

Population dynamics of grey wolves in the Bohemian Forest Ecosystem

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Abstract

The ongoing wolf's recolonization of continental Europe at the beginning of the 21st century recently reached many regions where wolves were absent for centuries, including several areas on the Czechia's borders. We observed the process of wolf recolonization across the Bohemian Forest Ecosystem from 2015 until April 2023. We adopted a multifaceted approach, based on systematic tracking surveys, camera traps, telemetry, and provoked howling. The first breeding pair with permanent occupation was detected in 2016, connecting two major European wolf populations – Alpine and Central European. We observed increasing amounts of confirmed wolf occurrence data from 2015 to April 2023, with camera trap pictures and scats being the most abundant data sources. The implications of intensive population expansion were confirmed by locating six activity centres in the wolf year 2022/2023, reflecting six existing packs in the Bohemian Forest Ecosystem. Although those wolf packs spread practically across the whole study area in eight wolf years, the intense phase of recolonization is probably finished, and only continuous small changes in population density are expected. Further observations on the population dynamics are essential, focusing on the transitions between existing packs and the establishment of new territories within the existing social structure.

Key words: *Canis lupus*, occurrence data, population growth, recolonization

INTRODUCTION

The ongoing recolonization of grey wolves (*Canis lupus*) to much of the previously inhabited European landscapes is now indisputable. The wolf's historical range in Europe spanned from warm Mediterranean habitats to arctic Scandinavian tundra. However, livestock depredation and the high habitat overlap of wolves' territories with humans resulted in long-term persecution of this large carnivore (FRITTS et al. 2003), leading to the complete eradication of wolves from almost all of continental Europe within the last two centuries. A few enduring populations were scattered among isolated refuges, characterized by remoteness, terrain inaccessibility, and low human density (CHAPRON et al. 2014). Central Europe provided an exemplary case of such development, where wolf populations declined from a relatively high abundance by the end of the 17th century to their historical minimums in the subsequent few centuries (BUFKA et al. 2005). The only permanent occurrences in the 20th century in Central Europe were principally in the remote Carpathian Mountains in Slovakia and in eastern Poland along the Belarus border (HELL et al. 2001, NOWAK & MYSLAJEK 2016).

Most European wolf populations started to recover at the end of the 20th century, with subsequent and accelerating recolonization of their former habitats (FABBRI et al. 2007, CHAPRON et al. 2014, REINHARDT et al. 2019). The main reasons for such an impressive recolonisation process were the enhancement of wolf protection across Europe (e.g. Council Directive 92/43/EEC) and further legislation changes at a national level in several European countries (NOWAK & MYSLAJEK 2016). Moreover, slow but constant improvements in public perception towards all large European carnivores (DRESSEL et al. 2015) lead to the regulation of legal hunting (NOWAK & MYSLAJEK 2016). Additionally, the ecological adaptability of wolves - a relatively rapid reproduction of the pack's leading pair (MECH & BOITANI 2003) with subsequent dispersal of both sexes (GESE & MECH 1991), and the high availability of their primary food source, free-living ungulates (CARPIO et al. 2021, DUEA et al. 2025), contributed to the possibility of wolf population expansion (JARAUSCH et al. 2021).

The two major remnant populations in Central Europe, the Carpathian (Slovakia; KUTAL et al. 2017) and the Baltic wolf populations (Eastern Poland; NOWAK & MYSLAJEK 2016), acted as a source population for further wolf spreading to surrounding countries and establishing stable occupations. At first, an increased wolf occurrence at the Poland-Germany border became a key area for the newly formed Central European wolf population (KACZENSKY et al. 2021), which shortly after developed into a source population for the recolonization of Germany, Czechia, Denmark, and the Netherlands (JARAUSCH et al. 2021, KACZENSKY et al. 2021). The Carpathian wolf population also expanded its range in the last century (SALVATORI et al. 2002), with wolves increasing in numbers in Slovakia. Subsequently, they began recolonizing border areas with Hungary, Poland, and Eastern Czechia (KUTAL et al. 2016). A third major wolf population, the Alpine (FABBRI et al. 2007), also played an essential role in the wolves' further reoccupation of Central European landscapes. Originating in central Italy, this population currently spreads across the Alps to Northern Italy, France, Switzerland, Austria, and Germany (FABBRI et al. 2007, MARUCCO et al. 2023a). Therefore, the recent recolonization of Central Europe provided an opportunity to reconnect three major wolf populations (Central European, Carpathian, and Alpine; HULVA et al. 2018, SZEWCZYK et al. 2021) through newly established and stable populations converging in Czechia, acting as a geographical crossroad of European wolf populations.

Wolves started to return to Czechia around 2012, after being absent since the end of the 19th century. They first occurred only sporadically and later started to establish territories in Czech areas less used by humans, similarly, to remote areas in Germany (post-mining or military areas; REINHARDT et al. 2019, VOREL et al. 2024) and mountainous border areas in Poland (KACZENSKY et al. 2021). The Bohemian Forest Ecosystem (BFE) is situated on the Czechia-Germany border and is the largest strictly protected woodland in Central Europe but did not show any signs of permanent wolf occurrence during the 20th century (a detailed summary is provided in studies by BUFKA et al. 2005, and BUFKA & ČERVENÝ 2021). However, in 2013, the first irregular indications of wolf presence became noticeable (BUFKA & ČERVENÝ 2021), although these occurrences were isolated and far from all edges of the wolf's known range in the wider area (KUTAL et al. 2017, HULVA et al. 2024). Subsequently, the increasing occurrence of wolf presence on camera traps became evident in 2015, corresponding to the first established territory (BUFKA & ČERVENÝ 2021). The first reproduction event was confirmed in the summer of 2017, marking a milestone in wolf recolonization of the BFE, as the Alpine male and a Central European female succeeded in connecting two major wolf populations (HULVA et al. 2024). At present, the recolonization of the entire BFE can be assessed using various methods, and the current occupation has become a source population for surrounding regions.

This article describes the formation and development of wolf recolonization in the BFE, starting from the first proven permanent occurrence in 2015 up until April 2023. We utilized diverse monitoring methods, including systematic tracking surveys for genetical data, camera trapping, telemetry, and provoked howling to broaden the understanding of the initial steps of wolf recolonization in the BFE.

MATERIAL AND METHODS

Study area

The article focuses on the areas belonging to the Bohemian Forest Ecosystem (49°06'45"N 13°08'09"E) on the borders of Czechia and Germany (Fig. 1). The BFE includes the Šumava National Park (NP; 685 km²) and the Bayerische Wald NP (250 km²) as its core, as well as the Šumava Protected Landscape Area (996 km²), belonging in administration and management under the Šumava NP. Areas in close proximity to the protected landscapes were also incorporated in the study, including areas in Northern Austria. The average elevations of the BFE range between 800 and 1 400 m, which influences the average temperature of approximately 3 to 6°C over the course of the year, depending on the altitude and structure of the terrain. The snow season spans approximately from November to April; however, in the current climate change, the snow cover is highly unpredictable and depends largely on the temperature, altitude, and local conditions. The BFE is characterized by heavily forested mountainous habitats, mostly dominated by Norway spruce (*Picea abies*), which is the most prevalent tree species. Numerous moors are scattered across higher elevations, which, together with grasslands, compose a mosaic of predominant habitats for wolves in the BFE (VOREL et al. 2024).

Data collection

We summarised and evaluated all occurrence data from 2015–2023 in the BFE. The data were collected under research and monitoring programs led by both NP administrations, while many collaborators, professionals, and volunteers contributed to the databases of both institutions.

We used a “wolf year” (WY; KACZENSKY et al. 2009) to divide the study period, a commonly used unit largely determined by wolf reproduction, spanning from the first of May until the end of April of the following year. Our data comprised the period from WY 2015/2016 (starting the first of May 2015) to WY 2022/2023. Until the WY 2017/2018, wolves were monitored opportunistically as part of general wildlife monitoring by NP rangers. Since May 2018, all wolf monitoring has been coordinated with regard to wolf ecology and previous occurrences, and data have been systematically recorded. We adopted two main approaches

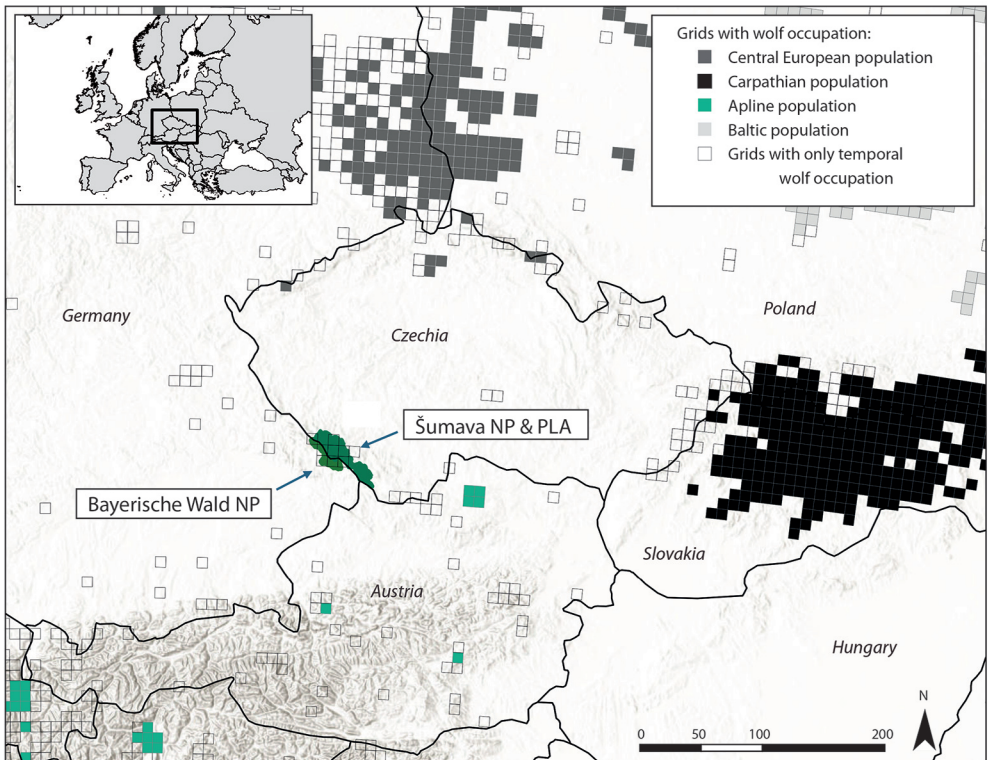


Fig. 1. The overall situation context of the study area, Bohemian Forest Ecosystem. Arrows indicate both National parks and Šumava Protected Landscape Area. Wolf presence data in Central Europe are shown in the EEA reference grid (10 × 10 km), with filled cells displaying permanent and empty cells indicating only sporadic occupation. The occupation level reflects the situation of early recolonization of the study area (grid data are according to KACZENSKY et al. 2021). The greyscale colouring indicates three important populations crucial to the region’s recolonization.

to monitor wolf occurrence in the BFE: systematic tracking surveys (conducted as regularly distributed walking routes) and camera trapping. We also used telemetry data from the autumn of 2020, however, it only covered certain seasons and a limited number of collared animals.

The essential part of systematic walking surveys is a comprehensive snow tracking event with a stable design of walking routes, conducted from January to March, in an attempt to cover the Czech part of the BFE in a single day. We started to launch these wide-scale events in 2019, as they act as important prerequisites for conducting subsequent monitoring methods used by rangers throughout the rest of the year. We evidenced all non-invasive occurrence data confirming the presence of wolves in a database during tracking surveys, which includes coordinates and fundamental information about the finding. Furthermore, samples of scats, hairs, urine, female oestrus blood, and swab samples from kill sites were collected and stored (acc. the approach of KACZENSKY et al. 2009 and REINHARDT et al. 2015).

Camera trapping was applied from the start of the observing period. However, most of the wolf occurrence data until 2019 originated from camera traps focused on regular lynx (*Lynx lynx*) monitoring (e.g., PALMERO et al. 2021). Although the subsequent design followed the wolves' behavioural ecology, the camera traps were primarily placed opportunistically on sites where signs of wolf presence (scats, tracks, prey, etc.) had been previously evidenced. The location of camera traps was adjusted based on the wolves' presence/absence or the experience of the rangers.

We used data automatically retrieved from GPS collars from individuals captured between 2020 and 2023 as part of ongoing trapping and collaring projects in Šumava NP. The detailed trapping methodology is described in VOREL et al. (2024). In summary, wolves were trapped using Belisle traps, sedated with a medetomidine–butorphanol–ketamine mixture mastered by a professional veterinarian, and equipped with GPS Plus collars (by Vectronic Aerospace). In regular mode, the collars retrieve fixes every 3 hours.

Data Analysis

Data validation

All collected occurrence data in the database were validated according to the guidelines published by KACZENSKY et al. (2009). Data were validated using three categories reflecting the relevance and probability of the occurrence mark being of wolf origin. Category C1 indicates hard evidence (e.g. telemetry data, genetic evidence, a wolf found dead, clear camera trap pictures), C2 confirmed data (e.g. wolf tracks or prey remains), and C3 is associated with unconfirmed data. The remaining data are evaluated as false (the categories follow the rules established by KACZENSKY et al. 2009 for wolf monitoring). In further analysis, only records of C1 and C2 categories were considered, which were subsequently converted to a point layer in the geographical information system (GIS) (ArcGIS 10.8 by ESRI). Thus, each given point represents one wolf occurrence record for the date of origin. The records were assigned to WYs.

Molecular analysis

All collected non-invasive samples were determined, and those with a reasonable chance of DNA sequencing were selected for molecular-based analysis. Applicable samples were

immediately fixed in 96% ethanol and, until the investigation, stored in fridges at -20°C or lower. Non-invasive samples were genotyped at a panel of 21 microsatellite loci and sex-determining genes (Amelogenin), as described in HULVA et al. (2024). Analysis in Structure (PRITCHARD et al. 2000) confirmed the species status of the final genotype. Later, we inserted each wolf's genotype identity and parental analyses into Cervus (KALINOWSKI et al. 2007) to evaluate the spatial context of separate groups and related individuals. The settings of the respective analyses were identical to those of HULVA et al. (2024). All successfully identified wolf-based samples were categorized as C1 and used in further spatial analysis.

Spatial analysis

We applied three distinct methods of spatial analysis to estimate wolf distribution patterns, each one of which was based on the specific properties of the different data types:

i. On telemetry data (based on three collared individuals), we applied autocorrelated kernel density estimators (AKDE, FLEMING et al. 2015). GPS telemetry data are inherently autocorrelated in both time and space due to the sequential nature of location fixes. Unlike traditional methods, AKDE specifically accounts for this spatiotemporal autocorrelation, resulting in more accurate home range estimations (SILVA et al. 2021).

ii. For occurrence data collected from field monitoring surveys (e.g. tracks, scat, and cameratraps pictures), we used standard kernel density estimators (KDE, WORTON 1989). KDE proved appropriate as our opportunistic observations were biologically independent and thus did not violate its basic premise. By biologically independent data, we mean data where the sampling interval is long enough for an animal to move from any point in its home range (or territory) to any other point (BARG 2005).

iii. To outline relations among individuals living in the same pack, we used the minimum convex polygon (MCP). This approach was selected due to its suitability for datasets with limited spatial points, allowing us to delineate pack territories by connecting all biological samples (e.g., scats, hairs, tissues) confirmed to belong to related individuals.

Telemetry data

Three collared wolf females provided adequate data for analysis, i.e. showed resident movement behaviour during tracking and a sufficient amount (>30) of tracking days (Table 1). We used telemetry data to quantify wolf home ranges and their core areas. Before estimating the home range, we first identified the movement strategies of the animals by the net squared displacement (NSD). NSD was calculated for each individual using centroids created for each day of tracking prior to calculating the NSD, and the most appropriate movement strategy was determined using the Akaike information criterion (SPITZ 2019, VOREL et al. 2024).

Subsequently, the data were segmented by a WY. We selected the most appropriate movement model for each segment. Consequently, the utilization distribution (UD) was calculated for each WY by using the AKDE method in the R environment; the package "ctmm" (CALABRESE et al. 2016). We then estimated the home ranges of resident animals for a given WY as 95% UD and core areas as 50% UD (FLEMING et al. 2015, CALABRESE et al. 2016, VOREL et al. 2024).

Table 1. List of wolves equipped with GPS collars in the Bohemian Forest Ecosystem from 2020 to 2022.

Individual (Collar ID)	Trapped status	Status Type	Sex	Age at capture (yr)	Weight (kg)	Capture date	Tracking days	No. fixes	Movement mode
f36930	subadult	non-breeding	f	~ 1.5	30.5	29. 11. 2020	221	2303	residential
f36931	subadult	non-breeding	f	~ 1.5	29.0	24. 10. 2021	85	1294	residential
f36927	subadult	breeding	f	~ 1.5	29.7	02. 11. 2021	320	4610	residential

Analysis of population Utilisation Distribution

To reveal the spatial context of the occurrence data and the theoretical distribution of wolves in the BFE, we applied kernel density estimation (KDE) to all occurrence data records collected in each WY. We used the KDE procedure in the R environment via the “adehabitatHR” package (CALENGE 2023) to estimate acquiring UD.

For the definition of core areas where collared animals occurred, we applied the acquired 50% UD AKDE (estimated from telemetry data; see VOREL et al. 2024 for details). We calculated centroids for all these 50% cores.

Definition of core areas within wolf activity

A specific subset of records with information about reproduction in the given WY contributed to the core area assessment and were converted into a separate GIS point layer. Three main sources were used for the GIS layer: camera trap pictures of pups from early summer, direct observations of pups by NP personnel, and provoked howling of pups (registered in summer as a response to the reproduced howl from an electronic caller). Furthermore, we separated the geolocation of individuals recognized by molecular analysis. Due to the isolation of DNA and consequent mSAT analysis, we revealed the structure of families (see HULVA et al. 2024) - either by connecting offspring with their parents or by identification of males and females that formed a pair. Points representing samples of related individuals were connected to areas as a 100% minimum convex polygon (MCP); to each such MCP, we calculated a centroid as the last step.

For some WYs and areas where we lacked both reproduction events and genetic samples to establish core areas, we relied on an alternative approach. We analyzed high-quality (C1) camera trap images showing multiple wolves captured simultaneously in the same frame. For these aggregated observations of multiple individuals occurring outside known core areas, we calculated a spatial centroid from their geographic coordinates. This method enabled us to identify potential pack activity centres even in the absence of traditional evidence such as reproduction data or genetic samples.

As a final step, we produced the utilisation distribution (UD) for each WY from all the obtained occurrence data, using 90% KDE UD. Into such UD we projected all centroids obtained in the appropriate WY. By combining these two datasets, we were able to identify the particular centres of wolves’ activity within the area occupied by wolves (for each WY) (the approach also applied by MARUCCO et al. 2023b).

RESULTS

Occurrence data

We collected a total of 2 625 records from WY 2015/2016 to WY 2022/2023, out of which 1 517 (57.8%) were classified as C1 or C2 evidence. The number of applicable occurrence data (C1 and C2) had a growing tendency during the study period (Fig. 2), ranging from 15 records in the first WY to 473 records in the last WY (Table 2). The only exception was a minor decrease in WY 2020/2021, with the most evident decline detected in camera trap occurrence data. Regardless, the most prevailing type of occurrence data in each distinguished WY originated from camera trapping with a cumulative number of 1 200 pictures (79.1% of all C1 and C2 data) containing at least one wolf individual. The second most represented type of occurrence data was wolf scats (10.2%), followed by footprints (3.0%). The remaining occurrence data: kill sites, urine, and howling were rather scarce and comprised only 7.7%. Besides telemetry data, the highest probability of classification as C1 or C2 had camera trap pictures (83.7%), whereas footprints, mostly collected with snow cover, had the lowest probability (17.0%) due to limited time for confident detection before the condition deteriorates.

Reproduction

Assessment of reproduction data revealed 31 records of C1 and C2 categories, with the highest number of records (18) coming from camera traps. Nine times, pups were identified by provoked howling conducted by experienced personnel. Finally, pups were directly observed by experienced rangers on four occasions. Out of 31 records, more than half (17) were recorded

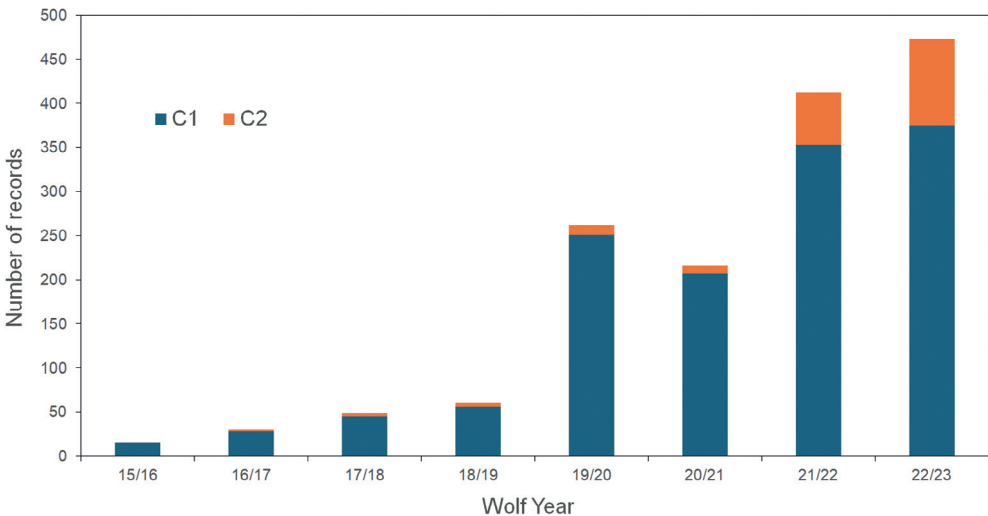


Fig. 2. Data collected during the study period in the Bohemian Forest Ecosystem. Total temporal trends of detected occurrences across the study area, with C1 represented by the blue part of the bar and C2 the orange.

Table 2. Observations of wolf occurrence in the studied period for each WY. Data are grouped according to collected type and categories: C1 – clear evidence, C2 – confirmed observation.

Type of occurrence data	15_16		16_17		17_18		18_19		
	C1	C2	C1	C2	C1	C2	C1	C2	
CameraTrap	10		23	1	29		53		
Tracks	5							4	
Prey									
Scats			5	1	14	2	2		
Others					1	2	1		
Urine					1				
Total	15	0	28	2	45	4	56	4	

Type of occurrence data	19_20		20_21		21_22		22_23		Total
	C1	C2	C1	C2	C1	C2	C1	C2	
CameraTrap	218		160		277	36	338	55	1 200
Tracks		1		2		1		33	46
Prey	2			2	4				8
Scats	26	10	23	4	26	6	25	10	154
Others	2		5	1	23	16	10		61
Urine	3		19		23		2		48
Total	251	11	207	9	353	59	375	98	1 517

in summer (June-August), when pups remain close to their denning sites. Since reproduction was often confirmed multiple times through autumn and winter within one pack, our number of reproduction data (31) does not equal the number of individual reproductive events. Our detection of reproductive events has risen from a single record in the summer of 2017 to a total number of 14 events recorded until the summer of 2022 (Table 3).

Telemetry

We quantified the home ranges of three collared wolves for their three respective territories from 20/21 to 22/23 WYs, see Fig. 3. On average, the estimated 95% (\pm SD) home range of these wolves was 268.8 (\pm 260.7) km², while the average 50% home range was 59.4 (\pm 77.2) km² (Table 4). However, the average estimate of home range indicates a relatively large discrepancy between a f36931 female and two other captured individuals (f36927 and f36930). The discrepancy is clearly visible in the average size of the home range, but also in confidence intervals and the number of sampling days (Table 4).

Genetic sampling

We collected a total number of 725 genetical samples, out of which 232 (32%) were successfully attributed to a wolf origin. The rest of the samples were either too damaged or belonged to

Table 3. Packs and the minimum number of wolves (adults, subadults, and pups) detected by Wolf Year. The numbers represent the minimal number of recorded animals. We have divided the numbers into three age categories: pups, subadults, and adults. We also included separate animals that could not be assigned to any of the identified packs (these are most likely solitary animals within the population). Pup identification was only possible from camera trap images during either summer or autumn, based mainly on distinctive body size. Other individuals were identified either as adults or subadults. We classified adults as breeding parents and the remaining animals (except pups) within the pack as subadults. The abbreviations in boxes identify the following packs: SRN – Srní Pack, RUD – Železná Ruda Pack, PAN – Pancíř Pack, BOR – Borová Lada Pack, ZVO – Zvonková Pack.

Pack	Age class	15/16	16/17	17/18	18/19	19/20	20/21	21/22	22/23
SRN	adults	1	2	2	2	2	2	2	1
	subadults	0	0	0	0	4	1	0	2
	pups	0	0	5	0	1	4	3	0
RUD	adults	-	-	1	2	2	2	2	2
	subadults	-	-	0	0	0	0	3	2
	pups	-	-	0	0	1	3	2	4
ZVO	adults	-	-	-	-	1	2	2	2
	subadults	-	-	-	-	0	0	0	0
	pups	-	-	-	-	0	0	3	4
BOR	adults	-	-	-	-	1	2	2	2
	subadults	-	-	-	-	0	0	0	1
	pups	-	-	-	-	0	0	4	4
PAN	adults	-	-	-	-	-	-	2	2
	subadults	-	-	-	-	-	-	0	0
	pups	-	-	-	-	-	-	0	4
BOB	adults	-	-	-	-	-	-	2	2
	subadults	-	-	-	-	-	-	0	0
	pups	-	-	-	-	-	-	0	4
Others	unknown	-	-	1	1	2	1	4	6
Sum of terr. centres		1	1	1	2	4	4	6	6
Sum of subadults & adults		1	2	2	4	10	9	15	16
Sum of pups		0	0	5	0	2	7	12	20
Sum of reproductions		0	0	1	0	2	2	4	5
Total number of individuals		1	2	8	5	14	17	31	42

a different species. The first genetically proven wolf samples were analysed in WY 2016/2017 (breeding adults of the establishing pack). In subsequent years, the collected genetic records started to gradually increase in time, but the exact amounts in individual WYs fluctuated to the recent amount of 27 samples in 2022/2023. The most efficient WY in terms of genetical data collection was 2021/2022, with an overall proportion of 32.5% successfully identified samples, yet there was no visible trend throughout the observed period. The largest number of genetic samples (48) was recorded in 2021/2022, corresponding to 25 known identities. Although a lower number of collected samples (27), the highest amount of known wolf identities (26) was detected in the WY 2022/2023.

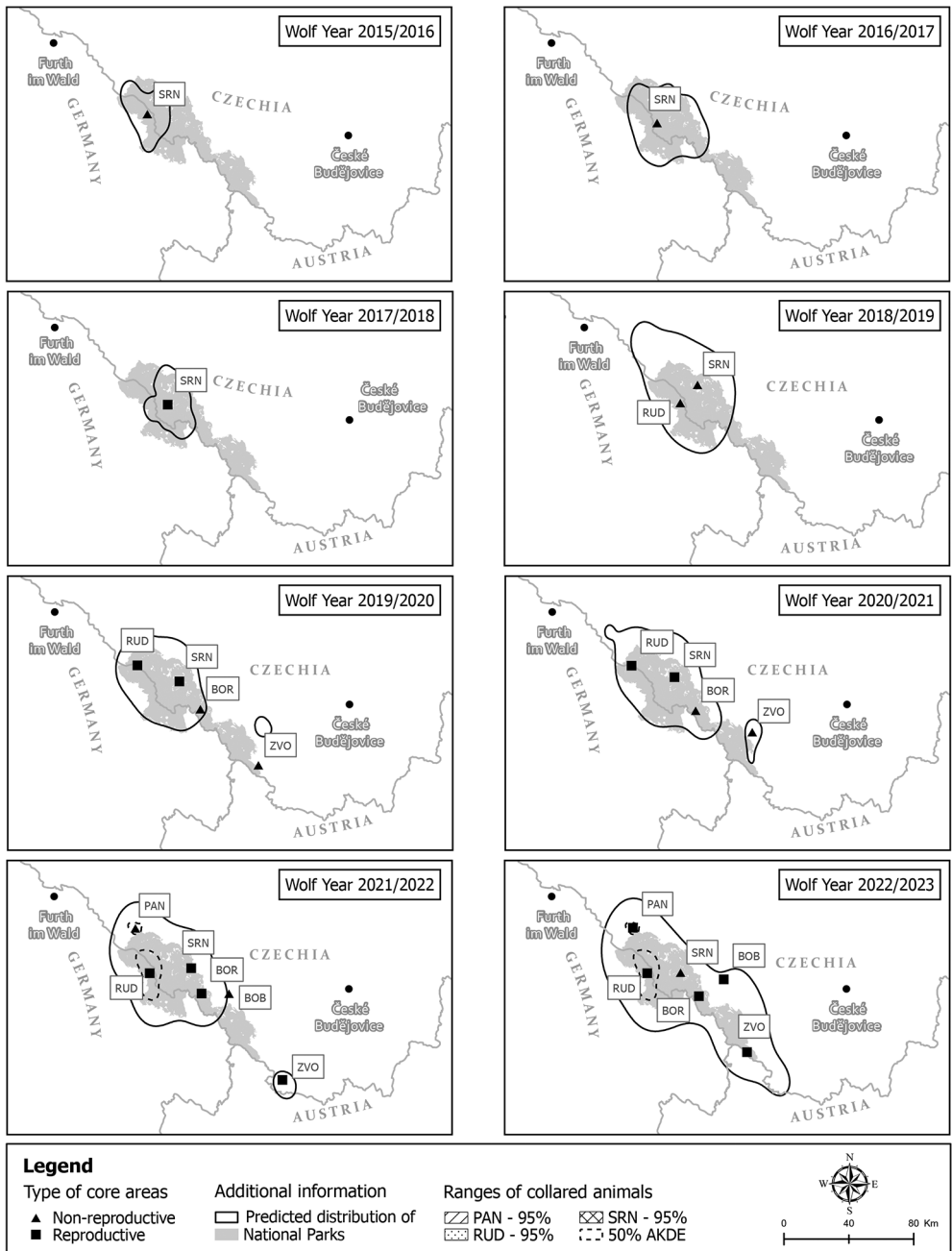


Fig. 3. The spatial predicted distribution of wolves – acquired Utilization Distributions of wolf occurrence data from WY 2015/2016 to 2022/2023. When relevant, the UD of data from GPS-collared wolves are applied. The labelled abbreviations in boxes identify the following packs: SRN – Srní Pack, RUD – Železná Ruda Pack, PAN – Pancíř Pack, BOR – Borová Lada Pack, ZVO – Zvonková Pack.

Table 4. Estimated sizes of home ranges in square kilometres (km²) acquired by the Autocorrelated Kernel Density Estimation (AKDE) procedure for 95% and 50% levels and the respective confidence interval (CI): lower (lwr) and upper (upr).

Animal	95% AKDE	CI 95%		50% AKDE	CI 50%	
		lwr	upr		lwr	upr
f36930	114.4	98.8	131.0	9.3	8.1	10.7
f36931	569.8	344.7	850.5	148.3	89.7	221.4
f36927	122.3	112.0	133.0	20.5	18.7	22.2

Population structure and development

The evidence of increasing wolf activity in the BFE occurred in WY 2015/2016 (Table 2) with ten C1 camera trap records and five C2 records of one solitary male who later established a permanent settlement in the area. The first proof of the permanent presence of a wolf pair in the BFE dates to a camera trap picture from 22. 11. 2016 (WY 2016/2017) near Hochschachten (in the Bavarian Forest NP). Finally, the first genetically detected individual, a female subsequently connected to the original breeding pair near Srní village (hereafter as pack “SRN”) was identified 6th December 2016. Her breeding male partner was identified on 13th January 2017 (documented in Supplementum of HULVA et al. 2024), while the first proof of the reproduction, and herewith the start of the first pack, was documented on 28th July 2017 (camera trap video with four pups). After the establishment of the first pack, the BFE was colonized by packs in Železná Ruda (RUD), Borová Lada (BOR), Pancíř (PAN), Zvonková (ZVO), and Boubín (BOB) in subsequent WYs, all of which currently have permanent occupation (Fig. 3, Table 3). A total of 36 individuals were detected in the BFE in the six packs with confirmed permanent occupation for WY 2022/2023. Furthermore, six more individuals were detected without clear affiliation to known packs, therefore, the estimated minimal wolf population size in the BFE for WY 2022/2023 was assessed on 42 wolves (Table 3).

The number of identified core areas within established territories increased throughout the study period, reflecting the progressive recolonization of the BFE. Starting with a single core area in WY 2016/2017 (corresponding to the SRN pack), we documented an expansion to two core areas in WY 2017/2018 with the establishment of the RUD pack. By WY 2019/2020, three core areas were identified with the addition of the BOR pack territory. A notable increase occurred in WY 2020/2021, when we identified five distinct core areas with the establishment of both PAN and ZVO packs. Finally, the addition of the BOB pack in WY 2021/2022 brought the total to six core areas, which remained stable through WY 2022/2023. The spatial predicted distribution of wolves, as measured by the total area of Utilization Distribution, increased dramatically from approximately 365.73 km² in WY 2015/2016 to 2713.37 km² in WY 2022/2023 (Fig. 3). This substantial increase in spatial distribution corresponds with the growth in pack numbers and total population size, demonstrating the successful reestablishment of wolves throughout the BFE.

DISCUSSION

In this study, we document the development of the wolf population in the BFE from the first occurrence of a single resident individual until six established packs in April 2023. We further describe an increasing trend of wolf observations in the BFE, based on wolf occurrence data. This trend is symbolic of the introductory stages of the recolonization process of the wolf population, mirroring initial stages of recolonization in Poland (NOWAK & MYSLAJEK 2016), Germany (JARAUSCH et al. 2021), Finland (KOJOLA et al. 2006), or Spain (BLANCO & CORTÉS 2007), and also reflects increased sampling effort over the years and a shift from opportunistic to systematic monitoring in the BFE. We also detected an increasing number of confirmed reproductions throughout the study period, which follows the successful and continuous establishment of wolf packs in the area. Overall, six different wolf packs inhabited the BFE, the majority of which had multiple successful reproductive events after establishing breeding pairs (Table 5). Based on camera trapping and genetic analyses, we identified 36 individuals associated with one of the six packs and six individuals without known affiliation in WY 2022/2023. Although it may seem the BFE wolf population is near saturation, our findings until the end of the observed period still indicate a possibility to establish new territories within the existing social structure.

Spatial analysis using the KDE procedure showed that wolf occurrence data were distributed with similar intensity across nearly the entire study area. This uniform distribution makes occurrence data alone inadequate for identifying concentrated areas of wolf activity. To accurately describe the population structure, we found it essential to supplement basic occurrence data with more specialized approaches: telemetry, genetic sampling, or direct identification of denning sites.

Recent studies have increasingly recognized the value of such multidisciplinary approaches for assessing population structure, especially for elusive species like wolves (MARUCCO et al. 2023b, PLANILLO et al. 2024). In our study, information on denning sites provided particularly valuable confirmation of pack territory cores, as these sites consistently fell within the 50% AKDE home range of collared animals (Fig. 3). With detailed genetic data or denning site information, we could reliably determine wolf population structure.

Locating denning sites—where wolves deliver and raise their pups—remains one of the most challenging aspects of wolf research and monitoring. This process demands not only precise, time-consuming field surveys but also comprehensive knowledge of the local environment (MARUCCO et al. 2023b). Researchers must also consider the potential negative effects of human disturbance on pup survival (CAPITANI et al. 2006), though some studies suggest that checking active denning sites does not significantly impact pack recruitment (GABLE et al. 2024).

Home ranges and their distribution can also be assessed from repeated collection of DNA samples (CANIGLIA et al. 2014). Although using the described technique can only reveal the presence of the same individual within the selected region, this complex approach can depict parental relations in the population and subsequently illustrate the presence of individual packs in the area (JARAUSCH et al. 2021). In other words, analysis of paternity using all DNA samples can indicate the appearance of animals that share the same home range. However, in our experience, the low number of repeated DNA samples can sometimes be misleading. Clear evidence of a relationship between animals can document two different aspects: either they

Table 5. List of all breeding individuals within six distinguished packs in the Bohemian Forest Ecosystem from the wolf year 2015/2016 to 2022/2023. The acronyms used for Males and Females are unique codes identifying each individual assessed in genetic analysis.

Pack	Wolf year	Male	Female
SRN	15/16	GW676m	
SRN	16/17	GW676m	GW665f
SRN	17/18	GW676m	GW665f
SRN	18/19	GW676m	GW665f
SRN	19/20	GW676m	GW665f
SRN	20/21	GW676m	GW665f
SRN	21/22	GW676m	GW665f
SRN	22/23		GW665f
RUD	17/18	GW1488m	
RUD	18/19	GW1488m	GW1112f
RUD	19/20	GW1488m	GW871f
RUD	20/21	GW1488m	GW871f
RUD	21/22	GW1488m	GW871f
RUD	22/23	GW1488m	GW871f
ZVO	19/20		CW20_123f
ZVO	20/21	CW21_395m	CW20_123f
ZVO	21/22	CW21_395m	CW20_123f
ZVO	22/23	CW21_395m	CW20_123f
BOR	19/20	CW20_120m	
BOR	20/21	CW20_120m	CW20_147f
BOR	21/22	CW20_120m	CW20_147f
BOR	22/23	CW20_120m	CW20_147f
PAN	21/22	CW21_418m	CW21_407f
PAN	22/23	CW21_418m	CW21_407f
BOB	21/22	CW22_120m	CW20_169f
BOB	22/23	CW22_120m	CW20_169f

are members of the same pack (and are reported to move within the packs at the given time and area), or they have only a clear relationship with the given pack (i.e. they sometimes visit their parents) and are sampled by chance during this visit. A surprisingly high number of dispersing individuals, or individuals moving within the area occupied by wolves without clear residence (i.e. floaters), was described by VOREL et al. (2024) in several Central European areas based on telemetry data. The actual quantity of dispersing wolves or floaters with conspecific contact with the resident wolves would probably be underestimated in a majority of wolf populations.

Our data reveals a continuous population development from the first confirmed occurrence in late 2015. During eight subsequent seasons, wolves recolonized almost the entire area of the BFE. The dynamic provided by our analysis is consistent with the trend shown by BUFKA & ČERVENÝ (2021), who predicted swift wolf recolonization. A rapid population development of wolves was recently described across many European areas (KOJOLA et al. 2006, BLANCO & CORTÉS 2007), including Central Europe (REINHARDT et al. 2019, KACZENSKY et al. 2021). There is evidence of almost exponential growth in areas of recent reoccupation, e. g. in Germany (JARAUSCH et al. 2021) or Poland (NOWAK & MYSLAJEK 2016) and therefore it is plausible to predict a similar process of population development within suitable habitats in Czechia (KUTAL & VOREL – unpubl.).

Overall, we can conclude that the wolf reoccupation of the BFE developed and expanded similarly to other Central European populations—on account of the random spread of individuals from two major wolf populations, the Alpine and the Central European (HULVA et al. 2024), meeting in the BFE and the subsequent dispersals of their newborn pups. Gradually, the first wolf settlement was established in 2015/16, followed by the carving of new territories in its immediate vicinity, thus increasing the number of packs, until the entire area of both national parks was occupied (some territories even partially exceeded NP borders). At the same time, the population became an important source of young dispersing individuals (VOJTĚCH & MOKRÝ 2024), providing an essential contribution to the recolonization of the remaining areas in Czechia and also being one of the important drivers of reconnecting three major wolf populations in Europe (Central European, Carpathian, and Alpine; HULVA et al. 2024). Within only eight WYs, the wolf population in the BFE reached stability through an increase in numbers and became one of the best-documented wolf populations in Czechia. This study highlights the power of bilateral collaborative efforts and multidisciplinary approaches in the acquisition of comprehensive insight into initial steps, dynamics, and the whole process of wolf recolonization.

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