 2013) indicates that other individuals of this population migrate over large distances in a southwesterly direction. A similar pattern has also been observed in the noctule in the Netherlands. In two studies, individuals ringed in a single summer roost were recovered in winter both at short distances (<10 km) from the summer roost and at sites more than 800 km to the southwest (Bels 1952, Van Heerdt & Sluiter 1965). Such partial migration is also found in Atlantic populations of bird species such as the European robin (*Erithacus rubecula*) (Adriaensen & Dhondt 1990) and the skylark (*Alauda arvensis*) (Hegemann et al. 2010). Another possible explanation is that the local summer population partly migrates southwest, while at the same time individuals from northern populations spend the winter here. Further research is needed to reveal

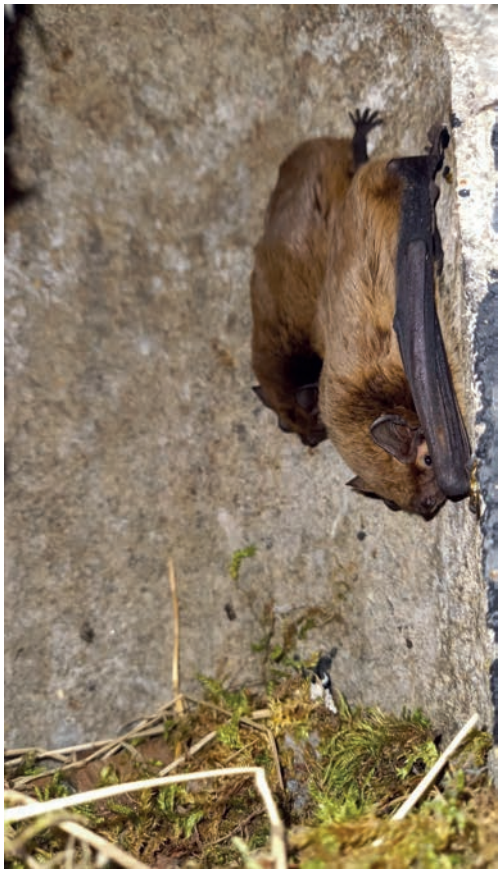

Figure 1. An adult female and an adult male in a bat box on 9 February 2023, Kolmont forest, Belgium. *Photo: René Janssen*.

if the migratory behaviour of Leisler's bat is linked to individual differences in age or sex, and is consistent between years.

At another bat box scheme in the Netherlands (Epe, Province of Gelderland), Leisler's bats were found only in autumn, spring and summer, but not during regular bat box checks in winter (personal communication Frans Bosch). As bat boxes often have a less stable microclimate than tree cavities, bat box checks likely underestimate the number of hibernating Leisler's bats (e.g. Ohlenforf et al. 2010).

In Leisler's bat, mating is thought to take place from August through the hibernation period (reviewed in Boston et al. 2020). Interestingly, the two individuals observed roosting together in Belgium were an adult female and a sexually active male with filled epididymis.

In conclusion, we report the first observations of hibernating Leisler's bats in Belgium and the Netherlands. These observations took place in forest fragments where maternity colonies of the species were known. Together with an earlier ringing record (Alcalde et al. 2013), this indicates that in this region, the species is likely to be partially migratory, with some individuals residing year-round. Bat box checks are an efficient way to record Leisler's bat, not only in spring, summer and autumn, but also in winter. Most individuals likely reside in tree cavities, and thus felling trees with cavities in winter could further endanger Leisler's bat populations already threatened by wind turbines (Voigt et al. 2012). Especially in forest plots where colonies are known in summer, the felling in winter of trees with cavities should be done with utmost care. In general, trees with cavities should be preserved as much as possible.

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Samenvatting

Eerste waarnemingen van overwinterende bosvleermuizen (*Nyctalus leisleri*) in België en Nederland

De bosvleermuis (*Nyctalus leisleri*) is een wijdverspreide, maar zeldzame soort in België en Nederland. In onze regio verblijft de soort typisch het hele jaar in boomholten, hoewel soms ook vleermuiskasten worden gebruikt. In Europa zijn waarnemingen bekend van migratie tussen Noordwest Europa en Zuidwest Europa, en wordt de soort dan ook als migrerend beschouwd. In België en Nederland is, op één ringwaarneming van een dier uit Spanje na, niets bekend over het wintergedrag van de bosvleermuis. In dit artikel beschrijven we de eerste winterwaarnemingen van de soort. Winterslapende dieren werden aangetroffen in vleermuiskasten in januari 2022 in de Nederlandse provincie Gelderland en in februari 2023 in de Belgische provincie Limburg. Het ging hier steeds om gebieden waar de soort ook in het zomerseizoen waargenomen wordt. Samen met de eerdere ringvondst suggereert dit dat een deel van de populatie bosvleermuizen in deze regio naar het zuiden trekt, maar een ander deel in de zomergebieden blijft overwinteren.

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