U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

PhD

3rd
CYCLE

FCUP
UM
FCUL
2024

U.PORTO

Wolf demography in human-dominated landscapes:
Insights for wolf conservation in the Anthropocene

Mónia Nakamura Mercier Real

FC

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

UNIVERSITÉ
DE MONTPELLIER

Ciências
ULisboa

Wolf demography in human-dominated landscapes:

Insights for wolf conservation in the Anthropocene

Mónia Nakamura Mercier Real
Tese de Doutoramento apresentada à
Faculdade de Ciências da Universidade do Porto
ao abrigo de acordo de cotutela com a Université de Montpellier
Biodiversidade, Genética e Evolução
2024

Wolf demography in human-dominated landscapes:

Insights for wolf conservation in the Anthropocene

Mónia Nakamura Mercier Real
Tese de Doutoramento apresentada à
Faculdade de Ciências da Universidade do Porto
ao abrigo de acordo de cotutela com a Université de Montpellier
Biodiversidade, Genética e Evolução
2024

Orientador
José Vicente López-Bao, Investigador, Biodiversity Research Institute
(University of Oviedo – CSIC – Principado de Asturias)

Co-orientadores
Olivier Gimenez, CNRS Senior Scientist, CEFE, CNRS

Pedro Monterroso, Investigador, CIBIO-InBIO, BIOPOLIS,
Universidade do Porto

THÈSE POUR OBTENIR LE GRADE DE DOCTEUR
DE L'UNIVERSITÉ DE MONTPELLIER

En Écologie, Évolution, Ressources Génétique, Paléobiologie

École doctorale GAIA

Unité de recherche CEFE (Centre d'Écologie Fonctionnelle et Evolutive)

En partenariat international avec Faculdade de Ciências da Universidade do Porto, Portugal

Wolf demography in human-dominated landscapes: Insights for wolf conservation in the Anthropocene

Démographie des loups dans les paysages dominés par l'homme: perspectives pour la conservation des loups dans l'Anthropocène

Présentée par Mónia NAKAMURA MERCIER REAL
Le 4 avril 2024

Sous la direction de José Vicente LÓPEZ-BAO,
Pedro MONTERROSO
et Olivier GIMENEZ

Devant le jury composé de

[Nuno FERRAND, Professeur, CIBIO-BIOPOLIS et Universidade do Porto]	Président du jury
[Barbara ZIMMERMANN, Professeure, Inland Norway University of Applied Sciences]	Rapporteure
[Francesca MARUCCO, Maître de conférences, Università degli studi di Torino]	Rapporteure
[Olivier GIMENEZ, Directeur de recherche, CEFE - CNRS]	Directeur de thèse
[Ana RODRIGUES, Directrice de recherche, CEFE - CNRS]	Membre du jury
[Paulo Célio ALVES, Maître de conférences (Equivalent), CIBIO-BIOPOLIS et Universidade do Porto]	Membre du jury
[Luís Miguel ROSALINO, Maître de conférences (Equivalent), CE3C et Universidade de Lisboa]	Membre du jury

UNIVERSITÉ
DE MONTPELLIER

Dedicated to my mother, Minami Nakamura

Foreword

In compliance with the no. 2 of article 4 of the General Regulation of Third Cyclesof the University of Porto and with the article 31 of the Decree-Law no. 74/2006, of March, with the alteration introduced by the Decree-Law no. 230.2009, of 14 September, the results of previously published work were totally used and included in some of the chapters of this dissertation. As this work was performed in collaboration with other authors, the candidate clarifies that he participated in obtaining, interpreting, analysing, and discussing the results, as well in the writing the published forms. The candidate was financially supported by Fundação para a Ciência e a Tecnologia (FCT) through the attribution of a PhD fellowship (SFRH/BD/144087/2019), funded by POPH-QREN funds from the European Social Fund. Candidate training was supported by the two doctoral schools: GAIA - UM and BioDiv – FCUP, and by the Project ‘Support for the operation of the Center for Research in Biodiversity and Evolutionary Biology’ (UIDP/50027/2020) of FCT; and the French National Research Agency (grant ANR-16-CE02-0007).

Support for the supervisors, and collaborators was also obtained from: the French National Research Agency (grant ANR-16-CE02-0007), FCT/MCTES through national funds (UID/BIA/50027/2021 and PPCDT/BIA-BDE/60296/2004); the Spanish Ministry of Economy, Industry and Competitiveness (CGL2017-87528-R AEI/FEDER EU), the Spanish Ministry of Science and Innovation (TED2021-132519B-I00), and by a GRUPIN research grant from the Regional Government of Asturias (AYUD/2021/51314); and from FCT with PhD grants (SFRH/BD/12291/2003 and XXI/BD/18326/98) and research contracts (IF/564/2012 and DL57/2016).

Field and lab work for wolf monitoring was supported by Grupo Lobo/Faculdade de Ciências da Universidade de Lisboa, ACHLI (Associação de Conservação do Habitat do Lobo Ibérico), VentoMinho - Energias Renováveis S.A, Empreendimentos Eólicos do Vale do Minho S.A., Bern Thies Foundation, Parque Nacional da Peneda-Gerês/ICNF, Associação Veranda (Associação para a Conservação e Divulgação do Património de Montanha).

fct Fundação para a Ciência e a Tecnologia

UNIÃO EUROPEIA
Fundo Social Europeu

REPÚBLICA PORTUGUESA | CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

PORTUGAL 2020

U.PORTO
FC FACULDADE DE CIÊNCIAS UNIVERSIDADE DO PORTO

UNIVERSITÉ DE MONTPELLIER

cibio

InBIO

BIOPOLIS

CENTRE D'ECOLOGIE FONCTIONNELLE & EVOLUTIVE

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Universidad de Oviedo
Universidá d'Uviéu
University of Oviedo

IMIB
Biodiversity Research Institute
(CSIC - Oviedo University - Principality of Asturias)

Principado de Asturias

anr agence nationale de la recherche
AU SERVICE DE LA SCIENCE

GRUPO LOBO

cE3c centre for ecology, evolution and environmental changes

CESAM
CENTRE FOR ENVIRONMENTAL AND MARINE STUDIES

BE BIOINSIGHT & ECOA

EEVM
Empreendimentos Eólicos do Vale do Minho, S.A.

VENTOMINHO Energias Renováveis SA

ACHLI
Associação de Conservação do Habitat do Lobo Ibérico

Publications

Published:

Chapter II - Nakamura M., López-Bao J.V., Rio-Maior H., Roque S., Gil P., Serronha A., García E., Palacios O.H., Ferrão da Costa, G., Álvares F., Petrucci-Fonseca F., Gimenez O. & Monterroso P. (2023) Insights into the dynamics of wolf occupancy in human-dominated landscapes. *Biological Conservation*, 286, October 2023, 110316. https://doi.org/10.1016/j.biocon.2023.110316

Chapter IV - Nakamura M, Rio-Maior H., Godinho R., Petrucci-Fonseca F. & Álvares F. (2021) Source-sink dynamics promote wolf persistence in human-modified landscapes: insights from long-term monitoring. *Biological Conservation*, 256, April 2021, 109075. https://doi.org/10.1016/j.biocon.2021.109075

In preparation:

Chapter III - Nakamura M., Rio-Maior H., Palacios, V., Garcia E., Sazatornil V., Sierra P., Ferrão da Costa G., Álvares F., Llaneza L., Lema C., Roque S., Gimenez O., Monterroso P & López-Bao J.V. (*in prep.*) Refuge and vulnerability at reproduction sites and beyond: wolf pup and adult numbers in anthropogenic landscapes.

Chapter V - Nakamura M., Monterroso P., Rio-Maior H., Godinho R., Álvares F., López-Bao J.V. & Gimenez O. (*in prep*.) An integrated population model to assess key vital rates and viability of a human-encroached wolf population.

This thesis should be cited as:

Nakamura, M. (2024). Wolf demography in human-dominated landscapes: Insights for wolf conservation in the Anthropocene. Ph.D. Dissertation, Université de Montpellier; Faculdade de Ciências da Universidade do Porto; Porto, Montpellier; Portugal, France.

Sworn Statement

I, Mónia Nakamura Mercier Real, enrolled in the Doctor’s Degree Biodiversity, Genetics and Evolution (BIODIV) at the Faculty of Sciences of the University of Porto hereby declare, in accordance with the provisions of paragraph a) of Article 14 of the Code of Ethical Conduct of the University of Porto, that the content of this thesis reflects perspectives, research work and my own interpretations at the time of its submission.

By submitting this thesis, I also declare that it contains the results of my own research work and contributions that have not been previously submitted to this or any other institution.

I further declare that all references to other authors fully comply with the rules of attribution and are referenced in the text by citation and identified in the bibliographic references section. This thesis does not include any content whose reproduction is protected by copyright laws.

I am aware that the practice of plagiarism and self-plagiarism constitute a form of academic offense.

Mónia Nakamura Mercier Real

7 th February 2024

Acknowledgements

First of all, I thank my Supervisors, Olivier Gimenez, Pedro Monterroso, and José Vicente López-Bao, for accepting this challenge. The most excellent combination I could have ever wished for in a supervision team. I learned a lot, and your help was of utmost importance.

Olivier, thank you for teaching me so many things during these years and for the easy and simple answers to questions I struggled with for days! I have learned so much and am convinced that 'R' is the way! The 'future me' will thank the 'past me' for what I've learned! I do not know how you do it, but thank you for always being available and for the prompt answers. Thank you for creating a fun "ambience" during meetings, team lunches, and coffee breaks! Montpellier was the best place and the right place to be, especially because of you.

Pedro, we started working much before the PhD, and the PhD was the only way I could ever have the time to learn and do something with all the field data. Thank you for the support and this opportunity to develop my skills in data analysis, for enlightening me with ideas and helping me see that I could go beyond fieldwork.

José Vicente, thank you for the support and the discussions, for making me see things more clearly and objectively, and for showing other points of view on wolf conservation. Thank you for sharing stories on collared wolves and for this great opportunity to work with you and improve my knowledge.

My journey with wolves started in August 2005 with **Helena Rio-Maior** when I was lucky enough to be the only volunteer available during the summer vacation to help her with fieldwork for two weeks. Lucky me, this chance was the first step that took me to where I am now. I still remember the first time I heard howls of pups and adults while we were eating dinner in a mountain cabin, making some time before hiking back to count cows and horses during the night. I had no idea this would be the study area I would live in from 2007 on. **Helena**, thank you for everything. I have learned much with you, and we have learned so much together. Thank you for always being available and for being the 'satellite': the only person who always knew where I was when I was alone in the mountains. For all the great, good, wonderful, hard, terrible, and fun moments we spent searching for the wolves and for our ways. I am sure the wolves found us many more times than we found them! We know it's like that, and that's why it is so wonderful.

Thank you to all the friends and colleagues who made available a lot of information to be included in this thesis: **Helena Rio-Maior, Pablo Sierra, Sara Roque, Francisco Álvares, Francisco Petrucci-Fonseca, José Vicente López-Bao, Patrícia Gil, Ana Serronha, Luis Llaneza, Vicente Palacios, Emilio Garcia, Victor Sazatornil, Isabel Ambrósio, Gonçalo Costa, Orencio Hernández Palacios, and Chisco Lema**. I also thank all the people involved in fieldwork. You all have been working hard to study wolves and improve their conservation. Thank you for that, and I hope we all can keep doing that!

To **Francisco Álvares**, thank you for all the years we worked together and for your enthusiasm in sharing your knowledge about the several components of the natural world: amphibians, reptiles, birds, wolves, other mammals, forests, etc. Thank you for the opportunity to work on the projects and for the support to continue and go further with this PhD.

Professor **Francisco Petrucci-Fonseca**, thank you for all the work you have been doing for wolves and your availability to help me whenever possible. The actual first step towards wolves started with you. Thank you.

To **Raquel Godinho**, thank you for your support and for making me understand more about the little but essential pieces of the puzzle: genetics!

I also thank **Diana Castro** and **Susana Lopes** for working at the lab and getting the most out of the scats and other stuff we bring!

Thank you, **José Miguel Oliveira**, for being so open to our ideas and doing the best you can to implement them! **Gonçalo Brotas**, **Cindy Loureiro** and **ACHLI,** thank you for all your efforts to improve our projects and wolf conservation in Portugal.

Thank you**, Juan Carlos Blanco,** for the availability to meet and discuss some interesting thoughts and share some experiences and stories of wolves.

To **Virgínia Pimenta, Inês Barroso, Javier Fernández-López, Sonia Illanas, Joaquin Vicente Baños, Carmen Ruiz Rodríguez**, thank you for all your help and your availability.

Thank you to all the people I met in the mountains of Alto Minho, including shepherds and hunters. Proud people who are hard to reach at first but are always open to offering a hot coffee and sharing great experiences and conversations.

To all the volunteers, students and colleagues who spent some time in Alto Minho for shorter or longer periods, making me connect with someone other than wolves and shepherds! **Ricardo Brandão,** for all the fun moments between trying and capturing wolves, by making us learn about birds and mushrooms! Thank you**, Carlos Barros,** for your great passion for wolves, for helping us in the field, and for Maria Rita, the best gift of all to Helena. I know you are watching for them.

I also want to thank my thesis committee at Montpellier, **Ana Rodrigues, Christophe Duchamp,** and **Pierre Boursot.** Thank you for the support and interesting discussions throughout the years.

To **Dora Bastos**, **João Pedro Marques**, and **Paulo Célio Alves** for the support and for helping me with all the bureaucratic and complicated procedures of the PhD in co-tutelle.

To the **WildEcol group** for the suggestions, comments and discussions to improve my work. To **Filipe Rocha**, in particular, for helping me when I was abroad and needed someone at CIBIO to solve some IT problems and for the ready availability whenever needed!

To **HAIR team**! What a team! Thank you all for the fun moments at CEFE, coffee breaks (yes, I will go back to 'remplir le réservoir'), lunch breaks (thank you, **Marwan Naciri**), beers, pizza, beers, fanfare, and more beers! Thank you, **Coline Canonne**, **Sarah Bauduin**, and **Maëlis Kervellec,** for the help with 'R' stuff and analysis.

Thank you, **Patrícia Rodrigues**, for your friendship, for reminding me to wear the helmet, checking if I have a light on my bicycle, and always bringing juice for me too! Thank you, **Patrícia, João** and **Carolina**, for the fun weekends, play days, lunches, and movie nights with popcorn at Montpellier!

Um agradecimento com grande carinho aos mais pequenos da minha vida: **Nozomi**, **Kazuki**, **Maria Rita** e **Carolina**, que me ajudaram a desligar da complicação do mundo real, a desfrutar das coisas mais simples e interpretar o mundo de uma forma mais inocente. Muitos sorrisos e risadas me ofereceram nestes anos! Não nos fazem sentir mais velhos, mas sim mais jovens!

To my best girls: **Fernanda Cipriano**, **Margarida Silva**, and **Rita Martins.** Thank you for being there for me even when I am away almost all the time. Let's continue with 'yoga'!

To my father, **Jaime Real,** for the support and help whenever needed. Thank you for letting me choose my way and being there.

To **Nuno Santos**, for being so patient with my dilemmas and for being there in my ups and downs. For being my friend and my partner. Learning, sharing and living. Thank you for all the moments and for your kindness. You make me carry the beer and useless rocks in my backpack, but that's the least I can do! It has been an incredible journey, and I cannot wait to take our next steps (finally, after this PhD is done!).

To my brother, **Dan Nakamura**, for all your strength, support, and advice. For telling me (25 years ago!) that "life is not that complicated. It is not as hard as it seems. You will not die from this. You will overcome this one too". I guess you keep helping people every day, saying it to many people, in other words. Thank you for making the world a better place for all around you with your daily actions.

To my mother, **Minami Nakamura**. No words can express my gratitude for all the support and letting me make my choices and mistakes. I still do not know how you did it, but you showed us (my brother and me) how to understand ourselves and others and deal with obstacles. Our mistakes are our own and no one else's, so I should count on me to solve them. People are there when I need them, but I try to solve it before reaching out. This was always very present during my life and my PhD. You are an inspiration. お母さん、どうもありがとうございます。

To the wolves. I have always followed my dream job: to be a biologist, to be close to nature and study it, and somehow 'save wildlife'. However, there are many puzzle pieces and players around this dream job that I had never imagined, especially when it comes to charismatic and iconic species such as wolves. I hope that piece by piece, we (players) will reach balanced and harmonized decisions and consensus.

Anyway, loving what you do makes a job a non-job. This is how it all started for me…

Photo credits

Chapter I: © Artur Vaz Oliveira

Chapter III: © Mónia Nakamura / CIBIO-InBIO

Chapter IV: © Mónia Nakamura / CIBIO-InBIO

Chapter V: © Artur Vaz Oliveira

Chapter VI: © Nuno Santos

Chapter VII: © Artur Vaz Oliveira / Mónia Nakamura / CIBIO-InBIO

Abstract

Large carnivore populations have been recovering and recolonizing several areas in recent decades. However, their predatory behaviour often leads to conflicts with humans, resulting in preemptive or retaliatory killing of large carnivores. Direct persecution and other anthropogenic disturbances make large carnivore conservation and management in human-dominated landscapes particularly challenging. This thesis's main objective is to investigate the impact of anthropogenic factors on large carnivore persistence in human-dominated landscapes. Among all large carnivores, wolves (*Canis lupus*) are remarkably resilient and able to adapt to various habitat conditions. Studies assessing how anthropogenic or environmental factors affect wolf persistence and the number of individuals and understanding wolf population dynamics and demography are lacking for human-dominated landscapes. Based on long-term wolf monitoring in human-dominated landscapes of Iberia, this thesis estimates wolf occupancy and identifies potential areas for recolonization; evaluates homesite (i.e. reproduction sites) characteristics and identifies factors influencing the number of individuals in packs; and addresses the status and viability of a population by assessing its spatiotemporal dynamics, demographic parameters, and extinction probabilities.

According to the developed dynamic occupancy model, wolf colonization probability was positively associated with altitude, livestock density, and unpaved road density, and negatively with the proportion of burned areas and national-regional and local road densities. Coherently, extinction probability decreased with higher unpaved road density. By evaluating the wolf range dynamics in the last twenty years in Iberia, we found occupancy and colonization probabilities to be higher in areas where wolves persisted or expanded than in areas where they went extinct or at the periphery of the species range. However, unexpected expansion fronts and stagnations observed in the last twenty years emphasize the possible effect of human-related social factors on the recovery of wolf populations and the need to assess such factors. Based on colonization and occupancy probabilities, a habitat suitability map was developed, in which priority areas for implementing conservation actions and mitigation measures are identified. By evaluating the characteristics around homesites (1, 2, and 5 km buffers) and the variations in the number of wolves, we show that refuge availability positively influences the number of adults at all buffer sizes. Human population density, paved road density, the proportion of burned areas, ruggedness, and pack density negatively influenced the number of adults, particularly at smaller homesite buffers. Although results indicate that higher refuge availability and lower unpaved road densities increase the number of pups, further research is needed to assess factors influencing the number of pups. This study reveals the importance of areas with sufficient refuge and low human disturbance for the number of wolves, particularly at homesites, since wolves can be particularly exposed and susceptible during the breeding season.

The long-term monitoring study in northwestern Portugal revealed a decline in the number of wolves between 1996 and 2005, with only two of the six packs remaining at the end of this period, arguably due to direct wolf persecution and low habitat connectivity between packs. However, since 2007, wolves have been recovering and recolonizing the study area, with two core packs playing a crucial role in recovering and maintaining several neighbouring sink packs. Subsequently, sink packs facilitated dispersal through a stepping-stone process. Core packs showed higher values of group persistence, breeding success and pack sizes compared to sink packs, which went extinct over several years. Similar to the findings elsewhere in Iberia, we found evidence of limited dispersal, underlining the likely impact of anthropogenic features on wolf dispersal and population structure. Furthermore, by combining multiple datasets, we developed an integrated population model that further supported a positive demographic trend between 2007 and 2019 and also allowed estimating adult survival rate at 72%, apparent adult survival rate at 62%, and pup survival rate at 53%. By forecasting population growth over ten years (2020-2029) under different survival rate scenarios, we emphasize that adult survival is a key factor shaping this population's growth and extinction risk.

Results show that livestock density strongly affects wolf occupancy and the number of individuals in packs, that dispersal plays a vital role in the persistence and recovery of wolf populations and that adult mortality rate strongly influences a population's viability. All approaches used in this thesis to understand wolf persistence in human-dominated landscapes highlight the need to assess human social factors, human dimensions, and illegal mortality causes and rates related to conflicts associated with livestock depredation. Thus, the priorities for wolf conservation in human-dominated landscapes should include improving landscape management and reducing human-large carnivore conflicts. In light of the conclusions of this thesis, several conservation actions, mitigation measures and lines of future research studies are proposed.

Keywords: *Canis lupus*, dispersal, dynamic occupancy model, human disturbance, integrated population model, population growth, reproduction site, source-sink dynamics, survival.

Resumé

Les populations de grands carnivores se sont reconstituées et ont recolonisé plusieurs régions au cours des dernières décennies. Cependant, leur comportement prédateur entraîne souvent des conflits avec l'homme, ce qui se traduit par l'abattage préventif ou en représailles des grands carnivores. La persécution directe et d'autres perturbations anthropogéniques rendent la conservation et la gestion des grands carnivores dans les paysages dominés par l'homme particulièrement difficiles. L'objectif principal de cette thèse est d'étudier l'impact des facteurs anthropogéniques sur la persistance des grands carnivores dans les paysages dominés par l'homme. Parmi tous les grands carnivores, les loups (*Canis lupus*) sont remarquablement résistants et capables de s'adapter à diverses conditions d'habitat. Les études évaluant la manière dont les facteurs anthropogéniques ou environnementaux affectent la persistance des loups et le nombre d'individus, ainsi que la compréhension de la dynamique et de la démographie des populations de loups, font défaut dans les paysages dominés par l'homme. Basée sur un suivi à long terme des loups dans les paysages ibériques dominés par l'homme, cette thèse estime la répartition spatiale des loups et identifie les zones potentielles de recolonisation ; évalue les caractéristiques des sites de reproduction et identifie les facteurs influençant le nombre d'individus dans les meutes ; et aborde le statut et la viabilité d'une population en évaluant sa dynamique spatio-temporelle, ses paramètres démographiques, et ses probabilités d'extinction.

Selon le modèle d'occupation dynamique développé, la probabilité de colonisation des loups était positivement associée à l'altitude, à la densité du bétail et à la densité des routes non asphaltées, et négativement à la proportion de zones brûlées et à la densité des routes nationales-régionales et locales. De manière cohérente, la probabilité d'extinction diminue avec l'augmentation de la densité des routes non asphaltées. En évaluant la dynamique de l'aire de répartition du loup au cours des vingt dernières années en Ibérie, nous avons constaté que les probabilités d'occupation et de colonisation étaient plus élevées dans les zones où les loups ont persisté ou se sont étendus que dans les zones où ils se sont éteints ou à la périphérie de l'aire de répartition de l'espèce. Cependant, les fronts d'expansion inattendus et les stagnations observées au cours des vingt dernières années soulignent l'effet possible des facteurs sociaux liés à l'homme sur le rétablissement des populations de loups et la nécéssité d'evaluer ces facteurs. Sur la base des probabilités de colonisation et d'occupation, une carte d'adéquation de l'habitat a été élaborée, dans laquelle sont identifiées les zones prioritaires pour la mise en œuvre d'actions de conservation et de mesures

d'atténuation. En évaluant les caractéristiques autour des sites de reproduction (zones tampons de 1, 2 et 5 km) et les variations du nombre de loups, nous montrons que la disponibilité des refuges influence positivement le nombre d'adultes à toutes les tailles de zones tampons. La densité de la population humaine, la densité des routes asphaltées, la proportion de zones brûlées, la rugosité et la densité des meutes influencent négativement le nombre d'adultes, en particulier dans les zones tampons les plus petites. Bien que les résultats indiquent qu'une plus grande disponibilité de refuges et une plus faible densité de routes non asphaltées augmentent le nombre de jeunes, des recherches supplémentaires sont nécessaires pour évaluer les facteurs qui influencent le nombre de jeunes. Cette étude révèle l'importance des zones avec suffisamment de refuges et peu de perturbations humaines pour le nombre d’individus, en particulier sur les sites de reproduction, car les loups peuvent être particulièrement exposés et sensibles pendant la saison de reproduction.

L’étude de suivi à long terme dans le nord-ouest du Portugal a révélé un déclin du nombre de loupsentre 1996 et 2005, avec seulement deux des six meutes restantes à la fin de cette période, sans doute en raison de la persécution directe des loups et à la faible connectivité de l'habitat entre les meutes. Cependent, depuis 2007, les loups se rétablissent et recolonisent la zone d'étude, deux meutes ‘source’ jouant un rôle crucial dans le rétablissement et le maintien de plusieurs meutes voisines. Par la suite, les meutes ont facilité la dispersion par le biais d'un processus en pas japonais. Les meutes ‘source’ ont montré des valeurs plus élevées de persistance de groupe, de succès de reproduction et de taille de meute par rapport aux meutes ‘puits’, qui se sont éteintes au cours de plusieurs années. A l’instar des résultats obtenus ailleur en Ibérie, nous avons trouvé des preuves d’une dispersion limitée soulignant l'impact probable des caractéristiques anthropogéniques sur la dispersion des loups et la structure de la population. En outre, en combinant plusieurs ensembles de données, nous avons développé un modèle de population intégré qui confirme une tendence démographique positive entre 2007 et 2019 et permet également d’estimerle taux de survie des adultes à 72%, le taux de survie apparent des adultes à 62% et le taux de survie des jeunes à 53%. En prévoyant la croissance de la population sur dix ans (2020-2029) selon différents scénarios de taux de survie, nous soulgnons que la survie des adultes est un facteur clé qui détermine la croissance et le risque d’extinction de cette population.

Les résultats montrent que la densité du bétail affecte fortement l'occupation des loups et le nombre d'individus dans les meutes, que la dispersion joue un rôle vital dans la persistance et la croissance des populations de loups et que le taux de mortalité des adultes influence fortement la viabilité d'une population. Toutes les approches utilisées

dans cette thèse pour comprendre la persistance des loups dans les paysages dominés par l'homme soulignent la nécessité d'évaluer les facteurs sociaux et les dimensions humaines, ainsi que les causes et les taux de mortalité illégale liés aux conflits associés à la déprédation du bétail. Ainsi, les priorités pour la conservation du loup dans les paysages dominés par l'homme devraient inclure l'amélioration de la gestion du paysage et la réduction des conflits entre l'homme et les grands carnivores. A la lumière des conclusions de cette thèse, plusieurs actions de conservation, des mesures d'atténuation et des lignes de recherche futures sont proposées.

Mots clés: *Canis lupus,* dispersion, modèle dynamique d'occupation, perturbation humaine, modèle intégré de population, croissance démographique, site de reproduction, dynamique source-puits, survie.

Resumo

Nas últimas décadas, as populações de grandes carnívoros têm vindo a recuperar e a recolonizar várias zonas. No entanto, o seu comportamento predatório conduz frequentemente a conflitos com os seres humanos, resultando no abate preventivo ou retaliatório de grandes carnívoros. A perseguição direta e outras perturbações antropogénicas tornam particularmente difícil a conservação e gestão dos grandes carnívoros em paisagens humanizadas. O principal objetivo desta tese é investigar o impacto dos factores antropogénicos na persistência dos grandes carnívoros em paisagens humanizadas. De entre todos os grandes carnívoros, o lobo (*Canis lupus*) é notavelmente resiliente e capaz de se adaptar a várias condições de habitat. São necessários estudos que avaliem a forma como os factores antropogénicos ou ambientais afectam a persistência do lobo e o número de indivíduos, e que explorem a dinâmica e demografia das populações de lobo em paisagens humanizadas. Com base na monitorização a longo prazo do lobo em paisagens humanizadas na Península Ibérica, esta tese estima a ocupação do lobo e identifica potenciais áreas de recolonização; avalia as caraterísticas dos locais de reprodução e identifica factores que influenciam o número de indivíduos nas alcateias; e aborda o estado e a viabilidade de uma população, avaliando a sua dinâmica espácio-temporal, parâmetros demográficos e probabilidades de extinção.

De acordo com o modelo dinâmico de ocupação desenvolvido, a probabilidade de colonização está positivamente relacionada com a altitude, a densidade de gado e a densidade de estradas não alcatroadas, e negativamente relacionada com a proporção de áreas ardidas e densidades de estradas nacionais-regionais e locais. Coerentemente, a probabilidade de extinção diminui com o aumento da densidade de estradas não alcatroadas. Ao avaliar a dinâmica da área de distribuição do lobo nos últimos vinte anos na Península Ibérica, as probabilidades de ocupação e colonização foram mais elevadas nas áreas onde a espécie persistiu ou se expandiu do que nas áreas onde a espécie se extinguiu ou na periferia da área de distribuição da espécie. No entanto, as inesperadas frentes de expansão e áreas de estagnação observadas nos últimos vinte anos enfatizam a possibilidade do efeito de factores sociais relacionados com o Homem na recuperação das populações de lobo e a necessidade de avaliar tais factores. Com base nas probabilidades de colonização e ocupação, foi elaborado um mapa de adequação de habitat, nos qual são identificadas as áreas prioritárias para a implementação de acções de conservação e medidas de mitigação. Ao avaliar as características em torno dos locais de reprodução (buffers de 1, 2, e 5

km) e as variações no número de lobos, demonstramos que a disponibilidade de refúgio influencia positivamente o número de adultos em todos os tamanhos de buffer considerados. A densidade populacional humana, a densidade de estradas alcatroadas, a proporção de áreas queimadas, o relevo acidentado e a densidade de alcateias influenciaram negativamente o número de adultos, particularmente em buffers menores dos locais de reprodução. Embora os resultados indiquem que a maior disponibilidade de refúgio e a menor densidade de estradas não alcatroadas aumentam o número de crias, são necessários estudos adicionais para avaliar os factores que influenciam o número de crias. Este estudo revela a importância de áreas com refúgio suficiente e pouca perturbação humana para o número de lobos, particularmente nos locais de repdoução, uma vez que os lobos podem estar particularmente expostos e susceptíveis durante a época de reprodução.

O estudo de longo prazo de monitorização no noroeste de Portugal, revelou uma diminuição no número de lobos entre 1996 e 2005, restando apenas duas das seis alcateias no final deste período, provavelmente devido à perseguição direta do lobo e à baixa conetividade do habitat entre alcateias. No entanto, desde 2007, os lobos têm vindo a recuperar e a recolonizar a área de estudo, com duas alcateias ‘core’ a desempenharem um papel crucial na recuperação e manutenção de várias alcateias ‘sink’ vizinhas. Subsequentemente, as alcateias facilitaram a dispersão através de um processo de ‘stepping-stone’. As alcateias ‘core’ apresentaram valores mais elevados de persistência do grupo, sucesso reprodutor e tamanho das alcateias em comparação com as alcateias ‘sink’, que estiveram extintas durante vários anos. À semelhança dos resultados obtidos noutros locais da Península Ibérica, encontrámos evidências de dispersão limitada, que realça o provável impacto das paisagens humanizadas na dispersão do lobo e na estrutura da população. Além disso, através da combinação de vários conjuntos de dados, desenvolvemos um modelo populacional integrado que confirmou a tendência demográfica positiva entre 2007 e 2019, e também permitiu estimar uma taxa de sobrevivência em adultos de 72%, uma taxa de sobrevivência aparente em adultos de 62% e uma taxa de sobrevivência em crias de 53%. Ao prever o crescimento da população ao longo de dez anos (2020-2029) com base em diferentes cenários de taxa de sobrevivência, enfatizamos que a sobrevivência dos adultos é um fator-chave que molda o crescimento e o risco de extinção desta população.

Os resultados mostram que a densidade do gado afecta fortemente a ocupação do lobo e o número de indivíduos nas alcateias, que a dispersão desempenha um papel

vital na persistência e recuperação das populações de lobo e que a taxa de mortalidade adulta influencia fortemente a viabilidade de uma população. Todas as abordagens utilizadas nesta tese, para compreender a persistência do lobo em paisagens humanizadas, salientam a necessidade de avaliar os factores sociais humanos, dimensão humana e as causas e taxas de mortalidade ilegal relacionadas com conflitos associados à predação de gado pelo lobo. Assim, as prioridades para a conservação do lobo em paisagens humanizadas devem incluir a melhoria da gestão da paisagem e a redução dos conflitos entre o Homem e os grandes carnívoros. À luz das conclusões desta tese, são propostas várias acções de conservação, medidas de mitigação e futuras linhas de investigação.

Palavras-chave: *Canis lupus*, dispersão, modelo dinâmico de ocupação, perturbação humana, modelo populacional integrado, crescimento populacional, local de reprodução, dinâmica source-sink, sobrevivência.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AM	Alto Minho
AST	Asturias
BCI	Bayesian Credible Interval
BreedS	Breeding Success
CEFE	Centre d'Écologie Fonctionelle et Evolutive
FCT	Fundação para a Ciência e a Tecnologia
FCUP	Faculty of Sciences of the University of Porto
GroupPR	Group Persistence Rate
IP	Iberian Peninsula
IPM	Integrated Population Model
LC	Large Carnivore
LOO	leave-one-out cross-validation
MCMC	Markov chain Monte Carlo
MLE	Maximum Likelihood Estimates
MR	Migrant Ratio
PVA	Population Viability Analysis
Rhat	Gelman-Rubin statistic

Chapter I

General introduction

1. General Introduction carnivore conservation in the Anthropocene

1.1. Large carnivore conservation in the Anthropocene

Large carnivores (LCs) are among the most controversial species for conservation since multiple ecological, cultural, legal, and socio-economic factors influence their conservation (Sillero-Zubiri et al., 2004). LCs are often of public interest due to potential negative interactions with human activities and land use, primarily associated with their predatory behaviour. Although humans and LCs have coexisted for millennia, conflicts have increased significantly due to the exponential increase of human populations and the resulting expansion of human activities (Linnell et al., 2001; Woodroffe, 2000). Human-carnivore conflicts often arise from the competition for game species or livestock depredation (Elofsson and Häggmark, 2021; Graham et al., 2005; Olson et al., 2015; Woodroffe et al., 2005). Despite the resilience and capacity of some LCs to persist in the presence of humans (Carter and Linnell, 2016), several studies have shown that LC population declines are linked to anthropogenic factors (e.g., Woodroffe, 2000). Over the past two centuries, LCs have suffered extensive range contractions and population declines due to direct persecution associated with conflicts and additional threats related to habitat loss, degradation, and prey depletion (Ripple et al., 2016, 2014; Wolf and Ripple, 2017). In recent decades, the implementation of conservation policies and changes in land-use management have contributed to the recovery of several LC populations and recolonization in areas where they had gone extinct (Chapron et al., 2014; Linnell et al., 2001; Weaver et al., 1996). However, much of this recovery is arguably linked to LCs' resilience and persistence to survive in human-dominated landscapes (Carter and Linnell, 2016; Lamb et al., 2020).

The concept and application of remote wilderness, in which wildlife is preserved in protected areas, have had successful results, particularly in North America (Packer et al., 2013; Venumière-Lefebvre et al., 2022). However, in human-dominated landscapes, LCs and humans need to share the same landscapes, making coexistence the way to manage and conserve LCs successfully (Carter and Linnell, 2016; López-Bao et al., 2017; Lute et al., 2018). Among all LC species in Europe (brown bear *Ursus arctos*, wolf *Canis lupus*, wolverine *Gulo gulo*, Eurasian lynx *Lynx lynx,* and Iberian lynx *L. pardinus*), the wolf is likely the most prone to conflict (Almarcha et al., 2022; Linnell and Cretois, 2018), but also the most resilient species since, as a generalist species (Mech and Boitani, 2003), it can persist in various environmental conditions, including highly anthropogenic landscapes.

1.2. Wolf conservation in human-dominated landscapes

The grey wolf (*Canis lupus*) was the world's most widely distributed mammal, living throughout the northern hemisphere, but it became extinct in much of Western Europe, Mexico and much of the United States of America (USA) and Japan (Boitani, 1995; IUCN, 2023; Mech, 1970). Although it is estimated that the species lost 26% of its historical range (Wolf and Ripple, 2017), several wolf populations have been recovering and returning to areas of their original range in the last decades. At present, wolves occur primarily in the wilderness and remote areas of North America (Canada, Alaska, and northern USA), Europe, and Asia (Mech and Boitani, 2003) but are also found in human-dominated landscapes, and the distribution is highly dynamic due to its currently increasing range and numbers in north-central and western USA and much of Europe (Boitani et al., 2022, 2018; Kaczensky, 2018) (Fig. 1-1).

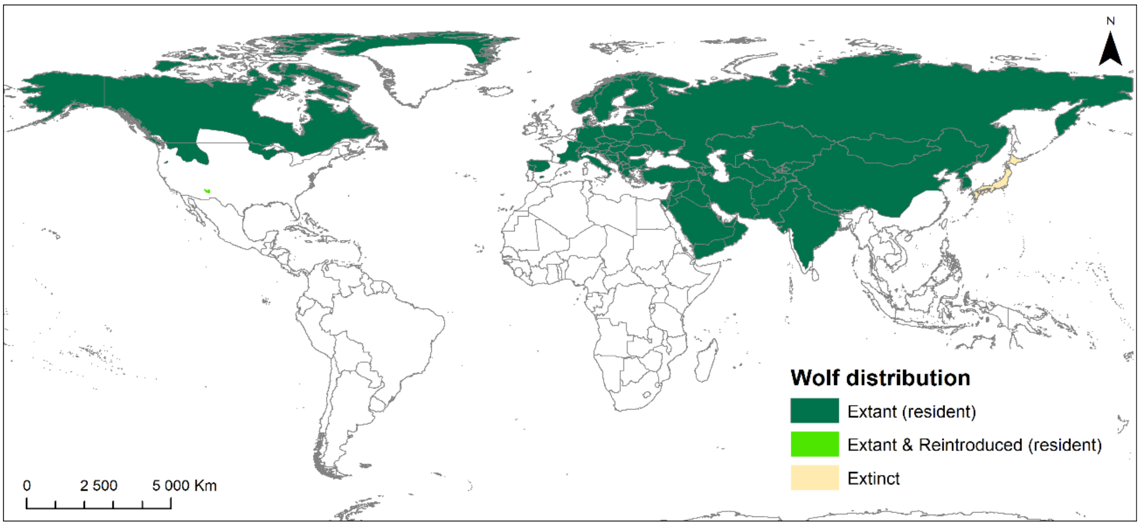


Fig. 1-1**:** Wolf (*Canis lupus*) distribution. Adapted from International Union for Conservation of Nature 2018 (The IUCN Red List of Threatened Species. Version 2021-2)

The wolf population trend is considered stable and of 'Least Concern' conservation status (IUCN, 2023). The species is included in CITES (Convention on International Trade in Endangered Species of the Wild Fauna and Flora) Appendix II (potentially endangered species), except populations from Bhutan, India, Nepal and Pakistan, which are more threatened and listed in Appendix I (species in danger of extinction). It is strictly protected under the Bern Convention of 1979 (Appendix II; need habitat conservation) and is listed in Annex IV (fully protected) of the Habitats Directive of 1992 in most EU Member States, with some populations entirely or partially listed in Annex V (species of community interest whose taking in the wild and exploitation may be subject to management measures, including regulated hunting). EU Member States are required to ensure that wolf populations reach or maintain a “favourable conservation status” under these Habitats Directive annexes (Epstein et al., 2016). Wolf hunting has been banned in several EU Member States (e.g., Poland, Romania, Portugal, Slovenia, and, recently, in Spain – since 2021 Instruction No. TED/980/2021 of the Ministry for the Ecological Transition). Derogations to the strict status of protection of the species listed in Appendix II of the Bern Convention or Annex IV of the Habitats Directive are possible under certain conditions according to Article 6 or Article 12, respectively. Thus, if livestock depredations persist despite non-lethal mitigation measures and livestock damage is high, lethal removals can be authorized for livestock protection (e.g., France, Slovenia).

In North America, the wolf is subject to hunting, trapping, and culling for the protection of livestock and wild ungulates, disease control and other individual conflicts dealt with by government authorities on a case-by-case basis. Wolf management is highly variable across Canadian provinces and states in the USA. Unlike wolf populations in southern Canada and the contiguous United States, which have been extirpated or reduced, wolves are widely distributed and abundant in Alaska and northern Canada. The federal Endangered Species Act has protected all wolves in the conterminous US states (i.e. excluding Alaska) since 1974. However, wolves have recurrently been delisted from endangered to threatened in several states by the US Fish and Wildlife Service, which allows wolf hunting (Cluff and Murray, 1995; Hayes and R, 1995; Musiani and Paquet, 2004). In North America, wolf introductions were carried out successfully in Idaho and Yellowstone National Park (Bangs et al., 1998; Fritts et al., 1997), in Arizona and New Mexico (Hedrick and Fredrickson, 2008; Parsons, 1998), and have been recently reintroduced in Colorado (Colorado Parks and Wildlife, 2020; Ditmer et al., 2022). In the USA, government protection of wolves and rehabilitation efforts through reintroduction programs have facilitated natural recolonization (e.g., Jimenez et al., 2017; Smith and Bangs, 2009). No reintroduction programmes have been conducted in Europe, but improving conservation policies and land use management have facilitated several wolf populations to recolonize naturally (e.g., Chapron et al., 2014; Fabbri et al., 2007; Reinhardt et al., 2019).

Although wolves were primarily present in protected and remote areas with low human presence (López-Bao et al., 2017), the return of wolves to human-dominated landscapes has increased conflicts (Kuijper et al., 2019; Lute et al., 2018; Musiani and Paquet, 2004; Redpath et al., 2013). Livestock depredation is globally recognized as one of the most widespread barriers to increasing public acceptance of carnivores (Baker et al., 2008; Lute et al., 2018). These conflicts are aggravated in human-dominated landscapes, often leading to the illegal killing of wolves. The coexistence between people and wolves is possible when both adapt (Carter and Linnell, 2016; Chapron and López-Bao, 2016). Wolves adapt by behavioural mechanisms of spatial or temporal avoidance of humans (e.g., Ahmadi et al., 2014; Carricondo-Sanchez et al., 2020; Gaynor et al., 2018; Smith et al., 2022) or demographic mechanisms to compensate for human-related mortality with high reproductive success or immigration rates (e.g., Adams et al., 2008; Gude et al., 2012; Murray et al., 2010). Shifting social attitudes toward wolf tolerance and co-adaptation can facilitate wolf recolonization and persistence (Arbieu et al., 2019; Carter et al., 2019; Carter and Linnell, 2016). Additionally, several forms of management have been undertaken to reduce conflicts resulting from livestock depredations, including compensation programs for livestock losses, subsidies for damage prevention tools such as electric fences and livestock guarding dogs (Ciucci and Boitani, 1998; Eklund et al., 2017; Iliopoulos et al., 2009; Karlsson and Sjöström, 2011; Salvatori and Mertens, 2012). Nevertheless, conflicts must be anticipated in human-dominated landscapes, particularly in areas where wolves are recolonizing and people are not used to their presence (López-Bao et al., 2017; Mech, 2017).

Knowledge of species distribution is vital for informing and prioritizing conservation actions and effective conservation planning (Guisan and Thuiller, 2005). By understanding how wolves use the landscape and how they recolonize new areas, we would expect to predict wolf occurrences in human-dominated landscapes and identify the areas prone to future conflicts with humans (Behr et al., 2017; Ditmer et al., 2022; Falcucci et al., 2013; Szewczyk et al., 2019). However, wolf habitat selection can be very different depending on the context and landscape features. In Eurasia, the long history of the coexistence of wolves and humans and continuous wolf persecution suggests that wolves have a higher tolerance of humans and human-made structures, which may force wolves to be more cautious at critical places within their territories, such as reproduction sites (Sazatornil et al., 2016). On the contrary, wolves were severely depleted in North America shortly after European settlers expanded into the territory, and intensive persecution began during the 18th century (Frank and Woodroffe, 2001). These different histories of the coexistence of wolves and humans have likely resulted in different tolerance and strength of human avoidance among wolves in North America and Eurasia and have also resulted in different management and conservation strategies. In fact, wolves in Eurasia are prone to inhabit cultural landscapes, select more human-disturbed areas, and share their territories with humans in closer proximity than in North America (Chapron et al., 2014).

1.2.1. Wolf spatial use: species distribution and homesite selection

Predictive species distribution models have been increasingly developed in studies of conservation biology, ecology, and wildlife management (Araújo and Guisan, 2006; Guisan and Thuiller, 2005). Suitable and unsuitable areas are more straightforward to differentiate in specialized species than in generalist species (Manel et al., 2001). As generalists, wolves may have an opportunistic habitat use, so observed habitat preferences are expected to differ between areas (Fechter and Storch, 2014). This difference can be highly pronounced between areas with low human interference and human-dominated landscapes (Sazatornil et al., 2016). In Europe, coexistence with humans in rural and urban landscapes may explain why wolves may show pronouncedly different habitat associations in different countries (Fechter and Storch, 2014).

Most species distribution models rely on the assumption that the species is detected everywhere it is present (e.g., Phillips et al., 2006; Yackulic et al., 2013), though the failure to detect a species when present can easily occur due to various reasons such as species behaviour, habitat characteristics, observer experience or weather conditions (Kéry, 2011; Kéry et al., 2010). Disregarding imperfect detection is a common source of survey bias (Linkie et al., 2007) that can result in biased distribution maps with underestimated presences (Kéry and Schaub, 2011; Lahoz-Monfort et al., 2014) and, unless properly accounted for, the heterogeneous detectability confounds inference of spatial and temporal patterns (MacKenzie et al., 2006, 2002). Furthermore, species distribution is not static and can vary through time and space, especially in expanding populations and invasive species. Although the limitations of models based on presence-only or presence-absence data, several studies on wolves have been carried out worldwide to predict species distribution, identify potential areas where the species is still absent, or even detect ecological corridors (Falcucci et al., 2013; Grilo et al., 2018; Kabir et al., 2017; Marboutin et al., 2011; Ronnenberg et al., 2017; Votsi et al., 2016). Single-season or static site-occupancy models have been implemented to overcome such issues and to assess patterns and determinants of the occurrence of a broad range of LCs (e.g., Linkie et al., 2007; Long et al., 2011; Rich et al., 2017; Thorn et al., 2009). By correcting for imperfect detection (false absence), it is possible to obtain unbiased occupancy estimates and more accurate distribution maps, which are relevant for conservation and management implications (MacKenzie et al., 2006, 2002). Covariates are included in models to infer relationships between observed patterns and the underlying processes that cause them, thereby projecting patterns in un-surveyed areas (MacKenzie et al., 2006). Multi-season or dynamic site-occupancy models have also been developed for LCs (e.g., Louvrier et al., 2018; Miller et al., 2013), which account for the influence of dynamic processes such as colonization and extinction on the species range dynamics, likelihood of presence, or spatial use (MacKenzie et al., 2003; Royle and Kéry, 2007). Thus, these modelling approaches have the potential to more accurately estimate the species distribution and predict areas of future recolonization.

The variables for species occupancy analysis are often selected based on previous habitat selection or species distribution studies since these can give an idea of possible factors influencing the species' presence. However, new variables should and have been increasingly added with innovative ideas depending on the aim of the study. Most studies on wolf habitat suitability and species distribution models mainly include environmental variables (e.g., altitude, slope, ruggedness, land cover, wild prey densities, water areas) and anthropic variables (e.g., human population density, settlements, infrastructures, roads, trails, land cover related to agricultural activity, livestock density). Some studies indicate that altitude influences wolf occupancy, though this could also be related to high anthropogenic activity in lower altitudes (<900m a.s.l., Grilo et al., 2018, 2002; Llaneza et al., 2012; Zlatanova and Popova, 2013), to lack of prey or severe geo-morphological conditions higher altitudes (Glenz et al., 2001). Thus, in areas with low human interference, altitude may influence wolf occupation due to prey availability, movement, migrations, and other climatic and habitat conditions (Ballard et al., 1997; Fritts et al., 1981). Slope is included in most studies predicting wolf distribution or habitat suitability. However, no significant differences are detected between high and low slope values, although wolves slightly prefer areas with slopes of 20 – 50º, which can also be related to less disturbance by human activities (Zlatanova and Popova, 2013). Overall, wolves use a wide range of habitat types but show particular preferences for forest cover in most parts of their range (Ciucci et al., 1997; Jedrzejewski et al., 2008; Mladenoff et al., 1999; Theuerkauf et al., 2003). Several studies conclude that road density, human population density or forest cover are the key variables associated with wolf occurrence and wolf habitat suitability (Blanco et al., 2005; Ciucci et al., 1997; Fuller et al., 1992; Jaeger et al., 2005; Jedrzejewski et al., 2008; Kaartinen et al., 2005; Llaneza et al., 2012; Mech et al., 1988; Thiel, 1985; Thurber et al., 1994; Whittington et al., 2005). Some studies failed to detect a negative influence of road density on wolf habitat use (Jędrzejewski et al., 2005; Thiel, 1985; Zlatanova and Popova, 2013), though primary roads can still cause habitat fragmentation and be significant barriers to dispersal (Zlatanova and Popova, 2013). In more disturbed regions such as northwest Iberia, wolves strongly avoid roads since densities reach 1.6 km/km^2 of paved roads and 1.8km/km^2 of trails (Dennehy et al., 2021; Rio-Maior et al., 2019).

In Europe, coexistence with humans in rural and even urban landscapes may explain why wolves show pronouncedly different habitat associations across their range. For instance, wolves in Poland use meadows and wetlands in addition to forests (Jedrzejewski et al., 2008). In Portugal, the presence of wolves appears closely linked to livestock abundance rather than a certain land cover type, and in Russia, wolves occupy mosaic habitats of forest and agricultural areas (Eggermann et al., 2011). In Spain, wolves frequently use agricultural fields (Blanco et al., 1992), while in Italy and Romania, wolves use shrubland and take advantage of garbage dumps (Ciucci et al., 1997; Corsi et al., 1999). Wolves tend to establish their home ranges in areas with the least human disturbance by avoiding human settlements (Kaartinen et al., 2005; Karlsson et al., 2007; Zlatanova and Popova, 2013) or areas with higher human population density. The effects of anthropic pressure and prey abundance were studied in a number of studies carried out in North America (Mech, 1970; Thurber and Peterson, 1993). In areas where wolves mainly feed on wild ungulates, prey density is often an insignificant variable for wolf habitat suitability modelling, though this may be related to the overall high densities observed in the study areas (Fritts and Carbyn, 1995; Karlsson et al., 2007; Mech et al., 1988; Mladenoff et al., 1999, 1995; Thiel, 1985; Thurber et al., 1994). In contrast, in areas with low prey densities or with a large variation of prey density throughout the landscape, this variable can significantly influence wolf occurrence (Fuller, 1989; Messier, 1994; Zlatanova and Popova, 2013). Information on wild prey densities is scarce. Thus, several studies consider variables that could reflect this parameter, such as number of ungulates harvested. Moreover, in areas with high human interference, in which wolves prey or scavenge on livestock in high proportions, prey density may greatly influence wolf habitat use (Ciucci et al., 2020). Responses to livestock density are expected to reflect trade-offs between human-related risks and access to prey because wolves can positively select areas where prey are abundant or avoid these areas due to proximity to humans (Rio-Maior et al., 2019).

Wolves have a well-defined breeding season, and their movements become temporarily and spatially predictable around breeding sites (Llaneza et al., 2018, 2014; Sazatornil et al., 2016). These sites, known as homesites (Joslin, 1967), represent a small area within wolf home ranges (1-13 km^2) for up to 6 months, where they become particularly vulnerable to human disturbance (Llaneza et al., 2018, 2016, 2012; Rio-Maior et al., 2018; Sazatornil et al., 2016). Similar to habitat selection predicted by species distribution models, homesite selection is variable across contexts and is influenced by human-modified features (Iliopoulos et al., 2014; Person et al., 2009; Sazatornil et al., 2016; Unger et al., 2009). Wolves often minimize the risk of exposure at breeding sites by avoiding anthropogenic features such as roads, settlements, and agricultural lands (Capitani et al., 2006; Sazatornil et al., 2016) and selecting areas with vegetation cover, rough terrains, or higher altitude (Ciucci et al., 2018; Sazatornil et al., 2016; Trapp et al., 2008). However, due to the higher vulnerability, wolves are possibly more demanding when selecting homesites than the remnant territory.

Although wolves show some tolerance for human disturbance at homesites (Thiel et al., 1998), homesite shifts are more likely to occur in areas with high disturbance, which can result in higher pup mortality (Argue et al., 2008; Ballard et al., 1987; Frame et al., 2007; Habib and Kumar, 2007). Selecting homesites with better conditions can potentially influence adult and young survival during the breeding season. In this context, selecting homesites can be extremely important for packs to succeed (Ciucci et al., 2018; Kaartinen et al., 2010; Sazatornil et al., 2016). Nevertheless, studies evaluating the influence of resource selection at homesites on the survival of individuals are lacking. It is unclear if and how this selection affects the number of adults, the reproductive success of packs, or the survival of pups. Less suitable homesites can likely have a relevant impact on population demography and viability (higher survival).

1.2.2. Wolf population dynamics and demography

Management of LCs is informed using a variety of population parameters. Therefore, population monitoring is integral to their management, providing valuable information for ground management actions and devising conservation strategies (Bled et al., 2017). Anthropogenic impacts on habitats and animal populations result in worldwide species range contractions and population decreases, and such heterogeneous landscapes can affect the distribution of organisms and produce habitat-specific population demography (Cardillo et al., 2005; Kristan, 2003). However, reliable demographic estimates are difficult to obtain in such elusive species since detecting groups, reproduction, mortality, and dispersers is challenging. Based on a systematic literature review on several parameters of population dynamics and demography in wolves along a gradient of humanization, we compare overall life history traits and identify the main knowledge gaps.

I searched for studies published in peer-reviewed journals until December 2020 using ‘Scopus’ and ‘Web of Science’, and extracted the average values of wolf parameters in each identified study area per study: i) density, growth rate, carrying capacity, and proportion of residents; ii) number of individuals per pack (pack size, litter size, number of pups, subadults, and adults) and age structure; iii); sex ratio (population, pack, dispersers, and by age), iv) mortality rate (population, by age, for dispersers, and loners); v) dispersal distance, duration, and probability (the two latter by age); vi) recruitment rate; vii) and migration rates (migration, immigration, emigration, and the number of immigrants and emigrants per generation). See Sup. Table 2, and Sup. Fig. 1 in Appendix I for the keywords search used, the parameters extracted, and the PRISMA flow chart with the number of studies screened and included in the final list of studies.

The number of published studies with information on wolf population and demographic parameters was 332. The number of publications abruptly increased since the beginning of the 2000s (Fig.1-2). The number of studies (N) highly varies across areas, with more than half conducted in North America (N = 196), followed by Europe (N = 123; including all Turkey and Russia), and lastly, Asia (N = 15). Consequently, there is also a high variation in the number of studies among countries (Fig. 1-3; See Sup. Fig. 2 in Appendix I for a detailed number of studies by country).

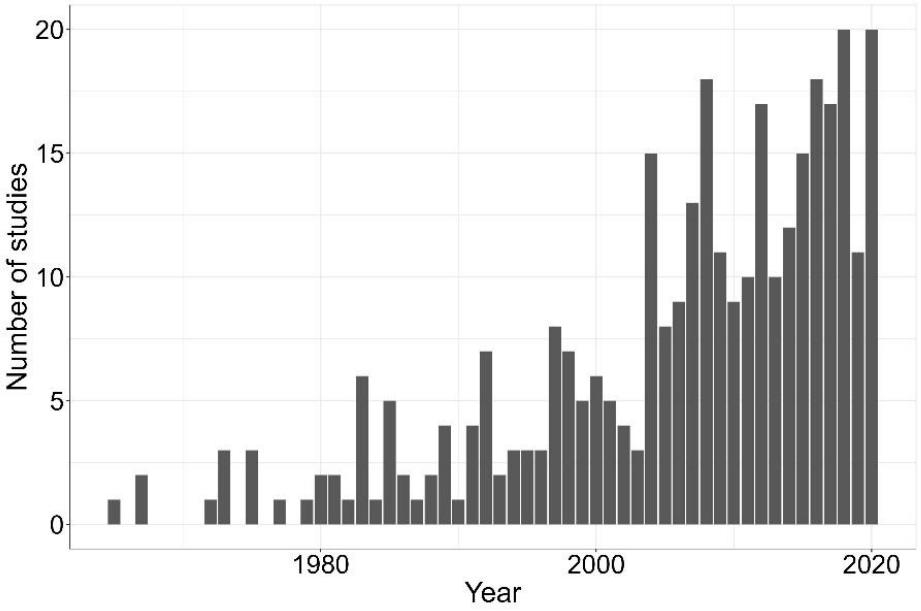


Fig. 1-2: Annual number of studies published in peer-reviewed journals between 1965 and 2020, with information on wolf (*Canis lupus*) population dynamics and demographic parameters.

Among demographic parameters, the number of individuals in packs by age and pack size are the most recurrent studied parameters, followed by the population-level density and growth rate parameters. The population sex ratio, proportion of residents, age structure, and overall mortality rate are frequently assessed, although most studies were conducted in North America. Parameters structured by individual age or related to dispersal and migration are less frequently assessed in North America and Europe and are utterly unknown in Asia (Fig. 1-4).

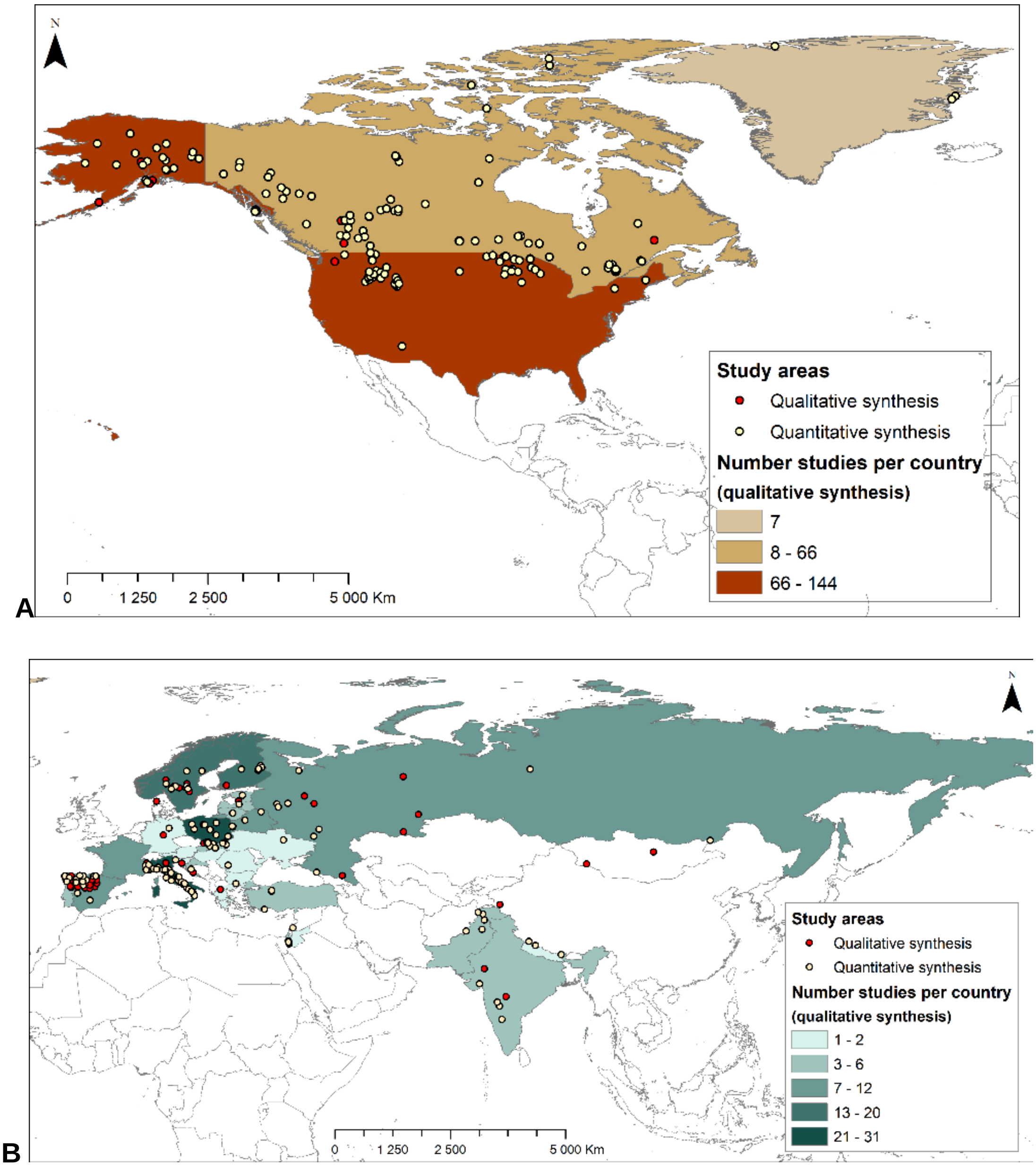


Fig. 1-3: Number of studies per country with information considered for the quantitative synthesis on wolf population dynamics and demographic parameters between 1965 and 2020 in A) North America and B) Eurasia. The points represent the approximate location of the study areas considered for the qualitative (with some information on the parameter) and quantitative (with mean values of the parameter) synthesis.

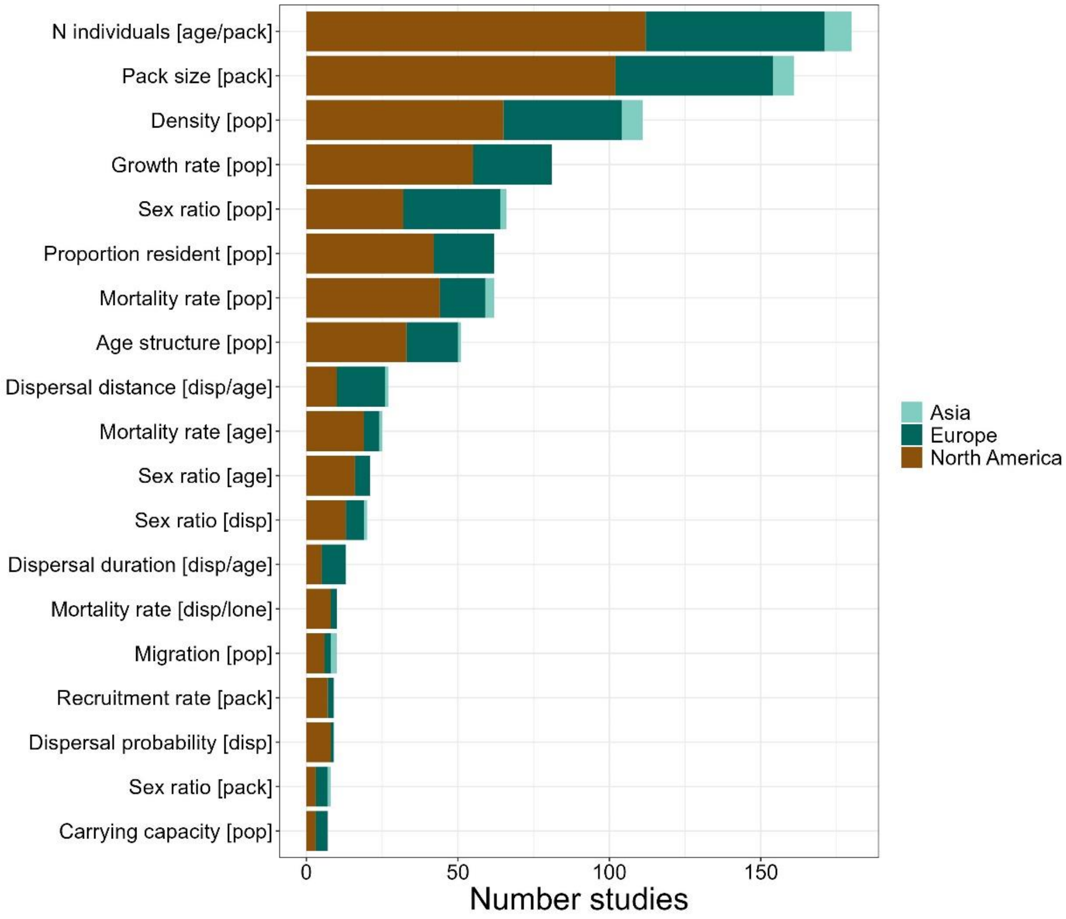


Fig. 1-4: Number of studies per parameter group by continent: Asia, Europe and North America. Each parameter group is included in the parameters levels of population [pop], pack [pack], age [age], disperser [disp] and lone wolves [lone].

Hereafter, we joined the information obtained for Europe and Asia, considering the few studies available in Asia, the overall human-dominated landscapes, and the similar history of coexistence in Europe and Asia (the intensity of wolf persecution in Eurasia increased in parallel with the expansion of livestock husbandry after domestication around 11,000 years BP; Vigne, 2011), compared to the landscapes of North America. To identify the knowledge gaps on wolf population dynamics and demography, we summarize the existing information and detect differences in each parameter between North America and Eurasia (performing generalized linear models). We further make some parameter descriptions by country when relevant.

Wolf density and growth rate: Wolf density and population growth rates are often determined to understand population status and trend. These parameters can be highly variable depending on wolf management strategies (e.g., wolf culling or hunting) or the type of wolf area (e.g., expansion fronts). The median wolf density in Eurasia (21.4 wolves/ 1000 km^2) was significantly higher than in North America (11.5 wolves/ 1000 km^2, β = -14.78, p = 0.04; Fig. 1-5A). A higher wolf density in human-dominated landscapes of Eurasia may be expected since wolves may benefit from multiple anthropogenic food sources (e.g., Ciucci et al., 2020; Hosseini-Zavarei et al., 2013; Meriggi et al., 1996; Vos, 2000). Exceptionally, the highest wolf densities were obtained for Pakistan, with 455–625 wolves/1000 km^2 (Hamid et al., 2019; Khan and Ling, 2020), and for Minnesota, with 182–308 wolves/1000 km^2 (Mech and Tracy, 2004). These outlier density estimates should be cautiously considered since these were calculated for very small sampling areas (22-24 km^2 in Pakistan) or small territory sizes obtained from VHF-collared wolves (20-33 km^2 in Minnesota), which are areas smaller than regular wolves' territory sizes (Mech and Boitani, 2003).

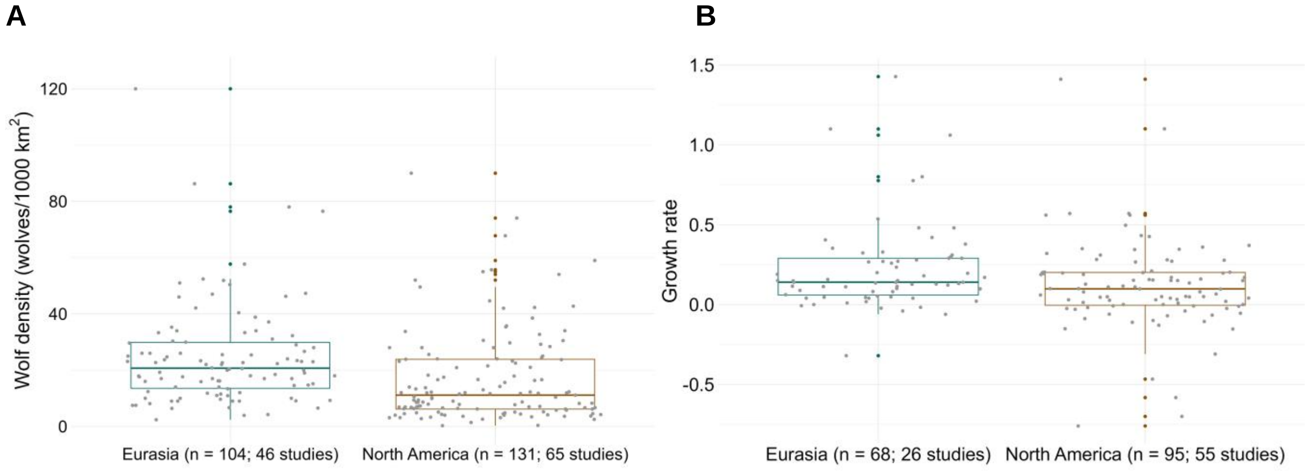


Fig. 1-5: Boxplots for estimated wolf density (A) and growth rate (B) per study area for each continent (n is the number of study areas). We removed outliers from four studies (range: 120-625 wolves/ 1000km^2).

The median reported wolf growth rate in Europe (14%) was significantly higher than in North America (10%, β = -0.11, p = 0.02; Fig. 1-5B), and no information was available for Asia. Several areas where wolf populations are subject to culling and hunting had low growth rates (e.g., Bjorge and Gunson, 1985; Hayes et al., 2003). Conversely, newly recolonized areas have low wolf densities and show increasing population growth rates. The USA showed the lowest median growth rate (9%), and Canada had a median growth rate of 15%. However, vast differences between study areas resulted in highly variable values of growth rates within both countries (USA: range -46–56%; n = 41 study areas; Canada: range -76–141%, n = 18 study areas). The European countries with the highest number of studies (Poland, Italy, Sweden, and Norway) had a median growth rate of 14%, and most had a positive trend (range: -6–110%). The countries with the highest median growth rates were Russia, Hungary, Lithuania, and France (range: 27–55%); however, these were based on a few studies.

The study areas with the highest growth rates were located in British Columbia (Canada) and some regions of Finland and Sweden (range: 106–143%) (Bergerud and Elliot, 1986; Bischof et al., 2020; Kojola et al., 2004), but corresponded to areas where wolf harvest stopped or with areas of recent recolonization. The lowest wolf growth rates were obtained in studies conducted in the USA and Canada: in Isle Royale, Michigan (-47%), Alaska (-31%; Boertje et al., 1996), and Alberta (range: -76% to -58%; Bjorge and Gunson, 1985). In Isle Royale, the low growth rate was due to a population crash in 1980–1982, possibly due to starvation and intraspecific killing (Peterson and Page, 1988). The studies in Alaska and Alberta were cases of short periods of intense wolf control to reduce depredation on cattle or wild ungulates (Bjorge and Gunson, 1985; Boertje et al., 1996).

Carrying capacity: This parameter is defined as the maximum number of individuals an area can sustain indefinitely without degrading it (Odum, 2017). When a population reaches the carrying capacity, the abundance of the population stabilizes. According to the literature review, few studies assess the carrying capacity of wolf populations (e.g., Klaczek et al., 2016; Mladenoff et al., 1997). Overall, the positive trend of growth rates observed in Europe suggests that most populations are below carrying capacity. The estimation of this parameter at the country level can be relevant, and the interest in assessing this parameter is increasing in human-dominated landscapes for management purposes, especially where wolves are recolonizing (Baliauskas et al., 2009; Fechter and Storch, 2014; Kawata, 2008).

Proportion of residents: Overall, the proportion of estimated resident individuals was significantly higher ($\beta = 8.82$, $p = 0.02$) in populations of North America (83%) than in Eurasia (78%) (Sup. Fig. 3 in Appendix I). The low proportion of residents could be related to recently recolonized areas and expansion fronts where dispersers and vagrant wolves are more frequently present. Of the countries in Eurasia with more available information, Italy and Finland had the lowest median proportion of estimated residents (55%) (Fabbri et al., 2018; Imbert et al., 2016; Kojola et al., 2009), and Spain and Poland had the highest values (79% and 93%, respectively)(Blanco and Cortés, 2007; López-Bao et al., 2018b; Nowak and Mysłajek, 2016; Silva et al., 2018; Śmietana and Wajda, 1997). Belarus (95%) and Portugal (97%) had the highest proportion of residents, though these were based on one study each (Jędrzejewska et al., 1996; Silva et al., 2018).

Number of individuals in packs: Pack sizes were broadly available, obtained from 59 studies in Eurasia (n = 99 study areas) and 102 studies in North America (n = 171 study areas). Overall, pack sizes were significantly larger (β = 2.91, p = 0.00) in North America (6.8) than in Eurasia (4.3)(Fig. 1-6), which could be related to different factors, including the effect of different methodological approaches, snow cover, type of prey, higher human disturbance and human-related mortalities (e.g., Llaneza et al., 2023). Accordingly, the largest pack sizes were observed in the Northern Rocky Mountains and the Yellowstone National Park (USA/Canada), with 37 wolves, and in the Denali National Park and Preserve (Alaska), with 29 wolves (Adams et al., 2010; Jimenez et al., 2017; Smith, 2005). Typically, packs consist of the breeding pair, the pups of the year, and some yearlings (i.e. subadults of 1-2 years old). However, pack and age structure can vary depending on intrinsic or external factors, such as mortality rates, intraspecific competition or food availability (e.g., Ballard et al., 1987; Mech, 2006). For instance, larger packs can be more advantageous for hunting larger prey or protecting the pups (Mech and Boitani, 2003).

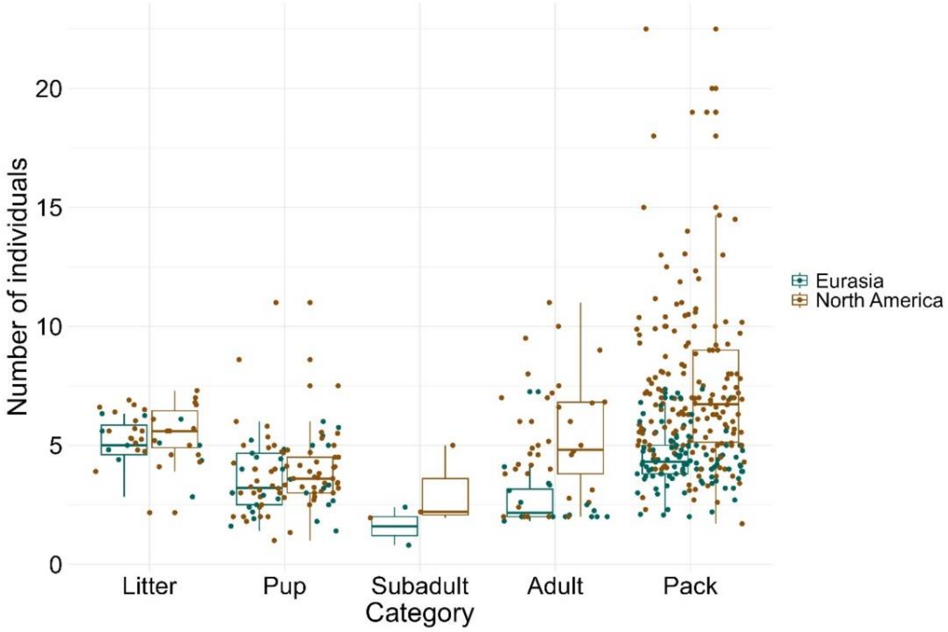


Fig. 1-6: Boxplots for the estimated number of individuals per pack by age and pack size (‘Pack’) for each continent. Ages are defined as: ‘litter’ ≤ 2 months old; ‘pup’ ≤1 year old; ‘subadult’ 1–2 years old; and ‘adult’ ≥ 2 years old.

According to the literature review, litter sizes were similar in Eurasia (5.0 pups) and North America (5.6 pups; β = -0.40, p = 0.31). The largest median litter sizes were observed in Poland, Belarus, USA, and Canada (range: 5.6–6.3; e.g., Hayes and Harestad, 2000; Rausch, 1967; Sidorovich et al., 2017; Śmietana and Wajda, 1997; Uboni et al., 2017; Webb et al., 2011). There was information available for Sweden, Norway, Italy, Spain and Finland, with median litter sizes ranging between 2.8 and 4.9 (e.g., Chapron et al., 2016; Fernández and Ruiz de Azua, 2010; Lovari et al., 2007; Pulliainen et al., 1965) (Fig. 1-6). The median number of pups per pack was similar (β = 0.29, p = 0.39) between Eurasia (3.2 pups) and North America (3.5 pups) (Fig. 1-6). In contrast to values obtained for litter sizes, Finland and Russia had the largest median number of pups (5.4), and Belarus and Poland had the lowest (2.0). There was information available for Greenland, Italy, Nepal, Pakistan, Spain, Sweden, USA, Canada, India, Israel, and Slovakia (ordered by increasing mean number of pups per pack per country, from 2.5 to 5.0 pups). The largest number of pups in packs were observed in the Northwest Territories in Canada, with 15 pups (Frame et al., 2008), and in Wyoming and Israel, with 12 pups (Cassidy et al., 2015; Reichmann and Saltz, 2005). Such a high number of pups in packs may result from multiple breeding (i.e. more than one female breeds in a pack). Although some studies present cases of multiple breeders (Ausband, 2018; Mech, 2000; Rio-Maior et al., 2018), this information is challenging to obtain, and the extent to which such cases occur in wolf populations is unknown.

The median number of subadults per pack was similar (β = 1.45, p = 0.38) between Eurasia (1.6 subadults) and North America (2.2 subadults). However, very few studies distinguished the number of subadults from adults in packs (e.g., Caniglia et al., 2014; Cassidy et al., 2015; Habib and Kumar, 2007)(Fig. 1-6). Packs in North America had a significantly higher (β = 2.43, p = 0.00) number of adults (4.8) compared to Eurasia (2.2 adults)(Fig. 1-6). The largest number of adults were observed in Alaska and Idaho (15-16 adults) (Peterson et al., 1984; Stansbury et al., 2016), though large packs with 11-12 adults were also observed in Michigan, Minnesota, and India (Kumar and Rahmani, 2008; Mech et al., 2019; Peterson et al., 1998). In areas where wolf populations are stable, pack composition should generally reflect the overall age structure of the population. However, age structure can be variable in wolf range expansion fronts or recently recolonized areas where there are many dispersers or lone wolves in the population (Mech and Boitani, 2003).

Sex ratio: The sex ratio of populations was significantly different (β = -0.39, p = 0.01) between continents, with male-biased (1.19) populations in Eurasia and a tendency to be female-biased (0.96) in North America (Fig. 1-7). This sex ratio difference could be related to differences in habitat quality, wolf population densities, sex-biased anthropogenic mortality or between stable or expanding populations with a higher number of dispersal individuals (e.g., Ausband, 2022; Mech, 1975). The highest sex ratio of the populations were obtained in Italy and Israel (4.5 and 3.7, respectively), and the lowest in some studies conducted in Canada, USA, Latvia, Hungary, and Slovakia (e.g., Boyd and Pletscher, 1999; Hausknecht et al., 2010; Pletscher et al., 1997; Žunna et al., 2009), which ranged between 0.39 and 0.50. There were no significant differences between continents in sex ratios within ages, packs or dispersers. The median sex ratio was the highest for dispersers in North America (1.32) (Fig. 1-7).

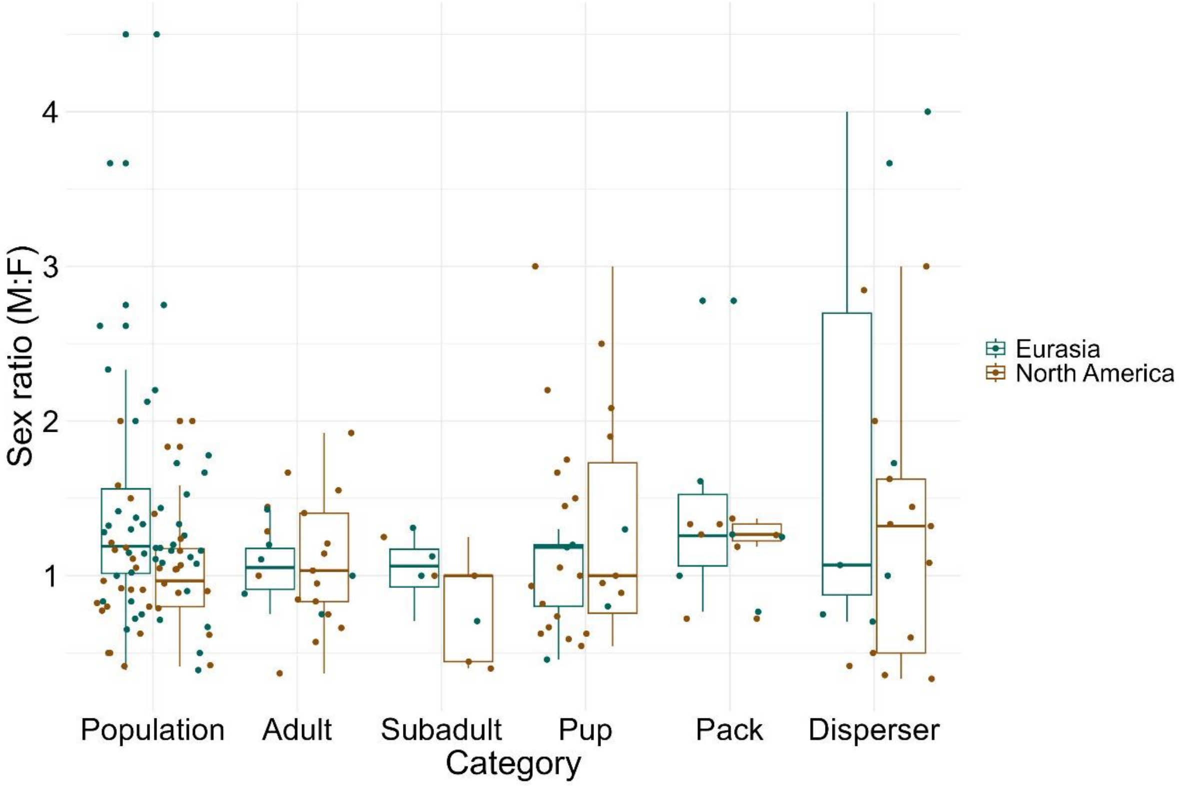


Fig. 1-7: Boxplots for sex ratio (male:female) of wolf populations, packs, by age and of dispersers. Ages are defined as: ‘pup’ ≤1 year old; ‘subadult’ 1–2 years old; and ‘adult’ ≥ 2 years old.

Mortality rate: The overall mortality rate was significantly higher (β = 14.21, p = 0.00) in populations of North America (30%; n = 44 study areas) than in Eurasia (18%, n =18 study areas)(Fig. 1-8), which is mainly due to wolf control and harvest policies conducted in several areas of North America (Creel et al., 2015). Few studies had information on mortality rates by age or for dispersers and lone wolves. As expected, estimated mortality rates of pups and subadults were higher compared to adults. The mortality rate of adults was 20% in Eurasia (n = 3 study areas) and 23% in North America (n = 14 study areas); and of subadults was 39% in Eurasia (n = 3 study areas) and 29% in North America (n= 7 study areas) (Fig. 1-8). The annual mortality rate of pups was the highest among all ages, with 61% in Eurasia (n = 2 study areas) and 40% in North America (n = 12 study areas). Some studies estimated pup mortality rates until autumn (i.e. the first eight months of age), of which the median was 50% in Eurasia (n = 4 study areas) and 43% in North America (n = 7 study areas). The pup mortality rate from autumn until the following spring (i.e. from 8 to 12 months of age) was 15% in Eurasia (n = 1 study areas) and 41% in North America (n = 4 study areas) (Fig. 1-8).

Non-resident individuals (dispersers and lone wolves) are expected to have higher mortality rates since they often travel through unknown landscapes and are more prone to anthropogenic disturbance and mortality. The mortality rate of lone wolves was significantly higher (β = -24.38, p = 0.04) in Eurasia (58%, n = 2 study areas) than in North America (31%, n = 1 study area), while of dispersers was 31% in Eurasia (n = 1 study area) and 44% in North America (n = 7 study areas) although not significant (β = 13.00, p = 0.47) (Fig. 1-8).

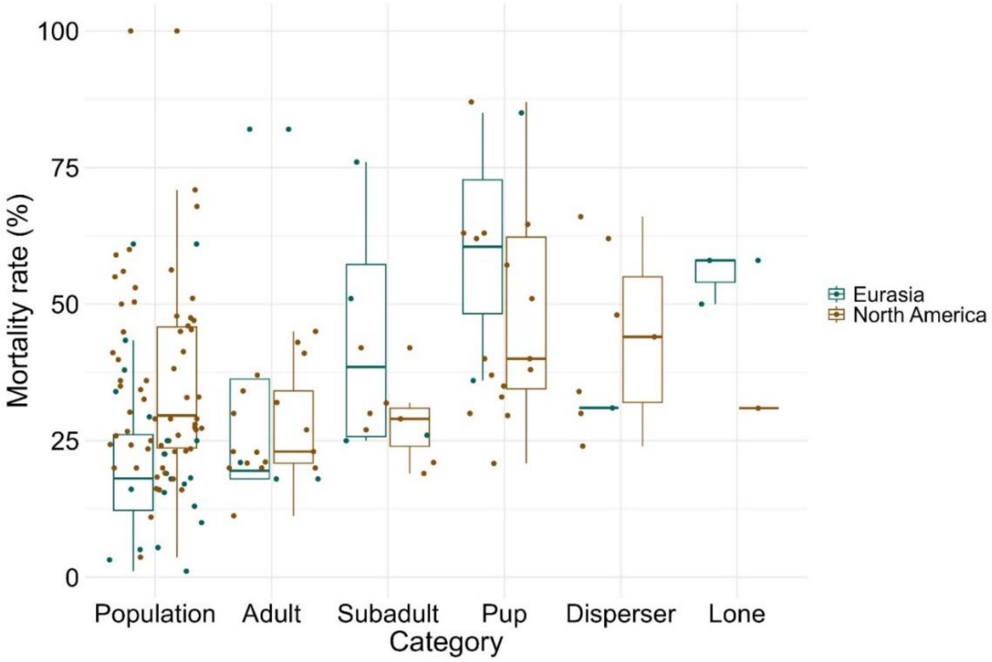


Fig. 1-8: Boxplots for mortality rate of the overall population, by age, and of dispersers and lone wolves. Ages are defined as: ‘pup’ ≤1 year old; ‘subadult’ 1–2 years old; and ‘adult’ ≥ 2 years old.

Dispersal: The median dispersal distance was 97 km in Eurasia and 225 km in North America (Sup. Fig. 3 in Appendix I). The longest dispersal distances were recorded in Scandinavia (1092 km), Minnesota (886 km) and Alberta (670 km) (Fritts, 1983; Van Camp and Gluckie, 1979; Wabakken et al., 2007). Several dispersal distances beyond 200 km have also been recorded in Central-Eastern Europe, including Ukraine, Belarus, Croatia, Russia, Slovenia and Austria (Byrne et al., 2018; Kirilyuk et al., 2020; Ražen et al., 2016). Dispersal distances of wolves in Spain, Poland, Portugal and Israel were shorter than 50 km (Blanco and Cortés, 2007; Gula et al., 2009; Reichmann and Saltz, 2005; Rio-Maior et al., 2016). Few studies in Eurasia included information on dispersal duration or distance by age. Dispersal probability was highest for subadults (55%), followed by adults (15%) and pups (9%)(Fuller, 1989; Gese and Mech, 1991; Jimenez et al., 2017; Kojola et al., 2006; Peterson et al., 1984; Schmidt et al., 2017) (Sup. Fig. 3 in Appendix I).

***Recruitment, migration, and pack dynamics*:** The definition of recruitment in demography is the process by which new individuals are added to a population by birth and maturation or by immigration. In some wolf studies, this term is used only for the number of pups in packs (Ausband et al., 2015; Klaczek et al., 2016; Parker and Luttich, 1986). However, the number of studies considering birth and immigration is increasing, with annual recruitment rates between 25% and 65% in studies conducted in the USA, France, Norway and Sweden (Adams et al., 2011; Bischof et al., 2020; Marescot et al., 2011; Webb et al., 2011). Recent studies based on the movement of individuals through GPS-collaring or non-invasive sampling have allowed the obtention of migration rates between wolf areas, populations, or genetic clusters. These migration rates can be determined in different ways, such as the proportion of individuals that immigrate or emigrate (Adams et al., 2008; Cubaynes et al., 2014; Hefner and Geffen, 1999; Jansson et al., 2012; Peterson et al., 1984; Reichmann and Saltz, 2005; Rick et al., 2017; Webb et al., 2011) or the number immigrants or emigrants per generation (Aspi et al., 2009; Pletscher et al., 1997). Very few studies included information on the dynamics of individuals in packs, such as pair bond duration, the probability of adoption, budding, and pack dissolvement (Ausband, 2019; Borg et al., 2015; Milleret et al., 2017).

1.3. Knowledge gaps

Several long-term wolf monitoring studies have been conducted, especially in wild areas of North America. However, in the human-dominated landscapes of Eurasia, most wolf studies are based on a few years of monitoring and studies on population dynamics may not reflect the actual fluctuation of populations. Although species distribution models have been developed for several countries in Europe, dynamic occupancy models based on long-term surveys and accounting for the observation process are still lacking. With such models, additional information on colonization, extinction, and occupancy probabilities can offer more insights into wolf spatial dynamics in human-dominated landscapes. Additionally, it would allow the identification of areas for potential recolonization and areas to prioritize conflict mitigation measures.

The selection of sites for wolf reproduction has often been evaluated in North America and Eurasia (Sazatornil et al., 2016), and several studies have included some assessment of human disturbance on homesites to understand wolf behaviour and tolerance towards human presence. Nevertheless, it is yet to be understood if exposure risk levels at homesites affect the number of adults and pups in packs. Although wolves can have a high tolerance to humans in human-dominated landscapes, the degree to which human disturbance affects pack sizes and, consequently, its demography, is yet to be explored.

According to the literature review of peer-reviewed journals on wolf population dynamics and demography, we identified several knowledge gaps. Overall, studies on wolf monitoring assessing population dynamics and demographic parameters have been increasing in the last twenty years but not equitably distributed throughout the wolf range. Most of the studies were conducted in North America and some European countries (e.g., Italy, Poland, Sweden, Norway and Finland), but information is lacking in most countries of Eurasia. As a social and elusive species, wolf monitoring is usually pack-based, not individual-based. Thus, pack size and number of individuals by age are the parameters for which more information is available. Consequently, growth rate and wolf density are obtained mainly by monitoring resident individuals, which may result in underestimating the number of individuals in the population. Studies assessing individual-level parameters are increasingly achievable with new technologies, such as GPS-collaring and molecular analysis of non-invasive sampling, combined with new statistical approaches to obtain reliable estimates. For example, capture-recapture models based on non-invasive genetic sampling have increasingly been used to obtain demographic parameters (López-Bao et al., 2018b; Marucco et al., 2012), and GPStagged animals offer reliable data on survival rates and mortality causes (Andrén et al., 2006; Mills et al., 2008; Treves et al., 2017) though most often these data are considered independently. Integrated population models have been progressively developed for several species to obtain higher accuracy and precision in estimating demographic parameters, even those for which there is no direct information (Bled et al., 2017; Schaub and Abadi, 2011). These models have enormous potential to identify the most critical limiting factors affecting wildlife and to help managers make informed decisions about population management (Arnold et al., 2018). Such methodologies at a large scale would better understand the wolf population status, identify limiting factors affecting wolf demographic parameters, and identify the main issues related to conflicts with humans. Moreover, in areas where wolves are recolonizing, the estimates of demographic parameters can be very different from areas where wolf populations are stable. Thus, parameters related to age structure, sex ratio, and dispersal or mortality rates need additional focus in future studies, particularly in human-dominated landscapes.

Molecular individual identification of non-invasive samples is often used to estimate several population dynamic parameters, including population size or dispersal distances. Recently, such tools, combined with structure and genealogy analysis, have allowed to determine further relevant parameters to understand wolf population dynamics better. For instance, Sweden and Norway have one of the best examples of continuous molecular monitoring of the wolf population, with most individuals identified and known genealogies. Continuous effort on non-invasive population surveys has made possible the estimation of further estimates, such as effective population size (i.e. the number of individuals in the population that reproduce), pack structure, probability of dispersal, adoption, budding or migration. In such a complex and dynamic system, these estimates allow one to understand better the spatial, temporal, and social dynamics of wolf populations, particularly in human-dominated landscapes, where anthropogenic mortality often disrupts population and pack dynamics.

Wolf mortality causes and overall mortality rates have frequently been assessed for wolf populations in North America, and such studies are often related to population management with wolf control (i.e. culling) or legal harvest. Studies on wolf mortality are also increasing in Europe based on opportunistically found dead wolves, molecular identification, and GPS-collaring. Nevertheless, cryptic mortality is thought to occur frequently in human-dominated landscapes, and wolf mortality rates are probably much higher than what has been obtained. Furthermore, although it is known that mortality rates are higher for younger individuals, studies on mortality causes and rates of pups (particularly for pups less than two months old) and subadults are still lacking. In this regard, the generalization of the use of GPS-collars in Eurasia, like in North America during the last decades, will shed light on survival and the main causes of mortality of wolves across different human-dominated landscapes.

Further studies with GPS-collaring and population genetic structure are needed to determine migration rates between monitored populations to know the success of migratory and dispersal events, particularly for expanding populations in human-dominated landscapes. Studies assessing parameters on pack dynamics through individual identification and social dynamics are still lacking. Additional studies assessing parameters such as pair bond duration, the probability of adoption, budding, and pack dissolvement are needed to understand further the social dynamics of packs and wolf populations (Sand et al., 2022).

Finally, additional information on several demographic parameters assessed here is often available in grey literature. Such information may also be valuable for obtaining improved estimates and should be considered for future studies when a detailed description of the used methodologies is presented.

1.4. Objectives

In this thesis, I aim to contribute to understanding the impact of anthropogenic and environmental factors on large carnivore persistence in human-dominated landscapes. I use wolf as a model species to address how different humanization levels can influence wolf habitat selection and ultimately affect their demography, status and viability. By investigating wolf occupancy, reproduction site selection, population dynamics, and demography, this thesis fills in some of the knowledge gaps identified in the literature review described in the previous section.

This research contributes to large carnivore conservation by providing additional knowledge on the populations and proposing management and conservation actions for areas with different levels of suitability for wolves.

The main research objectives of this thesis were defined as:

1. To estimate species dynamic occupancy probabilities to better understand the spatial structure of wolf populations and identify potential areas of recolonization in human-dominated landscapes;

2. To evaluate homesite selection in areas with different human disturbance levels and to infer the influence of exposure risk at homesite on pack size;
3. To provide insights into the population dynamics of a wolf population in human-dominated landscapes;
4. To estimate demographic parameters based on an integrated population model and address the status and viability of a wolf population in a human-dominated landscape.

1.5. The study system: Iberian wolf

We conducted our research in the human-dominated landscapes of Portugal and Spain. We gathered information obtained from several study areas within the Iberian wolf range, including Alto Minho, South of Douro, Vila Real, Asturias, Galicia, Castilla y Léon, and Cantabria (Fig. 1-9). The Iberian wolf population is distributed in the Northwestern Iberian Peninsula and is isolated from the remaining European wolf populations (Chapron et al., 2014; Silva et al., 2020).

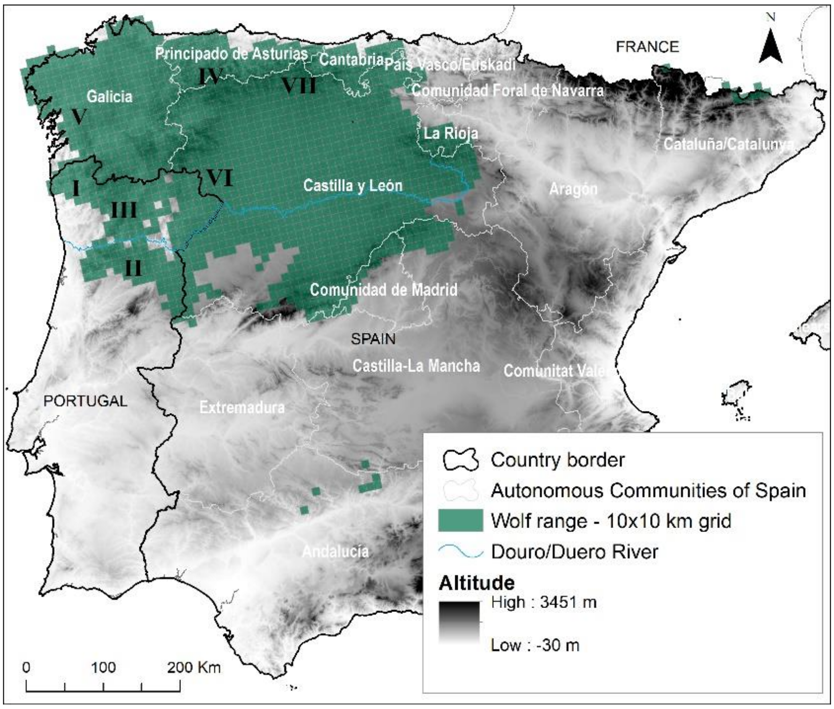


Fig. 1-9: Current wolf distribution in the Iberian Peninsula (adapted from Kaczensky, 2018) and location of the study areas where wolf surveys were conducted and included in this thesis: in Portugal: I- Alto Minho, II- South of Douro, and III- Vila Real; and Spain: IV- Asturias, V- Galicia; VI- Western Castilla y León, and VII – Picos de Europa (Asturias, Castilla y León and Cantabria).

The intense persecution at the beginning of the 20th century resulted in a severe decline in the Iberian wolf population (Nores and López-Bao, 2022; Valverde, 1972), but in recent decades, the population recovered, and range expansion occurred in a few areas of Spain (Blanco and Cortés, 2009; López-Bao et al., 2018a). The population is approximately 2,000 individuals in at least 350 packs (Blanco and Cortés, 2012; Chapron et al., 2014; MAGRAMA, 2016; Pimenta et al., 2005). The Iberian wolf has been strictly protected by law since 2021 in Spain and 1988 in Portugal. In Spain, the wolf population in the South of Douro River is listed in Annex IV of the EU Habitats Directive, whereas north of Duero River wolves is listed in Annex V. Widespread wolf predation on livestock is one of the major sources of conflict between wolves and humans in Iberia (Lagos and Bárcena, 2018; Pimenta et al., 2017). Despite the protection status, the Iberian wolf is subject to frequent illegal killing, which is probably a major source of wolf mortality, although not properly quantified (Rio-Maior et al., 2018). The deficient protection of domestic animals in general and the lack of wild prey in some particular contexts are probably the leading causes of such levels of intense livestock depredation (Pimenta et al., 2018; Singer et al., 2023; Torres et al., 2015; Vos, 2000). In this context, the Iberian wolf is an excellent example of species persistence in human-dominated landscapes.

Several genetic clusters have been identified within the Iberian wolf population, which are highly structured and have low dispersal rates between them (Silva et al., 2018). Such a structured population may result from highly human-dominated landscapes, with high anthropogenic mortality rates translating into high turn-over rates within packs and hampering successful dispersal and further population expansion. Although some studies have been conducted in Iberia to predict areas of higher habitat suitability for wolves (e.g., Grilo et al., 2018; Llaneza et al., 2012; Rio-Maior et al., 2019; Sazatornil et al., 2016), research based on long-term surveys is needed. The general distribution of the Iberian wolf is known based on disarticulated local studies or census surveys based on information from short time frames (e.g., Eggermann et al., 2011; Llaneza and Blanco, 2005; Pimenta et al., 2005), which may not reflect the true potential for species and breeding occupancy. Furthermore, the combination of such surveys with population dynamics and the estimation of demographic parameters is still lacking for Iberia. According to the literature review (Section 1.2.2.), some information on the sex ratio of wolf populations was available, based on non-invasive genetic sampling, captured wolves for collaring, or historical bounty information of harvested wolves (Blanco et al., 2005; Blanco and Cortés, 2007; Echegaray and Vilà, 2010; Fernández and Ruiz de Azua, 2010; Silva et al., 2018). Some studies with the proportion of residents were also available, with populations comprising between 73 and 97% of residents (Blanco and Cortés, 2007; López-Bao et al., 2018b; Silva et al., 2018). Wolf population density in Iberia is only known for a few areas in Spain, ranging between 10 and 26 wolves/1000 km^2 (Blanco and Cortés, 2007; López-Bao et al., 2018), and there was some information on the number of individuals in packs, including pack sizes, litter sizes, and the number of pups and adults (Fernández-Gil et al., 2020; Fernández and Ruiz de Azua, 2010; Llaneza et al., 2023, 2005). Beyond that, few studies with sex ratio by age, the mortality rate of the population and dispersers or lone wolves, dispersal duration by age, dispersal distance, and age structure of the population were available (Blanco and Cortés, 2007; Campos, 2018; Fernández and Ruiz de Azua, 2010; Rio-Maior et al., 2016). Information was utterly unavailable for the remnant parameters assessed in the literature review, such as population growth rates, mortality rate by age, sex ratio in packs or dispersal probabilities. The knowledge gaps identified for Portugal and Spain are similar to those of other countries in Eurasia. This thesis aims to fill in some of these gaps and propose executable measures to improve wolf conservation in human-dominated landscapes.

1.6. Thesis organization

I have organized the thesis into seven chapters:

The current **Chapter I** provides a general introduction to large carnivore conservation, particularly wolves. It includes a description of the current worldwide wolf population conservation status, a general literature review, and knowledge gaps on wolf population dynamics and demography. This chapter also describes the study system used in Chapters II and III, which include the Iberian wolf as a model species in several study sites of the Iberian Peninsula; and in Chapters IV and V, which focus on wolves in Alto Minho, in NW Portugal. Finally, Chapter I also highlights the main research aims of the thesis.

Chapter II includes a study on wolf occupancy in Iberia, based on transects of sign surveys conducted between 2005 and 2022 in four areas (Alto Minho, South of Douro, Vila Real, and Asturias). Based on the probabilities of colonization, extinction and occupancy obtained by the dynamic occupancy model and the two last known wolf distributions in Iberia, we propose priority areas for implementing conservation actions and mitigation measures.

Chapter III evaluates the relationship between anthropogenic disturbance, environmental resources, and intraspecific competition at wolf homesites and territories and the observed number of pups and adults from several packs of Iberia detected between 1986 and 2021. We used the observed number of individuals as a proxy of survival, when controlled by several covariates.

Chapter IV includes the main results of the wolf monitoring in Alto Minho, divided into two periods (A: 1996-2005 and B: 2007-2016). This chapter presents several general population dynamic parameters, such as the annual number of packs, average pack sizes, and number of adults and pups. It describes the extinction of packs in period A and their recovery in period B through source-sink dynamics.

Chapter V includes a study on the demography of wolves in Alto Minho between 2007 and 2019. In this chapter, I develop an integrated population model to estimate the number of individuals and growth rates with higher precision than naïve counts and survival rates of adults and pups. This chapter also includes a population viability analysis for this wolf population under different scenarios until 2029.

Chapter VI recapitulates the main conclusions of this thesis and future directions to wolf conservation in human-dominated landscapes by improving the methods to obtain higher accuracy demographic parameters to inform managers and implement better-suited conservation strategies.

Chapter VII includes a synthesis of the thesis in French, with the objectives, main results, discussion, and a general conclusion.

1.7. References

Adams, J.R., Vucetich, L.M., Hedrick, P.W., Peterson, R.O., Vucetich, J.A., 2011. Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. Proc. R. Soc. B Biol. Sci. 278, 3336–3344. https://doi.org/10.1098/rspb.2011.0261

Adams, L.G., Farley, S.D., Stricker, C.A., Demma, D.J., Roffler, G.H., Miller, D.C., Rye, R.O., 2010. Are inland wolf–ungulate systems influenced by marine subsidies of Pacific salmon? Ecol. Appl. 20, 251–262. https://doi.org/10.1890/08-1437.1

Adams, L.G., Stephenson, R.O., Dale, B.W., Ahgook, T.R., Demma, D.J., 2008. Population dynamics and harvest characteristics of wolves in the Central Brooks Range, Alaska. Wildl. Monogr. 170, 1–25. https://doi.org/10.2193/2008-012

Ahmadi, M., López-Bao, J.V., Kaboli, M., Lopez-Bao, J. V, Kaboli, M., López-Bao, J.V., Kaboli, M., 2014. Spatial heterogeneity in human activities favors the persistence of wolves in agroecosystems. PLoS One, 9, 1–10. https://doi.org/10.1371/journal.pone.0108080

Almarcha, F., Ferrández, T., López-Bao, J.V., 2022. Symbols, wolves and conflicts. Biol. Conserv. 275. https://doi.org/10.1016/j.biocon.2022.109756

Andrén, H., Linnell, J.D.C., Liberg, O., Andersen, R., Danell, A., Karlsson, J., Odden, J., Moa, P.F., Ahlqvist, P., Kvam, T., Franzén, R., Segerström, P., 2006. Survival rates and causes of mortality in Eurasian lynx (Lynx lynx) in multi-use landscapes. Biol. Conserv. 131, 23–32. https://doi.org/10.1016/j.biocon.2006.01.025

Araújo, M.B., Guisan, A., 2006. Five (or so) challenges for species distribution modelling. J. Biogeogr. 33, 1677–1688. https://doi.org/10.1111/j.1365-2699.2006.01584.x

Arbieu, U., Mehring, M., Bunnefeld, N., Kaczensky, P., Reinhardt, I., Ansorge, H., Böhning-Gaese, K., Glikman, J.A., Kluth, G., Nowak, C., Müller, T., 2019. Attitudes towards returning wolves (*Canis lupus*) in Germany: Exposure, information sources and trust matter. Biol. Conserv. 234, 202–210. https://doi.org/10.1016/j.biocon.2019.03.027

Argue, A.M., Mills, K.J., Patterson, B.R., 2008. Behavioural response of eastern wolves (Canis lycaon) to disturbance at homesites and its effects on pup survival. Can. J. Zool. 86, 400–406. https://doi.org/10.1139/Z08-013

Arnold, T.W., Clark, R.G., Koons, D.N., Schaub, M., 2018. Integrated population models facilitate ecological understanding and improved management decisions. J. Wildl. Manage. 82, 266–274. https://doi.org/10.1002/jwmg.21404

Aspi, J., Roininen, E., Kiiskilä, J., Ruokonen, M., Kojola, I., Bljudnik, L., Danilov, P., Heikkinen, S., Pulliainen, E., 2009. Genetic structure of the northwestern Russian wolf populations and gene flow between Russia and Finland. Conserv. Genet., 10, 815–826. https://doi.org/10.1007/s10592-008-9642-x

Ausband, D.E., 2022. Offspring sex ratios are male-biased reflecting sex-biased dispersal in Idaho, USA, wolves. Behav. Ecol. Sociobiol. 76, 134. https://doi.org/10.1007/s00265-022-03243-0

Ausband, D.E., 2019. Pair bonds, reproductive success, and rise of alternate mating strategies in a social carnivore. Behav. Ecol. 30, 1618–1623. https://doi.org/10.1093/beheco/arz126

Ausband, D.E., 2018. Multiple breeding individuals within groups in a social carnivore. J. Mammal. 99, 836–844. https://doi.org/10.1093/jmammal/gyy051

Ausband, D.E., Stansbury, C.R., Stenglein, J.L., Struthers, J.L., Waits, L.P., 2015. Recruitment in a social carnivore before and after harvest. Anim. Conserv., 18, 415–423. https://doi.org/10.1111/acv.12187

Baker, P.J., Boitani, L., Harris, S., Saunders, G., White, P.C., 2008. Terrestrial carnivores and human food production: impact and management. Mamm. Rev. 38, 123–166. https://doi.org/10.1111/j.1365-2907.2008.00122.x

Baliauskas, L., Kawata, Y., Balčiauskas, L., Kawata, Y., Baliauskas, L., Kawata, Y., 2009. Estimation of carrying capacity and growth rate of wolf in Lithuania. Acta Zool. Litu., 19, 79–84. https://doi.org/10.2478/v10043-009-0018-3

Ballard, W.B., Ayres, L.A., Krausman, P.R., Reed, D.J., Fancy, S.G., 1997. Ecology of wolves in relation to a migratory Caribou Herd in Northwest Alaska. J. Wildl. Manage. 61, 1–47. https://doi.org/10.2307/3830776

Ballard, W.B., Whitman, J.S., Gardner, L, C., 1987. Ecology of an exploited wolf population in south-central Alaska. Wildl. Monogr. 98, 3–54.

Bangs, E.E., Fritts, S.H., Fontaine, J.A., Smith, D.W., Murphy, K.M., Mack, C.M., Niemeyer, C.C., 1998. Status of gray wolf restoration in Montana, Idaho, and Wyoming. Wildl. Soc. Bull., 26, 785–798.

Behr, D.M., Ozgul, A., Cozzi, G., 2017. Combining human acceptance and habitat suitability in a unified socio-ecological suitability model: a case study of the wolf in Switzerland. J. Appl. Ecol., 54, 1919–1929. https://doi.org/10.1111/1365-2664.12880

Bergerud, A.T., Elliot, J.P., 1986. Dynamics of caribou and wolves in northern British Columbia. Can. J. Zool. 64, 1515–1529. https://doi.org/10.1139/z86-226

Bischof, R., Milleret, C., Dupont, P., Chipperfield, J., Tourani, M., Ordiz, A., de Valpine, P., Turek, D., Royle, J.A., Gimenez, O., Flagstad, Ø., Åkesson, M., Svensson, L., Brøseth, H., Kindberg, J., 2020. Estimating and forecasting spatial population dynamics of apex predators using transnational genetic monitoring. Proc. Natl. Acad. Sci. 117, 30531–30538. https://doi.org/10.1073/pnas.2011383117

Bjorge, R.R., Gunson, J.R., 1985. Evaluation of Wolf Control to Reduce Cattle Predation in Alberta. J. Range Manag. 38, 483. https://doi.org/10.2307/3899736

Blanco, J.C., Cortés, Y., 2012. Surveying wolves without snow: a critical review of the methods used in Spain. Hystrix 23, 35–48. https://doi.org/10.4404/hystrix-23.1-4670

Blanco, J.C., Cortés, Y., 2009. Ecological and social constraints of wolf recovery in Spain, in: Musiani, M., Boitani, L., Paquet, P.C. (Eds.), A New Era for Wolves and People: Wolf Recovery, Human Attitudes, and Policy. University of Calgary Press, Calgary, pp. 41–66.

Blanco, J.C., Cortés, Y., 2007. Dispersal patterns, social structure and mortality of wolves living in agricultural habitats in Spain. J. Zool. 273, 114–124. https://doi.org/10.1111/j.1469-7998.2007.00305.x

Blanco, J.C., Cortés, Y., Virgós, E., 2005. Wolf response to two kinds of barriers in an agricultural habitat in Spain. Can. J. Zool. 83, 312–323. https://doi.org/10.1139/z05-016

Blanco, J.C., Reig, S., De La Cuesta, L., 1992. Distribution, status and conservation problems of the wolf *Canis lupus* in Spain. Biol. Conserv. 60, 73–80. https://doi.org/10.1016/0006-3207(92)91157-N

Bled, F., Belant, J.L., Van Daele, L.J., Svoboda, N., Gustine, D., Hilderbrand, G., Barnes, V.G., 2017. Using multiple data types and integrated population models to improve our knowledge of apex predator population dynamics. Ecol. Evol. 7, 9531–9543. https://doi.org/10.1002/ece3.3469

Boertje, R.D., Valkenburg, P., McNay, M.E., 1996. Increases in Moose, Caribou, and Wolves Following Wolf Control in Alaska. J. Wildl. Manage. 60, 474. https://doi.org/10.2307/3802065

Boitani, L., 1995. Ecological and cultural diversities in the evolution of wolf-human relationships, in: Carbyn, L.N., Fritts, S.H., Seip, D.. (Eds.), Ecology and Conservation of Wolves in a Changing World. Canadian Circumpolar Institute, Edmonton, Canada, pp. 3–12.

Boitani, L., Kaczensky, P., Alvares, F., Andrén, H., Balys, V., Blanco, J.C., Chapron, G., Chiriac, S., Cirovic, D., Drouet-Houguet, N., Groff, C., Huber, D., Iliopoulos, Y., Ionescu, O., Kojola, I., Krofel, M., Kutal, M., Linnell, J., Majic, A., Mannil, P., Marucco, F., D. Melovski, Mengüllüoğlu, D., Mergeay, J., Nowak, S., Ozolins, J., Perovic, A., Rauer, G., Reinhardt, I., Rigg, R., Salvatori, V., Sanaja, B., Schley, L., Shkvyria, M., Sunde, P., Tirronen, K., Trajce, A., Trbojevic, I., Trouwborst, A., Arx, M. von, Wolfl, M., Zlatanova, D., Patkó, L., 2022. Assessment of the conservation status of the Wolf (*Canis lupus*) in Europe.

Boitani, L., Phillips, M., Jhala, Y., 2018. *Canis lupus* (errata version published in 2020). The IUCN Red List of Threatened Species 2018, The IUCN Red List of Threatened Species 2018.

Borg, B.L., Brainerd, S.M., Meier, T.J., Prugh, L.R., 2015. Impacts of breeder loss on social structure, reproduction and population growth in a social canid. J. Anim. Ecol. 84, 177–187. https://doi.org/10.1111/1365-2656.12256

Boyd, D.K., Pletscher, D.H., 1999. Characteristics of Dispersal in a Colonizing Wolf Population in the Central Rocky Mountains. J. Wildl. Manage. 63, 1094. https://doi.org/10.2307/3802828

Byrne, M.E., Webster, S.C., Lance, S.L., Love, C.N., Hinton, T.G., Shamovich, D., Beasley, J.C., 2018. Evidence of long-distance dispersal of a gray wolf from the Chernobyl Exclusion Zone. Eur. J. Wildl. Res., 64, 1-5. https://doi.org/10.1007/s10344-018-1201-2

Campos, R., 2018. Survival and Population Dynamics of the Iberian Wolf in Portugal. MSc Thesis. Universidade do Porto.

Caniglia, R., Fabbri, E., Galaverni, M., Milanesi, P., Randi, E., 2014. Noninvasive sampling and genetic variability, pack structure, and dynamics in an expanding wolf population. J. Mammal. 95, 41–49. https://doi.org/10.1644/13-MAMM-A-039

Capitani, C., Mattioli, L., Avanzinelli, E., Gazzola, A., Lamberti, P., Mauri, L., Scandura, M., Viviani, A., Apollonio, M., 2006. Selection of rendezvous sites and reuse of pup raising areas among wolves *Canis lupus* of north-eastern Apennines , Italy. Acta Theriol. (Warsz). 51, 395–404.

Cardillo, M., Mace, G.M., Jones, K.E., Bielby, J., Bininda-Emonds, O.R.P., Sechrest, W., Orme, C.D.L., Purvis, A., 2005. Evolution: Multiple causes of high extinction risk in large mammal species. Science (80-.). 309, 1239–1241. https://doi.org/10.1126/science.1116030

Carricondo-Sanchez, D., Zimmermann, B., Wabakken, P., Eriksen, A., Milleret, C., Ordiz, A.A., Sanz-Perez, A., Wikenros, C., 2020. Wolves at the door? Factors influencing the individual behavior of wolves in relation to anthropogenic features. Biol. Conserv., 244. https://doi.org/10.1016/j.biocon.2020.108514

Carter, N.H., Bruskotter, J.T., Vucetich, J., Crabtree, R., Jaicks, H., Karns, G., Nelson, M.P., Smith, D., Linnell, J.D.C., 2019. Towards Human–Wildlife Coexistence through the Integration of Human and Natural Systems, in: Human-Wildlife Interact: Turning Conflict into Coexistence. Cambridge University Press, College of Innovation and Design, Boise State University, Boise, ID, United States and School of Environment and Natural Resources, The Ohio State University, Columbus, OH, United States and School of Forest Resources and Environmental Sciences, Michigan, pp. 384–413. https://doi.org/10.1017/9781108235730.021

Carter, N.H., Linnell, J.D.C., 2016. Co-Adaptation Is Key to Coexisting with Large Carnivores. Trends Ecol. Evol. 31, 575–578. https://doi.org/10.1016/j.tree.2016.05.006

Cassidy, K.A., MacNulty, D.R., Stahler, D.R., Smith, D.W., Mech, L.D., 2015. Group composition effects on aggressive interpack interactions of gray wolves in Yellowstone National Park. Behav. Ecol., 26, 1352–1360. https://doi.org/10.1093/beheco/arv081

Chapron, G., Kaczensky, P., Linnell, J.D.C., von Arx, M., Huber, D., Andren, H., López-Bao, J.V., Adamec, M., Álvares, F., Anders, O., Balciauskas, L., Balys, V., Bed, P., Bego, F., Blanco, J.C., Breitenmoser, U., Broseth, H., Bufka, L., Bunikyte, R., Ciucci, P., Dutsov, A., Engleder, T., Fuxjager, C., Groff, C., Holmala, K., Hoxha, B., Iliopoulos, Y., Ionescu, O., Jeremi, J., Jerina, K., Kluth, G., Knauer, F., Kojola, I., Kos, I., Krofel, M., Kubala, J., Kunovac, S., Kusak, J., Kutal, M., Liberg, O., Maji, A., Mannil, P., Manz, R., Marboutin, E., Marucco, F., Melovski, D., Mersini, K., Mertzanis, Y., Mysłajek, R.W., Nowak, S., Odden, J., Ozolins, J., Palomero, G., Paunovi, M., Persson, J., Poto nik, H., Quenette, P.-Y., Rauer, G., Reinhardt, I., Rigg, R., Ryser, A., Salvatori, V., Skrbin ek, T., Stojanov, A., Swenson, J.E., Szemethy, L., Trajce, A., Tsingarska-Sedefcheva, E., Va a, M., Veeroja, R., Wabakken, P., Wolfl, M., Wolfl, S., Zimmermann, F., Zlatanova, D., Boitani, L., 2014. Recovery of large carnivores in Europe’s modern human-dominated landscapes. Science (80-.). 346, 1517–1519. https://doi.org/10.1126/science.1257553

Chapron, G., López-Bao, J.V., 2016. Coexistence with Large Carnivores Informed by Community Ecology. Trends Ecol. Evol. 31, 578–580. https://doi.org/10.1016/j.tree.2016.06.003

Chapron, G., Wikenros, C., Liberg, O., Wabakken, P., Flagstad, Ø., Milleret, C., Månsson, J., Svensson, L., Zimmermann, B., Åkesson, M., Sand, H., 2016. Estimating wolf (*Canis lupus*) population size from number of packs and an individual based model. Ecol. Modell. 339, 33–44. https://doi.org/10.1016/j.ecolmodel.2016.08.012

Ciucci, P., Boitani, L., 1998. Wolf and dog depredation on livestock in central Italy. Wildl. Soc. Bull. 26, 504–514.

Ciucci, P., Boitani, L., Falco, M., Maiorano, L., 2018. Hierarchical, multi-grain rendezvous site selection by wolves in southern Italy. J. Wildl. Manage. 82, 1049–1061. https://doi.org/10.1002/jwmg.21440

Ciucci, P., Boitani, L., Francisci, F., Andreoli, G., 1997. Home range, activity and movements of a wolf pack in central Italy. J. Zool. 243, 803–819. https://doi.org/10.1111/j.1469-7998.1997.tb01977.x

Ciucci, P., Mancinelli, S., Boitani, L., Gallo, O., Grottoli, L., 2020. Anthropogenic food subsidies hinder the ecological role of wolves: Insights for conservation of apex predators in human-modified landscapes. Glob. Ecol. Conserv. 21, e00841. https://doi.org/10.1016/j.gecco.2019.e00841

Cluff, H., Murray, D., 1995. Review of wolf control methods in North America, in: Carbyn LN, Fritts, S., Seip, D. (Eds.), Ecology and Conservation of Wolves in a Changing World. Canadian Circumpolar Institute, Occasional Publication no. 35, Edmonton, Canada, pp. 491–607.

Colorado Parks and Wildlife, 2020. Colorado Wolf Restoration and Management Plan. Denver, CO.

Corsi, F., Dupr, E., Boitani, L., Duprè, E., Boitani, L., Dupr, E., Boitani, L., 1999. A large-scale model of wolf distribution in Italy for conservation planning. Conserv. Biol. 13, 150–159. https://doi.org/10.1046/j.1523-1739.1999.97269.x

Creel, S., Becker, M., Christianson, D., Dröge, E., Hammerschlag, N., Hayward, M.W.W., Karanth, U., Loveridge, A., Macdonald, D.W.W., Matandiko, W., Rosenblatt, E., Schuette, P., Drge, E., Hammerschlag, N., Hayward, M.W.W., Karanth, U., Loveridge, A., Macdonald, D.W.W., Matandiko, W., M’Soka, J., Murray, D., Rosenblatt, E., Schuette, P., 2015. Questionable policy for large carnivore hunting : U.S. Wolf-hunting policies do not align with ecological theory or data. Science (80-.). 350, 1473–1475. https://doi.org/10.1126/science.aac4768

Cubaynes, S., MacNulty, D.R., Stahler, D.R., Quimby, K.A., Smith, D.W., Coulson, T.,

2014. Density-dependent intraspecific aggression regulates survival in northern Yellowstone wolves (*Canis lupus*). J. Anim. Ecol. 83, 1344–1356. https://doi.org/10.1111/1365-2656.12238

Dennehy, E., Llaneza, L., López-Bao, J.V., 2021. Contrasting wolf responses to different paved roads and traffic volume levels. Biodivers. Conserv. 30, 3133–3150. https://doi.org/10.1007/s10531-021-02239-y

Ditmer, M.A., Wittemyer, G., Breck, S.W., Crooks, K.R., 2022. Defining ecological and socially suitable habitat for the reintroduction of an apex predator. Glob. Ecol. Conserv. 38, e02192. https://doi.org/10.1016/j.gecco.2022.e02192

Echegaray, J., Vilà, C., 2010. Noninvasive monitoring of wolves at the edge of their distribution and the cost of their conservation. Anim. Conserv. 13, 157–161. https://doi.org/10.1111/j.1469-1795.2009.00315.x

Eggermann, J., Ferrão da Costa, G., Guerra, A.M., Kirchner, W.H., Petrucci-Fonseca, F., da Costa, G.F., Guerra, A.M., Kirchner, W.H., Petrucci-Fonseca, F., 2011. Presence of Iberian wolf (*Canis lupus signatus*) in relation to land cover, livestock and human influence in Portugal. Mamm. Biol. 76, 217–221. https://doi.org/10.1016/j.mambio.2010.10.010

Eklund, A., López-Bao, J.V., Tourani, M., Chapron, G., Frank, J., 2017. Limited evidence on the effectiveness of interventions to reduce livestock predation by large carnivores. Sci. Rep. 7, 2097. https://doi.org/10.1038/s41598-017-02323-w

Elofsson, K., Häggmark, T., 2021. The impact of lynx and wolf on roe deer hunting benefits in Sweden. Environ. Econ. Policy Stud. 23, 683–719. https://doi.org/10.1007/s10018-020-00299-y

Epstein, Y., López-Bao, J.V., Chapron, G., 2016. A Legal-Ecological Understanding of Favorable Conservation Status for Species in Europe. Conserv. Lett. 9, 81–88. https://doi.org/10.1111/conl.12200

Fabbri, E., Miquel, C., Lucchini, V., Santini, A., Caniglia, R., Duchamp, C., Weber, J.-M., Lequett, B., Marucco, F., Boitani, L., Fumagali, L., Taberlet, P., Randi, E., 2007. From the Apennines to the Alps: colonization genetics of the naturally expanding Italian wolf (*Canis lupus*) population. Mol. Ecol., 16, 1661–1671. https://doi.org/10.1111/j.1365-294X.2007.03262.x

Fabbri, E., Velli, E., D'Amico, F., Galaverni, M., Mastrogiuseppe, L., Mattucci, F., Caniglia, R., 2018. From predation to management: monitoring wolf distribution and understanding depredation patterns from attacks on livestock. Hystrix-Italian J. Mammal., 29, 101. https://doi.org/10.4404/hystrix-00070-2018

Falcucci, A., Maiorano, L., Tempio, G., Boitani, L., Ciucci, P., 2013. Modeling the potential distribution for a range-expanding species: Wolf recolonization of the Alpine range. Biol. Conserv. 158, 63–72. https://doi.org/10.1016/j.biocon.2012.08.029

Fechter, D., Storch, I., 2014. How many wolves (*Canis lupus*) fit into Germany? The role of assumptions in predictive rule-based habitat models for habitat generalists. PLoS One 9. https://doi.org/10.1371/journal.pone.0101798

Fernández-Gil, A., Quevedo, M., Barrientos, L.M., Nuño, A., Naves, J., de Gabriel, M., Ordiz, A., Revilla, E., 2020. Pack size in humanized landscapes: the Iberian wolf population. Wildlife Biol., 2020. https://doi.org/10.2981/wlb.00594

Fernández, J.M., Ruiz de Azua, N., 2010. Historical dynamics of a declining wolf population: persecution vs. prey reduction. Eur. J. Wildl. Res. 56, 169–179. https://doi.org/10.1007/s10344-009-0300-5

Frame, P.F., Cluff, H.D., Hik, D.S., 2008. Wolf reproduction in response to caribou migration and industrial development on the central barrens of Mainland Canada. Arctic 61, 134–142.

Frame, P.F., Dean Cluff, H., Hik, D.S., 2007. Response of Wolves to Experimental Disturbance at Homesites. J. Wildl. Manage. 71, 316–320. https://doi.org/10.2193/2005-744

Frank, L., Woodroffe, R., 2001. Behaviour ofcarnivores in exploited and controlled populations. Carnivore Conservation, in: Gittleman, J.L., Funk, S.M., Macdonald, D.W., Wayne, R.K. (Eds.), Carnivore Conservation. Cambridge University Press, Cambridge, pp. 419–442.

Fritts, S.H., 1983. Record Dispersal by a Wolf from Minnesota. J. Mammal., 64, 166–167. https://doi.org/10.2307/1380772

Fritts, S.H., Bangs, E.E., Fontaine, J.A., Johnson, M.R., Phillips, M.K., Koch, E.D., Gunson, J.R., 1997. Planning and implementing a reintroduction of wolves to Yellowstone National Park and central Idaho. 5.

Fritts, S.H., Carbyn, L.N., 1995. Population viability, nature reserves, and the outlook for gray wolf conservation in North America. Restor. Ecol. 3, 26–38. https://doi.org/10.1111/j.1526-100X.1995.tb00072.x

Fritts, S.H., Mech, L.D., Roberts, K., 1981. Dynamics, movements and feeding ecology of a newly protected wolf population in northwestern Minnesota. Wildl. Monogr. 80, 3–79.

Fuller, T.K., 1989. Population dynamics of wolves in north-central Minnesota. Wildl. Monogr. 105, 1–41.

Fuller, T.K., Berg, W.E., Radde, G.L., Lenarz, M.S., Joselyn, G.., 1992. A history and current estimate of wolf distribution and numbers in Minnesota. Wildl. Soc. Bull. 20, 42–55. https://doi.org/10.2307/3782759

Gaynor, K.M., Hojnowski, C.E., Carter, N.H., Brashares, J.S., 2018. The influence of human disturbance on wildlife nocturnality. Science (80-.). 360, 1232–1235. https://doi.org/10.1126/science.aar7121

Gese, E.M., Mech, L.D., 1991. Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969–1989. Can. J. Zool. 69, 2946–2955. https://doi.org/10.1139/z91-415

Glenz, C., Massolo, A., Kuonen, D., Schlaepfer, R., 2001. A wolf habitat suitability prediction study in Valais (Switzerland). Landsc. Urban Plan. 55, 55–65. https://doi.org/10.1016/s0169-2046(01)00119-0

Graham, K., Beckerman, A.P., Thirgood, S., 2005. Human–predator–prey conflicts: ecological correlates, prey losses and patterns of management. Biol. Conserv. 122, 159–171. https://doi.org/10.1016/j.biocon.2004.06.006

Grilo, C., Lucas, P.M., Fernández-Gil, A., Seara, M., Costa, G., Roque, S., Rio-Maior, H., Nakamura, M., Álvares, F., Petrucci-Fonseca, F., Revilla, E., 2018. Refuge as major habitat driver for wolf presence in human-modified landscapes. Anim. Conserv. 22, 59–71. https://doi.org/10.1111/acv.12435

Grilo, C., Moço, G., Cândido, A.T., Alexandre, A.S., Petrucci-Fonseca, F., 2002. Challenges for the recovery of the Iberian wolf in the Douro river south region. Rev. Biol. 20, 121–133.

Gude, J.A., Mitchell, M.S., Russell, R.E., Sime, C.A., Bangs, E.E., Mech, L.D., Ream, R.R., 2012. Wolf population dynamics in the U.S. Northern Rocky Mountains are affected by recruitment and human-caused mortality. J. Wildl. Manage., 76.

Guisan, A., Thuiller, W., 2005. Predicting species distribution: Offering more than simple habitat models. Ecol. Lett. 8, 993–1009. https://doi.org/10.1111/j.1461-0248.2005.00792.x

Gula, R., Hausknecht, R., Kuehn, R., 2009. Evidence of wolf dispersal in anthropogenic habitats of the Polish Carpathian Mountains. Biodivers. Conserv. 18, 2173–2184. https://doi.org/10.1007/s10531-009-9581-y

Habib, B., Kumar, S., 2007. Den shifting by wolves in semi-wild landscapes in the Deccan Plateau, Maharashtra, India. J. Zool. 272, 259–265. https://doi.org/10.1111/j.1469-7998.2006.00265.x

Hamid, A., Mahmood, T., Fatima, H., Hennelly, L.M., Akrim, F., Hussain, A., Waseem, M., 2019. Origin, ecology and human conflict of gray wolf (*Canis lupus*) in Suleman Range, South Waziristan, Pakistan. Mammalia 83, 539–551. https://doi.org/10.1515/mammalia-2018-0167

Hausknecht, R., Szabó, Á., Firmánszky, G., Gula, R., Kuehn, R., 2010. Confirmation of wolf residence in Northern Hungary by field and genetic monitoring. Mamm. Biol., 75, 348–352. https://doi.org/10.1016/j.mambio.2009.10.001

Hayes, R.D., Farnell, R., Ward, R.M.P., Carey, J., Dehn, M., Kuzyk, G.W., Baer, A.M., Gardner, C.L., O'Donoghue, M., 2003. Experimental reduction of wolves in the Yukon: Ungulate responses and management implications, Wildlife Monographs.

Hayes, R.D., R, G.J., 1995. Status and management of wolves in Canada, in: Carbyn, L.N., Fritts, S.H., Seip, D.R. (Eds.), Ecology and Conservation of Wolves in a Changing World. pp. 21–33.

Hayes, R.D.D., Harestad, A.S.S., 2000. Demography of a recovering wolf population in the Yukon. Can. J. Zool. 78, 36–48. https://doi.org/10.1139/z99-186

Hedrick, P.W., Fredrickson, R.J., 2008. Captive breeding and the reintroduction of Mexican and red wolves. Mol. Ecol., 17, 344–350. https://doi.org/10.1111/j.1365-294X.2007.03400.x

Hefner, R., Geffen, E., 1999. Group Size and Home Range of the Arabian Wolf (*Canis lupus*) in Southern Israel. J. Mammal. 80, 611–619. https://doi.org/10.2307/1383305

Hosseini-Zavarei, F., Farhadinia, M.S., Beheshti-Zavareh, M., Abdoli, A., 2013. Predation by grey wolf on wild ungulates and livestock in central Iran. J. Zool. 290, 127–134. https://doi.org/10.1111/jzo.12022

Iliopoulos, Y., Sgardelis, S., Koutis, V., Savaris, D., 2009. Wolf depredation on livestock in central Greece. Mammal Res., 54, 11–22. https://doi.org/10.1007/BF03193133

Iliopoulos, Y., Youlatos, D., Sgardelis, S., 2014. Wolf pack rendezvous site selection in Greece is mainly affected by anthropogenic landscape features. Eur. J. Wildl. Res., 60, 23–34. https://doi.org/10.1007/s10344-013-0746-3

Imbert, C., Caniglia, R., Fabbri, E., Milanesi, P., Randi, E., Serafini, M., Torretta, E., Meriggi, A., 2016. Why do wolves eat livestock? Factors influencing wolf diet in northern Italy. Biol. Conserv. 195, 156–168. https://doi.org/10.1016/j.biocon.2016.01.003

IUCN, 2023. The IUCN Red List of Threatened species: Grey wolf (*Canis lupus*) [WWW Document]. URL https://www.iucnredlist.org/species/3746/247624660

Jaeger, J.A.G., Bowman, J., Brennan, J., Fahrig, L., Bert, D., Bouchard, J., Charbonneau, N., Frank, K., Gruber, B., Von Toschanowitz, K.T., 2005. Predicting when animal populations are at risk from roads: An interactive model of road avoidance behavior. Ecol. Modell. 185, 329–348. https://doi.org/10.1016/j.ecolmodel.2004.12.015

Jansson, E., Ruokonen, M., Kojola, I., Aspi, J., 2012. Rise and fall of a wolf population: genetic diversity and structure during recovery, rapid expansion and drastic decline. Mol. Ecol. 21, 5178–5193. https://doi.org/10.1111/mec.12010

Jędrzejewska, B., Jędrzejewski, W., Bunevich, A.N., Mikowski, L., Okarma, H., 1996. Population dynamics of wolves *Canis lupus* in Bialowieza primeval forest (Poland and Belarus) in relation to hunting by humans, 1847-1993. Mamm. Rev. 26, 103–126. https://doi.org/10.1111/j.1365-2907.1996.tb00149.x

Jedrzejewski, W., Jedrzejewska, B., Zawadzka, B., Borowik, T., Nowak, S., Mysłajek, R.W., Myslajek, R.W., Mysłajek, R.W., 2008. Habitat suitability model for Polish wolves based on long-term national census. Anim. Conserv. 11, 377–390. https://doi.org/10.1111/j.1469-1795.2008.00193.x

Jędrzejewski, W., Niedziałkowska, M., Mysłajek, R.W., Nowak, S., Jędrzejewska, B., 2005. Habitat selection by wolves *Canis lupus* in the uplands and mountains of southern Poland. Acta Theriol. (Warsz). 50, 417–428. https://doi.org/10.1007/BF03192636

Jimenez, M.D., Bangs, E.E., Boyd, D.K., Smith, D.W., Becker, S.A., Ausband, D.E., Woodruff, S.P., Bradley, E.H., Holyan, J., Laudon, K., 2017. Wolf dispersal in the Rocky Mountains, Western United States: 1993–2008. J. Wildl. Manage. 81, 581–592. https://doi.org/10.1002/jwmg.21238

Joslin, P.W.B., 1967. Movements and homesites of timber wolves in Algonquin Park. Am. Zool. 7, 279–288.

Kaartinen, S., Colpaert, A., Kojola, I., Colpaert, A., 2005. Finnish wolves avoid roads and settlements. Ann. Zool. Fennici 42, 523–532.

Kaartinen, S., Luoto, M., Kojola, I., 2010. Selection of den sites by wolves in boreal forests in Finland. J. Zool. 281, 99–104. https://doi.org/10.1111/j.1469-7998.2009.00678.x

Kabir, M., Hameed, S., Ali, H., Bosso, L., Din, J.U., Bischof, R., Redpath, S., Nawaz, M.A., 2017. Habitat suitability and movement corridors of grey wolf (*Canis lupus*) in Northern Pakistan. PLoS One 12, e0187027. https://doi.org/10.1371/journal.pone.0187027

Kaczensky, P., 2018. IUCN Red List Mapping for the regional assessment of the Wolf (*Canis lupus*) in Europe.

Karlsson, J., Brøseth, H., Sand, H., Andrén, H., Broseth, H., Sand, H., Andren, H., 2007. Predicting occurrence of wolf territories in Scandinavia. J. Zool. 272, 276–283. https://doi.org/10.1111/j.1469-7998.2006.00267.x

Karlsson, J., Sjöström, M., 2011. Subsidized Fencing of Livestock as a Means of Increasing Tolerance for Wolves. Ecol. Soc. 16, art16. https://doi.org/10.5751/ES-03878-160116

Kawata, Y., 2008. Estimation of Carrying Capacities of Large Carnivores in Latvia. Acta Zool. Litu., 18, 3–9. https://doi.org/10.2478/v10043-008-0001-4

Kéry, M., 2011. Towards the modelling of true species distributions. J. Biogeogr. 38, 617–618. https://doi.org/10.1111/j.1365-2699.2011.02487.x

Kéry, M., Gardner, B., Monnerat, C., 2010. Predicting species distributions from checklist data using site-occupancy models. J. Biogeogr. 37, 1851–1862. https://doi.org/10.1111/j.1365-2699.2010.02345.x

Kéry, M., Schaub, M., 2011. Bayesian population analysis using WinBUGS – a hierarchical perspective. Academic Press.

Khan, T.A., Ling, S.H., 2020. A survey of the state-of-the-art swarm intelligence techniques and their application to an inverse design problem. *Journal of Computational Electronics*, *19*(4), 1606-1628.

Kirilyuk, A., Kirilyuk, V.E., Ke, R., 2020. Long-distance dispersal of wolves in the Dauria ecoregion. Mammal Res., 65, 639–646. https://doi.org/10.1007/s13364-020-00515-w

Klaczek, M.R., Johnson, C.J., Cluff, H.D., 2016. Wolf–caribou dynamics within the central Canadian Arctic. J. Wildl. Manage., 80, 837–849. https://doi.org/10.1002/jwmg.1070

Kojola, I., Aspi, J., Hakala, A., Heikkinen, S., Ilmoni, C., Ronkainen, S., 2006. Dispersal in an expanding wolf population in Finland. J. Mammal., 87, 281–286. https://doi.org/10.1644/05-MAMM-A-061R2.1

Kojola, I., Huitu, O., Toppinen, K., Heikura, K., Heikkinen, S., Ronkainen, S., 2004. Predation on European wild forest reindeer (*Rangifer tarandus*) by wolves (*Canis lupus*) in Finland. J. Zool. 263, 229–235. https://doi.org/10.1017/S0952836904005084

Kojola, I., Kaartinen, S., Hakala, A., Heikkinen, S., Voipio, H.-M.H.-M., 2009. Dispersal behavior and the connectivity between wolf populations in Northern Europe. J. Wildl. Manage., 73, 309–313. https://doi.org/10.2193/2007-539

Kristan, W.B.I., 2003. The role of habitat selection behavior in population dynamics: source-sink systems and ecological traps. Oikos 103, 457–468. https://doi.org/10.1034/j.1600-0706.2003.12192.x

Kuijper, D.P.J.J., Churski, M., Trouwborst, A., Heurich, M., Smit, C., Kerley, G.I.H.H., Cromsigt, J.P.G.M.P.G.M., 2019. Keep the wolf from the door: How to conserve wolves in Europe's human-dominated landscapes? Biological Conservation, 235, 102-111, https://doi.org/10.1016/j.biocon.2019.04.004

Kumar, S., Rahmani, A.R., 2008. Predation by wolves (*Canis lupus pallipes*) on blackbuck (*Antilope cervicapra*) in the Great Indian Bustard Sanctuary, Nannaj, Maharashtra, India. Int. J. Ecol. Environ. Sci. 34, 99–112.

Lagos, L., Bárcena, F., 2018. Spatial variability in wolf diet and prey selection in Galicia (NW Spain). Mammal Res., 63, 125–139. https://doi.org/10.1007/s13364-018-0352-6

Lahoz-Monfort, J.J., Guillera-Arroita, G., Wintle, B.A., 2014. Imperfect detection impacts the performance of species distribution models. Glob. Ecol. Biogeogr. 23, 504–515. https://doi.org/10.1111/geb.12138

Lamb, C.T., Ford, A.T., McLellan, B.N., Proctor, M.F., Mowat, G., Ciarniello, L., Nielsen, S.E., Boutin, S., 2020. The ecology of human–carnivore coexistence. Proc. Natl. Acad. Sci. U. S. A. 117, 17876–17883. https://doi.org/10.1073/pnas.1922097117

Linkie, M., Dinata, Y., Nugroho, A., Haidir, I.A., 2007. Estimating occupancy of a data deficient mammalian species living in tropical rainforests: Sun bears in the Kerinci Seblat region, Sumatra. Biol. Conserv. 137, 20–27. https://doi.org/10.1016/j.biocon.2007.01.016

Linnell, J.D.C., Cretois, B., 2018. Research for AGRI Committee – The revival of wolves and other large predators and its impact on farmers and their livelihood in rural regions of Europe, European Parliament, Policy Department for Structural and Cohesion Policies, Brussels.

Linnell, J.D.C., Swenson, J.E., Andersen, R., 2001. Predators and people: conservation of large carnivores is possible at high human densities if management policy is favourable. Anim. Conserv. 4, 345–349. https://doi.org/10.1017/s1367943001001408

Llaneza, L., Blanco, J.C., 2005. Situación del lobo (*Canis lupus* L.) en Castilla y León en 2001. Evolución de sus poblaciones. Galemys Boletín Inf. la Soc. Española para la Conserv. y Estud. los mamíferos 17, 15–28.

Llaneza, L., García, E.J., López-Bao, J.V., 2014. Intensity of Territorial Marking Predicts Wolf Reproduction: Implications for Wolf Monitoring. PLoS One 9, e93015. https://doi.org/10.1371/journal.pone.0093015

Llaneza, L., García, E.J., Palacios, V., Sazatornil, V., López-Bao, J.V., 2016. Resting in risky environments: the importance of cover for wolves to cope with exposure risk in human-dominated landscapes. Biodivers. Conserv. 25, 1515–1528. https://doi.org/10.1007/s10531-016-1134-6

Llaneza, L., López-Bao, J.V., Sazatornil, V., 2012. Insights into wolf presence in human-dominated landscapes: the relative role of food availability, humans and landscape attributes. Divers. Distrib. 18, 459–469. https://doi.org/10.1111/j.1472-4642.2011.00869.x

Llaneza, L., Palacios, V., Urzal, A., Ordiz, A., Sazatornil, V., Sierra, P., Álvares, F., 2005. Distribuición y aspectos poblacionales del lobo ibérico (*Canis lupus signatus*) en las provincias de Pontevedra y A Coruña (Galicia). Galemys 17, 61–80.

Llaneza, L., Sazatornil, V., García, E.J., Palacios, V., López-Bao, J.V., 2023. Minimum average pack size in Iberian wolves. Eur. J. Wildl. Res. 69, 1–6. https://doi.org/10.1007/s10344-023-01695-x

Llaneza, L., Sazatornil, V., López-Bao, J.V., 2018. The importance of fine-scale breeding site selection patterns under a landscape-sharing approach for wolf conservation. Biodivers. Conserv. 27, 1239–1256. https://doi.org/10.1007/s10531-017-1491-9

Long, R.A., Donovan, T.M., MacKay, P., Zielinski, W.J., Buzas, J.S., 2011. Predicting carnivore occurrence with noninvasive surveys and occupancy modeling. Landsc. Ecol. 26, 327–340. https://doi.org/10.1007/s10980-010-9547-1

López-Bao, J.V., Bruskotter, J., Chapron, G., 2017. Finding space for large carnivores. Nat. Ecol. Evol. 1, 139–141. https://doi.org/10.1038/s41559-017-0140

López-Bao, J.V., Fleurke, F., Chapron, G., Trouwborst, A., 2018a. Legal obligations regarding populations on the verge of extinction in Europe: Conservation, Restoration, Recolonization, Reintroduction. Biol. Conserv. 227, 319–325. https://doi.org/10.1016/j.biocon.2018.09.027

López-Bao, J.V., Godinho, R., Pacheco, C., Lema, F.J., García, E., Llaneza, L., Palacios, V., Jiménez, J., 2018b. Toward reliable population estimates of wolves by combining spatial capture-recapture models and non-invasive DNA monitoring. Sci. Rep. 8, 2177. https://doi.org/10.1038/s41598-018-20675-9

Louvrier, J., Duchamp, C., Lauret, V., Marboutin, E., Cubaynes, S., Choquet, R., Miquel, C., Gimenez, O., 2018. Mapping and explaining wolf recolonization in France using dynamic occupancy models and opportunistic data. Ecography (Cop.). 41, 647–660. https://doi.org/10.1111/ecog.02874

Lovari, S., Sforzi, A., Scala, C., Fico, R., 2007. Mortality parameters of the wolf in Italy:

Does the wolf keep himself from the door? J. Zool. 272, 117–124. https://doi.org/10.1111/j.1469-7998.2006.00260.x

Lute, M.L., Carter, N.H., López-Bao, J.V., Linnell, J.D.C., 2018. Conservation professionals agree on challenges to coexisting with large carnivores but not on solutions. Biol. Conserv., 218, 223–232. https://doi.org/10.1016/j.biocon.2017.12.035

MacKenzie, D.I., Nichols, J.D., Hines, J.E., Knutson, M.G., Franklin, A.B., 2003. Estimating Site Occupancy, Colonization, and Local Extinction When a Species Is Detected Imperfectly. Ecology 84, 2200–2207. https://doi.org/10.1890/02-3090

MacKenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Andrew, J., Langtimm, C. a, Ecology, S., Aug, N., 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83, 2248–2255. https://doi.org/10.2307/3072056

MacKenzie, D.I., Nichols, J.D., Royle, J.A., Pollock, K.H., Bailey, L.L., Hines, J.E., 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence., Wildlife Biology. Elsevier Inc.

MAGRAMA, 2016. Spanish Ministry of Agriculture, Food and Environment. Censo Nacional Lobo ibérico 2012-2014 (*Canis lupus*, Linnaeus, 1758) en España.

Manel, S., Williams, H.C., Ormerod, S.J., 2001. Evaluating presence – absence models in ecology : the need to account for prevalence. J. Appl. Ecol. 38, 921–931.

Marboutin, E., Pruszek, M., Calenge, C., Duchamp, C., 2011. On the effects of grid size and shape when mapping the distribution range of a recolonising wolf (*Canis lupus*) population. Eur. J. Wildl. Res. 57, 457–465. https://doi.org/10.1007/s10344-010-0453-2

Marescot, L., Pradel, R., Duchamp, C., Cubaynes, S., Marboutin, E., Choquet, R., Miquel, C., Gimenez, O., 2011. Capture-recapture population growth rate as a robust tool against detection heterogeneity for population management. Ecol. Appl. 21, 2898–2907. https://doi.org/10.1890/10-2321.1

Marucco, F., Vucetich, L.M., Peterson, R.O., Adams, J.R., Vucetich, J.A., 2012. Evaluating the efficacy of non-invasive genetic methods and estimating wolf survival during a ten-year period. Conserv. Genet. 13, 1611–1622. https://doi.org/10.1007/s10592-012-0412-4

Mech, L.D., 2017. Where can wolves live and how can we live with them? Biol. Conserv. 210, 310–317. https://doi.org/10.1016/j.biocon.2017.04.029

Mech, L.D., 2006. Estimated Age Structure of Wolves in Northeastern Minnesota. J. Wildl. Manage. 70, 1481–1483. https://doi.org/10.2193/0022-541X(2006)70[1481:EASOWI]2.0.CO;2

Mech, L.D., 2000. Leadership in wolf, *Canis lupus*, Packs. Can. Field-Naturalist 114, 259–263. https://doi.org/10.1139/cjz-77-8-1196

Mech, L.D., 1975. Disproportionate Sex Ratios of Wolf Pups. J. Wildl. Manage., 39, 737. https://doi.org/10.2307/3800235

Mech, L.D., 1970. The wolf: the ecology and behavior of an endangered species, 1st ed. Natural History Press, New York, USA.

Mech, L.D., Boitani, L., 2003. Wolves: Behavior, Ecology, and Conservation. The University of Chicago Press.

Mech, L.D., Fritts, S.H., Radde, G.L., Paul, W.J., 1988. Wolf distribution and road density in Minnesota. Wildl. Soc. Bull. 16, 85–87.

Mech, L.D., Isbell, F., Krueger, J., Hart, J., 2019. Gray Wolf (*Canis lupus*) recolonization failure: a Minnesota case study. Can. Field-Naturalist, 133, 60–65. https://doi.org/10.22621/cfn.v133i1.2078

Mech, L.D., Tracy, S., 2004. Record High Wolf, *Canis lupus*, Pack Density. Can. Field-Naturalist 118, 127. https://doi.org/10.22621/cfn.v118i1.896

Meriggi, A., Brangi, A., Matteucci, C., Sacchi, O., 1996. The feeding habits of wolves in relation to large prey availability in northern Italy. Ecography (Cop.). 19, 287–295. https://doi.org/10.1111/j.1600-0587.1996.tb01256.x

Messier, F., 1994. Ungulate Population Models with Predation: A Case Study with the North American Moose. Ecology 75, 478–488. https://doi.org/10.2307/1939551

Miller, D.A.W., Nichols, J.D., Gude, J.A., Rich, L.N., Podruzny, K.M., Hines, J.E., Mitchell, M.S., 2013. Determining Occurrence Dynamics when False Positives Occur: Estimating the Range Dynamics of Wolves from Public Survey Data. PLoS One 8. https://doi.org/10.1371/journal.pone.0065808

Milleret, C., Wabakken, P., Liberg, O., Åkesson, M., Flagstad, Ø., Andreassen, H.P., Sand, H., kesson, M., Flagstad, Andreassen, H.P., Sand, H., 2017. Let's stay together? Intrinsic and extrinsic factors involved in pair bond dissolution in a recolonizing wolf population. J. Anim. Ecol. 86, 43–54. https://doi.org/10.1111/1365-2656.12587

Mills, K.J., Patterson, B.R., Murray, D.L., 2008. Direct Estimation of Early Survival and Movements in Eastern Wolf Pups. J. Wildl. Manage. 72, 949–954. https://doi.org/10.2193/2006-457

Mladenoff, D.J., Haight, R.G., Sickley, T.A., Wydeven, A.P., 1997. Causes and implications of species restoration in altered ecosystems. Bioscience, 47, 21–31.

Mladenoff, D.J., Sickley, T.A., Haight, R.G., Wydeven, A.P., 1995. A Regional Landscape Analysis and Prediction of Favorable Gray Wolf Habitat in the Northern Great Lakes Region. Conserv. Biol. 9, 279–294. https://doi.org/10.1046/j.1523-1739.1995.9020279.x

Mladenoff, D.J., Sickley, T.A., Wydeven, A.P., 1999. Predicting gray wolf landscape recolonization: Logistic regression models vs. new field data. Ecol. Appl. 9, 37–44. https://doi.org/10.1890/1051-0761(1999)009[0037:PGWLRL]2.0.CO;2

Murray, D.L., Smith, D.W., Bangs, E.E., Mack, C., Oakleaf, J.K., Fontaine, J., Boyd, D., Jiminez, M., Niemeyer, C., Meier, T.J., Stahler, D., Holyan, J., Asher, V.J., 2010. Death from anthropogenic causes is partially compensatory in recovering wolf populations. Biol. Conserv. 143, 2514–2524. https://doi.org/10.1016/j.biocon.2010.06.018

Musiani, M., Paquet, P.C., 2004. The Practices of Wolf Persecution, Protection, and Restoration in Canada and the United States. Bioscience 54, 50–60. https://doi.org/10.1641/0006-3568(2004)054[0050:TPOWPP]2.0.CO;2

Nores, C., López-Bao, J.V., 2022. Historical data to inform the legal status of species in Europe: An example with wolves. Biol. Conserv. 272. https://doi.org/10.1016/j.biocon.2022.109639

Nowak, S., Mysłajek, R.W., 2016. Wolf recovery and population dynamics in Western Poland, 2001-2012. Mammal Res. 61, 83–98. https://doi.org/10.1007/s13364-016-0263-3

Odum, E.P., 2017. Fundamentals of Ecology (1953), in: The Future of Nature. Yale University Press, pp. 233–244. https://doi.org/10.12987/9780300188479-022

Olson, E.R., Van Deelen, T.R., Wydeven, A.P., Ventura, S.J., Macfarland, D.M., 2015.

Characterizing wolf-human conflicts in Wisconsin, USA. Wildl. Soc. Bull., 39, 676–688. https://doi.org/10.1002/wsb.606

Packer, C., Loveridge, A., Canney, S., Caro, T., Garnett, S.T., Pfeifer, M., Zander, K.K., Swanson, A., MacNulty, D., Balme, G., Bauer, H., Begg, C.M., Begg, K.S., Bhalla, S., Bissett, C., Bodasing, T., Brink, H., Burger, A., Burton, A.C., Clegg, B., Dell, S., Delsink, A., Dickerson, T., Dloniak, S.M., Druce, D., Frank, L., Funston, P., Gichohi, N., Groom, R., Hanekom, C., Heath, B., Hunter, L., Deiongh, H.H., Joubert, C.J., Kasiki, S.M., Kissui, B., Knocker, W., Leathem, B., Lindsey, P.A., MacLennan, S.D., McNutt, J.W., Miller, S.M., Naylor, S., Nel, P., Ng'weno, C., Nicholls, K., Ogutu, J.O., Okot-Omoya, E., Patterson, B.D., Plumptre, A., Salerno, J., Skinner, K., Slotow, R., Sogbohossou, E.A., Stratford, K.J., Winterbach, C., Winterbach, H., Polasky, S., 2013. Conserving large carnivores: Dollars and fence. Ecol. Lett. 16, 635–641. https://doi.org/10.1111/ele.12091

Parker, G.R., Luttich, S., 1986. Characteristics of the Wolf (*Canis lupus labradorius* Goldman) in Northern Quebec and Labrador. ARCTIC 39, 145–149. https://doi.org/10.14430/arctic2062

Parsons, D.R., 1998. Green Fire Returns to the Southwest: Reintroduction of the Mexican Wolf. Wildl. Soc. Bull. 26, 799–809.

Person, D.K., Russell, A.L., Person, David, K., Russell, Amy, L., 2009. Reproduction and Den Site Selection by Wolves in a Disturbed Landscape. Northwest Sci. 83, 211–224. https://doi.org/10.3955/046.083.0305

Peterson, R.O., Page, R.E., 1988. The rise and fall of Isle Royale wolves, 1975-1986. J. Mammal. 69, 89–99. https://doi.org/10.2307/1381751

Peterson, R.O., Thomas, N.J., Thurber, J.M., Vucetich, J.A., Waite, T.A., 1998. Population Limitation and the Wolves of Isle Royale. J. Mammal. 79, 828. https://doi.org/10.2307/1383091

Peterson, R.O., Woolington, J.D., Bailey, T.N., 1984. Wolves of the Kenai Peninsula, Alaska. Wildl. Monogr. 88, 3–52.

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Modell. 190, 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Pimenta, V., Barroso, I., Álvares, F., Correia, J., Ferrão da Costa, G., Moreira, L., Nascimento, J., Petrucci-Fonseca, F., Roque, S., Santos, G., 2005. Situação do lobo em Portugal: resultados do censo nacional 2002/2003. Relatório Técnico. Instituto da Conservação da Natureza/Grupo Lobo, Lisboa.

Pimenta, V., Barroso, I., Boitani, L., Beja, P., 2018. Risks a la carte: Modelling the occurrence and intensity of wolf predation on multiple livestock species. Biol. Conserv. 228, 331–342. https://doi.org/10.1016/j.biocon.2018.11.008

Pimenta, V., Barroso, I., Boitani, L., Beja, P., 2017. Wolf predation on cattle in Portugal : assessing the effects of husbandry systems. Biol. Conserv. 207, 17–26. https://doi.org/10.1016/j.biocon.2017.01.008

Pletscher, D.H., Ream, R.R., Boyd, D.K., Fairchild, M.W., Kunkel, K.E., 1997. Population dynamics of a recolonizing wolf population. J. Wildl. Manage. 61, 459–465. https://doi.org/10.2307/3802604

Pulliainen, E., Annales, S., Fennici, Z., Introduction, I., 1965. Studies on the wolf (*Canis lupus* L .) in Finland. Ann. Zool. Fennici 2, 215–259.

Rausch, R.A., 1967. Some Aspects of the Population Ecology of Wolves, Alaska. Am. Zool., 7, 253–265.

Ražen, N., Brugnoli, A., Castagna, C., Groff, C., Kaczensky, P., Kljun, F., Knauer, F., Kos, I., Krofel, M., Luštrik, R., Majić, A., Rauer, G., Righetti, D., Potočnik, H., 2016. Long-distance dispersal connects Dinaric-Balkan and Alpine grey wolf (*Canis lupus*) populations. Eur. J. Wildl. Res. 62, 137–142. https://doi.org/10.1007/s10344-015-0971-z

Redpath, S.M., Young, J., Evely, A., Adams, W.M., Sutherland, W.J., Whitehouse, A., Amar, A., Lambert, R.A., Linnell, J.D.C., Watt, A., Gutiérrez, R.J., 2013. Understanding and managing conservation conflicts. Trends Ecol. Evol. 28, 100–109. https://doi.org/10.1016/j.tree.2012.08.021

Reichmann, A., Saltz, D., 2005. The Golan wolves, the ecology, and management of an endangered pest. Isr. J. Zool. 51, 87–133. https://doi.org/10.1560/1BLK-B1RT-XB11-BWJH

Reinhardt, I., Kluth, G., Nowak, C., Szentiks, C.A., Krone, O., Ansorge, H., Müller, T., Mueller, T., 2019. Military training areas facilitate the recolonization of wolves in Germany. Conserv. Lett., 12. https://doi.org/10.1111/conl.12635

Rich, L.N., Davis, C.L., Farris, Z.J., Miller, D.A., Tucker, J.M., Hamel, S., Farhadinia, M.S., Steenweg, R., Di Bitetti, M.S., Thapa, K., Kane, M.D., Sunarto, S., Robinson, N.P., Paviolo, A., Cruz, P., Martins, Q., Gholikhani, N., Taktehrani, A., Whittington, J., Widodo, F.A., Yoccoz, N.G., Wultsch, C., Harmsen, B.J., Kelly, M.J., 2017. Assessing global patterns in mammalian carnivore occupancy and richness by integrating local camera trap surveys. Glob. Ecol. Biogeogr. 26, 918–929. https://doi.org/10.1111/geb.12600

Rick, J.A., Moen, R.A., Erb, J.D., Strasburg, J.L., 2017. Population structure and gene flow in a newly harvested gray wolf (*Canis lupus*) population. Conserv. Genet. 18, 1091–1104. https://doi.org/10.1007/s10592-017-0961-7

Rio-Maior, H., Beja, P., Nakamura, M., Álvares, F., 2018. Use of space and homesite attendance by Iberian wolves during the breeding season. Mamm. Biol. 92, 1–10. https://doi.org/10.1016/j.mambio.2018.03.014

Rio-Maior, H., Nakamura, M., Álvares, F., Beja, P., 2019. Designing the landscape of coexistence: Integrating risk avoidance, habitat selection and functional connectivity to inform large carnivore conservation. Biol. Conserv. 235, 178–188. https://doi.org/10.1016/j.biocon.2019.04.021

Rio-Maior, H., Beja, P., Nakamura, M., Santos, N., Brandão, R., Sargo, R., Dias, I., Silva, F., Álvares, F., 2016. Rehabilitation and post-release monitoring of two wolves with severe injuries. J. Wildl. Manage., 80, 729–735. https://doi.org/10.1002/jwmg.1055

Ripple, W.J., Chapron, G., López-Bao, J.V., Durant, S.M., Macdonald, D.W., Lindsey, P.A., Bennett, E.L., Beschta, R.L., Bruskotter, J.T., Campos-Arceiz, A., Corlett, R.T., Darimont, C.T., Dickman, A.J., Dirzo, R., Dublin, H.T., Estes, J.A., Everatt, K.T., Galetti, M., Goswami, V.R., Hayward, M.W., Hedges, S., Hoffmann, M., Hunter, L.T.B., Kerley, G.I.H., Letnic, M., Levi, T., Maisels, F., Morrison, J.C., Nelson, M.P., Newsome, T.M., Painter, L., Pringle, R.M., Sandom, C.J., Terborgh, J., Treves, A., Van Valkenburgh, B., Vucetich, J.A., Wirsing, A.J., Wallach, A.D., Wolf, C., Woodroffe, R., Young, H., Zhang, L., 2016. Saving the World's Terrestrial Megafauna. Bioscience 66, 807–812. https://doi.org/10.1093/biosci/biw092

Ripple, W.J., Estes, J.A., Beschta, R.L., Wilmers, C.C., Ritchie, E.G., Hebblewhite, M., Berger, J., Elmhagen, B., Letnic, M., Nelson, M.P., Schmitz, O.J., Smith, D.W., Wallach, A.D., Wirsing, A.J., 2014. Status and Ecological Effects of the World's Largest Carnivores. Science (80-.). 343, 1241484.

https://doi.org/10.1126/science.1241484

Ronnenberg, K., Habbe, B., Gräber, R., Strauß, E., Siebert, U., 2017. Coexistence of wolves and humans in a densely populated region (Lower Saxony, Germany). Basic Appl. Ecol. 25, 1–14. https://doi.org/10.1016/j.baae.2017.08.006

Royle, J.A., Kéry, M., 2007. A Bayesian State-space Formulation of Dynamic Occupancy Models. Ecol. Soc. Am. 88, 1813–1823.

Salvatori, V., Mertens, A.D., 2012. Damage prevention methods in Europe: Experiences from LIFE nature projects. Hystrix, 23. https://doi.org/10.4404/hystrix-23.1-4548

Sand, H., Liberg, O., Wikenros, C., Åkesson, M., Zimmermann, B., Wabakken, P., 2022. Spatial dynamics in the Scandinavian wolf population – effects of increased population size and culling.

Sazatornil, V., Rodríguez, A., Klaczek, M., Ahmadi, M., Álvares, F., Arthur, S., Blanco, J.C., Borg, B.L., Cluff, D., Cortés, Y., García, E.J., Geffen, E., Habib, B., Iliopoulos, Y., Kaboli, M., Krofel, M., Llaneza, L., Marucco, F., Oakleaf, J.K., Person, D.K., Potočnik, H., Ražen, N., Rio-Maior, H., Sand, H., Unger, D., Wabakken, P., López-Bao, J.V., 2016. The role of human-related risk in breeding site selection by wolves. Biol. Conserv. 201, 103–110. https://doi.org/10.1016/j.biocon.2016.06.022

Schaub, M., Abadi, F., 2011. Integrated population models: A novel analysis framework for deeper insights into population dynamics. J. Ornithol. 152, S227–S237. https://doi.org/10.1007/s10336-010-0632-7

Schmidt, J.H., Burch, J.W., MacCluskie, M.C., 2017. Effects of control on the dynamics of an adjacent protected wolf population in interior Alaska. Wildl. Monogr., 198, 1–30. https://doi.org/10.1002/wmon.1026

Sidorovich, V., Schnitzler, A., Schnitzler, C., Rotenko, I., 2017. Wolf denning behaviour in response to external disturbances and implications for pup survival. Mamm. Biol. 87, 89–92. https://doi.org/10.1016/j.mambio.2016.11.011

Sillero-Zubiri, C., Hoffmann, M., Macdonald, D.W., 2004. Canids: Foxes, Wolves, Jackals and Dogs, Canids: Foxes, Wolves, Jackals and Dogs. IUCN/SSC Canid Specialist Group.

Silva, P., Galaverni, M., Ortega-Del Vecchyo, D., Fan, Z., Caniglia, R., Fabbri, E., Randi, E., Wayne, R., Godinho, R., 2020. Genomic evidence for the Old divergence of Southern European wolf populations: Divergence of Southern European Wolves. Proc. R. Soc. B Biol. Sci., 287. https://doi.org/10.1098/rspb.2020.1206rspb20201206

Silva, P., López-Bao, J.V., Llaneza, L., Álvares, F., Lopes, S., Blanco, J.C., Cortés, Y., García, E., Palacios, V., Rio-Maior, H., Ferrand, N., Godinho, R., 2018. Cryptic population structure reveals low dispersal in Iberian wolves. Sci. Rep. 8, 14108. https://doi.org/10.1038/s41598-018-32369-3

Singer, L., Wietlisbach, X., Hickisch, R., Schoell, E.M., Leuenberger, C., Van den Broek, A., Désalme, M., Driesen, K., Lyly, M., Marucco, F., Kutal, M., Pagon, N., Papp, C.R., Milioni, P., Uzdras, R., Zihmanis, I., Zimmermann, F., Marsden, K., Hackländer, K., López-Bao, J.V., Klenzendorf, S., Wegmann, D., 2023. The spatial distribution and temporal trends of livestock damages caused by wolves in Europe. Biol. Conserv. 282. https://doi.org/10.1016/j.biocon.2023.110039

Śmietana, W., Wajda, J., 1997. Wolf number changes in Bieszczady National Park, Poland. Acta Theriol. (Warsz)., 42, 241–252.

Smith, A.F., Ciuti, S., Shamovich, D., Fenchuk, V., Zimmermann, B., Heurich, M., 2022. Quiet islands in a world of fear: Wolves seek core zones of protected areas to escape human disturbance. Biol. Conserv. 276, 109811. https://doi.org/10.1016/j.biocon.2022.109811

Smith, D.W., 2005. Ten years of Yellowstone wolves, 1995–2005. Yellowstone Sci. 13, 7–33. https://doi.org/10.5860/choice.38-5208

Smith, D.W., Bangs, E.E., 2009. Reintroduction of Wolves to Yellowstone National Park: History, Values and Ecosystem Restoration, in: Hayward, M.W., Somers, M.J. (Eds.), Reitroduction of Top-Order Predators, pp. 92–125.

Stansbury, C.R., Ausband, D.E., Zager, P., Mack, C.M., Waits, L.P., 2016. Identifying gray wolf packs and dispersers using noninvasive genetic samples. J. Wildl. Manage. 80, 1–12. https://doi.org/10.1002/jwmg.21136

Szewczyk, M., Nowak, S., Niedźwiecka, N., Hulva, P., Špinkytė-Bačkaitienė, R., Demjanovičová, K., Bolfíková, B.Č., Antal, V., Fenchuk, V., Figura, M., Tomczak, P., Stachyra, P., Stępniak, K.M., Zwijacz-Kozica, T., Mysłajek, R.W., 2019. Dynamic range expansion leads to establishment of a new, genetically distinct wolf population in Central Europe. Sci. Rep., 9, 19003. https://doi.org/10.1038/s41598-019-55273-w

Theuerkauf, J., 2003. Impact of man on wolf behaviour in the Białowieża Forest, Poland (PhD dissertation). Technische Universität München.

Theuerkauf, J., Rouys, S., Jedrzejewski, W., 2003. Selection of den, rendezvous, and resting sites by wolves in the Bialowieza Forest, Poland. Can. J. Zool. 81, 163–167. https://doi.org/10.1139/z02-190

Thiel, R.P., 1985. Relationship between road densities and wolf habitat suitability in Wisconsin. Am. Midl. Nat. 113, 404–407. https://doi.org/10.2307/2425590

Thiel, R.P., Merrill, S.B., Mech, L.D., 1998. Tolerance by Denning Wolves, *Canis lupus*, to Human Disturbance. Can. Field-Naturalist 122, 340–342.

Thorn, M., Scott, D.M., Green, M., Bateman, P.W., Cameron, E.Z., 2009. Estimating brown hyaena occupancy using baited camera traps. African J. Wildl. Res. 39, 1–10. https://doi.org/10.3957/056.039.0101

Thurber, J.M., Peterson, R.O., 1993. Effects of Population Density and Pack Size on the Foraging Ecology of Gray Wolves. J. Mammal. 74, 879–889. https://doi.org/10.2307/1382426

Thurber, J.M., Peterson, R.O., Drummer, T.D., Thomasma, S.A., 1994. Gray Wolf Response to Refuge Boundaries and Roads in Alaska. Wildl. Soc. Bull. 22, 61–68.

Torres, R.T., Silva, N., Brotas, G., Fonseca, C., 2015. To Eat or Not To Eat? The Diet of the Endangered Iberian Wolf (*Canis lupus signatus*) in a Human- Dominated Landscape in Central Portugal. PLoS One 10, e0129379. https://doi.org/10.1371/journal.pone.0129379

Trapp, J.R., Beier, P., Mack, C., Parsons, D.R., Paquet, P.C., 2008. Wolf, *Canis lupus*, den site selection in the Rocky Mountains. Can. Field-Naturalist, 122, 49–56. https://doi.org/10.22621/cfn.v122i1.543

Treves, A., Langenberg, J.A., López-Bao, J. V., Rabenhorst, M.F., 2017. Gray wolf mortality patterns in Wisconsin from 1979 to 2012. J. Mammal., 98, 17–32. https://doi.org/10.1093/jmammal/gyw145

Uboni, A., Smith, D.W., Stahler, D.R., Vucetich, J.A., 2017. Selecting habitat to what purpose? The advantage of exploring the habitat-fitness relationship. Ecosphere, 8. https://doi.org/10.1002/ecs2.1705

Unger, D.E., Keenlance, P.W., Kohn, B.E., Anderson, E.M., 2009. Factors influencing Homesite selection by gray wolves in Northwestern Wisconsin and east-central Minnesota, in: Recovery of Gray Wolves in the Great Lakes Region of the United States: An Endangered Species Success Story. Springer New York, Division of Natural Sciences, Alderson Broaddus College, Philippi, WV 26416, United States and Biology Department, Grand Valley State University, Allendale, MI 49401, United States and Wisconsin Department of Natural Resources, Rhinelander, WI 54501, Unit, pp. 175–189. https://doi.org/10.1007/978-0-387-85952-1_11

Valverde, J.A., 1972. El lobo español, Montes.

Van Camp, J., Gluckie, R., 1979. A Record Long-Distance Move by a Wolf (*Canis lupus*). J. Mammal., 60, 236–237. https://doi.org/10.2307/1379788

Venumière-Lefebvre, C.C., Breck, S.W., Crooks, K.R., 2022. A systematic map of human-carnivore coexistence. Biol. Conserv. 268. https://doi.org/10.1016/j.biocon.2022.109515

Vigne, J.D., 2011. The origins of animal domestication and husbandry: A major change in the history of humanity and the biosphere. Comptes Rendus - Biol. 334, 171–181. https://doi.org/10.1016/j.crvi.2010.12.009

Vos, J., 2000. Food habits and livestock depredation of two Iberian wolf packs (*Canis lupus signatus*) in the north of Portugal. J. Zool. 251, 457–462. https://doi.org/10.1111/j.1469-7998.2000.tb00801.x

Votsi, N.E.P., Zomeni, M.S., Pantis, J.D., 2016. Evaluating the Effectiveness of Natura 2000 Network for Wolf Conservation: A Case-Study in Greece. Environ. Manage. 57, 257–270. https://doi.org/10.1007/s00267-015-0621-y

Wabakken, P., Sand, H., Kojola, I., Zimmermann, B., Arnemo, J.M., Pedersen, H.C., Liberg, O., 2007. Multistage, long-range natal dispersal by a global positioning system-collared Scandinavian wolf.

Weaver, J.L., Paquet, P.C., Ruggiero, L.F., 1996. Resilience and Conservation of Large Carnivores in the Rocky Mountains. Conserv. Biol. 10, 964–976. https://doi.org/10.1046/j.1523-1739.1996.10040964.x

Webb, N.F., Allen, J.R., Merrill, E.H., 2011. Demography of a harvested population of wolves (*Canis lupus*) in west-central Alberta, Canada. Can. J. Zool. 89, 744–752. https://doi.org/10.1139/z11-043

Whittington, J., St. Clair, C.C., Mercer, G., 2005. Spatial responses of wolves to roads and trails in mountain valleys. Ecol. Appl. 15, 543–553. https://doi.org/10.1890/03-5317

Wolf, C., Ripple, W.J., 2017. Range contractions of the world's large carnivores. R. Soc. Open Sci. 4, 170052. https://doi.org/10.1098/rsos.170052

Woodroffe, R., 2000. Predators and people: Using human densities to interpret declines of large carnivores. Anim. Conserv. 3, 165–173. https://doi.org/10.1017/S136794300000086X

Woodroffe, R., Lindsey, P., Romanach, S., Stein, A., Oleranah, S., ole Ranah, S.M.K., 2005. Livestock predation by endangered African wild dogs (Lycaon pictus) in northern Kenya. Biol. Conserv. 124, 225–234. https://doi.org/10.1016/j.biocon.2005.01.028

Yackulic, C.B., Chandler, R., Zipkin, E.F., Royle, J.A., Nichols, J.D., Campbell Grant, E.H., Veran, S., 2013. Presence-only modelling using MAXENT: When can we trust the inferences? Methods Ecol. Evol. 4, 236–243. https://doi.org/10.1111/2041-210x.12004

Zlatanova, D., Popova, E., 2013. Habitat variables associated with wolf (*Canis lupus* L.) distribution and abundance in Bulgaria. Bulg. J. Agric. Sci. 19, 262–266.

Žunna, A., Ozoliņš, J., Pupila, A., 2009. Food habits of the wolf *Canis lupus* in Latvia based on stomach analyses. Est. J. Ecol., 58, 141. https://doi.org/10.3176/eco.2009.2.07

Chapter II

Insights into the dynamics of wolf occupancy in human-dominated landscapes

2. Insights into the dynamics of wolf occupancy in human-dominated landscapes

Biological Conservation 286 (2023) 110316

doi: https://doi.org/10.1016/j.biocon.2023.110316

Accepted: 8 September 2023

Mónia Nakamura [a,b,c,d,*], José Vicente López-Bao [e], Helena Rio-Maior [a,b,c], Sara Roque [f,g], Patrícia Gil[a,c], Ana Serronha [a,c], Emilio García [e], Gonçalo Ferrão da Costa [h,i], Francisco Álvares [a,c], Francisco Petrucci-Fonseca [f,g], Olivier Gimenez [d,1], Pedro Monterroso [a,c,1]

[a] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

[b] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

[c] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

[d] CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

[e] Research Unit of Biodiversity (UO/CSIC/PA), Oviedo University, Mieres, Spain

[f] Grupo Lobo, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

[g] cE3c, Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

[h] Bioinsight Lda., Odivelas, Portugal

[i] CESAM - Centre for Environmental and Marine Studies. University of Aveiro, Portugal

[1]co-senior authorship

* Corresponding author: Mónia Nakamura, CIBIO/InBIO, Campus Agrário de Vairão, 4485-661 Vairão, Portugal; moniayui@gmail.com

2.1. Highlights

- Occupancy model to assess the effects of anthropogenic factors on wolf spatial use
- Colonization increases with altitude, livestock density and unpaved road density.
- Wolf occupancy is negatively affected by burned areas and paved roads.
- Conflict management associated with livestock depredation is a priority.
- Other ecological and social factors may be shaping wolf range change in Iberia.

2.2. Abstract

Among large carnivores, wolves show a remarkable capability to persist in human-dominated landscapes. However, the temporal dynamics of variation in spatial use of these landscapes remains poorly understood. Considering the relevance of spatio-temporal variations of territorial marking on wolf behaviour, either to defend territory boundaries and core areas or to expand into new areas, the location of wolf signs should reflect the dynamics of spatial use. Taking advantage of a long-term non-invasive wolf monitoring dataset spanning from 2005 to 2022 we fit a dynamic occupancy model to investigate the effects of environmental and anthropogenic factors on the dynamics of wolf spatial use in human-dominated landscapes. We focused on two dynamic parameters – colonization and extinction – and developed a wolf habitat suitability map for Iberia. Colonization probability increased with higher altitude, livestock density, and unpaved road density, and with the decrease of burned areas, national-regional, and local road densities. Extinction probability decreased with higher unpaved road density.

In addition, we evaluated the wolf range dynamics in Iberia to understand if the ecological traits explained the expansion, stagnation or extinction sites observed since the beginning of the 2000s. Our results contribute to a sound understanding of wolf spatial use in human-dominated landscapes and its ability to adapt to these heterogeneous environments, allowing us to support adequate mitigation measures and conservation actions. The strong influence of livestock on the dynamics of wolf occupancy highlights the need to assess social factors, human dimensions, and direct wolf mortality causes for conflict management associated with livestock depredation.

Keywords: Anthropogenic variables; *Canis lupus*; Dynamic occupancy model; Human disturbance; Sign survey; Spatial use.

2.3. Introduction

Large carnivores (LCs) are often at the core of public concerns because of the potentially negative interactions with humans and human activities. Their predatory behaviour (e.g., competition for game species or livestock depredation) and the impact this might cause on human activities is probably one of the most critical factors driving opposition to sharing the landscape with these species (López-Bao et al., 2017; Wolf and Ripple, 2016). These difficulties are aggravated in human-dominated landscapes and often result in the preemptive or retaliatory killing of LCs. Consequently, legal killing or poaching influences species persistence, particularly in human-dominated landscapes (Carter and Linnell, 2016; Lamb et al., 2020). These factors, together with other anthropogenic pressures, such as roadkills, habitat disturbance and fragmentation or food availability (Lovari et al., 2007; Zimmermann et al., 2014), may lead to adapted dynamics of LC occupancy in human-dominated landscapes. LCs have demographic or behavioural mechanisms to adapt and coexist with humans, such as spatial or temporal avoidance of human-disturbed areas, high reproduction rates and immigration to compensate for mortality in sink areas (Pease and Mattson, 1999; Pulliam, 1988). Co-adaptation is needed for successful coexistence in such landscapes (Carter and Linnell, 2016). Thus, by understanding the spatial dynamics of LCs adapted to human-dominated landscapes, we can also adapt by prioritizing actions to favour colonization, avoid extinction, and maintain LC persistence.

The wolf (*Canis lupus*) is a valuable model species to address LCs' dynamic occupancy in human-dominated landscapes due to its ability to persist in a wide range of environmental conditions. Wolves occur from the most remote landscapes with very low human interference, such as Ellesmere Island, in the Canadian Article Circle (Mech and Cluff, 2011) up to areas with high human population and road densities in Eurasia (Fechter and Storch, 2014; Sazatornil et al., 2016). Several studies addressed how habitat and anthropogenic features affect wolf distribution, and results often point to an increase in wolf occurrence with higher refuge availability and lower anthropogenic infrastructures such as roads (e.g., Llaneza et al., 2012). Some studies have used species distribution modelling approaches – based on different survey methods, such as sign surveys, camera trapping or citizen science – to predict the distribution of suitable habitats for the species or detect ecological corridors (e.g., Grilo et al., 2018; Louvrier et al., 2018; Rich et al., 2017). Though, most studies have short sampling periods and do not account for the dynamic effects of temporal variation of habitat or anthropogenic covariates on wolf distribution and spatial use, such as burned areas and human population density. Since species distribution is not static and can vary through time and space, particularly in expanding populations (Marucco and McIntire, 2010), dynamic occupancy models can be a powerful tool to address wolf spatial dynamics.

Here, we used a dynamic occupancy model to study the factors determining the dynamics of wolf persistence in human-dominated landscapes, taking advantage of wolf surveys (transects of sign surveys) carried out in different areas within the Iberian wolf range. With this approach, we accounted for the influence of dynamic processes such as colonization and extinction on the species range dynamics (MacKenzie et al., 2003; Royle and Kéry, 2007). Although wolves are well known for their long-distance dispersal ability (e.g., 233 km in Ražen et al., 2016), evidence in the Iberian Peninsula (IP) suggest low dispersal in Iberian wolves (e.g., 32km in Blanco and Cortés, 2007; 24.8 km in Nakamura et al., 2021; see also Silva et al., 2018). After the 1970s, the north-western wolf population expanded in Spain (Blanco and Cortés, 2009; Chapron et al., 2014; López-Bao et al., 2018b), though it showed a remarkable regression pattern in Portugal, particularly in the south of Douro River (Monteiro, 2015). In the late 1990s, the species reached south of Castilla y León, north of Castilla-La Mancha (Guadalajara province) and Madrid (Blanco and Cortés, 2009, 2001). Genetic analyses have revealed that such expansion towards central Spain resulted from the expansion of wolves from the south-eastern Cantabrian Mountains (Silva et al., 2018). Currently, the population appears to have stagnated in eastern Castilla y León, the Basque Country, and north of Castilla-La Mancha (Guadalajara province) (Blanco and Cortés, 2009; López-Bao et al., 2018b) as well as in Portugal (Monteiro, 2015). For a detailed wolf distribution change since 1970, see Figure 1.2.1. in Blanco and Cortés (2009) and Sup. Fig. 4 in Appendix II. Even though the wolf range in NW Iberia has been relatively continuous in recent times (Chapron et al., 2014; López-Bao et al., 2018a; Nores and López-Bao, 2022), the habitat can vary throughout the range. Here, we aim to understand how anthropogenic features influence wolf spatial use. As such, we expect low wolf occupancy and colonization probabilities and high extinction probabilities where human-related features are more abundant.

We hypothesize that high paved road densities negatively affect wolf colonization and occupancy since the persistence of wolves may be lower in areas with high road densities (Jedrzejewski et al., 2008; Mladenoff et al., 1995). However, we hypothesize that unpaved road density positively affects wolf colonization and occupancy because wolves often use forest roads less used by humans as travel corridors (Weaver et al., 1996; Zimmermann et al., 2014). We also hypothesize that higher human population density and higher proportions of human settlements, agricultural lands, and burned areas have a negative effect on wolf colonization and occupancy and a positive effect on extinction probability (Ballard et al., 2000; Mladenoff et al., 1995; Sazatornil et al., 2016). Even though livestock depredation can promote direct persecution and increase extinction probability (DeCesare et al., 2018), we hypothesize that livestock density positively affects occupancy and local colonization probability due to higher food availability (Fuller et al., 2003), both in terms of depredation or scavenging events on livestock (e.g., Planella et al., 2016). We also include environmental features related to wolf ecology and hypothesize that wolf colonization and occupancy increase with higher elevations and refuge availability (Grilo et al., 2018; Jedrzejewski et al., 2008; Llaneza et al., 2012; Stenglein et al., 2011). Even though we expected that anthropogenic variables (e.g., proportion of burned areas and population density) would have a generalized negative effect on wolf spatial use (e.g., Ballard et al., 2000; Sazatornil et al., 2016), previous studies have shown that some have a positive or no apparent effect (e.g., Geary et al., 2020; Lino et al., 2019). Table 2-1 presents several results from previous studies in which environmental and anthropogenic variables positively or negatively affected wolf distribution. We present several hypotheses for each covariate and possible explanations according to possible expected effects on colonization, extinction and occupancy probabilities.

Furthermore, we assess the parameters of extinction, colonization, and occupancy probabilities for the current wolf range in the IP and its surroundings to understand better the potential of wolf occupancy from an ecological perspective. By distinguishing areas of wolf persistence, expansion, regression, and potential recolonization – obtained from differences between the wolf range estimated at the beginning of the 2000s (Álvares et al., 2005) and the estimated current range (Kaczensky, 2018) – we compare the average probabilities of extinction, colonization, and occupancy among the areas considered. With the recent range expansion of most of the wolf populations in Europe (Boitani et al., 2022; Chapron et al., 2014), a better knowledge of wolf landscape use changes and tolerance between wolves and people are crucial to improve and guide management actions. By identifying areas with a higher probability of colonization and extinction for wolves, we can predict future recolonization sites to carry out actions ahead and help avoid and mitigate conflicts. Based on our findings, we propose mitigation measures and conservation actions and locate the areas where such actions should be prioritized within the current wolf distribution and potential areas of recolonization.

2.4. Material and Methods

2.4.1. Wolf sampling areas

We used information collected between 2005 and 2022 from three areas in Portugal – Alto Minho (AM; 1,075 km^2), South of Douro (SD; 1,400 km^2), and Vila Real (VR; 1,700km^2) – and one in Spain – Asturias (AST; 5,700 km^2) within the Iberian wolf range (Fig. 2-1). The sampling years differed between study areas: 2007 through 2019 in AM, 2011 through 2020 in SD, 2005 through 2013 in VR, and 2019 through 2022 in AST. The number of packs detected across areas was: from 2 to 7 in AM, from 2 to 3 in SD, from 4 to 8 in VR, and around 40 packs in AST (Álvares et al., 2015; Nakamura et al., 2021; Regional Government of Asturias, 2022; Rio-Maior et al., 2020).

Across the study areas, there is a wide variation in human population density: AM 40.8 ± 48.1 inhabitants/km^2, SD 28.7 ± 26.2 inhabitants /km^2, VR 34.9 ± 64.4 inhabitants /km^2, AST 20.6 ± 36.3 inhabitants /km^2 (mean ± sd), reaching a maximum of 513.9 inhabitants /km^2 in VR (CIESIN 2018); as well as in road densities: AM 0.77 ± 0.37 km/km^2, SD 0.58 ± 0.28 km/km^2, VR 0.71 ± 0.54 km/km^2, AST 0.42 ± 0.35 km/km^2, reaching a maximum of 2.89 km/km^2 in VR (OpenStreetMap contributors, 2022). Wolves feed mainly on livestock in all the study areas due to low wild prey availability and high livestock densities with inadequate husbandry practices or inefficient damage prevention measures (Álvares et al., 2015; Llaneza et al., 1996; Pimenta et al., 2018). Even though livestock depredations are compensated across the study areas, wolf predation on livestock is not properly adressed and managed, leading to major conflicts and retaliatory killing of wolves (Álvares et al., 2015; Blanco and Cortés, 2009; Fernández-Gil et al., 2016; Pimenta et al., 2017).

2.4.2. Wolf data collection

In all study areas, transects were carried out on foot or by car (<10 km/h) along unpaved roads and paths in order to detect wolf faeces. Particular attention was given to usual wolf scent marking places such as junctions (Barja et al., 2004; Llaneza et al., 2005). Most transects were conducted monthly or seasonally with year-round surveys or more focused on summer-autumn, depending on the year and study area. Seasons were defined as 'spring' (March-May), 'summer' (June-August), 'autumn' (September-November), and 'winter' (December-February). Sampling units were 5x5 km cells (hereafter referred to as sites) adapted from the 10x10 km European Environment

Agency Reference Grid. The study areas differed in sampling coverage: 43, 56, 68, and 228 sites for AM, SD, VR, and AST, respectively (Fig. 2-1).

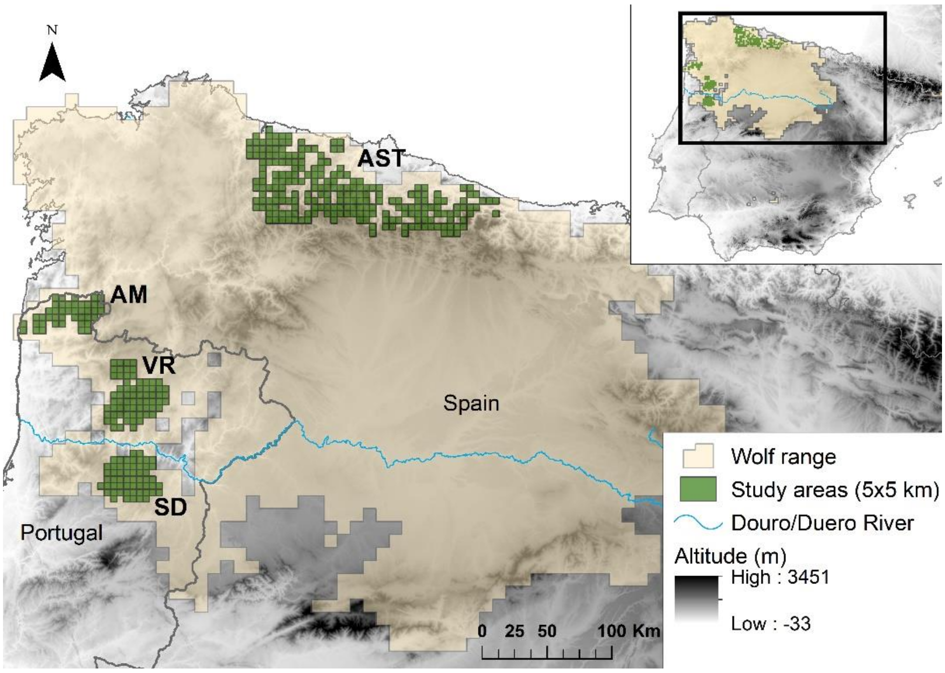


Fig. 2-1: Location of the study areas (AM - Alto Minho, SD - South Douro, VR- Vila Real, and AST – Asturias; 5x5km sites) in the context of the estimated wolf range in north-western Iberia in recent times (adapted from Kaczensky, 2018)

We summed transect lengths (i.e. total distance) to obtain the transect effort (km) per site and season (hereafter denominated 'site-season'). The effort varied over time and between study areas, with some transect changes throughout the sampling years. From autumn 2005 to autumn 2022, transects of sign survey effort was 8.72 ± 6.74 km (range 0.05-55.00km) per site-season, which comprised 5672 site-seasons sampled. The dataset consisted of 68 seasons surveyed, with a sampling average of 78.8 ± 48.4 sites per season (range 2-233) and 14.4 ± 13.9 seasons per site (range: 2-41) (see Sup. Fig. 5 in Appendix II for sampled sites). We submitted the general protocol used from sampling faeces to molecular analysis. The success of wolf assignment for AM and SD until 2012 was 83.3% (Nakamura et al. 2017).

Considering that wolf territory sizes are very variable between study areas (average minimum convex polygon of 408 km^2 for the IP, ranging between 14 and 2,810 km^2; Silva et al., 2018) – the scale of 5x5 km used in this study is adequate for our analysis since it allows to detect variation in wolf spatial use within wolf territories as well as in inter-territorial areas, allowing to detect colonization and extinction patterns at a local level. A 10x10 km scale would be too large to detect spatial use variations within areas where wolves have smaller territories (e.g., Alto Minho 135 km^2; Álvares et al., 2015). Moreover, a smaller scale analysis (e.g., 2x2 km) is rarely used in long-term wolf monitoring studies in the IP and could result in high spatial autocorrelation.

2.4.3. Environmental and anthropogenic covariates

We selected predictor covariates based on factors important to wolf spatial use and distribution based on previous knowledge of the species and worldwide studies mostly conducted in areas with some anthropogenic disturbance (Table 2-1). We obtained the covariates for each 5x5 km site. As environmental covariates, we considered: 'Altitude' as average altitude (a.s.l.); 'Ruggedness' as average Terrain Ruggedness Index (Riley et al., 1999); and 'Refuge' as the proportion of refuge availability for wolves. According to local habitat specificities, we considered bare rocks as a refuge since these frequently have cavities for wolf refuge. As such, we joined the habitats of forest, shrubland, and bare rocks into a single covariate reflecting refuge availability. For anthropogenic variables, we considered: i) densities of three paved road types (from high to low traffic levels: 'highway', 'national-regional', and 'local' roads), ii) unpaved road density, iii) human population density, iv) livestock unit (LU) density, v) proportion of human settlements, vi) proportion of agricultural lands and vii) proportion of burned areas. We calculated road densities by obtaining road type length per site (OpenStreetMap contributors, 2022). Livestock availability was quantified considering LU density (1 LU of horse and cattle; 0.15 LU of goat and sheep), which represents the primary food resource for wolves in several areas of the IP (Blanco et al., 1992; Llaneza and López-Bao, 2015; López-Bao et al., 2013; Pimenta et al., 2017; Torres et al., 2015). We calculated the annual proportion of burned areas from the sum of monthly burned area per site from MODIS (Moderate Resolution Imaging Spectroradiometer). We determined the proportion of area covered by settlements, agricultural lands, and refuge per site from Corine Land Cover (CLC, 2018) and the European Settlement Map (Corbane and Sabo, 2019). For a detailed covariate description, calculation, and source of information, see Sup. Table 3 in Appendix II.

Table 2-1: Covariates included in the dynamic occupancy model, rationale and underlying hypothesis: a higher covariate value has a positive (↑) or negative (↓) effect on colonization **(γ)**, extinction **(ε)**, and occupancy (ψ) parameters. For the occupancy parameter, a hypothesis with no clear effect was added (↔) when there may not be a clear positive or negative effect or when the effects are of a wide range. The considered hypotheses for the colonization and occupancy parameters are explained with reference to previous studies.

Covariate Type	**Covariate (units)**	**Hypothesis**			**Hypothesis explanation**	**References**
		Col (γ)	**Ext (ε)**	**Occ (ψ)**		
Anthropogenic	Paved road - Highway density (km/km^2)	↓	↑	↔ ↓	• Highway density has no clear effect on wolves in areas with well-established territories; • Wolves are less abundant in areas with higher road densities; • Wolves avoid high-level roads due to human disturbance (high traffic intensity).	Blanco et al., 2005; Dennehy et al., 2021; Jedrzejewski et al., 2008; Mladenoff et al., 1995; Rio-Maior et al., 2019; Zlatanova and Popova, 2013
	Paved road - National and regional road density (km/km^2)	↑	↓	↑ ↔ ↓	• Wolves are more abundant in areas with higher road densities; • Wolves frequently use mid-level roads for dispersal travelling and avoidance of resident wolves.	Jedrzejewski et al., 2008; Kabir et al., 2017; Mladenoff et al., 1995; Weaver et al., 1996; Zimmermann et al., 2014
		↓	↑		• Wolves are less abundant in areas with higher road densities; • Wolves avoid mid-level roads due to human disturbance (high traffic intensity); • Mid or low-level roads or high road densities increase mortality risk (roadkill) and decrease habitat connectivity that precludes dispersal.	Blanco et al., 2005; Dennehy et al., 2021; Jedrzejewski et al., 2008; Mladenoff et al., 1995; Rio-Maior et al., 2019; Zlatanova and Popova, 2013
	Paved road -Local road density (km/km^2)	↑	↓	↑ ↔ ↓	• Wolves frequently use low-level roads as travelling routes with less effort (both resident and dispersers);	Gurarie et al., 2011; Kabir et al., 2017; Mattisson et al., 2013; Weaver et al., 1996; Zimmermann et al., 2014
		↓	↑		• Wolves avoid low-level roads due to human disturbance (traffic and human activities). • Mid or low-level roads or high road densities increase mortality risk (roadkill) and decrease habitat connectivity that precludes dispersal.	Dennehy et al., 2021
	Unpaved road density (km/km^2)	↑	↓	↑ ↔ ↓	• Wolves frequently use low-level roads as travelling routes with less effort • Wolves frequently use forest/gravel roads as travelling routes as least-cost path; • Wolves frequently use unpaved roads and crossroads as territorial marking sites.	Barja et al., 2004; Gurarie et al., 2011; Kabir et al., 2017; Llaneza et al., 2014; Mattisson et al., 2013; Weaver et al., 1996; Whittington et al., 2005; Zimmermann et al., 2014
		↓	↑		• Wolves avoid potential human disturbance, resulting from higher accessibility for humans (e.g., 4x4 cars, hunting activities).	Rio-Maior et al., 2019
	Livestock Unit density (LU/km^2)	↑	↓	↑ ↔ ↓	• High wolf productivity, survival, and/or densities due to high prey availability: a) high conflict/poaching but the wolf population can strive; or b) low conflict/poaching;	-
		↓	↑		• Low wolf survival and/or wolf densities due to high conflict and poaching.	Blanco and Cortés, 2009; DeCesare et al., 2018
	Annual proportion of agricultural lands (%)	↑	↓	↑ ↔ ↓	• Wolves have higher access to prey (livestock or wild).	-
		↓	↑		• Wolves avoid human disturbance (human presence due to agricultural activities); • Low wolf survival and/or densities due to high conflict and poaching.	Mladenoff et al., 1995; Sazatornil et al., 2016

Table 2-1 (cont.) Covariates included in the dynamic occupancy model, rationale and underlying hypothesis: a higher covariate value has a positive (↑) or negative (↓) effect on colonization **(γ)**, extinction ***(ε)***, and occupancy (ψ) parameters. For the occupancy parameter, a hypothesis with no clear effect was added (↔) when there may not be a clear positive or negative effect or when the effects are of a wide range. The considered hypotheses for the colonization and occupancy parameters are explained with reference to previous studies.

Covariate Type	**Covariate (units)**	**Hypothesis**			**Hypothesis explanation**	**References**
		Col (γ)	**Ext (ε)**	**Occ (ψ)**		
Anthropogenic	Annual proportion of burned areas (%)	↑	↓	↑ ↔ ↓	• Wolves opportunistically select burned areas due to higher prey availability after fires. • Wolves positively select burned areas or there is no apparent effect.	Geary et al., 2020; Lewis et al., 2022; Lino et al., 2019
		↓	↑		• Wolves have lower prey availability (absence of prey); • Wolves avoid high human disturbance: easier accessibility to humans and higher exposure of wolves to humans due to the absence of refuge.	Ballard et al., 2000
	Annual human population density (nº inhabitants/km^2)	↑	↓	↑ ↔ ↓	• Wolves have higher access to resources • Wolf population near carrying capacity (saturated population).	-
		↓	↑		• Wolves avoid human activity/disturbance	Mladenoff et al., 1995; Sazatornil et al., 2016
Environmental	Altitude (m) a.s.l.	↑	↓	↑	• Wolves select areas with higher elevations to avoid human activities.	Glenz et al., 2001; Llaneza et al., 2018, 2012; Rio-Maior et al., 2019
		↓	↑	↓	• Dispersers select lower elevations to avoid resident wolves present in higher elevations.	Rio-Maior et al., 2019
	Annual proportion of refuge (forest, shrubland and bare rocks) (%)	↑	↓	↑ ↔ ↓	• Wolves select areas with higher refuge availability; • Wolves select areas with higher prey density.	Grilo et al., 2018; Jedrzejewski et al., 2008; Llaneza et al., 2012; Mladenoff et al., 1995; Oakleaf et al., 2006
		↓	↑		• Wolves use areas with less refuge (e.g., meadows) due to the high availability of prey.	Jedrzejewski et al., 2008

2.4.4. Dynamic occupancy model: extinction and recolonization probabilities

We fitted a dynamic occupancy model (MacKenzie et al., 2006) to scat detection data to identify anthropogenic and environmental variables potentially affecting wolf space use. Data on wolf scat detection and non-detection were organized per 395 sites in 72 surveys (i.e. seasons) from spring 2005 to winter 2022 (i.e. 18 years or primary occasions with four seasons each or secondary occasions).

We were interested in the dynamics of wolf occurrence in a given area. To do this, we assume that: i) the detection of scat markings confirms the presence of the species and reflects sites that wolves preferentially use or scent mark, and ii) the non-detection of scat markings reflects unused or non-preferentially used nor scent marked sites. Thus, we highlight that the meaning of the terms' colonization' and 'extinction' used for interpreting the occupancy model results are not actual colonization and extinction of the species but instead refer to a probability of a site becoming, respectively, used and unused from one year to another.

By correcting for imperfect detection (i.e. the species is undetected in occupied sites), occupancy approaches facilitate obtaining unbiased estimates of variables relevant to species conservation and management implications (MacKenzie et al., 2006, 2002). Covariates can be modelled to infer relationships between observed patterns and the underlying processes that cause them, thereby projecting patterns in un-surveyed areas (MacKenzie et al., 2006). Occupancy models rely on a spatial closure assumption, i.e. the ecological state of a site (occupied vs not occupied) remains unchanged over seasons j (from spring to winter) within a year. Considering the sampling methodology and wolf scent-marking behaviour, we included effort (transect length), unpaved road density, and average ruggedness as covariates for the detection parameter. For a detailed description of the dynamic occupancy model and covariates considered for the colonization, extinction, initial occupancy, and detection parameters, see Appendix A1.

We estimated posterior distributions of initial occupancy, colonization, and extinction parameters, considering detection probability using Markov Chain Monte Carlo (MCMC) implemented in JAGS (version 4.3.0) using R2jags (Plummer, 2011) in RStudio (Posit team, 2022). We generated two chains of 40,000 iterations after a burn-in of 3,000 iterations. We assessed model convergence visually by inspecting the chains and by checking the Gelman-Rubin statistic (Rhat < 1.1) (Gelman et al., 2004). We used posterior means and 50% and 95% Bayesian credible intervals (BCI's) to summarise parameter posterior distributions. Additionally, we considered the mass of the posterior distribution on the negative or positive side to interpret the results. To assess the effect of a covariate on a given parameter, we set the other covariates to their mean value. Considering the posterior estimate distributions of the model and the first 5000 iterations after burn-in, we obtained the annual colonization, extinction, and occupancy probabilities of all sites in the study areas.

We obtained the annual detection rate estimates from the model. Moreover, to ensure that wolf detection by transects of sign survey is adequate to assess wolf spatial use, and considering that the locations of GPS-collared wolves give the most accurate information on spatial use, we compared an available dataset from AM study area to calculate the seasonal proportion of sites with presence confirmed by GPS-collared wolves that had successful detection by transects (details in Appendix A2).

2.4.5. Assessing changes in estimated wolf range in the last two decades

Considering the posterior estimate distributions of the model, we predicted the annual parameter estimates for sites within the latest known wolf range (Kaczensky, 2018) to identify areas with higher probabilities of colonization, extinction and persistence. We also predicted these parameter estimates for the sites out of the current distribution range to identify areas with ecological potential for recolonization in the IP.

Moreover, we evaluated the wolf dynamics on a broad scale for the last two decades in the IP to better understand if the ecological traits of the model are in concordance with the species' range expansion progression, stagnation or extinction observed since 2000 (Álvares et al., 2015; López-Bao et al., 2015, 2018a). To do this, considering the last estimates for the entire wolf range in the IP, we attributed an occurrence area type for each 5x5 km site based on wolf presence or absence at the beginning of the 2000s (1999-2003, Álvares et al., 2005) and in recent times (Kaczensky, 2018). We considered four area types regarding wolf occurrence: i) persistence (a site with wolf presence in both periods; 129,475 km^2); ii) expansion (wolves became present from 2000s to present; 20,724 km^2); iii) regression (wolves became absent; 13,497 km^2); and iv) potential recolonization (i.e. 100 km buffer around the latest wolf range as the most potential recolonization area for wolves in the near future; 271,811 km^2). We defined this buffer considering the short dispersal distances observed in the IP (Blanco and Cortés, 2007; Nakamura et al., 2021; Silva et al., 2018) and the average dispersal distance of wolves in Europe (Morales-González et al., 2021; excluding outliers of >1,000 km and wolves from Scandinavia that are in expansion and have very high dispersal distances compared to the rest of Europe) (see Sup. Fig. 4 in Appendix II for a detailed map with the four area types). To understand if our dynamic occupancy model predicts differences between area types, we obtained the average annual probabilities of colonization, extinction, and occupancy estimated by the model over the 5x5 km sites of each area type. Here, we assume that known wolf ranges are the most recent and accurate information for the IP and that no sites were mistakenly attributed.

2.5. Results

Wolves were detected in 1,481 out of 5,672 site-seasons, based on 9,672 wolf scats (AM 5,886; SD 509; VR 1,637; AST 1,640). For detailed results per site-season and study area, see Sup. Fig. 5 in Appendix II. The annual occupancy probabilities for each study area and the average annual detection probability (41.2 ± 8.8%; range: 22.7-68.1%) are presented in Fig. 2-2A. Wolf detection probability increased with transect effort ($\beta = 0.89 \pm 0.11$), ruggedness ($\beta = 0.89 \pm 0.14$), and unpaved road density ($\beta = 0.20 \pm 0.14$) (Fig. 2-3A). Comparatively, in AM, the detection estimated by transects of wolf signs and by GPS-collared wolf locations (n = 40,282 locations) was similar. We detected the species through transects of sign survey in most sites where GPS-collared wolves were present (seasonal average: 82% ± 22%; range: 17-100%), supporting the approach used here. Regarding the dynamic process of wolf occupation in these human-dominated landscapes, the probability that an area will become marked by wolves (i.e. colonization probability) increased with altitude ($\beta = 3.36 \pm 2.14$), livestock density ($\beta = 3.01 \pm 1.64$), and unpaved road density ($\beta = 1.86 \pm 1.62$). Conversely, linear infrastructure development (national/regional roads: $\beta = -1.49 \pm 1.99$, local roads: $\beta = -1.01 \pm 1.48$) and the proportion of burned areas ($\beta = -1.26 \pm 2.12$) influenced the probability of colonization negatively (Fig. 2-3B). In contrast, the probability that wolves will stop using an area (i.e. extinction probability) increased with the surface of burned areas ($\beta = 0.09 \pm 0.15$), and major linear infrastructures (national-regional roads: $\beta = 0.09 \pm 0.23$, highways: $\beta = 0.08 \pm 0.27$) (Fig. 2-3C). On the contrary, extinction probability decreased with unpaved road density ($\beta = -0.25 \pm 0.42$; 50% BCI) and, to a lesser extent, with higher human population densities ($\beta = -0.10 \pm 0.30$), altitude ($\beta = -0.06 \pm 0.31$), and local paved road density ($\beta = -0.09 \pm 0.25$) (Fig. 2-3C). For detailed model results, see Sup. Fig. 6 and Sup. Fig. 7, and Sup. Table 4 and Sup. Table 5 of Appendix II.

The initial occupancy probability for the overall sampled area was 89.5 ± 0.9% (95% BCI: 67.9-99.7%), and the average annual colonization and extinction probabilities were 63.1% and 5.6%, respectively. The average occupancy probability for the overall sampled areas was 85.2 ± 0.2% (range: 81.0-89.5%) and constant from 2005 to 2022 (Fig. 2-2A).

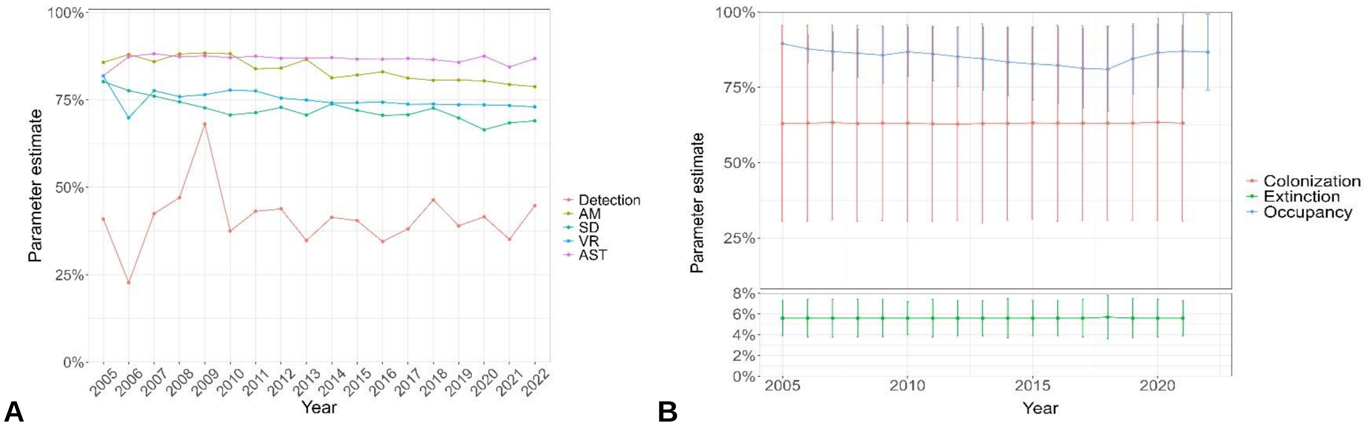


Fig. 2-2: A) Annual detection probability ('Detection') and annual occupancy probabilities for each study area (AM-Alto Minho, SD-South Douro, VR-Vila Real, AST-Asturias); B) Annual average parameter estimates for the overall study area (bars represent standard deviation values).

According to the predictions obtained for the overall IP, in the last year (2020-2021), 49% of the IP had a colonization probability higher than 50% (range 0-100%), 90% had an extinction probability higher than 10% (range: 3-58%), and 89% had an occupancy probability higher than 50% (range: 23-97%) (Fig. 2-4). The average annual colonization probability was relatively high in the areas of wolf expansion and persistence ($\gamma_{\text{expansion}}$ = 76.2 ± 0.3%; $\gamma_{\text{persistence}}$ = 66.9 ± 0.1%) but lower than 50% in the area of regression ($\gamma_{\text{regression}}$ = 43.1 ± 0.0%) (Fig. 2-4). The average annual extinction probability was generally low and similar across area types ($\boldsymbol{\varepsilon}_{\text{expansion}}$ = 5.8 ± 1.2%; $\boldsymbol{\varepsilon}_{\text{persistence}}$ = 5.8 ± 0.2%; $\boldsymbol{\varepsilon}_{\text{potential}}$ = 6.9 ± 0.1%; $\boldsymbol{\varepsilon}_{\text{regression}}$ = 6.4 ± 0.0%). The mean annual occupancy probability was always higher than 80% regardless of the area type ($\psi_{\text{expansion}}$ = 90.0 ± 3.6%; $\psi_{\text{persistence}}$ = 88.3 ± 3.4%; $\psi_{\text{regression}}$ =81.0 ± 4.5%) (Fig. 2-4). The colonization and occupancy probabilities for the potential recolonization area ($\gamma_{\text{potential}}$ = 49.5 ± 0.1%; $\psi_{\text{potential}}$ = 82.1 ± 4.7%) were relatively higher than the values obtained for the regression area. Additionally, 48% (103,141 km^2) of the considered potential recolonization area has over 50% of probability of being colonized (Fig. 2-4 and Fig. 2-5).

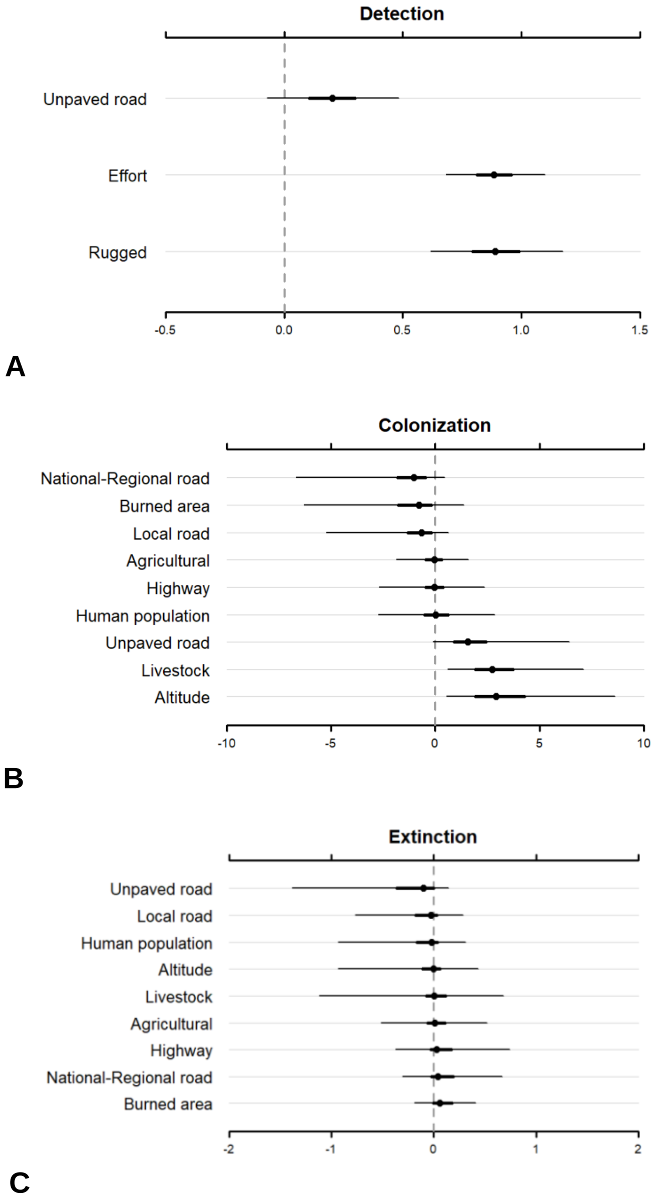


Fig. 2-3: Plots with mean covariate estimates of the dynamic occupancy model for each parameter (A-detection, B-colonization, and C-extinction), with 50% CI (thick bars) and 95% CI (thin bars). Covariates: 'Effort'- transect effort; 'Rugged'- ruggedness index;'Altitude' – average altitude (a.s.l); 'Highway', 'National-Regional road', 'Local road', and 'Unpaved road' densities; 'Burned area'- proportion of burned area; 'Agricultural: proportion of agricultural land; 'Livestock'- livestock unit density; and 'Human population'- human population density.

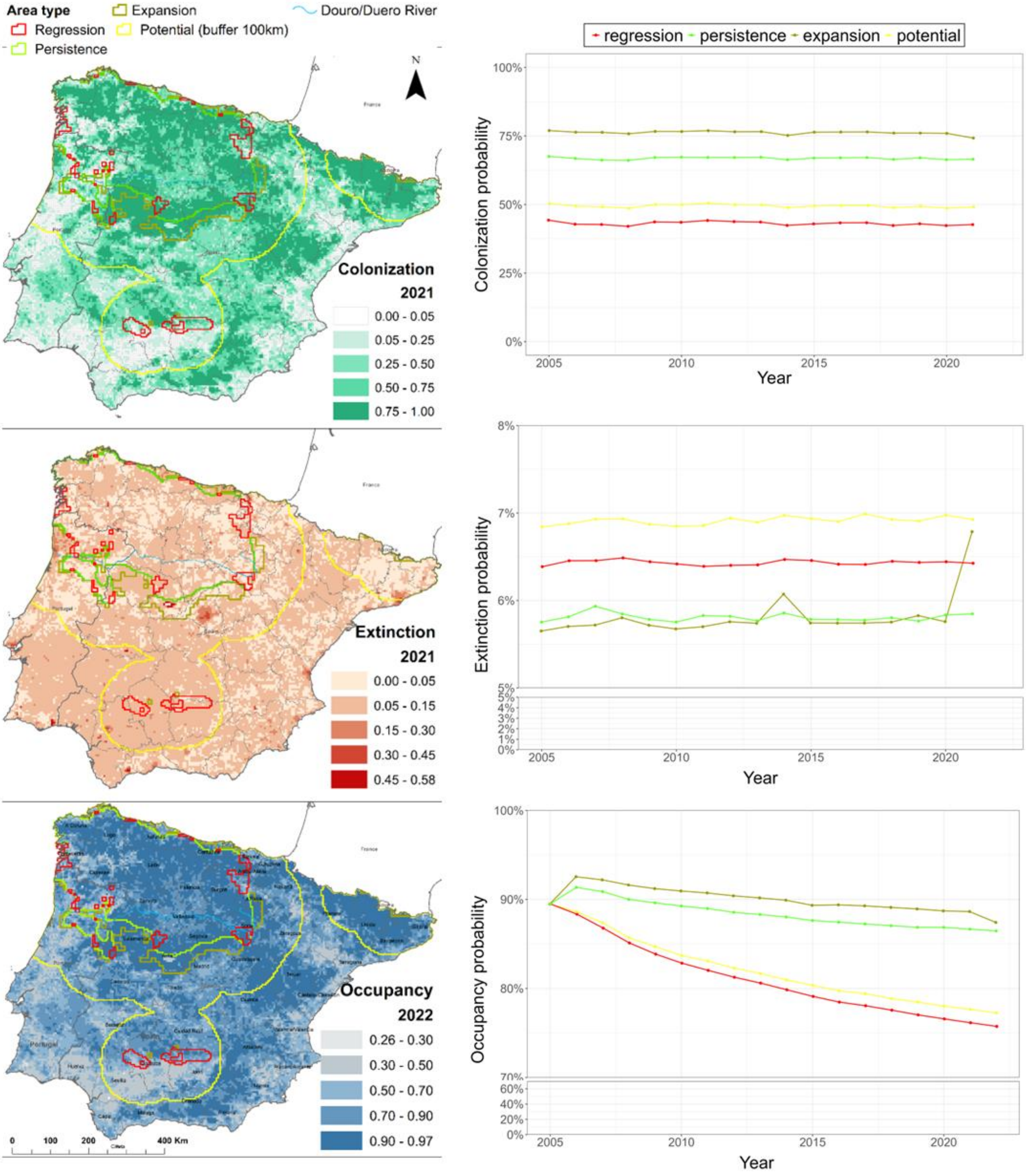


Fig. 2-4: Predicted wolf colonization, extinction and occupancy probabilities: i) of 2021-2022 for the Iberian Peninsula (left side maps); ii) from 2005 to 2022 in four area types, according to differences in wolf distributions between the beginning of the 2000s and recent years (right side graphs). Area types: regression, persistence, extinction and potential (i.e. 100 km buffer of the current wolf distribution)

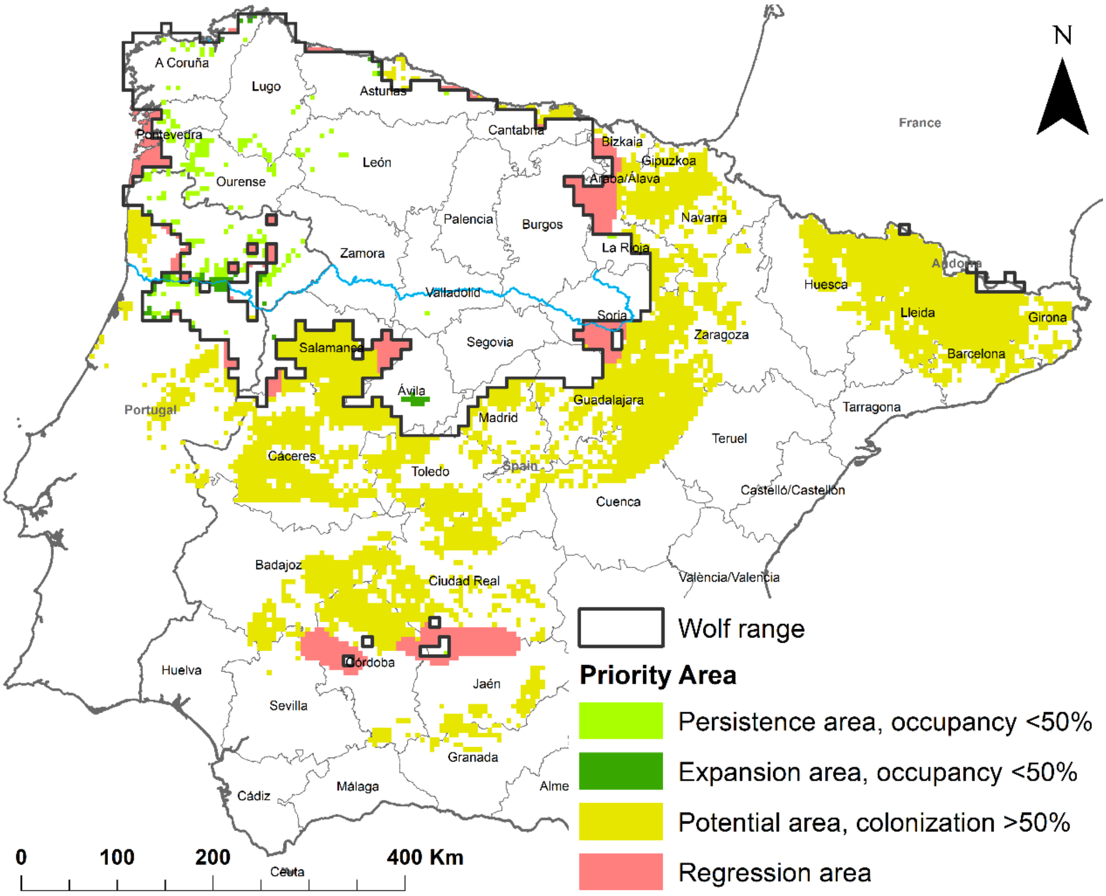


Fig. 2-5: Priority areas for implementing mitigation measures and conservation actions within the current wolf range (persistence and expansion areas with occupancy probability lower than 50%) and out of the range (potential recolonization area with colonization probability higher than 50% and regression area).

2.6. Discussion

By properly accommodating the detection process and landscape dynamics, our occupancy model helped us to identify environmental and anthropogenic variables influencing wolf spatial use in highly anthropogenic and heterogeneous landscapes of Western Europe. We obtained high average annual colonization probabilities (63%) and low extinction probabilities (6%) for the sampled study areas. The overall occupancy probabilities in the study areas were high (85%) throughout the study period, though they were generally higher in AM and AST compared with SD and VR, which indicates that the latter study areas have less suitable areas for wolves.

Our results suggest that higher altitude, livestock density, and unpaved road densities substantially increased the colonization probability. Our results also suggest that: the increase of burned areas and national-regional and local roads have potential negative effects on colonization; the increase of local and unpaved road densities have a potential negative effect on the extinction probability; and the increase of highway and national-regional road densities and the proportion of burned areas have a potential positive effect on the extinction probability. Our results indicate that human population density and the proportion of agricultural lands have no evident influence on wolf spatial dynamics.

Altitude was the covariate with the most decisive influence in the occupancy model, with areas over 750 m a.s.l. having >75% colonization probabilities (see also Llaneza et al., 2012). In human-dominated landscapes with small mountainous formations that can only encompass one or two packs, wolf territories are often bounded or surrounded by lower altitude areas and river valleys with higher human disturbance (Rio-Maior et al., 2019). Consequently, core areas of home ranges (e.g., breeding sites) are often located in mountainous and inaccessible areas with fewer human activities (Llaneza et al., 2012; Sazatornil et al., 2016), resulting in constant scent re-marking in such places (Barja et al., 2005; Llaneza et al., 2014). Unlike in core areas, space use and scent marking are potentially less constant on the territory edges with lower altitudes (Llaneza et al., 2014; Sazatornil et al., 2016), which can explain the tendency of higher extinction probability in these areas. Furthermore, colonization and extinction events are more likely to occur in territory edges due to annual territory shape differences and to elude intraspecific competition with neighbouring packs or dispersing wolves (Mech and Harper, 2002; Schlägel et al., 2017). On the other hand, wolves were absent or locally extinct in some sites at the beginning of the sampling period, even in areas with relatively high altitudes (e.g., Alto Minho; Nakamura et al., 2021). The recolonization of such areas throughout the sampling years can partly explain our results (Nakamura et al., 2021).

Wolves feed primarily on livestock in several regions of the IP (Blanco et al., 1992; Torres et al., 2015). Thus, as expected, wolves increasingly use areas where food availability is abundant, either in the form of live prey or carcasses (Llaneza and López-Bao, 2015; Mateo-Tomás et al., 2019). We obtained high colonization probabilities (>75%) when livestock density exceeds 60 LU/km^2. Livestock densities could positively affect extinction probabilities due to conflicts with humans, though we failed to detect such an effect. Nevertheless, poaching rates could be high since wolf productivity (e.g., Llaneza et al., 2023) can overcome poaching rates in this human-dominated landscape. In a population of 2,200-2,500 wolves of Iberia (Chapron et al., 2014) with remarkable annual wolf productivity (approximately 1,570 pups/year; see Sup. Table 6 in Appendix II), high extinction probabilities may be difficult to obtain even when mortality rates (mostly poaching) are high, as observed in the IP (Rio-Maior et al., 2018: poaching was the cause of death of 47% of 17 GPS collared wolves in Portugal between 2007 and 2017). This highlights the need to include a variable of wolf mortality probability in future occupancy studies to obtain better estimates of extinction probability and calls for increasing efforts in understanding wolf mortality causes in Iberia (mainly throughout GPS collaring).

Higher unpaved road densities increased the colonization probability and decreased extinction probability, possibly because wolves often use them to scent mark as territorial behaviour (Barja et al., 2004; Llaneza et al., 2004), for ease of travel (Whittington et al., 2005; Zimmermann et al., 2014), and because such roads are often associated to mountainous or forested areas, which can be more easily colonized. In contrast, wolves tend to colonize fewer areas with higher national-regional and local road densities, possibly due to high human disturbance since these roads often connect urban areas and have constant traffic or other human activities. Extinction probabilities tended to increase with national-regional road densities, which may be related to human disturbance and habitat fragmentation that preclude dispersal and to higher wolf mortality caused by traffic collisions on lower-level roads than highways (Dennehy et al., 2021).

Some studies have shown that predators can select burned areas due to the presence of prey (Geary et al., 2020; Lewis et al., 2022), avoid them immediately following disturbance and re-occupy relatively rapidly, or avoid them during the following years (Ballard et al., 2000). According to our model, wolves tended to avoid using areas with a higher proportion of burned areas and were less likely to colonize such areas. Since forest fires in the IP are often human-related (Nunes, 2012), our results indicate that wolves tend to avoid burned areas, likely due to direct human disturbance, low prey availability, or low refuge availability that increases their exposure to humans.

Taking advantage of wolves' high territoriality and marking behaviour, scat surveys are often used to assess spatial use and detect core areas (Barja et al., 2005; Llaneza et al., 2014). Our study identified some variables that explain habitat suitability for colonization of wolves, though it fails to detect factors clearly related to the extinction parameter. From an ecological perspective, this could be because of the species' overall expansion trend and its typical resilience in human-dominated landscapes (Blanco and Cortés, 2009; Weaver et al., 1996). Furthermore, extinction sites in our study areas are less frequent and may lack variability compared to sites with stable pack territories that are constantly used or colonized.

Considering the differences in the estimated wolf ranges for the IP in the last decades, higher mean colonization and occupancy probabilities were obtained for wolf expansion and persistence areas than for regression and potential recolonization areas. The opposite occurred for mean extinction probabilities, with higher values for regression and potential areas than for expansion and persistence areas. While the differences were not significant, the model still predicts a gradient of higher to lower wolf occupancy probabilities from expansion, persistence, potential and regression areas, in this order. However, the relatively high occupancy (81%) and low colonization (43-50%) in sites where wolves were absent in recent times (i.e. potential and regression areas) indicate that the recolonization may not occur easily or quickly and that other factors rather than those considered in this study may be operating.

According to the maps of parameter probabilities obtained for the IP, there are some large extensions of sites with low occupancy probabilities within the current wolf distribution. This is the case in southern Galicia (Pontevedra and Ourense) and northeastern Portugal. Wolf is believed to have gone extinct since the beginning of the 2000's in a large area at the boundaries of Álava, Burgos, and La Rioja, Soria, and eastern Salamanca (in Spain), and in the southwestern area of Vila Real and some areas of the southern range limit (in Portugal). The model also predicted part of this area as having low occupancy probabilities. According to the model, Sierra Morena (southern Spain) has some sites with high colonization probability. If wolves are still present in that area, colonization would likely occur in the surroundings. On the other hand, if this relict population did not resist, recolonization is unlikely to occur shortly due to low colonization probabilities or non-continuous areas with relatively high occupancy probability and considerable distance to the remnant wolf distribution.

The model predicted high colonization and occupancy probabilities for recently recolonized areas in Spain (La Rioja/Soria, northern Guadalajara, northern Madrid, Ávila and northern Salamanca). From an ecological point of view, we predict that in the recent future, wolf recolonization could proceed through the central mountainous massif of Portugal (Serra da Estrela), remnant Salamanca and into the area of Cáceres. In the eastern front of the wolf distribution, eastern Guadalajara and the borders with Teruel and Cuenca also have high probabilities of recolonization. However, over the last decades, population expansion has nearly stagnated in such areas, apparently caused by the wolf persecution triggered by high livestock damage (Blanco and Cortés, 2009). The wolf population has not expanded to several mountain areas free from man-made barriers, with high densities of wild ungulates or well-preserved *dehesa* areas (i.e. savannah-like wood pasture where livestock graze unguarded) (Blanco and Cortés, 2009). In opposition, since the 1970s, a significant expansion of the Spanish wolf population occurred into less suitable agricultural habitats with a low density of wild ungulates, little vegetation cover, and a high density of roads (Blanco and Cortés, 2009; López-Bao et al., 2018a). Since frequent damages to livestock alone are enough to prevent wolves from expanding into ecologically suitable habitats (Blanco and Cortés, 2009), such unexpected expansion fronts and stagnations emphasize the strong effect and the need to assess social factors on the recovery of wolf populations. Lastly, recent evidence indicate that wolf expansion from the Italian population is recolonizing the eastern Pyrenees (Louvrier et al., 2018), which is in concordance with the high colonization probabilities obtained by the model. Nevertheless, wide areas with low colonization probabilities between the expansion from France and the eastern front of the Iberian wolf range indicate that these populations are not expected to mix in the near future.

Wolf detectability increased with higher survey effort (see also Jiménez et al., 2016; Llaneza et al., 2014), landscape ruggedness, and density of unpaved roads. Wolves' seasonal variation of space use and marking behaviour may have influenced on lower detection rate than expected (Roda et al., 2022). Detection probability also increased with a higher density of unpaved roads, possibly because wolves often use these structures as preferred travel routes and marking sites (Stępniak et al., 2020). Furthermore, higher unpaved road density leads to a higher frequency of crossroads and intersections, known to be preferred scent-marking sites for wolves (Barja et al., 2004). The selection of conspicuous substrates at crossroads amplifies the visual component of deposited scats, increasing scat detectability for other wolves (Barja et al., 2004; Bojarska et al., 2020) and for the observer as well. In landscapes with lower ruggedness, wolves have more travelling route options and can travel more randomly across the landscape since effort is likely to be similar between untrailed paths or unpaved roads. Conversely, more rugged landscapes provide fewer options, and therefore wolves tend to travel through least cost paths that exist in limited availability (e.g., roads; Zimmermann et al., 2014). The regular use of such routes likely results in higher marking intensity and detectability of scats.

We propose long-term monitoring surveys in areas where packs are permanently present and in the neighbouring areas in order to detect recent wolf population expansions (see Nakamura et al., 2021). Transects of sign surveys, and camera trapping approaches, between known pack territories and close to the limit of wolf distribution can help detect dispersal events and recent recolonizations that would be harder to detect through other methods. Here, we performed a prediction based on the occupancy model to identify the potentially suitable areas where the wolf population could expand in the near future; although wolf dispersal patterns accounting for habitat availability and connectivity deserves further investigation. Considering the high wolf capabilities for dispersal, the sampling area for the national wolf population estimates should also include a buffer of 100 km of the last known distribution, particularly close to areas where wolves appear to be recolonizing (Fig. 2-5).

GPS-collared wolves in the expansion borders would facilitate detailed information on dispersal movements that is unobtainable through any other method. Additionally, intensive non-invasive sampling with molecular individual identification and genealogy analyses can also provide information on habitat connectivity and dispersal, especially relevant for recolonizing areas, particularly if included in a spatial capture-recapture framework (e.g., Caniglia et al., 2014; Kervellec et al., 2023). Integrating data on additional socio-ecological factors, such as wild prey density, hunting pressure, or other human infrastructures, could also benefit further investigations. It would be expected that wild prey availability, together with livestock vulnerability, influence on wolf diet, although detailed data on wild prey densities in the IP is limited. To address this knowledge gap, we recommend that more studies on wild ungulate density estimations should be conducted in the IP, such as wild boar density estimates obtained by ENETWILD-consortium et al. (2019) based on species occurrence and hunting bags or studies based on camera trap data (Gilbert et al., 2021). These density estimates could be incorporated into wolf occupancy studies to improve our understanding on the influence of both livestock and wild prey availability on wolf occurrence and persistence in such human-dominated landscapes. Based on our findings, the sites where the wolf was recently extinct or with low occupancy probabilities within its distribution range should be the main priority for implementing conservation actions and mitigation measures (Fig. 2-5). These areas are potential indicators of conflicts around the presence of wolves and poaching. Increasing awareness among the local communities, including livestock owners or hunters, would be highly relevant in such areas. Efforts should be focused on implementing livestock damage preventive methods (e.g., Eklund et al., 2017), developing patrolling activities, and improving law reinforcement to fight against poaching. We also recommend implementing roadkill mitigation measures (particularly on national-regional and local roads) and improving landscape management regarding habitats with a higher probability of large fires. Moreover, areas with high probabilities of colonization beyond the current wolf range should also be given priority (Fig. 2-5). Efforts should be intensified in these areas to raise awareness about facilitating human-wolf coexistence, promoting livestock damage preventive methods, and explaining how compensation schemes work. We also propose that future studies incorporate a social component, human dimensions, or estimated wolf mortality rates across space to shed light on the slow recovery and recolonization of the population in Iberia.

2.7. Funding

MN and SR worked under PhD grants [SFRH/BD/144087/2019] and [SFRH/BD/12291/2003], respectively, from Fundação para a Ciência e a Tecnologia. Field and lab work for wolf monitoring in Portugal was supported by Grupo Lobo/Faculdade de Ciências da Universidade de Lisboa, ACHLI (Associação de Conservação do Habitat do Lobo Ibérico), and Empreendimentos Eólicos do Vale do Minho S.A.. OG and MN were partly funded by the French National Research Agency [grant ANR-16-CE02-0007]. PM and SR were supported by UID/BIA/50027/2021 and PPCDT/BIA-BDE/60296/2004, respectively, with funding from FCT/MCTES through national funds. JVLB was supported by the Spanish Ministry of Economy, Industry and Competitiveness (CGL2017-87528-R AEI/FEDER EU), the Spanish Ministry of Science and Innovation (TED2021-132519B-I00), and by a GRUPIN research grant from the Regional Government of Asturias (AYUD/2021/51314).

2.8. Author contributions CRediT

Mónia Nakamura: Conceptualization, Data Curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing original draft, Writing review & editing. **José Vicente López-Bao**: Conceptualization, Data Curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing review & editing, Supervision. **Helena Rio-Maior**: Conceptualization, Data Curation, Funding acquisition, Investigation, Methodology, Writing review & editing, Project administration, Supervision. **Sara Roque**: Data Curation, Investigation, Methodology, Writing review & editing, Supervision. **Patrícia Gil:** Data Curation, Investigation, Writing review & editing. **Ana Serronha:** Data Curation, Investigation, Writing review & editing. **Emilio García**: Investigation, Writing review & editing. **Orencio Hernández Palacios:** Methodology, Writing review & editing. **Gonçalo Ferrão da Costa**: Data Curation, Investigation, Methodology, Writing review & editing. **Francisco Álvares**: Funding acquisition, Methodology, Writing review & editing, Project administration, Supervision. **Francisco Petrucci-Fonseca**: Funding acquisition, Writing review & editing, Project administration, Supervision. **Olivier Gimenez:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing review & editing, Supervision. **Pedro Monterroso**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing review & editing, Supervision.

2.9. Acknowledgements

We thank the staff of the Regional Government of Asturias, in particular all the rangers participating in wolf monitoring activities. We thank lab assistance for genetic data R Godinho, D Castro, S Lopes. We are grateful to A Guerra, J Eggermann, M Almeida, B Martí-Domken, J Pereira, D Cadete, S Pinto, V Ramiro, AS Pedro, J Bernardo, I Palmegiani, F Moreira, F Grilo, M Sampaio, and other field assistants, colleagues and volunteers. We also thank S Bauduin and J Gonçalves for the support and suggestions on programming procedures and selection of covariates. We are grateful to Instituto de Conservação da Natureza e Florestas (ICNF) and Serviço de Protecção da Natureza e do Ambiente – Guarda Nacional Republicana (SEPNA- GNR) for providing legal support for wolf monitoring in Portugal. This is contribution no. 32 from the Iberian Wolf Research Team (IWRT).

2.10. Research data

The processed data file (.Rdata) and code of the dynamic occupancy model (.Rmd) are available on the Zenodo Digital Repository (https://zenodo.org/record/8377581). The processed data files include the data frame with detection/non-detection results and standardized values of covariates included in the final model, per 5x5 km site (N=395) of the sampled study areas from March 2005 to February 2022.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2023.110316, and also in Appendix II of this thesis.

2.11. References

Álvares, F., Barroso, I., Blanco, J.C., Correia, J., Cortés, Y., Costa, G., Llaneza, L., Moreira, L., Nascimento, J., Palacios, V., Petrucci-Fonseca, F., Pimenta, V., Roque, S., Santos, E., 2005. Wolf status and conservation in the Iberian Peninsula, in: Conference Frontiers of Wolf Recovery: Southern US and the World. Colorado, pp. 76–77.

Álvares, F., Barroso, I., Costa, G.F. da, Espírito-Santo, C., Fonseca, C., Godinho, R., Nakamura, M., Petrucci-Fonseca, F., Pimenta, V., Rio-Maior, H., Santos, N., Torres, R.T., 2015. Situação de referência para o Plano de Ação para a Conservação do Lobo-ibérico em Portugal. Lisbon, Portugal.

Ballard, W.B., Krausman, P.R., Boe, S., Cunningham, S., Whitlaw, H.A., 2000. Short-term response of grey wolves, *Canis lupus*, to wildfire in Northeastern Alaska. Can. Field-Naturalist 114, 241–247.

Barja, I., de Miguel, F.J., Bárcena, F., 2005. Faecal marking behaviour of Iberian wolf in different zones of their territory. Folia Zool. 54, 21–29.

Barja, I., de Miguel, F.J., Bárcena, F., 2004. The importance of crossroads in faecal marking behaviour of the wolves (*Canis lupus*). Naturwissenschaften 91, 489–492. https://doi.org/10.1007/s00114-004-0557-1

Blanco, J.C., Cortés, Y., 2009. Ecological and social constraints of wolf recovery in Spain, in: Musiani, M., Boitani, L., Paquet, P.C. (Eds.), A New Era for Wolves and People: Wolf Recovery, Human Attitudes, and Policy. University of Calgary Press, Calgary, pp. 41–66.

Blanco, J.C., Cortés, Y., 2007. Dispersal patterns, social structure and mortality of wolves living in agricultural habitats in Spain. J. Zool. 273, 114–124. https://doi.org/10.1111/j.1469-7998.2007.00305.x

Blanco, J.C., Cortés, Y., 2001. Ecología, Censos, Percepción y Evolución del Lobo en España: Análisis de un Conflicto.

Blanco, J.C., Cortés, Y., Virgós, E., 2005. Wolf response to two kinds of barriers in an agricultural habitat in Spain. Can. J. Zool. 83, 312–323. https://doi.org/10.1139/z05-016

Blanco, J.C., Reig, S., De La Cuesta, L., 1992. Distribution, status and conservation problems of the wolf *Canis lupus* in Spain. Biol. Conserv. 60, 73–80. https://doi.org/10.1016/0006-3207(92)91157-N

Boitani, L., Kaczensky, P., Alvares, F., Andrén, H., Balys, V., Blanco, J.C., Chapron, G., Chiriac, S., Cirovic, D., Drouet-Houguet, N., Groff, C., Huber, D., Iliopoulos, Y., Ionescu, O., Kojola, I., Krofel, M., Kutal, M., Linnell, J., Majic, A., Mannil, P., Marucco, F., D. Melovski, Mengüllüoğlu, D., Mergeay, J., Nowak, S., Ozolins, J., Perovic, A., Rauer, G., Reinhardt, I., Rigg, R., Salvatori, V., Sanaja, B., Schley, L., Shkvyria, M., Sunde, P., Tirronen, K., Trajce, A., Trbojevic, I., Trouwborst, A., Arx, M. von, Wolfl, M., Zlatanova, D., Patkó, L., 2022. Assessment of the conservation status of the Wolf (*Canis lupus*) in Europe.

Bojarska, K., Sulich, J., Bachmann, S., Okarma, H., Theuerkauf, J., Gula, R., 2020. Opportunity and peril: how wolves use a dense network of forest roads. Mamm. Biol. 100, 203–211. https://doi.org/10.1007/s42991-020-00014-0

Caniglia, R., Fabbri, E., Galaverni, M., Milanesi, P., Randi, E., 2014. Noninvasive sampling and genetic variability, pack structure, and dynamics in an expanding wolf population. J. Mammal. 95, 41–49. https://doi.org/10.1644/13-MAMM-A-039

Carter, N.H., Linnell, J.D.C., 2016. Co-Adaptation Is Key to Coexisting with Large Carnivores. Trends Ecol. Evol. 31, 575–578. https://doi.org/10.1016/j.tree.2016.05.006

Center for International Earth Science Information Network - CIESIN - Columbia University, 2018. Gridded Population of the World, Version 4 (GPWv4): Population Density Adjusted to Match 2015 Revision UN WPP Country Totals, Revision 11.

Chapron, G., Kaczensky, P., Linnell, J.D.C., von Arx, M., Huber, D., Andren, H., López-

Bao, J.V., Adamec, M., Álvares, F., Anders, O., Balciauskas, L., Balys, V., Bed, P., Bego, F., Blanco, J.C., Breitenmoser, U., Broseth, H., Bufka, L., Bunikyte, R., Ciucci, P., Dutsov, A., Engleder, T., Fuxjager, C., Groff, C., Holmala, K., Hoxha, B., Iliopoulos, Y., Ionescu, O., Jeremi, J., Jerina, K., Kluth, G., Knauer, F., Kojola, I., Kos, I., Krofel, M., Kubala, J., Kunovac, S., Kusak, J., Kutal, M., Liberg, O., Maji, A., Mannil, P., Manz, R., Marboutin, E., Marucco, F., Melovski, D., Mersini, K., Mertzanis, Y., Mysłajek, R.W., Nowak, S., Odden, J., Ozolins, J., Palomero, G., Paunovi, M., Persson, J., Potonik, H., Quenette, P.-Y., Rauer, G., Reinhardt, I., Rigg, R., Ryser, A., Salvatori, V., Skrbinek, T., Stojanov, A., Swenson, J.E., Szemethy, L., Trajce, A., Tsingarska-Sedefcheva, E., Va a, M., Veeroja, R., Wabakken, P., Wolfl, M., Wolfl, S., Zimmermann, F., Zlatanova, D., Boitani, L., 2014. Recovery of large carnivores in Europe's modern human-dominated landscapes. Science (80-.). 346, 1517–1519. https://doi.org/10.1126/science.1257553

CLC, 2018. © European Union, Copernicus Land Monitoring Service 2018, European Environment Agency (EEA).

Corbane, C., Sabo, F., 2019. European Settlement Map from Copernicus Very High Resolution data for reference year 2015, Public Release 2019. https://doi.org/10.2905/8BD2B792-CC33-4C11-AFD1-B8DD60B44F3B

DeCesare, N.J., Wilson, S.M., Bradley, E.H., Gude, J.A., Inman, R.M., Lance, N.J., Laudon, K., Nelson, A.A., Ross, M.S., Smucker, T.D., 2018. Wolf-livestock conflict and the effects of wolf management. J. Wildl. Manage. 82, 711–722. https://doi.org/10.1002/jwmg.21419

Dennehy, E., Llaneza, L., López-Bao, J.V., 2021. Contrasting wolf responses to different paved roads and traffic volume levels. Biodivers. Conserv. 30, 3133–3150. https://doi.org/10.1007/s10531-021-02239-y

Eklund, A., López-Bao, J.V., Tourani, M., Chapron, G., Frank, J., 2017. Limited evidence on the effectiveness of interventions to reduce livestock predation by large carnivores. Sci. Rep. 7, 2097. https://doi.org/10.1038/s41598-017-02323-w

ENETWILD-consortium, Acevedo, P., Croft, S., Smith, G.C., Blanco-Aguiar, J.A., Fernandez-Lopez, J., Scandura, M., Apollonio, M., Ferroglio, E., Keuling, O., Sange, M., Zanet, S., Brivio, F., Podgórski, T., Petrović, K., Body, G., Cohen, A., Soriguer, R., Vicente, J., 2019. ENETwild modelling of wild boar distribution and abundance: update of occurrence and hunting data-based models. EFSA Support. Publ. 16. https://doi.org/10.2903/sp.efsa.2019.en-1674

Fechter, D., Storch, I., 2014. How many wolves (*Canis lupus*) fit into Germany? The role of assumptions in predictive rule-based habitat models for habitat generalists. PLoS One 9. https://doi.org/10.1371/journal.pone.0101798

Fernández-Gil, A., Naves, J., Ordiz, A., Quevedo, M., Revilla, E., Delibes, M., 2016. Conflict Misleads Large Carnivore Management and Conservation: Brown Bears and Wolves in Spain. PLoS One 11, e0151541. https://doi.org/10.1371/journal.pone.0151541

Fuller, T.K., Mech, L.D., Cochrane, J.F., 2003. Wolf population dynamics, in: Mech, L.D., Boitani, L. (Eds.), Wolves. Behaviour Ecology and Conservation. Chicago University Press, Chicago, pp. 161–191.

Geary, W.L., Doherty, T.S., Nimmo, D.G., Tulloch, A.I.T., Ritchie, E.G., 2020. Predator responses to fire: A global systematic review and meta-analysis. J. Anim. Ecol. 89, 955–971. https://doi.org/10.1111/1365-2656.13153

Gelman, A., Carlin, J.B., Stern, H.S., Rubin, D.B., 2004. Bayesian data analysis. Boca Raton FL: Chapman and Hall.

Gilbert, N.A., Clare, J.D.J., Stenglein, J.L., Zuckerberg, B., 2021. Abundance estimation of unmarked animals based on camera-trap data. Conserv. Biol. 35, 88–100. https://doi.org/10.1111/cobi.13517

Glenz, C., Massolo, A., Kuonen, D., Schlaepfer, R., 2001. A wolf habitat suitability prediction study in Valais (Switzerland). Landsc. Urban Plan. 55, 55–65. https://doi.org/10.1016/s0169-2046(01)00119-0

Grilo, C., Lucas, P.M., Fernández-Gil, A., Seara, M., Costa, G., Roque, S., Rio-Maior, H., Nakamura, M., Álvares, F., Petrucci-Fonseca, F., Revilla, E., 2018. Refuge as major habitat driver for wolf presence in human-modified landscapes. Anim. Conserv. 22, 59–71. https://doi.org/10.1111/acv.12435

Gurarie, E., Suutarinen, J., Kojola, I., Ovaskainen, O., 2011. Summer movements, predation and habitat use of wolves in human modified boreal forests. Oecologia 165, 891–903. https://doi.org/10.1007/s00442-010-1883-y

Jedrzejewski, W., Jedrzejewska, B., Zawadzka, B., Borowik, T., Nowak, S., Mysłajek, R.W., Myslajek, R.W., Mysłajek, R.W., 2008. Habitat suitability model for Polish wolves based on long-term national census. Anim. Conserv. 11, 377–390. https://doi.org/10.1111/j.1469-1795.2008.00193.x

Jiménez, J., García, E.J., Llaneza, L., Palacios, V., González, L.M., García-Domínguez, F., Múñoz-Igualada, J., López-Bao, J.V., 2016. Multimethod, multistate Bayesian hierarchical modeling approach for use in regional monitoring of wolves. Conserv. Biol. 30, 883–893. https://doi.org/10.1111/cobi.12685

Kabir, M., Hameed, S., Ali, H., Bosso, L., Din, J.U., Bischof, R., Redpath, S., Nawaz, M.A., 2017. Habitat suitability and movement corridors of grey wolf (*Canis lupus*) in Northern Pakistan. PLoS One 12, e0187027. https://doi.org/10.1371/journal.pone.0187027

Kaczensky, P., 2018. IUCN Red List Mapping for the regional assessment of the Wolf (*Canis lupus*) in Europe.

Kervellec, M., Milleret, C., Vanpé, C., Quenette, P.Y., Sentilles, J., Palazón, S., Jordana, I.A., Jato, R., Elósegui Irurtia, M.M., Gimenez, O., 2023. Integrating opportunistic and structured non-invasive surveys with spatial capture-recapture models to map connectivity of the Pyrenean brown bear population. Biol. Conserv. 278. https://doi.org/10.1016/j.biocon.2022.109875

Lamb, C.T., Ford, A.T., McLellan, B.N., Proctor, M.F., Mowat, G., Ciarniello, L., Nielsen, S.E., Boutin, S., 2020. The ecology of human–carnivore coexistence. Proc. Natl. Acad. Sci. U. S. A. 117, 17876–17883. https://doi.org/10.1073/pnas.1922097117

Lewis, J.S., LeSueur, L., Oakleaf, J., Rubin, E.S., 2022. Mixed-severity wildfire shapes habitat use of large herbivores and carnivores. For. Ecol. Manage. 506, 119933. https://doi.org/10.1016/j.foreco.2021.119933

Lino, S., Sillero, N., Torres, J., Santos, X., Álvares, F., Alvares, F., 2019. The role of fire on wolf distribution and breeding-site selection: Insights from a generalist carnivore occurring in a fire-prone landscape. Landsc. Urban Plan. 183, 111–121. https://doi.org/10.1016/j.landurbplan.2018.12.003

Llaneza, L., Álvares, F., Ordiz, A., Sierra, P., Uzal, A., 2004. Distribución y aspectos poblacionales del Lobo Ibérico en la provincia de Ourense. Ecología 18, 227–238.

Llaneza, L., Fernández, A., Nores, C., 1996. Dieta del lobo en dos zonas de Asturias (España) que difieren en carga ganadera. Acta Vertebr. 23, 201–213.

Llaneza, L., García, E.J., López-Bao, J.V., 2014. Intensity of Territorial Marking

Predicts Wolf Reproduction: Implications for Wolf Monitoring. PLoS One 9, e93015. https://doi.org/10.1371/journal.pone.0093015

Llaneza, L., López-Bao, J.V., Sazatornil, V., 2012. Insights into wolf presence in human-dominated landscapes: the relative role of food availability, humans and landscape attributes. Divers. Distrib. 18, 459–469. https://doi.org/10.1111/j.1472-4642.2011.00869.x

Llaneza, L., López-Bao, J. V., 2015. Indirect effects of changes in environmental and agricultural policies on the diet of wolves. Eur. J. Wildl. Res. 61, 895–902. https://doi.org/10.1007/s10344-015-0966-9

Llaneza, L., Ordiz, A., Palacios, V., Uzal, A., 2005. Monitoring wolf populations using howling points combined with sign survey transects. Wildl. Biol. Pract. 1, 108–117. https://doi.org/10.2461/wbp.2005.1.13

Llaneza, L., Sazatornil, V., García, E.J., Palacios, V., López-Bao, J.V., 2023. Minimum average pack size in Iberian wolves. Eur. J. Wildl. Res. 69, 1–6. https://doi.org/10.1007/s10344-023-01695-x

Llaneza, L., Sazatornil, V., López-Bao, J.V., 2018. The importance of fine-scale breeding site selection patterns under a landscape-sharing approach for wolf conservation. Biodivers. Conserv. 27, 1239–1256. https://doi.org/10.1007/s10531-017-1491-9

López-Bao, J.V., Blanco, J.C., Rodríguez, A., Godinho, R., Sazatornil, V., Alvares, F., García, E.J., Llaneza, L., Rico, M., Cortés, Y., Palacios, V., Chapron, G., 2015. Toothless wildlife protection laws. Biodivers. Conserv. 24, 2105–2108. https://doi.org/10.1007/s10531-015-0914-8

López-Bao, J.V., Bruskotter, J., Chapron, G., 2017. Finding space for large carnivores. Nat. Ecol. Evol. 1, 139–141. https://doi.org/10.1038/s41559-017-0140

López-Bao, J.V., Fleurke, F., Chapron, G., Trouwborst, A., 2018a. Legal obligations regarding populations on the verge of extinction in Europe: Conservation, Restoration, Recolonization, Reintroduction. Biol. Conserv. 227, 319–325. https://doi.org/10.1016/j.biocon.2018.09.027

López-Bao, J.V., Godinho, R., Pacheco, C., Lema, F.J., García, E., Llaneza, L., Palacios, V., Jiménez, J., 2018b. Toward reliable population estimates of wolves by combining spatial capture-recapture models and non-invasive DNA monitoring. Sci. Rep. 8, 2177. https://doi.org/10.1038/s41598-018-20675-9

López-Bao, J.V., Sazatornil, V., Llaneza, L., Rodríguez, A., 2013. Indirect Effects on Heathland Conservation and Wolf Persistence of Contradictory Policies that Threaten Traditional Free-Ranging Horse Husbandry. Conserv. Lett. 6. https://doi.org/10.1111/conl.12014

Louvrier, J., Duchamp, C., Lauret, V., Marboutin, E., Cubaynes, S., Choquet, R., Miquel, C., Gimenez, O., 2018. Mapping and explaining wolf recolonization in France using dynamic occupancy models and opportunistic data. Ecography (Cop.). 41, 647–660. https://doi.org/10.1111/ecog.02874

Lovari, S., Sforzi, A., Scala, C., Fico, R., 2007. Mortality parameters of the wolf in Italy: Does the wolf keep himself from the door? J. Zool. 272, 117–124. https://doi.org/10.1111/j.1469-7998.2006.00260.x

MacKenzie, D.I., Nichols, J.D., Hines, J.E., Knutson, M.G., Franklin, A.B., 2003. Estimating Site Occupancy, Colonization, and Local Extinction When a Species Is Detected Imperfectly. Ecology 84, 2200–2207. https://doi.org/10.1890/02-3090

MacKenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Andrew, J., Langtimm, C.

a, Ecology, S., Aug, N., 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83, 2248–2255. https://doi.org/10.2307/3072056

MacKenzie, D.I., Nichols, J.D., Royle, J.A., Pollock, K.H., Bailey, L.L., Hines, J.E., 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence., Wildlife Biology. Elsevier Inc.

Marucco, F., McIntire, E.J.B., 2010. Predicting spatio-temporal recolonization of large carnivore populations and livestock depredation risk: wolves in the Italian Alps. J. Appl. Ecol. 47, 789–798. https://doi.org/10.1111/j.1365-2664.2010.01831.x

Mateo-Tomás, P., Olea, P.P., López-Bao, J.V., González-Quirós, P., Peón, P., 2019. Different criteria for implementing sanitary regulations lead to disparate outcomes for scavenger conservation. J. Appl. Ecol. 56, 500–508. https://doi.org/10.1111/1365-2664.13293

Mattisson, J., Sand, H., Wabakken, P., Gervasi, V., Liberg, O., Linnell, J.D.C., Rauset, G.R., Pedersen, H.C., 2013. Home range size variation in a recovering wolf population: evaluating the effect of environmental, demographic, and social factors. Oecologia 173, 813–825. https://doi.org/10.1007/s00442-013-2668-x

Mech, L.D., Cluff, H.D., 2011. Movements of Wolves at the Northern Extreme of the Species' Range, Including during Four Months of Darkness. PLoS One 6, e25328. https://doi.org/10.1371/journal.pone.0025328

Mech, L.D., Harper, E.K., 2002. Differential use of a Wolf, *Canis lupus*, pack territory edge and core. Can. Field- Nat. 116, 315–316.

Mladenoff, D.J., Sickley, T.A., Haight, R.G., Wydeven, A.P., 1995. A Regional Landscape Analysis and Prediction of Favorable Gray Wolf Habitat in the Northern Great Lakes Region. Conserv. Biol. 9, 279–294. https://doi.org/10.1046/j.1523-1739.1995.9020279.x

Monteiro, R., 2015. Demographic history of the isolated and endangered wolf population in the south of the Douro river. MSc thesis. University of Porto, Portugal.

Morales-González, A., Fern, A., Quevedo, M., Revilla, E., Morales-gonz, A., 2021. Patterns and determinants of dispersal in grey wolves (*Canis lupus*). https://doi.org/10.1111/brv.12807

Nakamura, M., Godinho, R., Rio-Maior, H., Roque, S., Kaliontzopoulou, A., Bernardo, J., Castro, D., Lopes, S., Petrucci-Fonseca, F., Álvares, F., 2017. Evaluating the predictive power of field variables for species and individual molecular identification on wolf noninvasive samples. Eur. J. Wildl. Res. 63, 53. https://doi.org/10.1007/s10344-017-1112-7

Nakamura, M., Rio-Maior, H., Godinho, R., Petrucci-Fonseca, F., Álvares, F., 2021. Source-sink dynamics promote wolf persistence in human-modified landscapes: Insights from long-term monitoring. Biol. Conserv. 256, 109075. https://doi.org/10.1016/j.biocon.2021.109075

Nores, C., López-Bao, J.V., 2022. Historical data to inform the legal status of species in Europe: An example with wolves. Biol. Conserv. 272. https://doi.org/10.1016/j.biocon.2022.109639

Nunes, A.N., 2012. Regional variability and driving forces behind forest fires in Portugal an overview of the last three decades (1980–2009). Appl. Geogr. 34, 576–586. https://doi.org/10.1016/j.apgeog.2012.03.002

Oakleaf, J.K., Murray, D.L., Oakleaf, J.R.J., Bangs, E.E., Mack, C.M., Smith, D.W., Fontaine, J.A., Jimenez, M.D., Meier, T.J., Niemeyer, C.C., 2006. Habitat Selection by Recolonizing Wolves in the Northern Rocky Mountains of the United States. J. Wildl. Manage. 70, 554–563. https://doi.org/10.2193/0022-541X(2006)70

OpenStreetMap contributors, 2022. retrieved from https://download.geofabrik.de/.

Pease, C.M., Mattson, Da.J., 1999. Demography of the Yellowstone Grizzly Bears. Ecology 80, 957–975.

Pimenta, V., Barroso, I., Boitani, L., Beja, P., 2018. Risks a la carte: Modelling the occurrence and intensity of wolf predation on multiple livestock species. Biol. Conserv. 228, 331–342. https://doi.org/10.1016/j.biocon.2018.11.008

Pimenta, V., Barroso, I., Boitani, L., Beja, P., 2017. Wolf predation on cattle in Portugal : assessing the effects of husbandry systems Accepted in Biological Conservation. Biol. Conserv. 207, 17–26. https://doi.org/10.1016/j.biocon.2017.01.008

Planella, A., Palacios, V., García, E.J.E.J., Llaneza, L., García-Domínguez, F., Muñoz-Igualada, J., López-Bao, J.V., 2016. Influence of different GPS schedules on the detection rate of wolf feeding sites in human-dominated landscapes. Eur. J. Wildl. Res. 62, 471–478. https://doi.org/10.1007/s10344-016-1020-2

Plummer, M., 2011. JAGS: a program for the statistical analysis of Bayesian hierarchical models by Markov Chain Monte Carlo.

Posit team, 2022. RStudio: Integrated Development Environment for R. Posit Software, PBC.

Pulliam, R.H., 1988. Sources, sinks, and population regulation. Am. Nat. 132, 652–661. https://doi.org/10.1086/284880

Ražen, N., Brugnoli, A., Castagna, C., Groff, C., Kaczensky, P., Kljun, F., Knauer, F., Kos, I., Krofel, M., Luštrik, R., Majić, A., Rauer, G., Righetti, D., Potočnik, H., 2016. Long-distance dispersal connects Dinaric-Balkan and Alpine grey wolf (*Canis lupus*) populations. Eur. J. Wildl. Res. 62, 137–142. https://doi.org/10.1007/s10344-015-0971-z

Regional Government of Asturias, 2022. https://sede.asturias.es/ast/bopa-disposiciones?p_p_id=pa_sede_bopa_web_portlet_SedeBopaDispositionWeb&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&_pa_sede_bopa_web_portlet_SedeBopaDispositionWeb_mvcRenderCommandName=%2Fdisposition%2Fdetail&p_r_p_dispo.

Rich, L.N., Davis, C.L., Farris, Z.J., Miller, D.A., Tucker, J.M., Hamel, S., Farhadinia, M.S., Steenweg, R., Di Bitetti, M.S., Thapa, K., Kane, M.D., Sunarto, S., Robinson, N.P., Paviolo, A., Cruz, P., Martins, Q., Gholikhani, N., Taktehrani, A., Whittington, J., Widodo, F.A., Yoccoz, N.G., Wultsch, C., Harmsen, B.J., Kelly, M.J., 2017. Assessing global patterns in mammalian carnivore occupancy and richness by integrating local camera trap surveys. Glob. Ecol. Biogeogr. 26, 918–929. https://doi.org/10.1111/geb.12600

Riley, S.J., DeGloria, S.D., Elliot, R., 1999. A Terrain Ruggedness Index that Qauntifies Topographic Heterogeneity. Intermt. J. Sci. 5, 23–27.

Rio-Maior, H., Beja, P., Nakamura, M., Álvares, F., 2018. Use of space and homesite attendance by Iberian wolves during the breeding season. Mamm. Biol. 92, 1–10. https://doi.org/10.1016/j.mambio.2018.03.014

Rio-Maior, H., Nakamura, M., Álvares, F., Beja, P., 2019. Designing the landscape of coexistence: Integrating risk avoidance, habitat selection and functional connectivity to inform large carnivore conservation. Biol. Conserv. 235, 178–188. https://doi.org/10.1016/j.biocon.2019.04.021

Rio-Maior, H., Nakamura, M., Godinho, R., Álvares, F., 2020. Investigação Aplicada à Conservação do Lobo no Noroeste de Portugal: Plano de Monitorização do lobo no âmbito do Sobreequipamento dos Parques Eólicos do Alto Minho I e de Arga (abril 2019-março 2020) - Relatório Técnico.

Roda, F., Poulard, F., Ayache, G., Nasi, N., D'Antuoni, C., Mathieu, R., Cheylan, G., 2022. How do seasonal changes in adult wolf defecation patterns affect scat detection probabilities? J. Vertebr. Biol. 71. https://doi.org/10.25225/jvb.22043

Royle, J.A., Kéry, M., 2007. A Bayesian State-space Formulation of Dynamic Occupancy Models. Ecol. Soc. Am. 88, 1813–1823.

Sazatornil, V., Rodríguez, A., Klaczek, M., Ahmadi, M., Álvares, F., Arthur, S., Blanco, J.C., Borg, B.L., Cluff, D., Cortés, Y., García, E.J., Geffen, E., Habib, B., Iliopoulos, Y., Kaboli, M., Krofel, M., Llaneza, L., Marucco, F., Oakleaf, J.K., Person, D.K., Potočnik, H., Ražen, N., Rio-Maior, H., Sand, H., Unger, D., Wabakken, P., López-Bao, J.V., 2016. The role of human-related risk in breeding site selection by wolves. Biol. Conserv. 201, 103–110. https://doi.org/10.1016/j.biocon.2016.06.022

Schlägel, U.E., Merrill, E.H., Lewis, M.A., 2017. Territory surveillance and prey management: Wolves keep track of space and time. Ecol. Evol. 7, 8388–8405. https://doi.org/10.1002/ece3.3176

Silva, P., López-Bao, J.V., Llaneza, L., Álvares, F., Lopes, S., Blanco, J.C., Cortés, Y., García, E., Palacios, V., Rio-Maior, H., Ferrand, N., Godinho, R., 2018. Cryptic population structure reveals low dispersal in Iberian wolves. Sci. Rep. 8, 14108. https://doi.org/10.1038/s41598-018-32369-3

Stenglein, J.L., Waits, L.P., Ausband, D.E., Zager, P., Mack, C.M., 2011. Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. J. Mammal. 92, 784–795. https://doi.org/10.1644/10-MAMM-A-200.1

Stępniak, K.M., Niedźwiecka, N., Szewczyk, M., Mysłajek, R.W., 2020. Scent marking in wolves *Canis lupus* inhabiting managed lowland forests in Poland. Mammal Res. 65, 629–638. https://doi.org/10.1007/s13364-020-00514-x

Torres, R.T., Silva, N., Brotas, G., Fonseca, C., 2015. To Eat or Not To Eat? The Diet of the Endangered Iberian Wolf (*Canis lupus signatus*) in a Human- Dominated Landscape in Central Portugal. PLoS One 10, e0129379. https://doi.org/10.1371/journal.pone.0129379

Weaver, J.L., Paquet, P.C., Ruggiero, L.F., 1996. Resilience and Conservation of Large Carnivores in the Rocky Mountains. Conserv. Biol. 10, 964–976. https://doi.org/10.1046/j.1523-1739.1996.10040964.x

Whittington, J., St. Clair, C.C., Mercer, G., 2005. Spatial responses of wolves to roads and trails in mountain valleys. Ecol. Appl. 15, 543–553. https://doi.org/10.1890/03-5317

Wolf, C., Ripple, W.J., 2016. Prey depletion as a threat to the world's large carnivores. R. Soc. Open Sci. 3. https://doi.org/10.1098/rsos.160252

Zimmermann, B., Nelson, L., Wabakken, P., Sand, H., Liberg, O., 2014. Behavioral responses of wolves to roads: scale-dependent ambivalence. Behav. Ecol. 25, 1353–1364. https://doi.org/10.1093/beheco/aru134

Zlatanova, D., Popova, E., 2013. Habitat variables associated with wolf (*Canis lupus*

L.) distribution and abundance in Bulgaria. Bulg. J. Agric. Sci. 19, 262–266.

Chapter III

Refuge and vulnerability at reproduction sites and beyond: wolf pup and adult numbers in anthropogenic landscapes

3. Refuge and vulnerability at reproduction sites and beyond: wolf pup and adult numbers in anthropogenic landscapes

[Manuscript in preparation for submission in scientific journal]

Mónia Nakamura [a,b,c,d,*], Helena Rio-Maior [a,b,c], Luis Llaneza [e], Pablo Sierra [f], Francisco Javier Lema [g], Vicente Palacios [h], Emilio García [h], Orencio Hernández Palacios [i], Victor Sazatornil [j], Sara Roque [k,l], Patrícia Gil [a,c], Ana Serronha [a,c], Gonçalo Ferrão da Costa [m,n], Francisco Álvares [a,c], Francisco Petrucci-Fonseca [k,l], Pedro Monterroso [a,c], Olivier Gimenez [d,1], José Vicente López-Bao [o,1]

[a] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

[b] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

[c] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

[d] CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

[e] A.RE.NA, Asesores en Recursos Naturales, S.L, 27003 Lugo, Spain

[f] Pontevedra, Spain

[g] Fuentes Amarelle 6, 15130 Corcubion, A Coruña, Spain

[h] ARCA, People and Nature, SL, Oviedo, Spain

[i] Dirección General del Medio Natural y Planificación Rural, Gobierno del Principado de Asturias, E-33005 Oviedo, Spain

[j] Forest Science and Technology Center of Catalonia (CTFC), Km 2 Road From St. Llorenç de Morunys to Port del Comte, 25280 Solsona, Spain

[k] Grupo Lobo, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

[l] cE3c, Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

[m] BE Bioinsight & ECOA , Odivelas, Portugal

[n] CESAM - Centre for Environmental and Marine Studies. University of Aveiro, Portugal

[o] Biodiversity Research Institute (University of Oviedo – CSIC – Principado de Asturias), Mieres, Spain

[1]co-senior authorship

* Corresponding author: Mónia Nakamura, CIBIO/InBIO, Campus Agrário de Vairão, 4485-661 Vairão, Portugal, +351 252660411, moniayui@gmail.com

3.1. Abstract

Although wolves persist in human-dominated landscapes, minimizing exposure risk, it is unclear whether selection for lower human disturbance and human-related resources influence pack size. The minimum number of adults and pups observed at homesites (i.e. reproduction sites) may reflect different survival success modulated by exposure risk. Taking advantage of direct observations at wolf homesites in Iberia from 1986 to 2021, we assessed the influence of factors related to anthropogenic vulnerability, resource availability, and intraspecific competition within 1, 2 and 5 km homesite buffers on the minimum number of adults and pups observed in packs. Refuge availability in all buffers was positively correlated with the number of adults.

We found that human population density, paved road density, and the proportion of burned areas negatively influence the number of adults, particularly at smaller homesite buffers, suggesting that anthropogenic factors impact pack structure. We also found that higher ruggedness and intraspecific competition can also have a negative impact on pack composition. The negative influence of livestock density on the number of adults suggests an influence of poaching due to conflicts related to livestock depredations. Although the results are not as straightforward as for adults, our results indicate that increased refuge availability and decreased unpaved road density at homesites positively correlate with the number of pups observed. The relationship between different proxies of refuge availability and vulnerability to humans and pack size may reflect different human pressures on wolves across space within the same population. Integrating high-quality refuge areas in landscape planning becomes an important element to favour the persistence of wolves in human-dominated landscapes. Similarly, implementing measures to mitigate human disturbance and human-caused mortalities, particularly at homesites, is a priority for wolf conservation in human-dominated landscapes.

Keywords: human disturbance; *Canis lupus*; homesite; human-dominated landscapes; intraspecific competition; litter size; number of adults; resource availability

3.2. Introduction

Most large carnivores are resilient species, being habitat and diet generalists (Fechter and Storch, 2014; Mech, 1995), and able to recover and persist in human-dominated landscapes (Chapron et al., 2014). However, their predatory behaviour often triggers conflicts with humans, which may increase human pressure on these species, either legally or illegally (Carter and Linnell, 2016; Lamb et al., 2020). Direct persecution and exposure to other anthropogenic disturbances, such as linear infrastructures that result in road kills (Dennehy et al., 2021), make large carnivore persistence and coexistence with humans even more challenging.

Wolves (*Canis lupus*), in particular, can remarkably adapt to a wide range of environmental conditions, making them an example of high resilience and adaptability, which facilitates their coexistence with humans (Carter and Linnell, 2016; Theuerkauf et al., 2003a). Wolves often adapt by temporally or spatially shifting their activities within their territories to avoid human activities or features (Llaneza et al., 2016; e.g., Rio-Maior et al., 2019; Theuerkauf et al., 2007; Whittington et al., 2005). However, wolves are more exposed to risks during the breeding season, when their activity is more focused on reproduction sites (Linnell et al., 2017; Ruprecht et al., 2012; Sazatornil et al., 2016), hereafter referred to as 'homesites', which include the den site and rendezvous sites where the pups are left while older wolves forage (Joslin, 1967). Research on homesite selection has been conducted in North America (e.g., Norris et al., 2002; Person et al., 2009; Trapp et al., 2008) and increasingly in Europe and Asia (e.g., Ahmadi et al., 2014; Ciucci et al., 2018; Habib and Kumar, 2007; Iliopoulos et al., 2014; Theuerkauf et al., 2003b). Wolves often minimize the risk of exposure at breeding sites by avoiding anthropogenic features such as roads, settlements, and agricultural lands (Capitani et al., 2006; Sazatornil et al., 2016) and selecting refuge areas with either enough vegetation cover, located in more remote and rough terrains, or higher altitude (Ciucci et al., 2018; Llaneza et al., 2018; Sazatornil et al., 2016; Trapp et al., 2008). Despite the intense fire regimes observed in some areas, wolves can still use homesites with burned areas (Lino et al., 2019). However, in human-dominated areas, forest fires can often be human-related (Nunes, 2012), increasing the risk of exposure at breeding sites.

Some evidence is available on the response of wolves to close encounters with humans at homesites (Argue et al., 2008; Frame et al., 2007). When pups are small, with limited mobility (<6 weeks old, but see Mech, 2022) and are more vulnerable, adults may resist moving them to avoid the risk of predation or accident (Frame et al., 2007; Habib and Kumar, 2007). However, human activities and disturbance at homesites (Thiel et al., 1998) can lead to homesite shifting (Argue et al., 2008; Ballard et al., 1987; Frame et al., 2007). Homesite shifts seem less likely to occur in sites with low disturbance, and the pup mortality risk can be slightly elevated after disturbance at homesites (Argue et al., 2008; Frame et al., 2007; Habib and Kumar, 2007). Considering this, packs would be more successful (i.e. higher survival rates and number of individuals) when selecting homesites with lower human disturbance. For the young, the first six months of life are often critical, during which most young mortalities occur, though estimates of survival rate for the first months of life are lacking (Harrington and Mech, 1982; Jędrzejewska et al., 1996). However, few studies have evaluated the influence of habitat selection at homesites on the survival of individuals in packs (Benson et al., 2015; Sidorovich et al., 2017) and, consequently, on the number of adults and pups.

Although pack size is influenced by different factors, including mortality risk, prey availability, and intraspecific competition (Sells et al., 2022), the numbers of adults and pups are rarely considered separately in habitat selection studies. It is reasonable to assume that homesites with better conditions (i.e., higher levels of refuge translating into lower exposure risk) can potentially lead to higher adult and young survival during the breeding season. Furthermore, we expect the homesite selection and the resources available in different buffers around homesites to affect the number of adults and pups differently since the areas are used differently, and the wolves may require better conditions in smaller buffers (Llaneza et al., 2018). We expect packs to be more successful by selecting homesites with low risk, that is, lower probability of human encounter and disturbance, and better refuge quality (Llaneza et al., 2018, 2016). Consequently, during the breeding season, the number of observed adults and pups (i.e. minimum pack size) should be lower when less suitable and riskier homesites are selected, possibly due to lower survival.

Here, we aim to understand how the minimum number of wolves observed at homesites (separated by adults and pups) varies with factors related to vulnerability to anthropogenic pressures, refuge availability, and intraspecific competition. We hypothesize that higher anthropogenic disturbance and exposure decrease the number of adults and pups. We consider higher anthropogenic disturbance when the following covariates are higher: human population density, livestock density, paved and unpaved road density, trail density, and proportion of human settlements, agricultural land, and burned areas (e.g., Ahmadi et al., 2014; Ballard et al., 2000; Dennehy et al., 2021; Jedrzejewski et al., 2008; Mladenoff et al., 1995; Shepherd and Whittington, 2006). Moreover, higher ruggedness, slope, altitude, and refuge availability reflect lower anthropogenic exposure (e.g., Grilo et al., 2018; Llaneza et al., 2012; Oakleaf et al., 2006; Sazatornil et al., 2016). We hypothesize that higher resource availability – related to food, refuge and movement – increases the number of adults and pups. We considered the availability of food (livestock and wild prey densities), refuge (refuge habitat and rivers), and travelling paths (unpaved road and trail densities) as resources. We hypothesize that a higher pack density (i.e. higher intraspecific competition) may decrease the number of adults due to higher competition for resources and territory and intraspecific mortality (Messier, 1985; Rich et al., 2012; Sibly and Hone, 2002). Finally, we hypothesize that the covariates related to anthropogenic vulnerability and refuge availability strongly influence the number of pups and adults within the 1 km buffer more than larger buffers since wolves may require areas with lower exposure risk in smaller buffers (Llaneza et al., 2018).

3.3. Material and Methods

3.3.1. Study area and wolf data collection

We used data collected between 1986 and 2021 in several areas within the Iberian wolf range: Northwestern Portugal, Galicia, Asturias, and Western Castilla y León (Zamora) and Picos de Europa (encompassing Astúrias, León, and Cantabria) in Spain (Fig. 3-1). Wolf monitoring was conducted using several methods during pup-rearing season (from June to November). To locate homesites, we combined results from transects of sign survey, simulated howling, direct observation, GPS-collaring, and/or camera trapping (e.g., Llaneza et al., 2014; Llaneza and Blanco, 2005; Llaneza and Núñez-Quirós, 2009; Nakamura et al., 2021; Rio-Maior et al., 2018). After confirming the reproduction in a homesite, additional observational efforts were made at homesites to ascertain the value of the minimum pack size used here. For this study, we only considered the number of individuals obtained from observations at homesites to avoid methodological biases since, for instance, in howling surveys, the precise count of wolves in a large group is difficult and can be misestimated (Joslin, 1967; Llaneza et al., 2023; Palacios et al., 2017). Although observations at homesites could potentially offer reliable pack size estimates after sufficient sampling effort – since pack members regularly gather at homesites during pup-rearing season (Packard, 2003; Theuerkauf et al., 2003b), which increases the probability of observing all pack members – we acknowledge that the numbers of observed individuals used here are minimum numbers (Llaneza et al., 2023). Considering that results were obtained through one methodology (observations at homesites) during the breeding season and implementing the same protocols, we assume that the minimum numbers of individuals obtained here are comparable among the five areas. We conducted the observations with binoculars and telescopes, mostly with one to three observers, in the morning (arriving before sunrise) or in the evening (leaving after sunset) for 30 to 240 minutes and at a distance between 300 and 1000 m.

We counted the number of pups (<1 year old) or adults/subadults (>1 year old; hereafter referred to as 'adults') observed together and considered it as the minimum number of individuals belonging to that pack-year. We approximated the location of the homesites in orthoimages, and, when available, we combined information from the movements of animals recorded through GPS tracking (Llaneza et al., 2018; Rio-Maior et al., 2018).

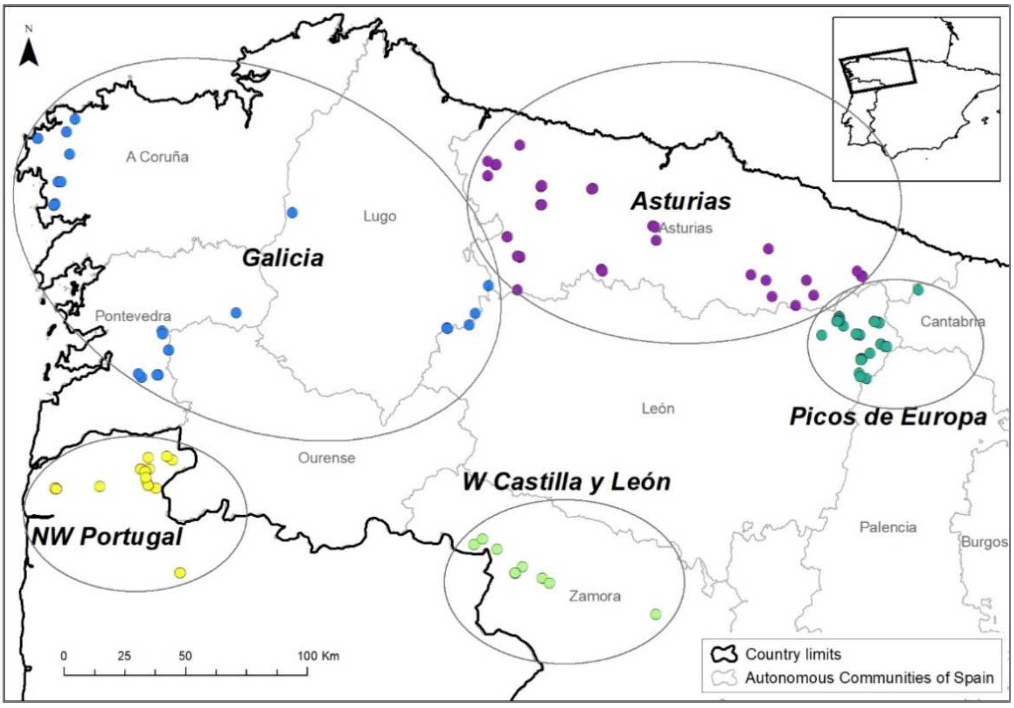


Fig. 3-1: Detected homesites in five areas: Northwestern Portugal (yellow); Spain: Galicia (blue), Asturias (purple), Western Castilla y León – Zamora (light green), and Picos de Europa - Asturias, León, and Cantabria (green).

3.3.2. Explanatory covariates

We gathered environmental and anthropogenic covariates expected to affect homesite quality and selection by wolves and additional covariates related to the species' social ecology and management. We obtained the values of each covariate for three buffers around the detected homesites: 1 km and 2 km buffers, which represent the core areas of adults and pups during the breeding season (Iliopoulos et al., 2014; Joly et al., 2018; Rio-Maior et al., 2018; Sazatornil et al., 2016); and a 5 km buffer (79 km^2), which represents the minimum area of the pack territories, considering that homesites are often found in central areas of the territories (Ciucci and Mech, 1992; Trapp et al., 2008). Average territory sizes can highly vary among sites within Europe, from 85 km^2 (e.g., Mancinelli et al., 2018; Śmietana and Wajda, 1997) to over 1000 km^2 (e.g., Kaartinen et al., 2005; Mattisson et al., 2013). However, within Iberia, average values are approximately 408-460 km^2, with several home ranges smaller than 150 km^2 (Álvares et al., 2015; Blanco et al., 2005; Silva et al., 2018). Since there is no available information on pack territory limits, the 5 km buffer used here assures that the buffer area considered is within the limits of the territory.

For anthropogenic covariates, we considered: i) 'humanpop' as the average human population density; ii) 'livestock' as livestock unit (LU) density (1 LU of horse and cattle; 0.15 LU of goat and sheep; according to the Portuguese legislation Decree-Law nº 81/2013 Annex II), which represents the primary livestock species predated by wolves in Iberia, and even the main food resource for them in several areas (e.g., Llaneza and López-Bao, 2015; Pimenta et al., 2017); iii) 'pavedroad', 'unpavedroad', and 'trail' as each type of road density; iv) 'sett', 'agric', and 'burn', as the proportion of human settlements, agricultural land and burned areas, respectively. We determined the proportion of human settlements from the European Settlement Map (Corbane and Sabo, 2019). We calculated the annual proportion of burned areas from the sum of monthly burned area per site from The European Space Agency Fire Disturbance Climate Change Initiative for the period between 1986 and 2001 (Otón et al., 2021) and from MODIS (Moderate Resolution Imaging Spectroradiometer) between 2002 and 2021.

As environmental covariates, we considered: 'altitude' as the average altitude (a.s.l.), 'slope' as the average slope (obtained from the Copernicus Digital Elevation Model), 'ruggedness' as the average Terrain Ruggedness Index (Riley et al., 1999), 'wild prey' as the average estimated wild ungulate density (Illanas et al., 2022; Linnell et al., 2020); 'river' as the waterway density, and 'refuge' as the proportion of refuge availability for wolves, from a functional point of view (Llaneza et al., 2016, 2012). Thus, we joined forests, shrublands, and bare rocks into a single covariate called refuge. According to local habitat specificities, we considered bare rocks as a refuge since these frequently have cavities used by wolves as refuges (Nakamura et al., 2023). We determined the proportion of area covered by agricultural lands and refuge per site from Corine Land Cover (CLC, 2012). To account for the intraspecific competition, we included a 'pack density' covariate as the average number of packs with overlapping buffers of 20 km, obtained from the known pack distribution in the Iberian Peninsula (Álvares et al., 2005) for the middle of the time frame considered (1986-2021). For a detailed covariate description, calculation, and source of information, see Sup. Table 7 of Appendix III.

3.3.3. Statistical analyses

To detect differences in pack size and the number of adults and pups between the five surveyed areas, we performed Kruskall-Wallis tests and Wilcoxon Rank Sum tests.

We fit generalized linear mixed effect models for the number of pups and adults separately in order to explore factors influencing each cohort since adults may also be affected by factors beyond the homesite buffers (e.g., mortality risk) as opposed to pups. We included random effects on the 'year' of the homesite detection to accommodate variation through sampling years. Before the analysis, we assessed pairwise covariate relationships using Spearman's correlation coefficient. Due to the high correlation ($r > |0.7|$), we removed the covariates of 'altitude', 'slope', and 'agric' from the analysis. All explanatory variables were standardized. We explored the explanatory power of different models.

Firstly, we created the full models for each buffer according to our hypotheses, in which the covariates could positively or negatively influence the number of individuals for the 1, 2 and 5 km buffers. Secondly, we created models that included only covariates related to anthropogenic vulnerability, resource availability, or intraspecific competition. We assumed that although pups only use the 1 and 2 km buffers in the first months of life, they benefit from food resources available at the pack territory level, supplied by the other pack members (Harrington and Mech, 1982). Hence, we constructed only one model for the number of pups with the 5 km buffer, which included covariates of food availability (livestock and wild prey densities). According to our initial hypotheses, we created two anthropogenic vulnerability submodels, each including covariates expected to positively or negatively influence the number of individuals. We also created two resource availability submodels, one related to food availability and another to refuge availability. All models for the number of pups ($n = 18$) and adults ($n = 27$), including the null models for each buffer, are presented in Sup. Fig. 8 and see Sup. Table 8 and Sup. Table 9 of Appendix III.

We fit all models with a negative binomial likelihood distribution using the 'brms' R package (Bürkner, 2017) in RStudio (Posit team, 2022). We generated two chains of

5000 iterations after a burn-in of 1000 iterations. We assessed models' convergence visually by inspecting the chains and by checking the Gelman-Rubin statistic (Rhat < 1.1) (Gelman et al., 2004). We performed a model comparison using the 'brms' R package to calculate the Watanabe-Akaike information criterion (WAIC), in which lower values reflect a superior model fit, leave-one-out cross-validation (LOO), and WAIC weights (Vehtari et al., 2023; Watanabe, 2018). We ranked the models by WAIC to understand the order of relevance of resource availability, anthropogenic vulnerability, and intraspecific competition on the number of pups and adults at different buffer areas. We interpreted the results mostly from the models ranked first and also considered other models, no matter the differences in WAIC, since these could also give insights into variations in the number of individuals in a Bayesian framework (McElreath, 2018). We used posterior means, SDs, and 95 % Bayesian Credible intervals (BCIs) to summarise parameter posterior distributions.

3.4. Results

We used the minimum number of adults and pups observed at 144 homesites for 63 packs, averaging 4.5 ± 3.2 packs per year (± SD; range: 1-14). The observed minimum pack size at the end of summer and early fall, was, on average, 7.9 ± 2.4 wolves (n = 131; range: 2-16), with 4.6 ± 1.6 pups (n = 137; range: 1-9) and 3.1 ± 1.6 adults (n = 138; range: 1-8). The number of adults was significantly lower (p=0.002; Wilcoxon Rank Sum test) in Asturias compared with Picos de Europa and Portugal. No significant differences were detected in pack sizes and the number of pups between the five areas (

Table 3-1 and Fig. 3-2).

Table 3-1: Average pack sizes and number of pups and adults observed per area and for the overall study area (mean ± standard deviation; minimum-maximum, and number of homesites; * The number of adults was significantly lower in Asturias, compared to Picos de Europa and Portugal (p = 0.002; Wilcoxon Rank Sum test).

	Pack size			**N Pup**			**N Adult**		
Area	**Mean±SD**	**range**	**n**	**Mean±SD**	**range**	**n**	**Mean±SD**	**range**	**n**
Asturias	7.2 ± 1.7	3-10	39	5.0 ± 1.3	2-7	39	2.2 ± 1.1*	1-5	39
Galicia	7.6 ± 2.6	2-13	27	4.4 ± 1.5	1-7	28	3.2 ± 1.6	1-7	27
Picos de Europa	8.3 ± 1.8	4-12	36	4.9 ± 1.0	1-6	33	3.4 ± 1.4*	1-7	36
W Castilla y León W	8.6 ± 3.0	4-13	9	4.9 ± 1.6	2-8	11	3.3 ± 1.9	1-6	9
NW Portugal	8.5 ± 3.6	2-16	20	3.9 ± 2.3	1-9	23	4.0 ± 2.0*	1-8	27

Overall	**7.9 ± 2.4**	**2-16**	**131**	**4.6 ± 1.6**	**1-9**	**137**	**3.1 ± 1.6**	**1-8**	**138**

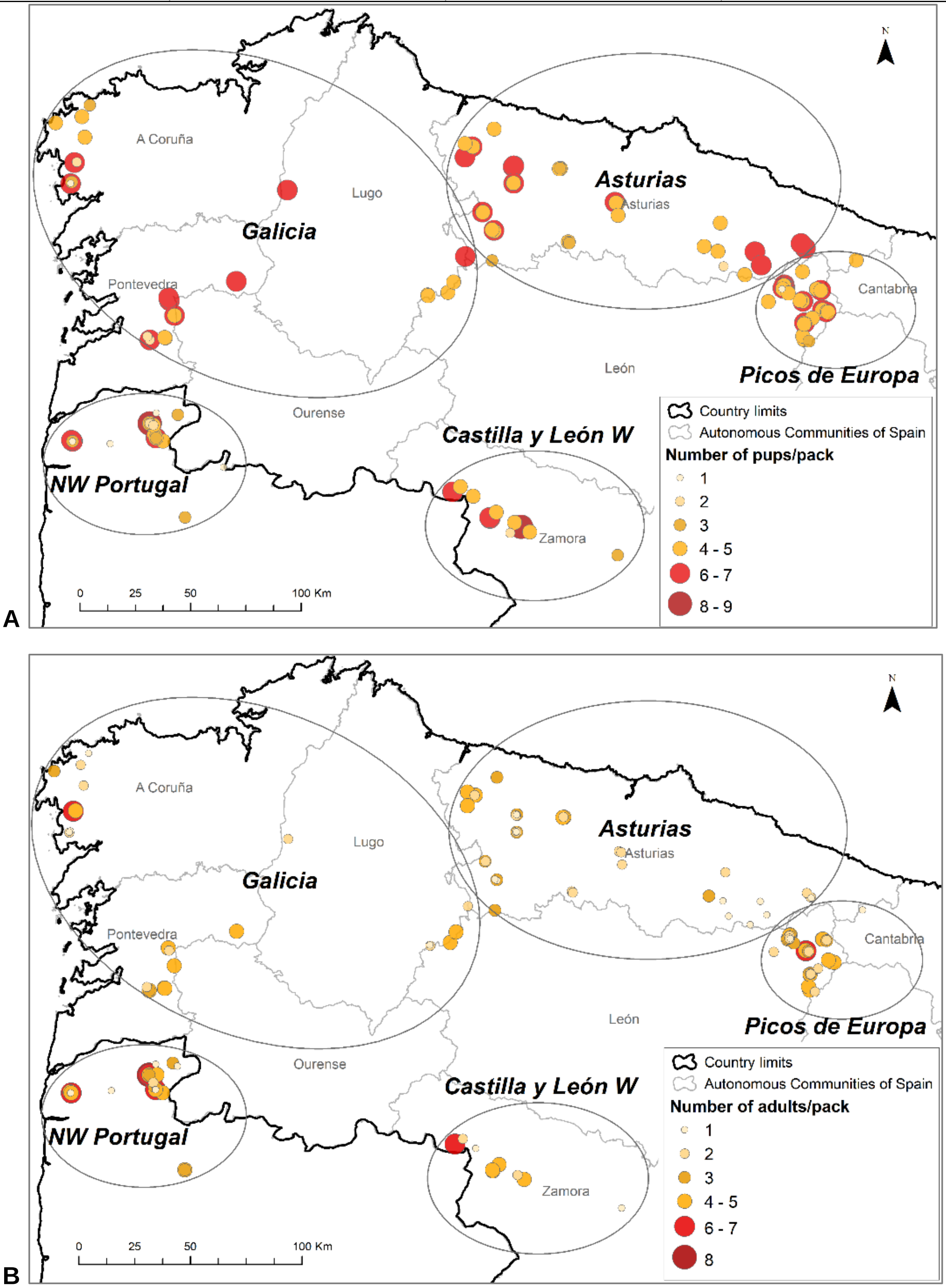


Fig. 3-2: Number of individuals observed per homesite between 1986 and 2021: A) number of pups B) and adults.

For pups, the null models for all buffers ranked first, and the constructed models had similar results between the 1 and 2 km buffers (for all models ranked, see Sup. Table 10 in Appendix III). Refuge availability emerged as a recurrent factor explaining the number of pups in several models (Fig. 3-3). Although not significant, the posterior distributions of the covariates in these models were very similar, with a tendency for a positive relationship between the number of observed pups and the following factors: ruggedness (β = 0.04; BCI: -0.05-0.12), the proportion of refuge (β = 0.04; BCI: -0.05-0.16), and river density (β = 0.02; BCI: -0.06-0.10) (Fig. 3-3A and B). The models related to food availability ranked next, and both livestock and wild prey densities had no effect on the number of pups (β = 0.00; BCI: -0.08-0.08) (Fig. 3-3C). Among all the models for pups, unpaved road density was the only covariate with a significant and negative relationship with the observed number of pups (Fig. 3-3). None of the covariates of human disturbance was significant (Fig. 3-3D). For detailed results with model ranking and posterior distributions of models for pups, see Sup. Table 10 and Sup. Fig. 9 of Appendix III.

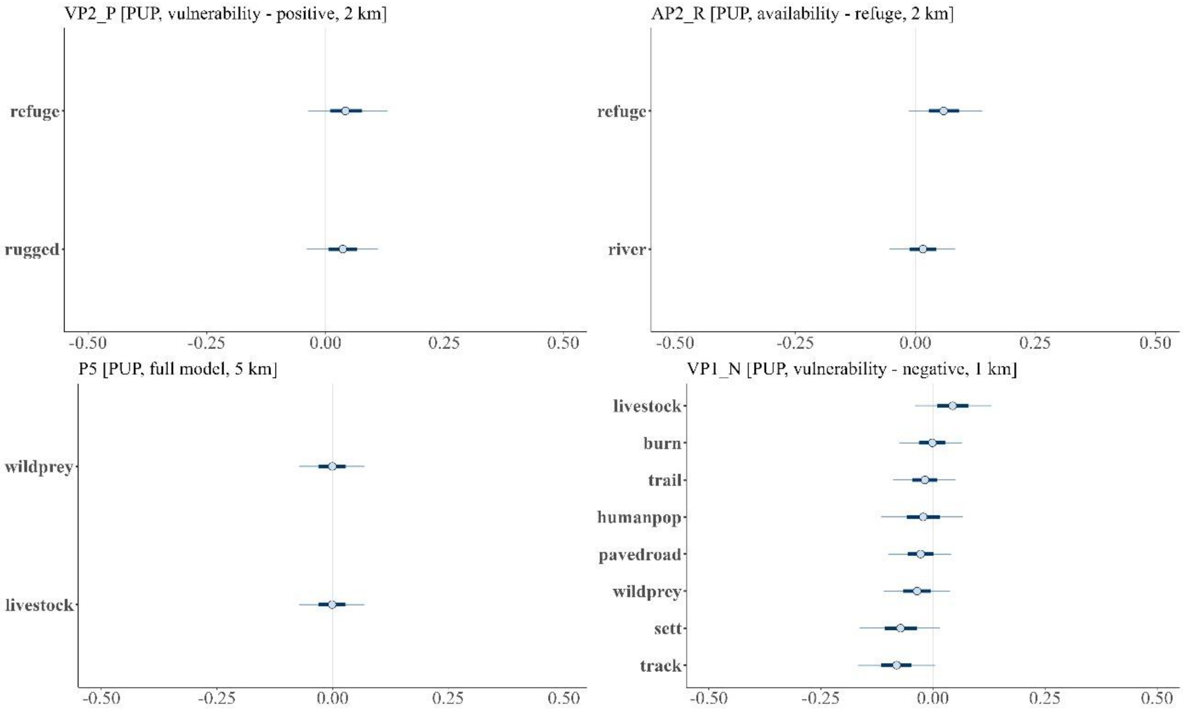


Fig. 3-3: Posterior distributions of four representative pup model results, with the average β estimate (point), standard deviation (± SD; thick bars), and 95% Bayesian Credible Intervals (± 95% BCI; thin bars) for each covariate.

For adults, the best two models explaining the observed number of adults were for the 2 and 5 km buffers and related to anthropogenic vulnerability (for all models ranked, see Sup. Table 11 in Appendix III). Ruggedness (β = -0.20; BCI: -0.31-0.09) and refuge (β = 0.19; BCI: 0.06-0.32) were positively correlated with the number of adults during the breeding season (Fig. 3-4A and B). The full model and the overall anthropogenic vulnerability model within the 1 km buffer were the third and fourth models that best explained the observed number of adults (Fig. 3-4C and D). These models had similar results, showing a negative relationship between the observed number of adults and human population density(β = -0.14; BCI: -0.29-0.01), ruggedness (β = -0.12; BCI: -0.25-0.01), pack density (β = -0.12; BCI: -0.25-0.01), paved road density (β = -0.11; BCI: -0.24-0.00), and burned areas (β = -0.10; BCI: -0.25-0.02); and a positive relationship with refuge (β= 0.13; BCI: -0.00-0.31) (Fig. 3-4C). For detailed results with model ranking and posterior distributions of models for adults, see Sup. Table 11 and Sup. Fig. 10 in Appendix III.

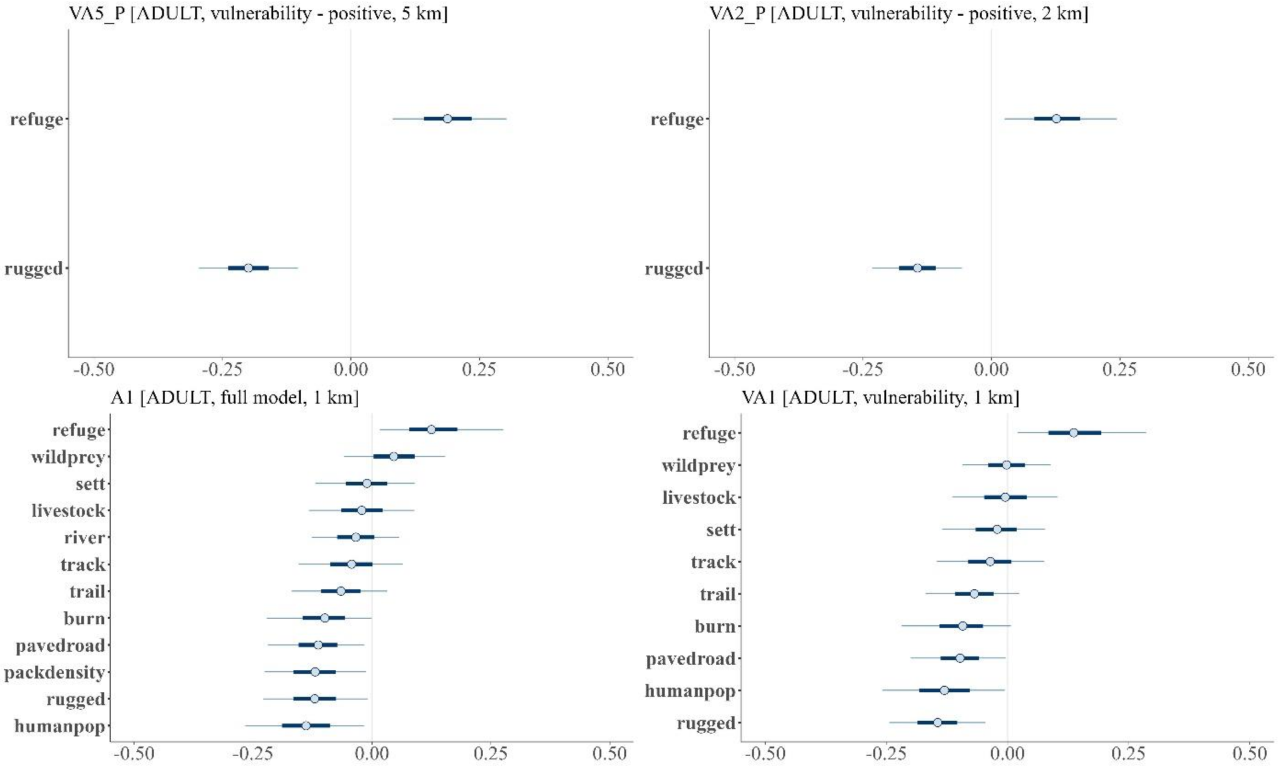


Fig. 3-4**:** Posterior distributions of the best four models of the number of adults (by rows), with the average β estimate (point), standard deviation (± SD; thick bars), and 95% Bayesian Credible Intervals (± 95% BCI; thin bars) for each covariate.

3.5. Discussion

Observations at wolf homesites over 35 years allowed us to obtain important insights into the drivers of the minimum number of pups and adults in wolf packs in human-dominated landscapes of Iberia. We identified several factors correlated with the number of adults observed in packs during the breeding season. Although there was some evidence that the refuge and density of unpaved roads were related to the number of pups, our models explain little of the observed variability. Our results suggest that more individuals are likely to persist at homesites with higher refuge availability and lower vulnerability to humans.

The number of observed adults increases with refuge availability at homesites, and this factor was also relevant in the larger buffer area considered, which may reflect the influence of refuge availability on adult survival along their territories. Furthermore, the number of observed adults decreases at homesites with higher human population density, rugged terrain, pack density, paved road density and proportion of burned areas within the 1 km buffer. Although wolves feed remarkably on livestock in the different study areas (Álvares et al., 2015; Lagos and Bárcena, 2018; Llaneza et al., 1996; Nores et al., 2008; Torres et al., 2015), livestock density negatively affected the observed number of adults, compared with the density of wild prey, which could indicate the influence of wolf persecution due to conflicts associated with livestock depredations.

Environmental factors

Similarly to previous studies on wolf habitat and homesite selection, refuge availability was the most relevant factor positively correlating with the observed number of adults (e.g., Grilo et al., 2018; Llaneza et al., 2018, 2016; Sazatornil et al., 2016). Wolves may select areas with enough refuge and fewer human activities or infrastructures throughout the territory to locate their homesites in order to reduce exposure risk (e.g., Hebblewhite and Merrill, 2007; Llaneza et al., 2018; Rio-Maior et al., 2019; Sazatornil et al., 2016; Theuerkauf, 2009). Our models also suggest that this selection by adults may have an influence on the number of observed pups. Although we expected that the density of waterways could affect the number of individuals at homesites – since the breeding female needs easy access to a reliable water source while denning and without requiring long absences that could put the pups at risk and increase mortality (Packard, 2003) –, our models did not show any effect on the number of pups or adults, possibly because this factor may not be a limiting factor in several study areas, or because a low resolution of the layers we used.

The observed influence of ruggedness on wolf habitat selection has been ambiguous over studies. At the home range scale, wolves often select rugged areas with lower accessibility to avoid human disturbance or avoid them while travelling through least cost paths or hunting in more accessible terrains (Cristescu et al., 2019; May et al., 2008; Oakleaf et al., 2006; Whittington et al., 2005). At the homesite level, dens and rendezvous sites can be located in lower and higher rugged terrains, and this selection is context-dependent (Llaneza et al., 2018; Sazatornil et al., 2016). The number of adults we observed decreased with higher ruggedness at both homesite and home range levels. This result could be related to increased sight lines for wolves, allowing for more time to retreat from eventual predators (Joly et al., 2018) or by an effect of the detectability of wolves by the observers, with lower detectability in rugged terrains.

Anthropogenic factors

Paved road density emerged as a relevant factor at the 1 km homesite buffer, with the observed number of adults decreasing with higher paved road density. The frequent human activity and high traffic intensity on such roads most probably facilitate direct persecution by humans and increase accidental road kills (Dennehy et al., 2021; Person and Russell, 2008). When paved roads cross the homesites, these risks can be significantly higher for adults during the breeding season, when they are highly active at homesites and often return daily for pup attendance, increasing the number of crossings of roads in time and space (Dennehy et al., 2021; Person and Russell, 2008; Rio-Maior et al., 2018). Such intense wolf activity may increase detectability by humans and, consequently, increase wolves' exposure risk. Although our models had low explanatory strength for the number of pups, unpaved road density was the only significant covariate negatively related to the number of observed pups. Unpaved roads can also increase pup mortality by direct persecution or accidental road-kill due to higher accessibility of humans (Nowak and Mysłajek, 2016). Although road-kills of pups or young are more commonly detected after the breeding season, when they start accompanying the other pack members, they also occur while still exploring the surrounding homesite (at least one pup was found hit by a car at a homesite; authors com. pess.)

The number of adult wolves observed decreased with human population density, which is in concordance with the negative influence of this covariate on wolf habitat in some areas and homesite selection (Mladenoff et al., 1995; Sazatornil et al., 2016). Such a result can be related to higher vulnerability to humans and higher mortality rates, similar to other anthropogenic vulnerability covariates. Although wolves can select burned areas opportunistically due to the presence of prey (Geary et al., 2020; Lewis et al., 2022), we detected a negative relationship with the proportion of burned areas in the observed number of adults. This could be due to low refuge availability or higher human disturbance and exposure (Ballard et al., 2000; Nakamura et al., 2023) that may increase adult mortality.

The negative effect of livestock density on the observed number of adults is the opposite of what would be expected in a density-dependence context (Solomon, 1949) since wolves mostly feed on livestock and it is not a limiting factor in our study areas. As such, a large number of adults in packs would be advantageous to help in pup care, protect the social unit, and increase hunting success (MacNulty et al., 2014; Peterson et al., 1984). However, the low number of adults in packs indicates that other factors may be operating. One plausible explanation would be that conflicts with humans may increase human pressure on wolves, either legally or illegally. Since the death of a breeder sometimes leads to unsuccessful reproduction, pack splitting or even pack dissolution (Borg et al., 2015; Jędrzejewski et al., 2004; Mech and Nelson, 1990), this factor could influence the number of adults and pups observed.

Joining anthropogenic vulnerability, refuge availability and intraspecific competition

According to the ranking of our models, selecting areas with higher refuge availability is the most relevant factor in observing higher numbers of adults in packs. The top four models also suggest more adults in areas with low pack densities and low vulnerability to humans (by avoiding areas with higher human population density, paved roads and burned areas). The strength of the correlation between the observed number of adults and refuge was higher at larger homesite buffers (5 km and 2 km). This result shows the relevance of improving refuge availability for wolf conservation by increasing areas with high and dense vegetation throughout the territories. Conversely, covariates directly related to human activities and infrastructures (human population density, paved road density, and burned areas) correlated more with the number of adults at the lower homesite buffer (1 km). This outcome evidences the importance of areas with low anthropogenic activities and interference, where wolves reproduce, with low disturbance and mortality risk (Sazatornil et al., 2016). Wolf-human conflict mitigation measures are of utmost importance since a large part of wolf mortality in Iberia possibly results from poaching (Campos, 2018) resulting from such conflicts.

Differences between areas

The average pack size (7.9) obtained in this study was higher than in other wolf populations in Europe for summer-autumn (Apollonio et al., 2004; Nowak et al., 2008) and within the average ranges reported for Iberia (6.5 - 9.3; Barrientos, 2000; Fernández-Gil et al., 2020; Llaneza et al., 2023). Compared to other studies in Europe that included results from direct observations, the average number of pups (4.6) was higher, and the number of adults (3.1) was similar (Fernández-Gil et al., 2020; Mattioli et al., 2018; Nowak et al., 2008). The number of adults was highest in Portugal and lowest in Asturias. Since unpaved road density, human population density and the proportion of burned areas are generally low in Asturias, the low number of adults is possibly related to pack density rather than the human-related covariates. The number of individuals may be underestimated in areas with lower detectability through observation or lower effort. We tried to reduce possible biases by assessing the number of individuals during summer and fall, implementing only one methodology (observation at homesites) with the same protocols. However, future studies at observation points should also include the effort (e.g., the duration or the number of observation points) to account for the detectability of all the individuals in a pack.

Final considerations

Our study confirmed that most factors affecting the number of observed adults were already known to influence habitat selection at a home range or homesite level. Adults select sites less exposed to human activities and disturbance, with more refuge available. Pup survival may be affected during the denning period, and factors other than those considered here may be operating, such as diseases, social competition or malnutrition (Mech, 1977; Nowak and Mysłajek, 2016). Although our study offers some insights into the relationship between exposure risk and the number of adults and pups observed at homesites, the mechanism operating on the number of individuals remains unexplained. Thus, further investigations into adult and pup mortality causes and rates are needed, including in the early stages of pup life. Such studies could be developed directly by GPS-collaring adults and older pups complemented by den site sampling and ear-tagging of young pups. The availability of sufficient refuge emerges as an important factor allowing wolf persistence in human-dominated landscapes. Real-time monitoring of wolf homesites in vulnerable and sensitive areas to human activities would facilitate the implementation of mitigation and conservation measures onsite to avoid disturbance, particularly 1 or 2 km around homesites (e.g., construction of infrastructures, leisure and hunting activities) (Sazatornil et al., 2016; Trouwborst, 2023). This would allow the implementation of temporal regulations of human activities around homesites in order to increase survival.

3.6. Funding

MN and SR worked under PhD grants [SFRH/BD/144087/2019] and [SFRH/BD/12291/2003], respectively, from Fundação para a Ciência e a Tecnologia. Field work for wolf monitoring in Portugal was supported by Grupo Lobo/Faculdade de Ciências da Universidade de Lisboa, ACHLI (Associação de Conservação do Habitat do Lobo Ibérico), and Empreendimentos Eólicos do Vale do Minho S.A. OG and MN were partly funded by the French National Research Agency [grant ANR-16- CE02-0007]. PM and SR were supported by UID/BIA/50027/2021 and PPCDT/ BIA-BDE/60296/2004, respectively, with funding from FCT/MCTES through national funds. JVLB was supported by the Spanish Ministry of Economy, Industry and Competitiveness (CGL2017-87528-R AEI/ FEDER EU), the Spanish Ministry of Science and Innovation (TED2021- 132519B-I00), and by a GRUPIN research grant from the Regional Government of Asturias (AYUD/2021/51314).

3.7. CRediT authorship contribution statement

Mónia Nakamura: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Helena Rio-Maior**: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing – review & editing, Project administration, Supervision. **Luis Llaneza:** Data curation, Investigation, Project administration, Supervision. Writing – review & editing. **Pablo Sierra**: Data curation, Investigation, Writing – review & editing. **Francisco Javier Lema**: Data curation, Investigation, Writing – review & editing. **Vicente Palacios:** Data curation, Investigation, Writing – review & editing. **Emilio García**: Data curation, Investigation, Writing – review & editing. **Orencio Hernández Palacios:** Methodology, Writing – review & editing. **Victor Sazatornil:** Data curation, Investigation, Writing – review &

editing. **Sara Roque:** Data curation, Investigation, Methodology, Writing – review & editing, Supervision. **Patrícia Gil:** Data curation, Investigation, Writing – review & editing. **Ana Serronha**: Data curation, Investigation, Writing – review & editing. **Gonçalo Ferrão da Costa:** Data curation, Investigation, Methodology, Writing – review & editing. **Francisco Álvares:** Funding acquisition, Methodology, Writing – review & editing, Project administration, Supervision. **Francisco Petrucci-Fonseca**: Funding acquisition, Writing – review & editing, Project administration, Supervision. **Pedro Monterroso:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing, Supervision. **Olivier Gimenez:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – review & editing, Supervision. **José Vicente López-Bao:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – review & editing, Supervision.

3.8. References

Ahmadi, M., López-Bao, J.V., Kaboli, M., Lopez-Bao, J. V, Kaboli, M., López-Bao, J.V., Kaboli, M., 2014. Spatial heterogeneity in human activities favors the persistence of wolves in agroecosystems. PLoS One, 9, 1–10. https://doi.org/10.1371/journal.pone.0108080

Álvares, F., Barroso, I., Blanco, J.C., Correia, J., Cortés, Y., Costa, G., Llaneza, L., Moreira, L., Nascimento, J., Palacios, V., Petrucci-Fonseca, F., Pimenta, V., Roque, S., Santos, E., 2005. Wolf status and conservation in the Iberian Peninsula, in: Conference Frontiers of Wolf Recovery: Southern US and the World. Colorado, pp. 76–77.

Álvares, F., Barroso, I., Costa, G.F. da, Espírito-Santo, C., Fonseca, C., Godinho, R., Nakamura, M., Petrucci-Fonseca, F., Pimenta, V., Rio-Maior, H., Santos, N., Torres, R.T., 2015. Situação de referência para o Plano de Ação para a Conservação do Lobo-ibérico em Portugal. Lisbon, Portugal.

Apollonio, M., Mattioli, L., Scandura, M., Mauri, L., Gazzola, A., Avanzinelli, E., 2004. Wolves in the Casentinesi Forests: insights for wolf conservation in Italy from a protected area with a rich wild prey community. Biol. Conserv. 120, 249–260. https://doi.org/10.1016/j.biocon.2004.02.021

Argue, A.M., Mills, K.J., Patterson, B.R., 2008. Behavioural response of eastern wolves (Canis lycaon) to disturbance at homesites and its effects on pup survival. Can. J. Zool. 86, 400–406. https://doi.org/10.1139/Z08-013

Ballard, W.B., Krausman, P.R., Boe, S., Cunningham, S., Whitlaw, H.A., 2000. Short-term response of grey wolves, *Canis lupus*, to wildfire in Northeastern Alaska. Can. Field-Naturalist 114, 241–247.

Ballard, W.B., Whitman, J.S., Gardner, L, C., 1987. Ecology of an exploited wolf population in south-central Alaska. Wildl. Monogr. 98, 3–54.

Barrientos, L.M., 2000. Tamaño y composición de diferentes grupos de lobos en castilla y león. Galemys 12, 249–256.

Benson, J.F., Mills, K.J., Patterson, B.R., 2015. Resource selection by wolves at dens and rendezvous sites in Algonquin park, Canada. Biol. Conserv. 182, 223–232. https://doi.org/10.1016/j.biocon.2014.12.010

Blanco, J.C., Cortés, Y., Virgós, E., 2005. Wolf response to two kinds of barriers in an agricultural habitat in Spain. Can. J. Zool. 83, 312–323. https://doi.org/10.1139/z05-016

Borg, B.L., Brainerd, S.M., Meier, T.J., Prugh, L.R., 2015. Impacts of breeder loss on social structure, reproduction and population growth in a social canid. J. Anim. Ecol. 84, 177–187. https://doi.org/10.1111/1365-2656.12256

Bürkner, P.-C., 2017. brms : An R Package for Bayesian Multilevel Models Using Stan. J. Stat. Softw. 80. https://doi.org/10.18637/jss.v080.i01

Campos, R., 2018. Survival and Population Dynamics of the Iberian Wolf in Portugal. MSc Thesis. Universidade do Porto.

Capitani, C., Mattioli, L., Avanzinelli, E., Gazzola, A., Lamberti, P., Mauri, L., Scandura, M., Viviani, A., Apollonio, M., 2006. Selection of rendezvous sites and reuse of pup raising areas among wolves *Canis lupus* of north-eastern Apennines , Italy. Acta Theriol. (Warsz). 51, 395–404.

Carter, N.H., Linnell, J.D.C., 2016. Co-Adaptation Is Key to Coexisting with Large Carnivores. Trends Ecol. Evol. 31, 575–578. https://doi.org/10.1016/j.tree.2016.05.006

Chapron, G., Kaczensky, P., Linnell, J.D.C., von Arx, M., Huber, D., Andren, H., López-Bao, J.V., Adamec, M., Álvares, F., Anders, O., Balciauskas, L., Balys, V., Bed, P., Bego, F., Blanco, J.C., Breitenmoser, U., Broseth, H., Bufka, L., Bunikyte, R., Ciucci, P., Dutsov, A., Engleder, T., Fuxjager, C., Groff, C., Holmala, K., Hoxha, B., Iliopoulos, Y., Ionescu, O., Jeremi, J., Jerina, K., Kluth, G., Knauer, F., Kojola, I., Kos, I., Krofel, M., Kubala, J., Kunovac, S., Kusak, J., Kutal, M., Liberg, O., Maji, A., Mannil, P., Manz, R., Marboutin, E., Marucco, F., Melovski, D., Mersini, K., Mertzanis, Y., Mysłajek, R.W., Nowak, S., Odden, J., Ozolins, J., Palomero, G., Paunovi, M., Persson, J., Poto nik, H., Quenette, P.-Y., Rauer, G., Reinhardt, I., Rigg, R., Ryser, A., Salvatori, V., Skrbin ek, T., Stojanov, A., Swenson, J.E., Szemethy, L., Trajce, A., Tsingarska-Sedefcheva, E., Va a, M., Veeroja, R., Wabakken, P., Wolfl, M., Wolfl, S., Zimmermann, F., Zlatanova, D., Boitani, L., 2014. Recovery of large carnivores in Europe's modern human-dominated landscapes. Science (80-.). 346, 1517–1519. https://doi.org/10.1126/science.1257553

Ciucci, P., Boitani, L., Falco, M., Maiorano, L., 2018. Hierarchical, multi-grain rendezvous site selection by wolves in southern Italy. J. Wildl. Manage. 82, 1049–1061. https://doi.org/10.1002/jwmg.21440

Ciucci, P., Mech, L.D., 1992. Selection of Wolf Dens in Relation to Winter Territories in Northeastern Minnesota. J. Mammal. 73, 899–905. https://doi.org/10.2307/1382214

CLC, 2012. © European Union, Copernicus Land Monitoring Service 2012, European Environment Agency (EEA).

Corbane, C., Sabo, F., 2019. European Settlement Map from Copernicus Very High Resolution data for reference year 2015, Public Release 2019. https://doi.org/10.2905/8BD2B792-CC33-4C11-AFD1-B8DD60B44F3B

Cristescu, B., Domokos, C., Teichman, K.J., Nielsen, S.E., 2019. Large carnivore habitat suitability modelling for Romania and associated predictions for protected areas. PeerJ 7, e6549. https://doi.org/10.7717/peerj.6549

Dennehy, E., Llaneza, L., López-Bao, J.V., 2021. Contrasting wolf responses to different paved roads and traffic volume levels. Biodivers. Conserv. 30, 3133–3150. https://doi.org/10.1007/s10531-021-02239-y

Fechter, D., Storch, I., 2014. How many wolves (*Canis lupus*) fit into Germany? The role of assumptions in predictive rule-based habitat models for habitat generalists. PLoS One 9. https://doi.org/10.1371/journal.pone.0101798

Fernández-Gil, A., Quevedo, M., Barrientos, L.M., Nuño, A., Naves, J., de Gabriel, M., Ordiz, A., Revilla, E., 2020. Pack size in humanized landscapes: the Iberian wolf population. Wildlife Biol., 2020. https://doi.org/10.2981/wlb.00594

Frame, P.F., Dean Cluff, H., Hik, D.S., 2007. Response of Wolves to Experimental Disturbance at Homesites. J. Wildl. Manage. 71, 316–320. https://doi.org/10.2193/2005-744

Geary, W.L., Doherty, T.S., Nimmo, D.G., Tulloch, A.I.T., Ritchie, E.G., 2020. Predator responses to fire: A global systematic review and meta-analysis. J. Anim. Ecol. 89, 955–971. https://doi.org/10.1111/1365-2656.13153

Gelman, A., Carlin, J.B., Stern, H.S., Rubin, D.B., 2004. Bayesian data analysis. Boca Raton FL: Chapman and Hall.

Grilo, C., Lucas, P.M., Fernández-Gil, A., Seara, M., Costa, G., Roque, S., Rio-Maior, H., Nakamura, M., Álvares, F., Petrucci-Fonseca, F., Revilla, E., 2018. Refuge as major habitat driver for wolf presence in human-modified landscapes. Anim. Conserv. 22, 59–71. https://doi.org/10.1111/acv.12435

Habib, B., Kumar, S., 2007. Den shifting by wolves in semi-wild landscapes in the Deccan Plateau, Maharashtra, India. J. Zool. 272, 259–265. https://doi.org/10.1111/j.1469-7998.2006.00265.x

Harrington, F.H., Mech, L.D., 1982. Patterns of homesites attendance in two Minnesota wolf packs, in: Harrington, F.H., Paquet, P.C. (Eds.), Wolves of the World-Perspective of Behaviour, Ecology, and Conservation. Noyes Publications, Parkridge, New Jersey, pp. 81–105.

Hebblewhite, M., Merrill, E., 2007. Modelling wildlife-human relationships for social species with mixed-effects resource selection models. J. Appl. Ecol., 45, 834–844. https://doi.org/10.1111/j.1365-2664.2008.01466.x

Iliopoulos, Y., Youlatos, D., Sgardelis, S., 2014. Wolf pack rendezvous site selection in Greece is mainly affected by anthropogenic landscape features. Eur. J. Wildl. Res., 60, 23–34. https://doi.org/10.1007/s10344-013-0746-3

Illanas, S., Croft, S., Smith, G.C., López-Padilla, S., Vicente, J., Blanco-Aguiar, J.A., Scandura, M., Apollonio, M., Ferroglio, E., Zanet, S., Vada, R., Keuling, O., Plis, K., Podgorski, T., Brivio, F., Fernández-López, J., Ruiz-Rodríguez, C., Soriguer, R.C., Acevedo, P., 2022. New models for wild ungulates occurrence and hunting yield abundance at European scale. EFSA Support. Publ. 19. https://doi.org/10.2903/sp.efsa.2022.EN-7631

Jędrzejewska, B., Jędrzejewski, W., Bunevich, A.N., Mikowski, L., Okarma, H., 1996. Population dynamics of wolves *Canis lupus* in Bialowieza primeval forest (Poland and Belarus) in relation to hunting by humans, 1847-1993. Mamm. Rev. 26, 103–126. https://doi.org/10.1111/j.1365-2907.1996.tb00149.x

Jedrzejewski, W., Jedrzejewska, B., Zawadzka, B., Borowik, T., Nowak, S., Mysłajek, R.W., Myslajek, R.W., Mysłajek, R.W., 2008. Habitat suitability model for Polish wolves based on long-term national census. Anim. Conserv. 11, 377–390. https://doi.org/10.1111/j.1469-1795.2008.00193.x

Jędrzejewski, W., Schmidt, K., Jędrzejewska, B., Theuerkauf, J., Kowalczyk, R., Zub, K., Jędrzejewski, W., Schmidt, K., Jędrzejewska, B., Theuerkauf, J., Kowalczyk, R., Zub, K., 2004. The process of a wolf pack splitting in Białowieża Primeval Forest, Poland. Acta Theriol. (Warsz)., 49, 275–280. https://doi.org/10.1007/BF03192527

Joly, K., Sorum, M.S., Cameron, M.D., 2018. Denning Ecology of Wolves in East-Central Alaska, 1993–2017. ARCTIC 71, 444–455. https://doi.org/10.14430/arctic4749

Joslin, P.W.B., 1967. Movements and homesites of timber wolves in Algonquin Park. Am. Zool. 7, 279–288.

Kaartinen, S., Colpaert, A., Kojola, I., Colpaert, A., 2005. Finnish wolves avoid roads and settlements. Ann. Zool. Fennici 42, 523–532.

Lagos, L., Bárcena, F., 2018. Spatial variability in wolf diet and prey selection in Galicia (NW Spain). Mammal Res., 63, 125–139. https://doi.org/10.1007/s13364-018-0352-6

Lamb, C.T., Ford, A.T., McLellan, B.N., Proctor, M.F., Mowat, G., Ciarniello, L., Nielsen, S.E., Boutin, S., 2020. The ecology of human–carnivore coexistence. Proc. Natl. Acad. Sci. U. S. A. 117, 17876–17883. https://doi.org/10.1073/pnas.1922097117

Lewis, J.S., LeSueur, L., Oakleaf, J., Rubin, E.S., 2022. Mixed-severity wildfire shapes habitat use of large herbivores and carnivores. For. Ecol. Manage. 506, 119933. https://doi.org/10.1016/j.foreco.2021.119933

Linnell, J.D.C., Cretois, B., Nilsen, E.B., Rolandsen, C.M., Solberg, E.J., Veiberg, V., Kaczensky, P., Van Moorter, B., Panzacchi, M., Rauset, G.R., Kaltenborn, B., 2020. The challenges and opportunities of coexisting with wild ungulates in the human-dominated landscapes of Europe's Anthropocene. Biol. Conserv. 244, 108500. https://doi.org/10.1016/j.biocon.2020.108500

Linnell, J.D.C., Swenson, J.E., Andersen, R., Barnes, B., Linnell, J.D.C., Swenson, J.E., Barnes, B., 2017. How vulnerable are denning bears to disturbance ? 28, 400–413. https://doi.org/10.2307/3783698

Lino, S., Sillero, N., Torres, J., Santos, X., Álvares, F., Alvares, F., 2019. The role of fire on wolf distribution and breeding-site selection: Insights from a generalist carnivore occurring in a fire-prone landscape. Landsc. Urban Plan. 183, 111–121. https://doi.org/10.1016/j.landurbplan.2018.12.003

Llaneza, L., Blanco, J.C., 2005. Situación del lobo (*Canis lupus* L.) en Castilla y León en 2001. Evolución de sus poblaciones. Galemys Boletín Inf. la Soc. Española para la Conserv. y Estud. los mamíferos 17, 15–28.

Llaneza, L., Fernández, A., Nores, C., 1996. Dieta del lobo en dos zonas de Asturias (España) que difieren en carga ganadera. Acta Vertebr. 23, 201–213.

Llaneza, L., García, E.J., López-Bao, J.V., 2014. Intensity of Territorial Marking Predicts Wolf Reproduction: Implications for Wolf Monitoring. PLoS One 9, e93015. https://doi.org/10.1371/journal.pone.0093015

Llaneza, L., García, E.J., Palacios, V., Sazatornil, V., López-Bao, J.V., 2016. Resting in risky environments: the importance of cover for wolves to cope with exposure risk in human-dominated landscapes. Biodivers. Conserv. 25, 1515–1528. https://doi.org/10.1007/s10531-016-1134-6

Llaneza, L., López-Bao, J.V., Sazatornil, V., 2012. Insights into wolf presence in human-dominated landscapes: the relative role of food availability, humans and landscape attributes. Divers. Distrib. 18, 459–469. https://doi.org/10.1111/j.1472-4642.2011.00869.x

Llaneza, L., López-Bao, J. V., 2015. Indirect effects of changes in environmental and agricultural policies on the diet of wolves. Eur. J. Wildl. Res. 61, 895–902. https://doi.org/10.1007/s10344-015-0966-9

Llaneza, L., Núñez-Quirós, P., 2009. Distribution of the Iberian Wolf (*Canis lupus signatus*) in Galicia (NW Spain): Concordance Between Field Sampling and Questionnaires. Wildl. Biol. Pract. 5, 23–32. https://doi.org/10.2461/wbp.2009.5.5

Llaneza, L., Sazatornil, V., García, E.J., Palacios, V., López-Bao, J.V., 2023. Minimum average pack size in Iberian wolves. Eur. J. Wildl. Res. 69, 1–6. https://doi.org/10.1007/s10344-023-01695-x

Llaneza, L., Sazatornil, V., López-Bao, J.V., 2018. The importance of fine-scale breeding site selection patterns under a landscape-sharing approach for wolf conservation. Biodivers. Conserv. 27, 1239–1256. https://doi.org/10.1007/s10531-017-1491-9

MacNulty, D.R., Tallian, A., Stahler, D.R., Smith, D.W., 2014. Influence of Group Size on the Success of Wolves Hunting Bison. PLoS One 9, e112884. https://doi.org/10.1371/journal.pone.0112884

Mancinelli, S., Boitani, L., Ciucci, P., 2018. Determinants of home range size and space use patterns in a protected wolf (*Canis lupus*) population in the central Apennines, Italy. Can. J. Zool. 96, 828–838. https://doi.org/10.1139/cjz-2017-0210

Mattioli, L., Canu, A., Passilongo, D., Scandura, M., Apollonio, M., 2018. Estimation of pack density in grey wolf (*Canis lupus*) by applying spatially explicit capture-recapture models to camera trap data supported by genetic monitoring. Front. Zool. 15, 1–15. https://doi.org/10.1186/s12983-018-0281-x

Mattisson, J., Sand, H., Wabakken, P., Gervasi, V., Liberg, O., Linnell, J.D.C., Rauset, G.R., Pedersen, H.C., 2013. Home range size variation in a recovering wolf population: evaluating the effect of environmental, demographic, and social factors. Oecologia 173, 813–825. https://doi.org/10.1007/s00442-013-2668-x

May, R., van Dijk, J., Wabakken, P., Swenson, J.E., Linnell, J.D.C., Zimmermann, B., Odden, J., Pedersen, H.C., Andersen, R., Landa, A., 2008. Habitat differentiation within the large-carnivore community of Norway's multiple-use landscapes. J. Appl. Ecol. 45, 1382–1391. https://doi.org/10.1111/j.1365-2664.2008.01527.x

McElreath, R., 2018. Statistical Rethinking, Statistical Rethinking. https://doi.org/10.1201/9781315372495

Mech, L.D., 2022. Newly Documented Behavior of Free-Ranging Arctic Wolf Pups. Arctic 75, 272–276. https://doi.org/10.14430/arctic75056

Mech, L.D., 1995. The Challenge and Opportunity of Recovering Wolf Populations. Conserv. Biol., 9, 270–278. https://doi.org/10.1046/j.1523-1739.1995.9020270.x

Mech, L.D., 1977. Productivity, mortality, and population trends of wolves in northeastern Minnesota. J. Mammal. 58, 559–574. https://doi.org/10.2307/1380004

Mech, L.D., Nelson, M.E., 1990. Non-Family Wolf, *Canis lupus*, Packs. Can. Field-Naturalist 104, 482–483.

Messier, F., 1985. Social organization, spatial distribution, and population density of wolves in relation to moose density. Can. J. Zool. 63, 1068–1077. https://doi.org/10.1139/z85-160

Mladenoff, D.J., Sickley, T.A., Haight, R.G., Wydeven, A.P., 1995. A Regional Landscape Analysis and Prediction of Favorable Gray Wolf Habitat in the Northern Great Lakes Region. Conserv. Biol. 9, 279–294. https://doi.org/10.1046/j.1523-1739.1995.9020279.x

Nakamura, M., López-Bao, J.V., Rio-Maior, H., Roque, S., Gil, P., Serronha, A., García, E., Hernández Palacios, O., Ferrão da Costa, G., Álvares, F., Petrucci-Fonseca, F., Gimenez, O., Monterroso, P., 2023. Insights into the dynamics of wolf occupancy in human-dominated landscapes. Biol. Conserv. 286. https://doi.org/10.1016/j.biocon.2023.110316

Nakamura, M., Rio-Maior, H., Godinho, R., Petrucci-Fonseca, F., Álvares, F., 2021. Source-sink dynamics promote wolf persistence in human-modified landscapes: Insights from long-term monitoring. Biol. Conserv. 256, 109075. https://doi.org/10.1016/j.biocon.2021.109075

Nores, C., Llaneza, L., Álvarez, Á., 2008. Wild boar Sus scrofa mortality by hunting and wolf *Canis lupus* predation : an example in northern Spain. Wildlife Biol. 14, 44–51. https://doi.org/10.2981/0909-6396(2008)14[44:WBSSMB]2.0.CO;2

Norris, D.R., Theberge, M.T., Theberge, J.B., 2002. Forest composition around wolf (*Canis lupus*) dens in eastern Algonquin Provincial Park , Ontario. Can. J. Zool. 80, 866–872. https://doi.org/10.1139/Z02-067

Nowak, S., Mysłajek, R.W., 2016. Wolf recovery and population dynamics in Western Poland, 2001-2012. Mammal Res. 61, 83–98. https://doi.org/10.1007/s13364-016-0263-3

Nowak, S., Mysłajek, R.W., Jędrzejewska, B., 2008. Density and demography of wolf, *Canis lupus* population in the western-most part of the Polish Carpathian Mountains, 1996-2003. Folia Zool. 57, 392–402.

Nunes, A.N., 2012. Regional variability and driving forces behind forest fires in Portugal an overview of the last three decades (1980–2009). Appl. Geogr. 34, 576–586. https://doi.org/10.1016/j.apgeog.2012.03.002

Oakleaf, J.K., Murray, D.L., Oakleaf, J.R.J., Bangs, E.E., Mack, C.M., Smith, D.W., Fontaine, J.A., Jimenez, M.D., Meier, T.J., Niemeyer, C.C., 2006. Habitat Selection by Recolonizing Wolves in the Northern Rocky Mountains of the United States. J. Wildl. Manage. 70, 554–563. https://doi.org/10.2193/0022-541X(2006)70

Otón, G., Lizundia-Loiola, J., Pettinari, M.L., Chuvieco, E., 2021. Development of a consistent global long-term burned area product (1982–2018) based on AVHRR-LTDR data. Int. J. Appl. Earth Obs. Geoinf. 103. https://doi.org/10.1016/j.jag.2021.102473

Packard, J.M., 2003. Wolf Behavior: Reproductive, Social, and Intelligent, in: Mech, L.D., Boitani, L. (Eds.), Wolf: Behavior, Ecology and Conservation. Chicago, IL, pp. 35–65.

Palacios, V., Font, E., García, E.J., Svensson, L., Llaneza, L., Frank, J., López-Bao, J.V., 2017. Reliability of human estimates of the presence of pups and the number of wolves vocalizing in chorus howls: implications for decision-making processes. Eur. J. Wildl. Res. 63, 1–8. https://doi.org/10.1007/s10344-017-1115-4

Person, D.K., Russell, A.L., 2008. Correlates of mortality in an exploited wolf population. J. Wildl. Manage. 72, 1540–1549. https://doi.org/10.2193/2007-520

Person, D.K., Russell, A.L., Person, David, K., Russell, Amy, L., 2009. Reproduction and Den Site Selection by Wolves in a Disturbed Landscape. Northwest Sci. 83, 211–224. https://doi.org/10.3955/046.083.0305

Peterson, R.O., Woolington, J.D., Bailey, T.N., 1984. Wolves of the Kenai Peninsula, Alaska. Wildl. Monogr. 88, 3–52.

Pimenta, V., Barroso, I., Boitani, L., Beja, P., 2017. Wolf predation on cattle in Portugal : assessing the effects of husbandry systems. Biol. Conserv. 207, 17–26. https://doi.org/10.1016/j.biocon.2017.01.008

Posit team, 2022. RStudio: Integrated Development Environment for R. Posit Software, PBC.

Rich, L.N., Mitchell, M.S., Gude, J.A., Sime, C.A., 2012. Anthropogenic mortality, intraspecific competition, and prey availability influence territory sizes of wolves in Montana. J. Mammal. 93, 722–731. https://doi.org/10.1644/11-MAMM-A-079.2

Riley, S.J., DeGloria, S.D., Elliot, R., 1999. A Terrain Ruggedness Index that Qauntifies Topographic Heterogeneity. Intermt. J. Sci. 5, 23–27.

Rio-Maior, H., Beja, P., Nakamura, M., Álvares, F., 2018. Use of space and homesite attendance by Iberian wolves during the breeding season. Mamm. Biol. 92, 1–10. https://doi.org/10.1016/j.mambio.2018.03.014

Rio-Maior, H., Nakamura, M., Álvares, F., Beja, P., 2019. Designing the landscape of coexistence: Integrating risk avoidance, habitat selection and functional connectivity to inform large carnivore conservation. Biol. Conserv. 235, 178–188. https://doi.org/10.1016/j.biocon.2019.04.021

Ruprecht, J.S., Ausband, D.E., Mitchell, M.S., Garton, E.O., Zager, P., 2012. Homesite attendance based on sex, breeding status, and number of helpers in gray wolf packs. J. Mammal., 93, 1001–1005. https://doi.org/10.1644/11-MAMM-A-330.1

Sazatornil, V., Rodríguez, A., Klaczek, M., Ahmadi, M., Álvares, F., Arthur, S., Blanco, J.C., Borg, B.L., Cluff, D., Cortés, Y., García, E.J., Geffen, E., Habib, B., Iliopoulos, Y., Kaboli, M., Krofel, M., Llaneza, L., Marucco, F., Oakleaf, J.K., Person, D.K., Potočnik, H., Ražen, N., Rio-Maior, H., Sand, H., Unger, D., Wabakken, P., López-Bao, J.V., 2016. The role of human-related risk in breeding site selection by wolves. Biol. Conserv. 201, 103–110. https://doi.org/10.1016/j.biocon.2016.06.022

Sells, S.N., Mitchell, M.S., Podruzny, K.M., Ausband, D.E., Emlen, D.J., Gude, J.A., Smucker, T.D., Boyd, D.K., Loonam, K.E., 2022. Competition, prey, and mortalities influence gray wolf group size. J. Wildl. Manage. 86, 1–17. https://doi.org/10.1002/jwmg.22193

Shepherd, B., Whittington, J., 2006. Response of wolves to corridor restoration and human use management. Ecol. Soc. 11, 1.

Sibly, R.M., Hone, J., 2002. Population growth rate and its determinants: An overview. Philos. Trans. R. Soc. B Biol. Sci. 357, 1153–1170. https://doi.org/10.1098/rstb.2002.1117

Sidorovich, V., Schnitzler, A., Schnitzler, C., Rotenko, I., 2017. Wolf denning behaviour in response to external disturbances and implications for pup survival. Mamm. Biol. 87, 89–92. https://doi.org/10.1016/j.mambio.2016.11.011

Silva, P., López-Bao, J.V., Llaneza, L., Álvares, F., Lopes, S., Blanco, J.C., Cortés, Y., García, E., Palacios, V., Rio-Maior, H., Ferrand, N., Godinho, R., 2018. Cryptic population structure reveals low dispersal in Iberian wolves. Sci. Rep. 8, 14108. https://doi.org/10.1038/s41598-018-32369-3

Śmietana, W., Wajda, J., 1997. Wolf number changes in Bieszczady National Park, Poland. Acta Theriol. (Warsz)., 42, 241–252.

Solomon, M.E., 1949. The Natural Control of Animal Populations. J. Anim. Ecol. 18, 1. https://doi.org/10.2307/1578

Theuerkauf, J., 2009. What Drives Wolves: Fear or Hunger? Humans, Diet, Climate and Wolf Activity Patterns. Ethology 115, 649–657. https://doi.org/10.1111/j.1439-0310.2009.01653.x

Theuerkauf, J., Gula, R., Pirga, B., Tsunoda, H., Eggermann, J., Brzezowska, B., Rouys, S., Radler, S., 2007. Human impact on wolf activity in the Bieszczady Mountains , SE Poland. Annu. Zool. Fenn. 44, 225–231.

Theuerkauf, J., Jędrzejewski, W., Schmidt, K., Gula, R., 2003a. Spatio temporal segregation of wolves from humans in the Bialowieza Forest Poland. J. Wildl. Manage. 67, 706–716.

Theuerkauf, J., Rouys, S., Jedrzejewski, W., 2003b. Selection of den, rendezvous, and resting sites by wolves in the Bialowieza Forest, Poland. Can. J. Zool. 81, 163–167. https://doi.org/10.1139/z02-190

Thiel, R.P., Merrill, S.B., Mech, L.D., 1998. Tolerance by Denning Wolves, *Canis lupus*, to Human Disturbance. Can. Field-Naturalist 122, 340–342.

Torres, R.T., Silva, N., Brotas, G., Fonseca, C., 2015. To Eat or Not To Eat? The Diet of the Endangered Iberian Wolf (*Canis lupus signatus*) in a Human- Dominated Landscape in Central Portugal. PLoS One 10, e0129379. https://doi.org/10.1371/journal.pone.0129379

Trapp, J.R., Beier, P., Mack, C., Parsons, D.R., Paquet, P.C., 2008. Wolf, *Canis lupus*, den site selection in the Rocky Mountains. Can. Field-Naturalist 122, 49–56. https://doi.org/10.22621/cfn.v122i1.543

Trouwborst, A., 2023. Waking up Bats, Hazing Wolves and Draining Snake Lakes – Exploring the EU Habitats Directive’s Prohibition of “Deliberate Disturbance.” J. Eur. Environ. Plan. Law 20, 217–235. https://doi.org/10.1163/18760104-20030003

Vehtari, A., Gelman, A., Gabry, J., 2023. loo: Efficient Leave-One-Out Cross-Validation and WAIC for Bayesian Models. R Package Version 2.6.0. https://doi.org/10.1007/s11222-016-9696-4

Watanabe, S., 2018. Asymptotic Equivalence of Bayes Cross Validation and Widely Applicable Information Criterion in Singular Learning Theory 11, 3571–3594.

Whittington, J., St. Clair, C.C., Mercer, G., 2005. Spatial responses of wolves to roads and trails in mountain valleys. Ecol. Appl. 15, 543–553. https://doi.org/10.1890/03-5317

Chapter IV

Source-sink dynamics promote wolf persistence in human-modified landscapes: insights from long-term monitoring

4. Source-sink dynamics promote wolf persistence in human-modified landscapes: insights from long-term monitoring

Biological Conservation, 256, 109075

doi: 10.1016/j.biocon.2021.109075

Accepted: 8 March 2021

Mónia Nakamura [a,b,*,1], Helena Rio-Maior [a,b,1], Raquel Godinho [a,b], Francisco Petrucci-Fonseca [c,d] and Francisco Álvares [a]

[a] CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

[b] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s⁄n. 4169-007 Porto, Portugal

[c] Grupo Lobo, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

[d] cE3c, Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

* Corresponding author: Mónia Nakamura, CIBIO/InBIO, Campus Agrário de Vairão, 4485-661 Vairão, Portugal, moniayui@gmail.com

[1] these authors should be considered joint first author

4.1. Highlights

- Pattern of pack extinctions and recoveries characterizes wolf population dynamics.
- Source-sink dynamics is unveiled with few core packs supporting a wolf population.
- Sink packs secured by few dispersers from core packs within the population nuclei.
- Pack assignment of molecularly identified individuals from noninvasive samples.

4.2. Abstract

Long-term monitoring studies assessing wolf population dynamics are scarce, particularly in human-dominated landscapes of southern Europe. In this work, we estimate wolf demographic parameters in northwest Portugal based on a multi-methodological approach over 20 years split into two periods (period A: 1996-2005; period B: 2007-2016). Period B takes advantage of methodological upgrades in wolf surveys, as GPS telemetry and the use of genetic noninvasive samples, to report dispersal events and identify core and sink packs. The average annual population size was 27.0 ± 2.1 (SE; range 13-43) individuals, with 2 to 6 annual packs and an average density of 1.7 ± 0.1 wolves/100km^2. The population showed a growth rate of 4.2 ± 7.6%, with a decreasing trend (period A, -8 ± 9%) followed by a recovery (period B, 16 ± 11%), mainly due to local extinction and reestablishment of sink packs. The average pack size was 6.2 ± 0.3 individuals, with a maximum of 16 individuals, the highest reported value for Iberian wolves. During period B, the percentage of dispersers detected in this population was 11% and the average dispersal distance was 24.8 ± 1.2km. Core packs showed higher group persistence, breeding success, and average pack sizes compared with sink packs. Results suggest a source-sink dynamics in this population, with few core packs promoting the maintenance and recovery of sink packs through a stepping-stone process. Our findings provide a comprehensive overview of wolf population dynamics in human-dominated landscapes and reinforce that wolf management and conservation planning should take into consideration population trends based on long-term studies, and spatial dynamics of demographic traits across packs.

Key-words: *Canis lupus*; Iberian wolf; Group size; Breeding success; Dispersal; Source-sink dynamics.

4.3. Introduction

Conservation of species that directly affect human activities by competing for resources is challenging and requires multifaceted approaches covering biological, socio-political, and economic issues (Treves and Karanth, 2003). Large carnivores are a special focus of attention in this regard because they often prey on livestock, which results in human-wildlife conflicts and on their legal control or illegal killing (Treves and Karanth, 2003; Woodroffe and Redpath, 2015). Population-level dynamics is a key component to develop suitable and effective management actions, particularly for species susceptible to intense human persecution (Fuller, 1995). Systematic monitoring for periods longer than a decade is not standard practice on large carnivores presumably because it requires heavy investment in human resources, time, funds, and standardized protocols to ensure comparability across long periods (Boitani and Powell, 2012). Still, a few available long-term studies on large carnivores have monitored demographic parameters focusing on areas with low human presence in North America and northern-central Europe (Benson et al., 2015; Mech, 2009, 1995; Nowak and Mysłajek, 2016; O'Neil et al., 2017; Peterson et al., 1984; Smith et al., 2017; Wabakken et al., 2001). However, such studies and the consequent understanding of population dynamics are lacking for most large carnivore populations, particularly in landscapes with high human interference, such as Southern Europe.

Population dynamics and extinction risk on wolves (*Canis lupus*) are largely affected by prey availability, mortality rates, and recruitment (Fuller, 1989; Mech and Boitani, 2003). Recruitment occurs through reproduction or dispersal (immigration) (Fuller, 1989; Hayes and Harestad, 2000) and its rate is highly variable and depends on source-sink dynamics (Fuller et al., 2003; Loreau et al., 2013; Pulliam, 1988). In source or core areas, reproduction rates or immigration exceed mortality rates so that individuals emigrate to new ranges, whereas in sink areas mortality rates or emigration exceed the levels of reproduction or immigration required to maintain or increase a population (Elmhagen and Angerbjorn, 2001; Howe et al., 1991; Minnie et al., 2018; Pulliam, 1988). Therefore, the role of source-sink dynamics between neighboring packs may be particularly relevant in heterogeneous human-dominated landscapes, where wolf populations may suffer intense and patchy human persecution, being particularly susceptible to pack local extinctions or population declines.

Assessment of source-sink dynamics in wolf populations requires estimating differences in density, survival, reproduction, and dispersal among habitats (Donker and Krebs, 2012), as well as collecting long-term data on demographic traits and evaluating the role of dispersal in colonization and population maintenance (Gese and Mech, 1991). Wolf dispersal, recolonization events, and pack structure have been studied using different combinations of methodologies, such as telemetry of collared wolves and noninvasive molecular methods (Blanco and Cortés, 2007; Gese and Mech, 1991; Kojola et al., 2009; Lucchini et al., 2002; Marucco et al., 2012). However, most available studies on wolf population dynamics, particularly in Europe, employ a limited number of methodological approaches that may hamper data collection across different ecological conditions or levels of human interference in the landscape (Jędrzejewska et al., 1996; Marucco et al., 2012; Nowak et al., 2008; Nowak and Mysłajek, 2016).

One of the largest wolf populations persisting in Europe is located in the northwest Iberian Peninsula, which is estimated in >2000 individuals comprising >350 packs distributed over ca. 140,000 km^2 (MAGRAMA, 2016; Pimenta et al., 2005). For the last decades, this population range has an increasing trend in Spain while it remains stable in Portugal (Álvares et al., 2015; Chapron et al., 2014), although according to Grilo et al. (Grilo et al., 2018) suitable habitat for range expansion is available. Portugal supports two wolf populations: one larger (≈51 packs) located in the north of Douro river (northern Portugal) in continuity with the wolf range in Spain, and another smaller (≈6 packs) in the south of the Douro river (central Portugal), which is isolated from the remaining Iberian wolf population (Álvares et al., 2015; Pimenta et al., 2005). Despite the high mobility of wolves, previous studies on Iberian wolves suggest reduced dispersal rates and distances, as well as high levels of population structure (Blanco and Cortés, 2007; Rio-Maior et al., 2016; Silva et al., 2018).

Wolves in the Iberian Peninsula are included in the Bern Convention (Annex II), CITES and Habitats Directive (92/43/CEE), and are subject to different protection regimes. In Spain, depending on each autonomous region, wolves are managed from hunting or administrative culling to full protection, while in Portugal are fully protected by law since 1988 and listed as “Endangered” in the Portuguese Red Data Book (Cabral et al., 2005). Studies based on Iberian wolf population monitoring, both at national and regional scales, have been restricted to a few years long and resort mostly to sign, visual and acoustic detection of wolves (Blanco et al., 1992; Blanco and Cortés, 2007; Eggermann et al., 2011; Llaneza et al., 2005; Pimenta et al., 2005). The lack of studies addressing wolf population dynamics is particularly critical as the Iberian wolf persists in highly heterogeneous and human-modified landscapes, showing a high trophic dependency on domestic animals and facing threats such as human persecution, habitat disturbance, and scarcity of natural prey (Blanco et al., 1992; Eggermann et al., 2011; Hindrikson et al., 2017; Pimenta et al., 2018; Rio-Maior et al., 2019). These evidences highlight the need for science-based information regarding Iberian wolf population dynamics based on long-term monitoring, which can then support adequate management and conservation actions.

In this study we investigate wolf population dynamics based on a monitoring study conducted in northwest Portugal over 20 years and by resorting to different methodologies, to address the following goals: i) to estimate population size, density, and growth rates over time; ii) to estimate pack persistence, breeding success, packs size, and sex ratios; iii) to assess dispersal patterns between packs; and iv) to identify core and sink packs. This study takes advantage of a long-term monitoring approach to characterize wolf population dynamics and to detect detailed annual variations of population estimates. This approach is expected to provide a comprehensive analysis of the spatial dynamics between packs as sources of individuals to sustain a wolf population at a regional scale. Therefore, based on our findings, we discuss practical management and conservation implications to assure wolf persistence in human-dominated landscapes.

4.4. Methods

4.4.1. Study area

This study was carried out in northwest Portugal, comprising approximately 1600 km^2 located in the western border of the Iberian wolf range (Fig. 4-1). The study area covers the Alto Minho wolf population, defined by Silva et al. (2018) as a distinct genetic cluster with limited evidence of exchanging individuals with other neighboring wolf populations both in Portugal (East Trás-os-Montes) and in Spain (West Galicia). The climate is temperate Atlantic with a strong oceanic influence characterized by a marked seasonality, with hot summers and rainy winters with little snow cover (monthly average temperature range: 6.3 ºC-21.7 ºC; IPMA 2014; annual average precipitation: 1357 mm; APA 2014). Altitude ranges from 180 m to 1416 m asl, and the landscape is very heterogeneous with areas of low elevation and river valleys occupied by human settlements and agricultural land, whereas mountainous areas consist predominantly of scrublands, oak (*Quercus* spp.) forest patches, and forest plantations. This area is characterized by a high human density (107.7 inhabitants/km^2; PORDATA 2014) with dispersed human settlements, and a high road density (1.60 km/km^2 of paved roads; INE 2014), including three highways that cross the western half of the study area (Fig. 4-1). Wolves feed mostly on extensively grazed livestock such as cattle, horses, goats, and sheep, comprising up to 80% of the wolf diet (Álvares, 2011). Wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*) are the main wild prey available for wolves representing up to 15% of the wolf diet (Álvares, 2011; Vingada et al., 2010). The intense wolf predation on domestic animals leads to major conflicts with livestock owners prompting illegal persecution towards wolves, even though the losses caused by wolves are fully compensated by the national authorities in Portugal (Álvares et al., 2015; Barroso et al., 2016; Pimenta et al., 2018). Poaching caused the deaths of 47% of GPS collared wolves tracked in the study area during 2007-2017 (Rio-Maior et al., 2018).

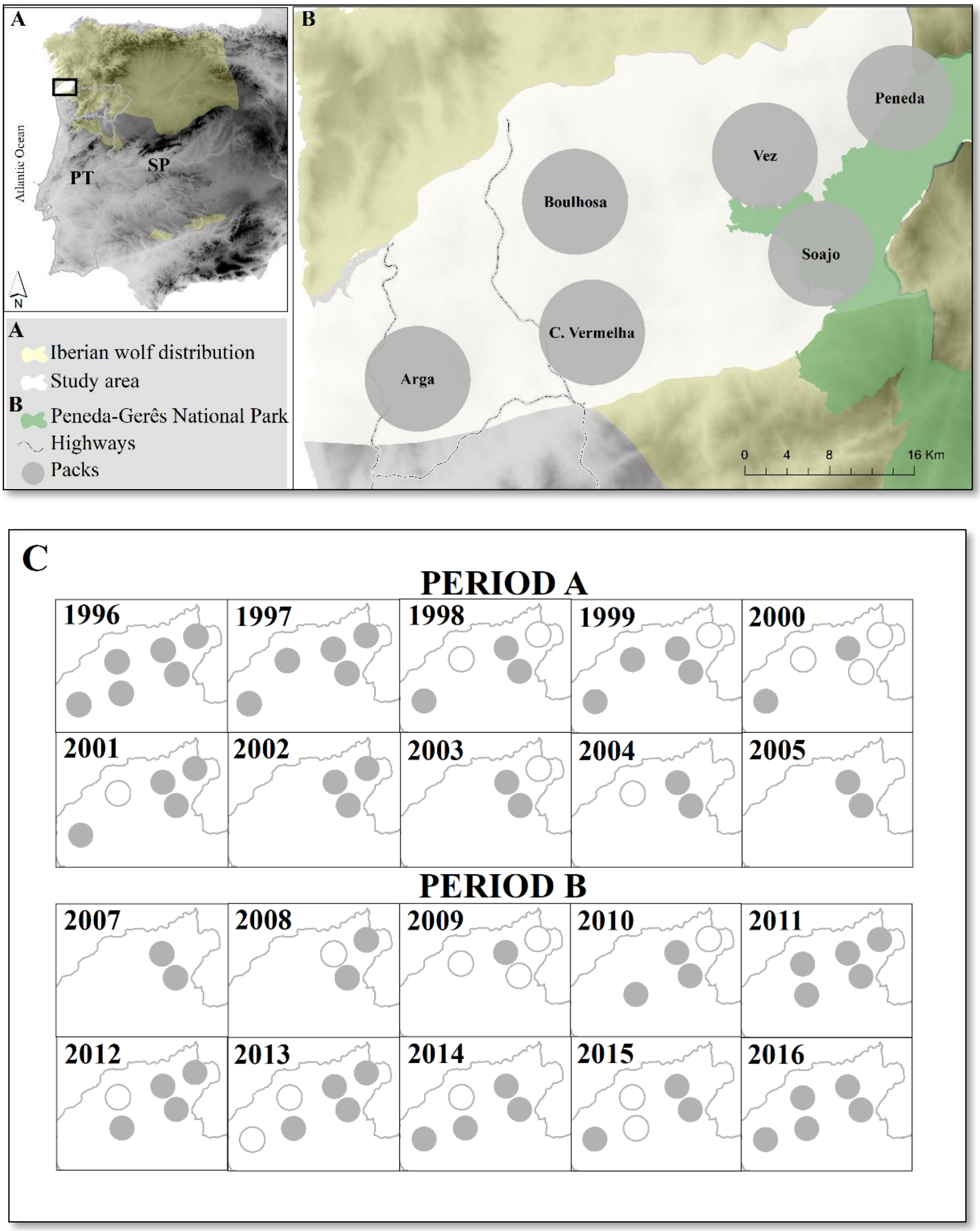


Fig. 4-1: A) Location of the study area in northwest Portugal in relation to wolf distribution in the Iberian Peninsula. B) Study area with the location of detected packs and other features including Peneda-Gerês National Park, highways, and altitude. C) Annual results for period A (1996-2005) and period B (2007-2016) regarding the detection of packs without reproduction (white circles) and with reproduction (grey circles).

4.4.2. Field methods

We monitored the Alto Minho wolf population over 20 years and framed this study in two periods, A (1996-2005) and B (2007-2016). We conducted wolf monitoring yearly, during wolf pup-rearing season (from late June to early November) primarily based on transects for sign surveys covered on foot or by car (<10 km/h), simulated howling, and visual detection of wolves (Harrington and Mech, 1982; Llaneza et al., 2014). These methods are largely used in the Iberian Peninsula to detect breeding packs by locating pups still at homesites and to estimate the number and composition of packs during summer and autumn (Blanco et al., 1992; Llaneza et al., 2014, 2005; Pimenta et al., 2005). In period A, wolf monitoring also relied upon the compilation of trustable wolf sightings from about 20 known informers among local people (park rangers, livestock owners, and hunters) distributed throughout all the study area, and that regularly provided information on pack size and breeding occurrence. We verified the information afterward through additional field effort on sign, howling, and observation surveys as an attempt to validate the data provided by the informers. In period B, taking advantage of the technological advances, we also used camera trapping, Global Positioning System (GPS) telemetry of collared wolves, and molecular individual identification of invasive and noninvasive samples (see Sup. Table 12 in Appendix IV for details on methods used for periods A and B).

During period A, we extensively surveyed the study area for yearly detection of packs and breeding evidence, although the sampling effort for each methodology was not quantified. During period B, we performed 6190 km of sign survey transects (average effort per pack-year: 95.2 km, range 3-513 km), 252 visual observation surveys (average effort per pack-year: 6, range 1-24), complemented with 1075 howling surveys (average effort per pack-year: 18, range 1-75). For pack sizes estimated from howling surveys, we considered a maximum of 3 adults and 3 pups joining a chorus in sequence or emitting from different directions because the precise count of wolves howling in a large group is difficult (Harrington and Mech, 1982; Joslin, 1967; Palacios et al., 2017). We used camera trapping (KG680V, Keepguard®, China) opportunistically and as a complement of remaining detection methods, mostly in packs in which howling and visual observation surveys had very low success. We carried out 1249 nights*camera trap (average effort per pack-year: 178 nights*camera trap, range 26-579). We obtained wolf positive results in 10% of howling surveys, 37% of visual observation surveys, and 5% of camera trapping nights. We captured a total of 21 wolves with Belisle® leg-hold snares (size 8, Edouard Belisle, Saint Veronique, PQ, Canada) and fitted individuals with more than 7 month-old (N = 18) with a GPS telemetry collar (650 g Model ProLight, Vectronic Aerospace GmBh, Berlin, Germany), following the methodological procedures described in (Rio-Maior et al., 2019, 2018). We collected blood samples from captured wolves (N = 21) and putative wolf genetic noninvasive samples (957 scats, 13 urine, 21 saliva, and 9 hair samples) in transects and opportunistically (e.g., homesites, wolf prey carcass, and snow tracking). We submitted the samples to molecular analysis for species, individual, and sex identification.

4.4.3. Molecular analysis

DNA from the blood of the captured wolves was extracted using the commercial DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer’s instructions, and samples were genotyped for a total of 50 microsatellites following Godinho et al. (2015). Scats, urine, saliva, and hair samples were DNA extracted and PCR amplified as described in Nakamura et al. (2017). Briefly, we followed procedures of Boom et al. (1990) and Frantz et al. (2003) for scats preserved in 96% ethanol and for urine, saliva, and hair samples preserved in silica-gel, whereas urine and saliva samples preserved in 96% ethanol were extracted using the commercial QIAamp DNA Micro Kit (QIAGEN). To monitor for potential DNA contaminations, negative controls were included throughout the entire process. All pre-PCR procedures were performed in dedicated laboratories used exclusively for low-quality DNA samples. Species identification was performed through mitochondrial (mtDNA) control region sequencing (see Table B1 in Appendix B) and samples exhibiting wolf mtDNA were genotyped for a set of 19 microsatellites selected among the most variable in Iberian wolves (Godinho et al., 2011, 2015), and for a sex identification marker (DBX/DBY, Seddon 2005). Microsatellites were amplified in four multiplex reactions (see Sup. Table 13 in Appendix IV) and separated by size on an ABI3130xl DNA analyzer. Alleles were scored using GENEMAPPER 5.0 (Applied Biosystems) and checked manually.

Quality control for noninvasive DNA was assessed by PCR replication as described in Nakamura et al. (2017) and consensus genotype over four replicas for each sample was achieved following rules defined in Godinho et al. (2015). Error rates (allelic dropout and false alleles) and the probability of identity (PID and PIDsibs) for our dataset were calculated using the software GIMLET 1.3.3 (Valière, 2002) (see Tables B1 and B2 in Appendix B). The same software was used to identify multiple samples of the same individual. Species identification was successful for 81% of the collected samples, of which 93% were correctly identified as wolves, being the remnant from dogs and red foxes (*Vulpes vulpes*). Among wolf samples, we achieved 476 individual genotypes corresponding to a total of 160 different wolves identified during period B (2007-2016).

4.4.4. Estimates on population, packs, individuals, and dispersal

We combined the results obtained from all monitoring methodologies (field and molecular methods) to estimate annual minimum counts of individuals and to estimate the following parameters for population and packs during periods A and B: i) annual population size; ii) population growth rates; iii) population density; iv) number of packs, and v) pack sizes. To assess variations on the population growth rate, we determined the annual finite rate of increase between two successive years (λ) as the ratio of their annual population sizes, and obtained the average values for the overall monitoring period and separately for periods A and B. We calculated the overall wolf density as the average of annual population sizes in the study area.

We obtained the annual maximum likelihood estimates (MLE) of the population size for period B by fitting a capture-recapture model to the data on molecular individual identification of wolves using the ‘capwire’ R package (Miller et al., 2005; Pennell et al., 2013; Pennell and Miller, 2015). We organized the data in capture classes (i.e. number of captures per individual; from 1 to 7), and the number of individuals in each capture class per year (Pennell et al., 2013). We performed a likelihood ratio test to select between the Equal Capture Model (ECM) and the Two-Innate Rates Model (TIRM) and obtained the 95% confidence intervals for the MLE (see Sup. Table 14 in Appendix IV).

We confirmed the occurrence of a pack when we detected at least two resident wolves (Mech and Boitani, 2003) or whenever we confirmed a breeding occurrence within the territory of a pack. We estimated the territories of the packs through the home ranges of 12 GPS collared resident wolves (as described in Rio-Maior et al., 2019) by using 95% Minimum Convex Polygon (MCP, Hayne 1949). We obtained these estimations over period B and considered several collared individuals from the same pack between 2008 and 2016. Since we do not own telemetry data every year for all the packs, we assumed that the territories were stable. Our estimates are likely to be reliable because the packs in the study area show high fidelity to the breeding sites and are spatially restricted to some extent since they occur in a wolf-saturated area with limited available habitat (Álvares, 2011; Blanco and Cortés, 2007; Rio-Maior et al., 2018). To characterize each pack, we assessed: i) the group persistence rate (GroupPR), defined as the proportion between the number of years in which the pack was detected and the number of monitoring years; and ii) the breeding success (BreedS), defined as the proportion of years that a pack had pups.

We categorized each wolf according to its social status as ‘residents’, ‘dispersers’, or ‘uncategorized’. For both periods, we considered an individual as ‘resident’ whenever detected in a pack territory through field methods. Moreover, for period B we used the territories primarily for the pack assignment of noninvasive samples, in which we considered ‘resident’ the individuals detected through noninvasive sampling based on the following criteria: when detected at least once in a homesite, as previously defined by Stenglein et al. (Stenglein et al., 2011) or when detected at least twice a year inside a pack territory. In period B, we identified dispersal events and ‘dispersers’ through the GPS locations of collared wolves (Rio-Maior et al., 2019), or by the detection of molecularly identified individuals in two different pack territories. For both periods, ‘uncategorized’ wolves included individuals detected: i) inside a pack territory with no evidence of group occurrence, ii) in overlapping pack territories, or iii) outside pack territories. We determined the annual sex ratios of the overall population, packs, and of dispersers based on the results of molecular analyses of both invasive and non-invasive samples.

We assessed core and sink dynamics based on the annual number of individuals per pack as a proxy of recruitment (reproduction or immigration) and losses (mortality or emigration). We also identified core packs as net exporters of wolves, whereas sink packs as net importers following Pulliam (1988). Thus, we estimated a migrant ratio per pack as $MR = (I - E)/(I + E)$, which is founded on the number of detected immigrants (I) and emigrants (E). Packs with an overall average $MR \leq 0$ were considered core packs, while the remnant packs with $MR > 0$ were considered sink packs. To better understand the recovery trend of sink packs during period B, we assigned dispersal events to three sub-periods, 2007-2010, 2011-2013, and 2014-2016, and obtained MR values per pack for each sub-period. We estimated the minimum distance traveled by each disperser, defined as the linear distance between the most recent homesite of the source pack to the farthest sample location in the sink pack. We performed chi-square or Wilcoxon tests in the R Stats package (R Core Team, 2019) to detect significant differences ($p < 0.05$) between: i) period A and B for population size, annual finite rate of population increase, and pack sizes; ii) population sizes obtained from minimum counts and capture-recapture models in period B; iii) core and sink packs for GroupPR, BreedS, pack size, number of adults, number of pups, sex ratio and MR; and iv) males and females for dispersal distances. We performed the statistical analyses in The R

Language for Statistical Programming (R Core Team, 2019) and RStudio software (RStudio Team, 2018).

4.5. Results

4.5.1. Population parameters

Population size estimated from minimum counts was on average 27.0 ± 2.1 (SE) wolves per year during summer-autumn (period A: 25.2 ± 2.4, range 14-40; period B: 28.7 ± 3.3, range 12-43; Fig. 4-2), which corresponds to an overall average wolf density of 1.7 ± 0.1 wolves/100km^2 (period A: 1.6 ± 0.2 wolves/100km^2, range 0.9-2.5; period B: 1.8 ± 0.2 wolves/100km^2, range 0.7-2.0). Population sizes were not significantly different between sampling periods ($\chi^2_{(15)} = 14$, $p = 0.526$; see also Sup. Table 16 in Appendix IV for population estimates). Employing the capture-recapture model for molecularly identified individuals during period B, we obtained an annual MLE population size estimate of 31.1 ± 3.9 individuals (95% CI: 23.7-43.4) (see also Sup. Table 15 in Appendix IV). We detected no statistical differences between annual population size estimates during period B obtained from minimum counts and capture-recapture of noninvasive sampling ($W = 36$, $p = 0.123$). The overall mean annual finite rate of increase (λ) of this wolf population was 1.042 ± 0.076 (growth rate: 4.2 ± 7.6%). Both periods had regular oscillation of λ with no statistical differences between them ($\chi^2_{(16)} = 17$, $p = 0.386$), although period A showed a global decreasing trend (λ=0.92 ± 0.09; growth rate: -8 ± 9%) while period B had an increasing trend (λ = 1.16 ± 0.11; growth rate: 16 ± 11%; Fig. 4-2).

During period B, the average number of individuals detected per year by field methods was 27.7 ± 3.2 (range 12-40) and by molecular methods was 23.7 ± 3.2 (range 11-43), which correspond, respectively, to an average of 96.3 ± 1.6% (range 88-100%) and 83.8 ± 5.5% (range 46-100%) of the estimated individuals in the population obtained by minimum counts (see also Sup. Table 16 in Appendix IV). The annual average number of detections per genetically identified individual was 1.8 ± 0.1, and most individuals (74%; n = 119) were detected only one year, 10% two years (n = 16), and 16% were detected from 3 to 5 years (n = 25). The overall population sex ratio (M:F) obtained was 1.1:1.0 (see also Sup. Table 16 in Appendix IV). Among the genetically identified individuals, 60% (n = 96) were considered as residents in a single pack, 11% (n = 17) as dispersers, and 29% (n = 47) as uncategorized individuals.

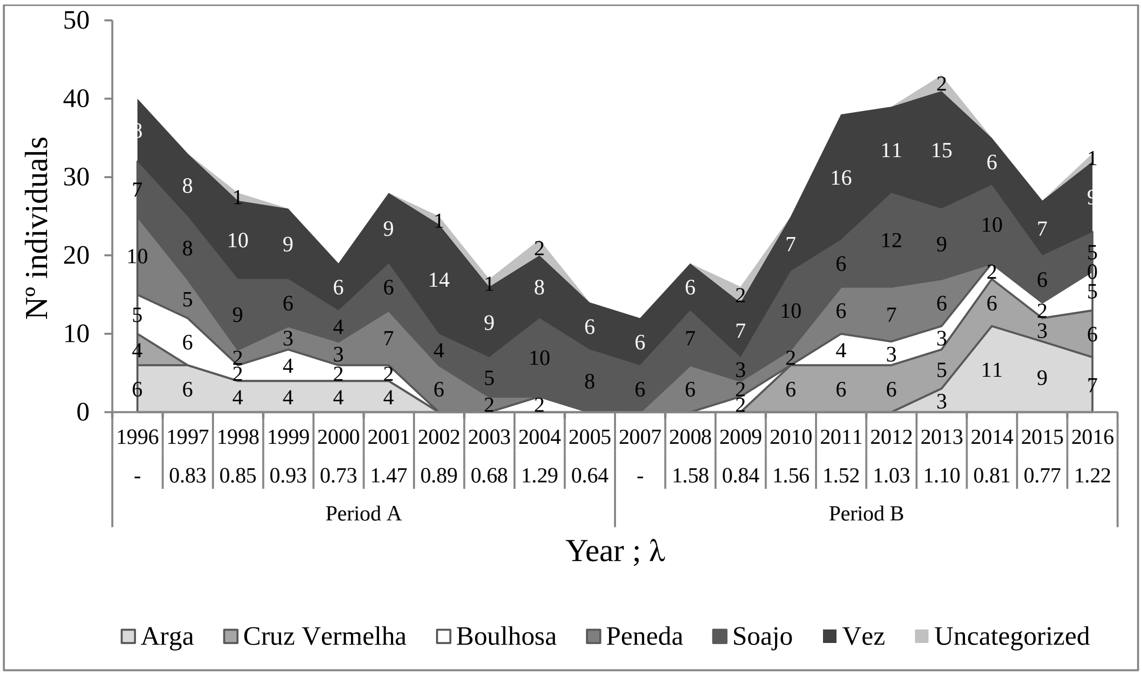


Fig. 4-2: Annual wolf population size estimated during summer-autumn and yearly finite rate of population increase (λ) in northwest Portugal during period A (1996-2005) and period B (2007-2016), including the number of individuals estimated per each pack (Arga, Cruz Vermelha, Boulhosa, Peneda, Soajo, and Vez), and the number of individuals not assigned to packs (Uncategorized).

4.5.2. Pack parameters

During period A, the average number of packs per year was 4.2 ± 0.4 (range 2-6 packs/year), involving 3.3 ± 0.4 breeding detections per year (range 2-6 breeding packs/year; Fig. 4-1C). During period B, the average number of packs per year was 4.4 ± 0.4 (range 2-6 packs/year), involving 3.3 ± 0.4 breeding detections (range 1-5 breeding packs/year) (Fig. 4-1C). On average, each pack or breeding detection was confirmed by 1.2 ± 0.1 methods (range:1-3) in period A, and 2.3 ± 0.3 methods (range:1-5) in period B. For period A, 24% of the sighting information reporting pack and breeding detections were also confirmed by howling or observation surveys (see Sup. Fig. 11and Sup. Table 12 in Appendix IV for further details on group and breeding detection per method). Considering all sampling period, two packs (Vez and Soajo) were detected every year (GroupPR: 100%; n = 20 years), while four packs (Peneda, Boulhosa, Cruz Vermelha, and Arga) were not detected during 6 to 12 years, which resulted in GroupPR's of 70%, 70%, 40%, and 50%, respectively (Fig. 4-1C and Fig. 4-2). BreedS for each pack ranged from 36% to 95%, with an overall average of 75.9 ± 9.8% (period A: 80.5 ± 10.9%; period B: 72.7 ± 9.6%) considering a total of 86 pack-years over 20 years (Fig. 4-3; see also Sup. Table 16 in Appendix IV).

We obtained an average pack size of 6.2 ± 0.3 wolves during summer-autumn (Fig. 4-4A), which showed no statistical differences between periods (period A: 5.9 ± 0.4, n=42 pack-years; period B: 6.4 ± 0.5, n = 44 pack-years; $\chi^2_{(15)} = 16$, p = 0.382). The average size of packs without reproduction (n = 20) was 2.7 ± 0.2 individuals. Considering only packs with reproduction (n = 66) the average pack size was 7.2 ± 0.3 wolves of which 3.8 ± 0.2 were adults, and 3.3 ± 0.2 were pups (Fig. 4-4B). The largest pack size of 16 individuals (8 adults and 8 pups) was detected in Vez pack during 2011 (Fig. 4-2 and Fig. 4-4A). Considering the 20-year sampling period, the average pack size was highest for Vez pack (8.9±0.7 individuals) and lowest for Boulhosa pack (3.1±0.4) (Fig. 4-3). Packs exhibited an overall sex ratio of 1.3:1.0 (M:F) although with a wide variation between packs (see Sup. Table 16 in Appendix IV).

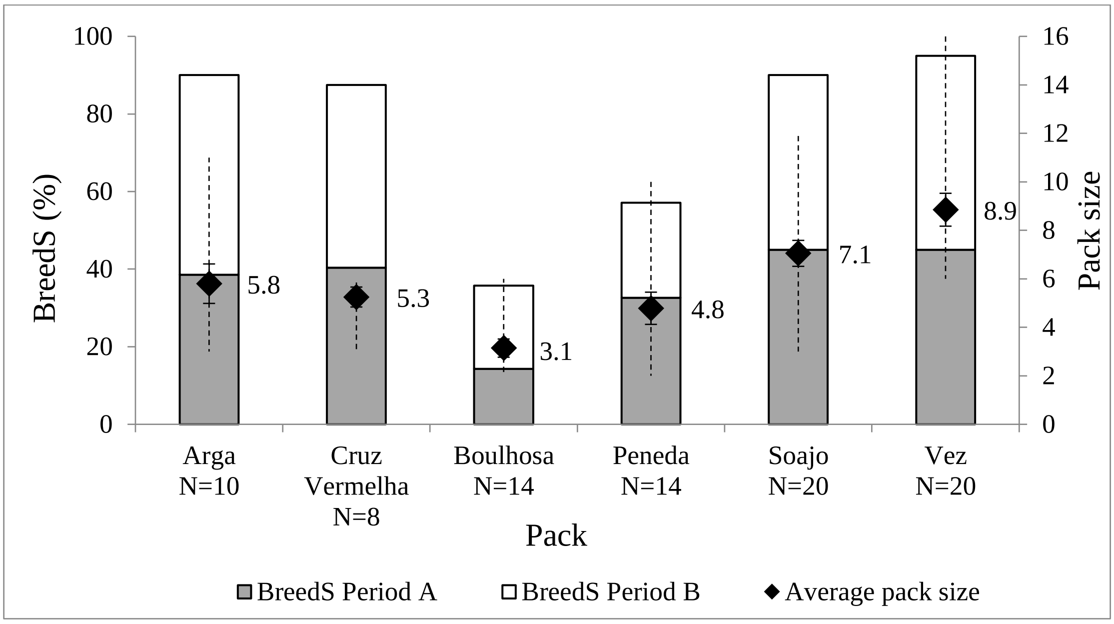


Fig. 4-3: Breeding success (BreedS) per pack in northwest Portugal for period A (1996-2005; grey bars) and period B (2007-2016; white bars), and overall average pack size with reference to the number of years (N) that each pack was detected. Error bars represent standard errors of average pack size (full line) and range of pack size values (dashed line).

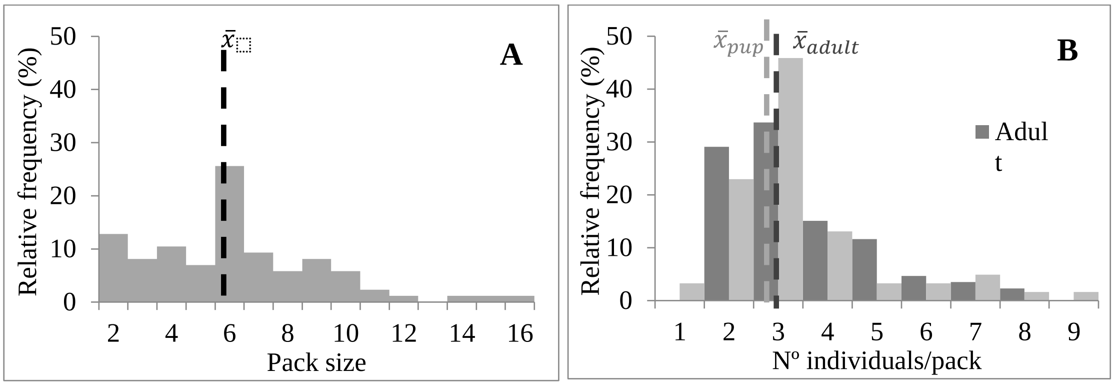


Fig. 4-4: Relative frequencies of the number of individuals estimated per pack yearly during summer-autumn in northwest Portugal between 1996 and 2016: A) pack size; B) number of adults per pack, and number of pups per pack with reproduction. Dashed lines represent average values ($\bar{x}$).

4.5.3. Dispersal patterns between packs

We detected a total of 18 dispersal events between monitored packs (Fig. 4-5), which involved 17 individuals, including 9 males and 8 females (sex ratio: 1.1:1.0). We detected five dispersers both by molecular analysis and GPS telemetry, and the remnant 12 solely by noninvasive molecular analysis (see Sup. Table 17 in Appendix IV for further details on detected dispersal events). In 9 (50%) dispersal events wolves were residents before and after dispersal, in 8 (44%) dispersal events wolves were resident in one of the packs (3 before and 5 after dispersal), and in 1 (6%) dispersal event it was not confirmed as a resident before or after dispersal. The estimated minimum distance travelled by dispersers averaged 24.8 ± 1.2 km (females: 21.6 ± 1.6 km, range 5.5-42.2; males: 27.4 ± 1.6km, range 7.4-59.6; Fig. 4-5), and did not differ between sexes (W = 35, p = 0.689). During sub-period 2007-2010, we only detected two dispersers, both originated from Vez pack and incorporated into Peneda and Boulhosa packs. In sub-period 2011-2013 Vez, Soajo and Peneda packs had a higher number of emigrants than immigrants (MR ≤ 0), while the remnant packs only had immigrants (MR = 1.0). In sub-period 2014-2016 Soajo, Cruz Vermelha, and Arga packs had a higher number of emigrants than immigrants (MR ≤ 0), while the remnant packs only had immigrants (MR = 1.0; Fig. 4-5). According to the average pack sizes and MR per pack, Vez and Soajo packs were classified as core packs and the remnant four packs as sink packs (Fig. 4-5). MR values between core (-0.43 ± 0.37) and sink packs (0.50 ± 0.22) were significantly different ($\chi^2(3)$ = 8.543, p = 0.014; see Sup. Table 18 in Appendix IV for further detailed values of MR per sub-periods). Values of GroupPR, BreedS, pack size, number of adults, and number of pups were also significantly different between core and sink packs (χ^2 tests; $p < 0.05$), with sink packs also presenting a male-biased average sex ratio (1.5:1) (Sup. Table 16 in Appendix IV).

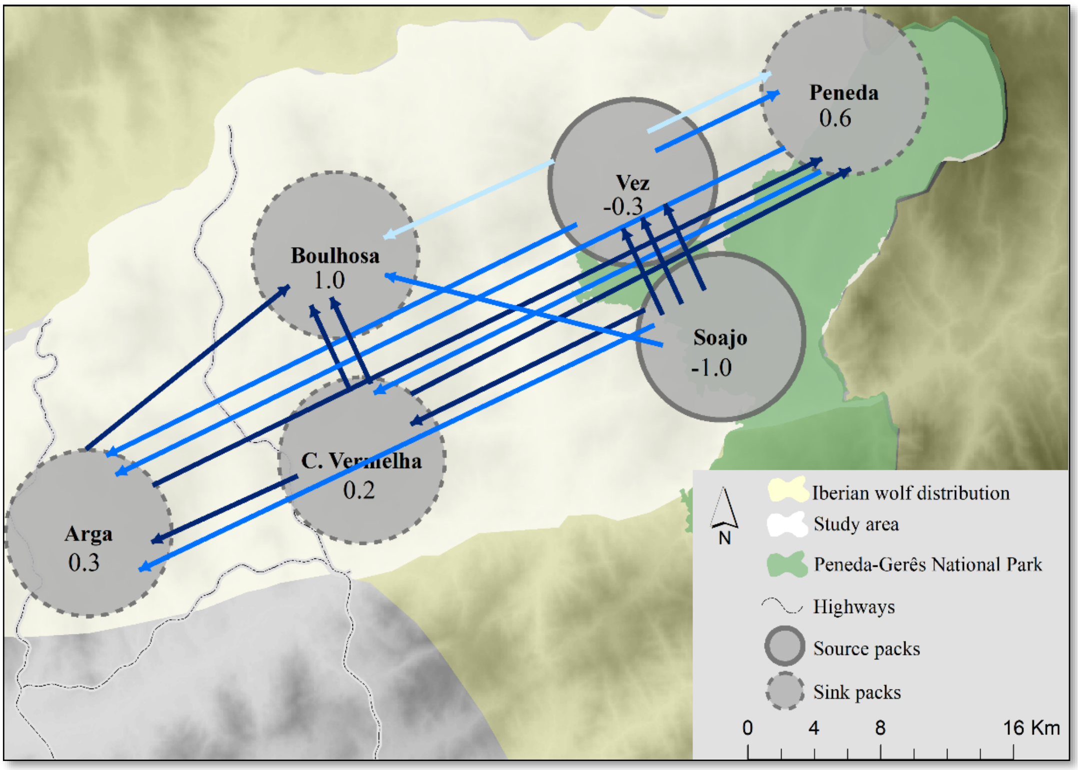


Fig. 4-5: Direction of the 18 detected dispersal events, considering the movement of each individual (arrows) between packs (circles) based on GPS telemetry and genetic recapture in noninvasive samples in northwest Portugal during sub-periods: 2007-2010 (light blue arrows), 2011-2013 (vivid blue arrows), and 2014-2016 (dark blue arrows). Average values of pack migrant ratios (MR) are presented inside the circles depicting the approximate territory for each pack. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.6. Discussion

Our 20-year wolf monitoring focused on population estimates, pack persistence, breeding rates, and dispersal patterns provided innovative insights on the population dynamics of this large carnivore in human-dominated landscapes, and the first long-term study available for the Iberian wolf population. We documented demographic and spatial patterns of pack extinctions and recoveries involving fluctuations in local population size that one-off studies would probably fail to detect.

Overall population size estimates revealed a stable trend from 1996 to 2016 with a 4.2% growth rate, which is lower than values reported for other wolf populations in Europe, either stable (5%, Caniglia et al. 2012) or recovering (29%, Wabakken et al. 2001; 38%, Nowak and Mysłajek 2016). Even though, population trends were contrasting across the sampling period. During period A (1996-2005) we observed a decrease in the number of detected packs, with only two packs remaining by the end of the period (Vez and Soajo), which resulted in a population size decline (growth rate: -8%). During period B (2007-2016), the four previously extinct packs re-established, which led to the recovery of the population (growth rate: 16%). The individual identification of residents and dispersers allowed for a more complete assessment of the population size, which was seldom used in previous assessments of wolf populations (e.g., López-Bao et al., 2018; Stansbury et al., 2016).

Our results suggest that the use of molecular methods on its own may not be the most adequate to estimate population size since the annual number of individuals detected by field methods was often higher than the one detected by molecular analysis. However, our results also unveil the important contribution of individual identification by the molecular methods to better assess the big picture of a wolf population, either in combination with field methods to obtain minimum counts or applying capture-recapture modelling. The similar estimates of annual population sizes obtained from these methods during period B suggest that the results can adequately reflect the actual number of wolves present in our study area. Additionally, individual identification was essential, in combination with telemetry data, to identify dispersal movements, otherwise hardly achievable.

The average population density of 1.7 wolves/100km^2 is within the range of values reported for the Iberian wolf in other regions (Llaneza and Ordiz, 2003; López-Bao et al., 2018; Pimenta et al., 2005). However, our long-term monitoring also allowed us to detect variations in wolf density over the years, ranging from 0.7 up to 2.7 wolves/100km^2. These findings highlight the relevance of monitoring studies across long time series to adequately detect density fluctuations over time, as population census conducted during few years can provide a misguided perception of the wolf density and population size. Although caution is needed to compare results across both monitoring periods, due to differences in available methodologies and the absence of sampling effort quantification during period A, no significant differences were detected between demographic parameters of both periods. This supports the results obtained in period A, even expecting that the probability of detecting a pack or reproduction during period B could be higher than in period A due to the availability of innovative and more reliable methodologies. Further support of our results during period A come from: i) the national wolf census held in 2002 and 2003, during which intensive effort on sampling methodologies was assured to detect packs and reproduction (Pimenta et al., 2005), and the results were largely in line with the results obtained in this study, including the absence of three packs (considered here as sink packs); ii) the near absence of livestock depredations registered by national authorities in the territories attributed to the sink packs during the years that those packs were not detected, which is a reliable indicator of pack absence as wolves feed mostly on livestock in our study area (Álvares et al., 2015). Overall, these evidences reinforce that sink packs were, in fact, extinct for several years.

The average pack size of 6.2 individuals during summer-autumn in our study was higher than other wolf populations in Europe for the same seasonal periods (Jędrzejewska et al., 1996; Nowak et al., 2008), although lower than average values reported in other Iberian studies (e.g., 6.5 to 9.3 wolves; Barrientos, 2000; Fernández-Gil, 2013). Nevertheless, we found one of the largest pack sizes (n = 16) reported for European wolf populations, particularly for the Iberian Peninsula where 15 individuals per pack was the maximum number previously reported (e.g., Barrientos, 2000). The large pack sizes and high wolf densities detected locally may result from specific conditions of high prey availability that promote pack persistence, high productivity, and low rates of wolf dispersal (Fuller et al., 2003; Fuller, 1989). In fact, in northwest Portugal occurs high numbers of free-grazing horses and cattle, which constitute the main prey for wolves (Álvares et al., 2015). However, this trophic dependency leads to high levels of illegal persecution towards wolves in our study area due to conflicts with livestock owners (Álvares, 2011; Barroso et al., 2016). During period B, all the GPS collared individuals that died (47%; Rio-Maior et al., 2018) were killed by human-related causes, including snaring (50.0%), shooting (37.5%), and poisoning (12.5%). This mortality data from collared wolves in our study area suggests an annual survival of 0.541 (95% CI: 0.352, 0.831; authors, unpublished data), which may explain the low growth rate obtained for our studied population although differences of survival among packs are yet to be investigated. The interplay between prey availability and wolf demography (e.g., productivity, mortality, and dispersal) is expected to influence pack sizes (Fuller, 1989; Hayes and Harestad, 2000). Thus, further studies including mortality rates are needed to disentangle the factors determining group persistence and group size across different packs in our study area.

Overall, we detected up to six packs per year with an average breeding success (76%) similar to other studies worldwide (Blanco et al., 1992; Fernández-Gil et al., 2010; Mech et al., 1998). Wolf packs in our study area showed several demographic differences among them, suggesting a source-sink dynamics between packs at a regional level (Pulliam, 1988). Based on our findings, core packs showed higher values of group persistence, breeding success, number of adults, and number of pups, acting as sources of dispersers to recover sink packs that were locally extinct during periods of 2 to 12 years. Core packs were located in the eastern part of the study area, partially inside the Peneda-Gerês National Park, and occupying areas with higher elevation, higher prey availability, less human disturbance, and greater connectivity to the remaining Iberian wolf range than sink packs (Álvares, 2011; Rio-Maior et al., 2019).

Despite high levels of direct wolf persecution, the population decline observed between 1996 and 2005 can also be related to low connectivity between packs. During this period two highways were constructed crossing north-south the study area, which eventually segregated wolves spatially in the west and the east for several years (Álvares, 2011). The construction of linear structures, such as highways, can have a high impact on wildlife space use, including population fragmentation and barrier effects although, after construction and habitat restoration, those areas can progressively be reused by wolves (Blanco et al., 2005; White et al., 2007). This was supported by our results, as we have found that GPS collared wolves crossed these highways during period B, by using passages constructed for human activities (e.g., viaducts and tunnels with paved or forest roads for agricultural activities). However, the habitat disturbance during the construction of these highways together with the absence of exclusive wildlife crossings may have constrained the immigration of wolves to sink packs in the central-western part of our study area, leading to their local extinction during period A as postulated in a framework of meta-population dynamics (Hanski and Gilpin, 1991). Accordingly, high levels of poaching together with anthropogenic landscape features that act as dispersal barriers have been reported as important drivers for wolf population declines worldwide (Fuller et al., 2003; Huck et al., 2010). Furthermore, dispersal wolves tend to have lower survival rates than resident individuals as they often travel across unfamiliar areas with higher levels of human activity (Peterson et al., 1984; Rio-Maior et al., 2019).

Despite the human-dominated landscape of northwest Portugal, the local wolf population recovered between 2007 and 2016 through a successful stepping-stone strategy of dispersal across the study area. This recovery was possibly promoted by increasing habitat connectivity and decreasing incidence of mortality causes with a strong demographic impact such as poison that can result in the death of several pack members at once (Álvares, 2011; authors, unpublished data). Additionally, the large sizes of core packs might have increased intraspecific competition triggering dispersal of subordinates towards sink areas (e.g., Rosenzweig, 1981). As a result, packs that were extinct started to re-establish in sequence from east to west, mostly by the contribution of dispersing individuals from the two core packs. Furthermore, newly established packs are known to produce large litters and, after 2 to 4 years of regular breeding can contribute to the recovery of populations where wolves had been almost eliminated (Hayes and Harestad, 2000). Accordingly, our results confirmed that recently established sink packs showing large pack sizes and high breeding success (Cruz Vermelha and Arga packs) also started to provide emigrants to other small packs with low breeding rates (Peneda and Boulhosa packs), promoting a generalized dispersal across the study area in the latter years of our sampling period.

The percentage of dispersers detected in our population (11%) was lower than the values reported for other wolf populations in North America and Europe (24-28%; e.g., Blanco and Cortés, 2007; Fuller et al., 2003; Mech et al., 1998). However, it was higher than the one obtained in a study in North America based on noninvasive sampling and spatial assignment criteria (5.9%, Stansbury et al. 2016). Dispersal rates can highly vary between areas and are triggered by several factors, such as intraspecific competition, mortality, or prey availability (Gese and Mech, 1991; Kojola et al., 2009). Dispersal may also be driven by uneven human-caused mortalities across the landscape (Minnie et al., 2018; Novaro et al., 2005), resulting in compensatory immigration (Pulliam, 1988). Although the mortality rates in each pack of our study area are unknown, higher mortality is expected in areas more disturbed and accessible by humans (e.g., Murray et al., 2010). Indeed, all GPS collared wolves resident in a sink pack (n = 3; Boulhosa pack) were killed from poaching, including shot and snare (authors, unpublished data). Thus, this particular pack can behave similarly to other heavily exploited wolf populations acting as a sink for immigrating wolves (Mech and Boitani, 2003).

Similarly to other studies, we detected no differences regarding sex ratio for the overall population (Lucchini et al., 2002; Stansbury et al., 2016), for dispersers (Fuller, 1989; Gese and Mech, 1991; Peterson et al., 1984), and for dispersal distances (Blanco and Cortés, 2007; Gese and Mech, 1991). The average dispersal distance obtained in this study (25 km) was similar to the only other reported value for the Iberian wolf (Blanco and Cortés, 2007), though much lower than values reported for other wolf populations in North America and Europe, where wolves frequently disperse across average distances of 50 to 300 km (Gese and Mech, 1991; Wabakken et al., 2001). Dispersal distances in our study were measured as minimum distances based on locations of noninvasive samples and pack homesites and may thus be slightly underestimated. Moreover, some wolves considered as dispersers exhibited short dispersal distances (6-8 km) that could eventually be attributed to extraterritorial or pre-dispersal movements. However, we believe it reflects successful dispersal because some of our studied packs have closely located territories, and we found no evidence of these individuals returning to their original pack, which would be detectable given our intensive sign survey. In fact, it is more likely to detect a scat of an individual in its home territory than a scat left during an excursion outside the territory. Nevertheless, we acknowledge that the few individuals not confirmed as pack residents after dispersal (i.e. that were detected only once by noninvasive sampling) could represent cases of extraterritorial movements and not effective dispersal events. Limited dispersal distances in northwest Portugal were also supported by the five dispersal events documented by GPS telemetry during the latter 10 years of our sampling period. Furthermore, Silva et al. (2018) combined genetic and tracking information from collared wolves to show strong evidence of a very limited number of dispersers (< 4% out of 218 wolves) among eleven genetic clusters identified in the Iberian wolf range. Our results on limited dispersal have implications on the genetic structure of wolf populations, further supporting the findings from Silva et al. (2018) that reported a well-defined genetic cluster for our study area, with high levels of differentiation and low levels of admixture with other Iberian wolf subpopulations. However, there are few evidences of wolves emigrating from neighboring regions during our sampling period: i) a GPS collared male wolf dispersing from Galicia (NW Spain) in winter of 2014-2015 was found dead inside our study area (Silva et al., 2018; L. Llaneza, com pess.), and ii) the molecular identification of a disperser in our study area coming from an adjacent genetic cluster (Silva et al. 2018).

Overall, our results provide compelling evidence that in a human-dominated landscape few core packs play a major role in the maintenance and recovery of a wolf population nuclei at a regional level while sink packs may act as stepping-stones to facilitate dispersal. Our findings also suggest differences in group persistence, breeding occurrence, and dispersal patterns among neighboring packs that may result from the heterogeneous ecological conditions in a human-dominated landscape. Furthermore, the source-sink dynamics with the combined effect of habitat fragmentation, high human density and disturbance, as well as high levels of human-caused mortality seem to limit long-distance dispersal (Blanco et al., 2005; Rio-Maior et al., 2019; Silva et al., 2018).

4.6.1. Conservation and management implications

The continuous monitoring developed in this 20-year study provides valuable knowledge on wolf population dynamics as well as important insights for wolf conservation, with implications both in a regional context and at a wider scale. In a regional context, we reported the first available data on wolf dispersal rates in Portugal and on population dynamics for Iberian wolves. In addition, we found a striking variability on the wolf demographic traits, both across years and neighboring packs, which raises important implications for the use of information from wolf population census that are often limited in time and space. In a wider context, we report one of the first comprehensive scenarios of the demographic and spatial dynamics of wolf populations in human-dominated landscapes, characterized by sharp source-sink dynamics between packs. Therefore, management actions should take into consideration the habitat suitability for both source and sink packs, by promoting connectivity as well as reducing human-caused mortality and disturbance to enhance dispersal movements and breeding rates. These management considerations should primarily target core packs, due to their ability to produce dispersing wolves that are crucial for sustaining wolf occurrence in a wider area (Mech and Boitani, 2003). Moreover, to allow pack re-colonization and recovery by dispersers from adjacent packs, habitat connectivity must be assured through ecological corridors and permeability of barriers (e.g., highways), particularly in landscapes highly disturbed by humans (Rio-Maior et al., 2019).

Furthermore, areas of recent wolf re-colonization leading to pack recovery after local extinction should be the target of awareness campaigns and management actions, particularly where the species was absent for several years. These actions should mostly promote livestock husbandry practices adequate to reduce predation risk, such as the use of livestock guarding-dogs, efficient surveillance to reduce poaching as well as an increase in abundance of wild prey. Such measures, particularly in human-dominated landscapes such as our study area could potentially minimize conflicts due to livestock depredations and decrease human-caused wolf mortality (Barroso et al., 2016; Pimenta et al., 2018). Finally, we propose that future studies targeting heterogeneous landscapes where demographic parameters and ecological conditions differ between neighboring packs, such as in the Iberian Peninsula and other southern

European regions, should apply a multi-method approach to properly assess wolf population dynamics. Besides visual and acoustic detections of wolves that provide valuable information on population estimates and breeding rates, the combined use of telemetry and noninvasive genetic sampling allow for additional demographic parameters as sex-ratio and dispersal. Thus, a step-forward on acquiring a broader view of a wolf population (e.g., genealogies, multiple breeding, dispersal) is conditional on a multi-method approach. In this context, the reliable methodological approach used in this study, with a long-term monitoring effort, should become a more common procedure to assure a better understanding of the demographic and spatial patterns related to wolf population dynamics in human-dominated landscapes.

4.7. Author contributions CrediT

Mónia Nakamura: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Helena Rio-Maior**: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization, Funding acquisition, Supervision. **Raquel Godinho**: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing**. Francisco Petrucci-Fonseca**: Writing - review & editing, Supervision, Funding acquisition, Project administration. **Francisco Álvares:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Funding acquisition, Project administration.

4.8. Acknowledgements

We thank lab assistance for genetic data (D Castro, S Lopes) and field assistants, colleagues and volunteers, particularly J Pereira, P Sierra, M Palma, S Roque, V Palacios, P Alarcão, A Moedas. We are grateful to the veterinaries N Santos, R Brandão, N Fandos and C Ferreira for assisting wolf trapping and capture for GPS-telemetry. We thank N Santos for revising earlier versions of the manuscript. We are grateful to Instituto de Conservação da Natureza e Florestas (ICNF) and Serviço de Protecção da Natureza e do Ambiente – Guarda Nacional Republicana (SEPNA-GNR) for providing legal support for wolf trapping and monitoring. Wolf trapping was conducted under permits from the national authority for Nature Conservation (ICNF, Instituto da Conservação da Natureza e das Florestas). A special thanks to C Barros

who, although no longer with us, is still watching wolves from another world. This is contribution no. 27 from the Iberian Wolf Research Team (IWRT).

4.9. Funding

MN and FA worked under PhD grants (SFRH/BD/144087/2019 and XXI/BD/18326/98, respectively) and RG under research contracts (IF/564/2012 and DL57/2016) from Fundação para a Ciência e a Tecnologia. Field and lab work for wolf monitoring was supported by Grupo Lobo/Faculdade de Ciências da Universidade de Lisboa, Bern Thies Foundation, Parque Nacional da Peneda-Gerês/ICNF, Associação Veranda (Associação para a Conservação e Divulgação do Património de Montanha), ACHLI (Associação de Conservação do Habitat do Lobo Ibérico), and VentoMinho Energias Renováveis S.A.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2021.109075, and in Appendix IV of this thesis.

4.10. References

Álvares, F., 2011. Ecologia e Conservação do Lobo (*Canis lupus*, L.) no Noroeste de Portugal. PhD thesis. Universidade de Lisboa - Faculdade de Ciências.

Álvares, F., Barroso, I., Costa, G.F. da, Espírito-Santo, C., Fonseca, C., Godinho, R., Nakamura, M., Petrucci-Fonseca, F., Pimenta, V., Rio-Maior, H., Santos, N., Torres, R.T., 2015. Situação de referência para o Plano de Ação para a Conservação do Lobo-ibérico em Portugal. Lisbon, Portugal.

APA, 2014. Sistema Nacional de Informação de Recursos Hídricos. Agência Portuguesa do Ambiente.

Barrientos, L.M., 2000. Tamaño y composición de diferentes grupos de lobos en castilla y león. Galemys 12, 249–256.

Barroso, I., Pimenta, V., Santos, N., Godinho, R., Pimenta, C., 2016. Sistema de monitorização de lobos mortos em Portugal: resultados entre 1999 e 2015, in: Abstracts of the III Iberian Wolf Congress. Instituto de Conservação da Natureza e Florestas, Lisbon, Portugal. https://doi.org/10.13140/RG.2.2.10907.82720

Benson, J.F., Mills, K.J., Patterson, B.R., 2015. Resource selection by wolves at dens and rendezvous sites in Algonquin park, Canada. Biol. Conserv. 182, 223–232. https://doi.org/10.1016/j.biocon.2014.12.010

Blanco, J.C., Cortés, Y., 2007. Dispersal patterns, social structure and mortality of wolves living in agricultural habitats in Spain. J. Zool. 273, 114–124. https://doi.org/10.1111/j.1469-7998.2007.00305.x

Blanco, J.C., Cortés, Y., Virgós, E., 2005. Wolf response to two kinds of barriers in an agricultural habitat in Spain. Can. J. Zool. 83, 312–323.

https://doi.org/10.1139/z05-016

Blanco, J.C., Reig, S., De La Cuesta, L., 1992. Distribution, status and conservation problems of the wolf *Canis lupus* in Spain. Biol. Conserv. 60, 73–80. https://doi.org/10.1016/0006-3207(92)91157-N

Boitani, L., Powell, R.A., 2012. Carnivore Ecology and Conservation: A Handbook of Techniques. Oxford University Press, New York, USA. https://doi.org/10.1093/acprof:oso/9780199558520.001.0001

Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Noordaa, J. Van Der, 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28, 495–503.

Cabral, M.J., Almeida, J., Almeida, P.R., Dellinger, T., Ferrand de Almeida, N., Oliveira, M.E., Palmeirim, J.M., Queirós, A.I., Rogado, L., Santos-Reis, M., 2005. Livro Vermelho dos Vertebrados de Portugal. Instituto da Conservação da Natureza, Lisboa, Portugal.

Caniglia, R., Fabbri, E., Cubaynes, S., Gimenez, O., Lebreton, J.-D.D., Randi, E., 2012. An improved procedure to estimate wolf abundance using non-invasive genetic sampling and capture–recapture mixture models. Conserv. Genet. 13, 53–64. https://doi.org/10.1007/s10592-011-0266-1

Chapron, G., Kaczensky, P., Linnell, J.D.C., von Arx, M., Huber, D., Andren, H., López-Bao, J.V., Adamec, M., Álvares, F., Anders, O., Balciauskas, L., Balys, V., Bed, P., Bego, F., Blanco, J.C., Breitenmoser, U., Broseth, H., Bufka, L., Bunikyte, R., Ciucci, P., Dutsov, A., Engleder, T., Fuxjager, C., Groff, C., Holmala, K., Hoxha, B., Iliopoulos, Y., Ionescu, O., Jeremi, J., Jerina, K., Kluth, G., Knauer, F., Kojola, I., Kos, I., Krofel, M., Kubala, J., Kunovac, S., Kusak, J., Kutal, M., Liberg, O., Maji, A., Mannil, P., Manz, R., Marboutin, E., Marucco, F., Melovski, D., Mersini, K., Mertzanis, Y., Mysłajek, R.W., Nowak, S., Odden, J., Ozolins, J., Palomero, G., Paunovi, M., Persson, J., Poto nik, H., Quenette, P.-Y., Rauer, G., Reinhardt, I., Rigg, R., Ryser, A., Salvatori, V., Skrbin ek, T., Stojanov, A., Swenson, J.E., Szemethy, L., Trajce, A., Tsingarska-Sedefcheva, E., Va a, M., Veeroja, R., Wabakken, P., Wolfl, M., Wolfl, S., Zimmermann, F., Zlatanova, D., Boitani, L., 2014. Recovery of large carnivores in Europe’s modern human-dominated landscapes. Science (80-.). 346, 1517–1519. https://doi.org/10.1126/science.1257553

Donker, S.A., Krebs, C.J., 2012. Evidence for source-sink dynamics in a regional population of arctic ground squirrels (*Urocitellus parryii plesius*). Wildl. Res. 39, 163–170. https://doi.org/10.1071/WR11167

Eggermann, J., Ferrão da Costa, G., Guerra, A.M., Kirchner, W.H., Petrucci-Fonseca, F., da Costa, G.F., Guerra, A.M., Kirchner, W.H., Petrucci-Fonseca, F., 2011. Presence of Iberian wolf (*Canis lupus signatus*) in relation to land cover, livestock and human influence in Portugal. Mamm. Biol. 76, 217–221. https://doi.org/10.1016/j.mambio.2010.10.010

Elmhagen, B., Angerbjorn, A., 2001. The applicability of metapopulation theory to large mammals. Oikos 94, 89–100. https://doi.org/10.1034/j.1600-0706.2001.11316.x

Fernández-Gil, A., 2013. Comportamiento y conservación de grandes carnívoros en ambientes humanizados. Osos y lobos en la Cordillera Cantábrica. PhD thesis. Universidad de Oviedo.

Fernández-Gil, A., Álvares, F., Vilá, C., Ordiz, A., 2010. Los Lobos de la Península Ibérica Propuestas para el diagnóstico de sus poblaciones. ASCEL, Palencia, Spain.

Frantz, A.C., Pope, L.C., Carpenter, P.J., Roper, T.J., Wilson, G.J., Delahay, R.J., Burke, T., 2003. Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Mol. Ecol. 12, 1649–1661. https://doi.org/10.1046/j.1365-294X.2003.01848.x

Fuller, T.K., 1995. Guidelines for gray wolf management in the northern Great Lakes region. Vol. 271. International Wolf Center.

Fuller, T.K., Mech, L.D., Cochrane, J.F., 2003. Wolf population dynamics, in: Mech, L.D., Boitani, L. (Eds.), Wolves. Behaviour Ecology and Conservation. Chicago University Press, Chicago, pp. 161–191.

Fuller, T.K.K.T.K., 1989. Population dynamics of wolves in north-central Minnesota. Wildl. Monogr. 105, 33–41.

Gese, E.M., Mech, L.D., 1991. Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969–1989. Can. J. Zool. 69, 2946–2955. https://doi.org/10.1139/z91-415

Godinho, R., Llaneza, L., Blanco, J.C., Lopes, S., Álvares, F., García, E.J., Palacios, V., Cortés, Y., Talegón, J., Ferrand, N., 2011. Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. Mol. Ecol. 20, 5154–5166. https://doi.org/10.1111/j.1365-294X.2011.05345.x

Godinho, R., López-Bao, J.V., Castro, D., Llaneza, L., Lopes, S., Silva, P., Ferrand, N., 2015. Real-time assessment of hybridization between wolves and dogs: combining noninvasive samples with ancestry informative markers. Mol. Ecol. Resour., 15, 317–328. https://doi.org/10.1111/1755-0998.12313

Grilo, C., Lucas, P.M., Fernández-Gil, A., Seara, M., Costa, G., Roque, S., Rio-Maior, H., Nakamura, M., Álvares, F., Petrucci-Fonseca, F., Revilla, E., 2018. Refuge as major habitat driver for wolf presence in human-modified landscapes. Anim. Conserv. 22, 59–71. https://doi.org/10.1111/acv.12435

Hanski, I., Gilpin, M., 1991. Metapopulation dynamics: brief history and conceptual domain. Biol. J. Linn. Soc. 42, 3–16. https://doi.org/10.1111/j.1095-8312.1991.tb00548.x

Harrington, F.H., Mech, L.D., 1982. An analysis of howling response parameters useful for wolf pack censusing. J. Wildl. Manage. 46, 686–693. https://doi.org/10.2307/3808560

Hayes, R.D.D., Harestad, A.S.S., 2000. Demography of a recovering wolf population in the Yukon. Can. J. Zool. 78, 36–48. https://doi.org/10.1139/z99-186

Hayne, D.W., 1949. Calculation of size of home range. J. Mammal. 30, 1–18.

Hindrikson, M., Remm, J., Pilot, M., Godinho, R., Stronen, A.V., Baltrūnaité, L., Czarnomska, S.D., Leonard, J.A., Randi, E., Nowak, C., Åkesson, M., López-Bao, J.V., Álvares, F., Llaneza, L., Echegaray, J., Vilà, C., Ozolins, J., Rungis, D., Aspi, J., Paule, L., Skrbinšek, T., Saarma, U., 2017. Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management. Biol. Rev., 92, 1601–1629. https://doi.org/10.1111/brv.12298

Howe, R.W., Davis, G.J., Mosca, V., 1991. The demographic significance of “sink” populations. Biol. Conserv. https://doi.org/10.1016/0006-3207(91)90071-G

Huck, M., Jedrzejewski, W., Borowik, T., Miłosz-Cielma, M.M., Schmidt, K., Jedrzejewska, B., Nowak, S., Mysłajek, R.W., Jędrzejewski, W., Borowik, T., Miłosz-Cielma, M.M., Schmidt, K., Jędrzejewska, B., Nowak, S., Mysłajek, R.W., 2010. Habitat suitability, corridors and dispersal barriers for large carnivores in Poland. Acta Theriol. (Warsz)., 55, 177–192. https://doi.org/10.4098/j.at.0001-

7051.114.2009

INE, 2014. Instituto Nacional de Estatística - Statistics Portugal - http://www.ine.pt/. Instituto Nacional de Estatística – Statistics Portugal.

IPMA, 2014. Normais climatológicas 1981-2010. Instituto Português do Mar e da Atmosfera http://www.ipma.pt/pt/oclima/normais.clima/.

Jędrzejewska, B., Jędrzejewski, W., Bunevich, A.N., Mikowski, L., Okarma, H., 1996. Population dynamics of wolves *Canis lupus* in Bialowieza primeval forest (Poland and Belarus) in relation to hunting by humans, 1847-1993. Mamm. Rev. 26, 103–126. https://doi.org/10.1111/j.1365-2907.1996.tb00149.x

Joslin, P.W.B., 1967. Movements and homesites of timber wolves in Algonquin Park. Am. Zool. 7, 279–288.

Kojola, I., Kaartinen, S., Hakala, A., Heikkinen, S., Voipio, H.-M.H.-M., 2009. Dispersal behavior and the connectivity between wolf populations in Northern Europe. J. Wildl. Manage., 73, 309–313. https://doi.org/10.2193/2007-539

Llaneza, L., Garca, E.J., Lpez-Bao, J. V, García, E.J., López-Bao, J.V., 2014. Intensity of territorial marking predicts wolf reproduction: implications for wolf monitoring. PLoS One 9, e93015. https://doi.org/10.1371/journal.pone.0093015

Llaneza, L., Ordiz, A., 2003. Distribuición y aspectos poblacionales del lobo ibérico (*Canis lupus signatus*) en la provincia de Lugo. Galemys 15, 55–66.

Llaneza, L., Ordiz, A., Palacios, V., Uzal, A., 2005. Monitoring wolf populations using howling points combined with sign survey transects. Wildl. Biol. Pract. 1, 108–117. https://doi.org/10.2461/wbp.2005.1.13

López-Bao, J.V., Godinho, R., Pacheco, C., Lema, F.J., García, E., Llaneza, L., Palacios, V., Jiménez, J., 2018. Toward reliable population estimates of wolves by combining spatial capture-recapture models and non-invasive DNA monitoring. Sci. Rep., 8, 2177. https://doi.org/10.1038/s41598-018-20675-9

Loreau, M., Daufresne, T., Gonzalez, A., Gravel, D., Guichard, F., Leroux, S.J., Loeuille, N., Massol, F., Mouquet, N., 2013. Unifying sources and sinks in ecology and Earth sciences. Biol. Rev. 88, 365–379. https://doi.org/10.1111/brv.12003

Lucchini, V., Fabbri, E., Maucco, F., Ricci, S., Boitani, L., Randi, E., 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. Mol. Ecol. 11, 857–868.

MAGRAMA, 2016. (Spanish Ministry of Agriculture, Food and Environment). Censo Nacional Lobo ibérico 2012-2014 (*Canis lupus*, Linnaeus, 1758) en España.

Marucco, F., Avanzinelli, E., Boitani, L., 2012. Non-invasive Integrated Sampling Design to Monitor the Wolf Population in Piemonte, Italian Alps. Hystrix, 23. https://doi.org/10.4404/hystrix-23.1-4584

Mech, L.D., 2009. Long-term reseach on wolves in the Superior National Forest, in: Recovery of Gray Wolves in the Great Lakes Region of the United States. Springer New York, New York, NY, pp. 15–34. https://doi.org/10.1007/978-0-387-85952-1_2

Mech, L.D., 1995. A ten-year history of the demography and productivity of an arctic wolf pack. Arctic, 48, 329–332.

Mech, L.D., Adams, L.G., Meier, T.J., Burch, J.W., Dale, B.W., 1998. The Wolves of Denali. University of Minnesotta Press, Minneapolis, London.

Mech, L.D., Boitani, L., 2003. Wolves: Behavior, Ecology, and Conservation. The University of Chicago Press.

Miller, C.R., Joyce, P., Waits, L.P., 2005. A new method for estimating the size of small populations from genetic mark-recapture data. Mol. Ecol. 14, 1991–2005. https://doi.org/10.1111/j.1365-294X.2005.02577.x

Minnie, L., Zalewski, A., Zalewska, H., Kerley, G.I.H., 2018. Spatial variation in anthropogenic mortality induces a source–sink system in a hunted mesopredator. Oecologia. https://doi.org/10.1007/s00442-018-4072-z

Murray, D.L., Smith, D.W., Bangs, E.E., Mack, C., Oakleaf, J.K., Fontaine, J., Boyd, D., Jiminez, M., Niemeyer, C., Meier, T.J., Stahler, D., Holyan, J., Asher, V.J., 2010. Death from anthropogenic causes is partially compensatory in recovering wolf populations. Biol. Conserv. 143, 2514–2524. https://doi.org/10.1016/j.biocon.2010.06.018

Nakamura, M., Godinho, R., Rio-Maior, H., Roque, S., Kaliontzopoulou, A., Bernardo, J., Castro, D., Lopes, S., Petrucci-Fonseca, F., Álvares, F., Petrucci-Fonseca F., lvares F., 2017. Evaluating the predictive power of field variables for species and individual molecular identification on wolf noninvasive samples. Eur. J. Wildl. Res., 63, 1–10. https://doi.org/10.1007/s10344-017-1112-7

Novaro, A.J., Funes, M.C., Walker, R.S., 2005. An empirical test of source-sink dynamics induced by hunting. J. Appl. Ecol. 42, 910–920. https://doi.org/10.1111/j.1365-2664.2005.01067.x

Nowak, S., Mysłajek, R.W., 2016. Wolf recovery and population dynamics in Western Poland, 2001-2012. Mammal Res. 61, 83–98. https://doi.org/10.1007/s13364-016-0263-3

Nowak, S., Mysłajek, R.W., Jędrzejewska, B., 2008. Density and demography of wolf, *Canis lupus* population in the western-most part of the Polish Carpathian Mountains, 1996-2003. Folia Zool. 57, 392–402.

O'Neil, S.T., Bump, J.K., Beyer, D.E., 2017. Spatially varying density dependence drives a shifting mosaic of survival in a recovering apex predator (*Canis lupus*). Ecol. Evol. 7, 9518–9530. https://doi.org/10.1002/ece3.3463

Palacios, V., Font, E., García, E.J., Svensson, L., Llaneza, L., Frank, J., López-Bao, J.V., 2017. Reliability of human estimates of the presence of pups and the number of wolves vocalizing in chorus howls: implications for decision-making processes. Eur. J. Wildl. Res. 63, 1–8. https://doi.org/10.1007/s10344-017-1115-4

Pennell, M.W., Miller, C.R., 2015. Package "Capwire" Estimates population size from non-invasive sampling - R package version 1.1.4.

Pennell, M.W., Stansbury, C.R., Waits, L.P., Miller, C.R., 2013. Capwire: A R package for estimating population census size from non-invasive genetic sampling. Mol. Ecol. Resour. 13, 154–157. https://doi.org/10.1111/1755-0998.12019

Peterson, R.O., Woolington, J.D., Bailey, T.N., 1984. Wolves of the Kenai Peninsula, Alaska. Wildl. Monogr. 88, 3–52.

Pimenta, V., Barroso, I., Álvares, F., Correia, J., Ferrão da Costa, G., Moreira, L., Nascimento, J., Petrucci-Fonseca, F., Roque, S., Santos, G., 2005. Situação do lobo em Portugal: resultados do censo nacional 2002/2003. Relatório Técnico. Instituto da Conservação da Natureza/Grupo Lobo, Lisboa.

Pimenta, V., Barroso, I., Boitani, L., Beja, P., 2018. Risks a la carte: Modelling the occurrence and intensity of wolf predation on multiple livestock species. Biol. Conserv. 228, 331–342. https://doi.org/10.1016/j.biocon.2018.11.008

PORDATA, 2014. Bases de dados Portugal Contemporâneo. http://www.pordata.pt/Portugal.

Pulliam, R.H., 1988. Sources, sinks, and population regulation. Am. Nat. 132, 652–661. https://doi.org/10.1086/284880

R Core Team, 2019. R: A language and environment for statistical computing.

Rio-Maior, H., Beja, P., Nakamura, M., Álvares, F., 2018. Use of space and homesite attendance by Iberian wolves during the breeding season. Mamm. Biol., 92, 1–10. https://doi.org/10.1016/j.mambio.2018.03.014

Rio-Maior, H., Beja, P., Nakamura, M., Santos, N., Brandão, R., Sargo, R., Dias, I., Silva, F., Álvares, F., 2016. Rehabilitation and post-release monitoring of two wolves with severe injuries. J. Wildl. Manage., 80, 1–7. https://doi.org/10.1002/jwmg.1055

Rio-Maior, H., Nakamura, M., Álvares, F., Beja, P., 2019. Designing the landscape of coexistence: Integrating risk avoidance, habitat selection and functional connectivity to inform large carnivore conservation. Biol. Conserv. 235, 178–188. https://doi.org/10.1016/j.biocon.2019.04.021

Rosenzweig, M.L., 1981. A Theory of Habitat Selection. Ecology 62, 327–335. https://doi.org/10.2307/1936707

RStudio Team, 2018. RStudio: Integrated Development for R RStudio, Inc., Boston, MA. http://www.rstudio.com.

Seddon, J.M., 2005. Canid-specific primers for molecular sexing using tissue or non-invasive samples. Conserv. Genet. 6, 147–149. https://doi.org/10.1007/s10592-004-7734-9

Silva, P., López-Bao, J. V, Llaneza, L., Álvares, F., Lopes, S., Blanco, J.C., Cortés, Y., García, E., Palacios, V., Rio-Maior, H., Ferrand, N., Godinho, R., 2018. Cryptic population structure reveals low dispersal in Iberian wolves. Sci. Rep., 8, 1–14. https://doi.org/10.1038/s41598-018-32369-3

Smith, J.E., Lehmann, K.D.S., Montgomery, T.M., Strauss, E.D., Holekamp, K.E., 2017. Insights from long-term field studies of mammalian carnivores. J. Mammal. 98, 631–641. https://doi.org/10.1093/jmammal/gyw194

Stansbury, C.R., Ausband, D.E., Zager, P., Mack, C.M., Waits, L.P., 2016. Identifying gray wolf packs and dispersers using noninvasive genetic samples. J. Wildl. Manage. 80, 1–12. https://doi.org/10.1002/jwmg.21136

Stenglein, J.L., Waits, L.P., Ausband, D.E., Zager, P., Mack, C.M., 2011. Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. J. Mammal. 92, 784–795. https://doi.org/10.1644/10-MAMM-A-200.1

Treves, A., Karanth, K.U., 2003. Human-carnivore conflict and perspectives on carnivore management worldwide. Conserv. Biol. 17, 1491–1499. https://doi.org/10.1111/j.1523-1739.2003.00059.x

Valière, N., 2002. GIMLET: a computer program for analysing genetic individual identification data. Mol. Ecol. Notes 2, 377–379. https://doi.org/10.1046/j.1471-8278

Vingada, J., Fonseca, C., Cancela, J., Ferreira, J., Eira, C., 2010. Ungulates and their management in Portugal, in: M. Apollonio, R. Andersen, and R. J. Putman, Editors. European Ungulates and Their Management in the 21st Century. Cambridge University Press, Cambridge, UK, pp. 392–419.

Wabakken, P., Sand, H., Liberg, O., Bjärvall, A., Bjrvall, A., 2001. The recovery, distribution, and population dynamics of wolves on the Scandinavian peninsula, 1978-1998. Can. J. Zool., 79, 710–725. https://doi.org/10.1139/z01-029

White, C.A., Hurd, T.E., Hebblewhite, M., Pengelly, I.R., 2007. Mitigating Fire Suppression , Highway , and Habitat Fragmentation Effects in the Bow Valley Ecosystem , Banff National Park : Preliminary Evaluation of a Before-After-Control-Impact (BACI) Design with Path Analysis, in: “Monitoring the Effectiveness of Biological Conservation” Conference,. pp. 165–185.

Woodroffe, R., Redpath, S.M., 2015. Hunted predators: intrinsic value—response. Science (80-.). 349, 1295. https://doi.org/10.1126/science.349.6254.1295

Chapter V

An integrated population model to assess key vital rates and viability of a human-encroached wolf population

5. An integrated population model to assess key vital rates and viability of a human-encroached wolf population

[Manuscript in preparation for submission in scientific journal]

Nakamura, M [a,b,c,d]; Monterroso, P [a,c]; Rio-Maior, H [a,b,c]; Godinho, R [a,b,c]; Álvares, F [a,b]; López-Bao, J V [e]*; Gimenez, O [d]*

[a] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

[b] Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, 4099-002 Porto, Portugal

[c] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

[d] CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

[e] Biodiversity Research Institute (University of Oviedo – CSIC – Principado de Asturias), Oviedo University, Mieres, Spain

* co-senior authorship

5.1. Abstract

The persistence of large carnivores in human-dominated landscapes is particularly modulated by the impact of humans on different demographic parameters, such as survival, heavily influenced by retaliatory killing. Robust demographic estimates are seldomly obtained in such elusive species since reproduction, mortality, and dispersal rates can be challenging to estimate, requiring remarkable logistical and economic efforts. We develop an integrated population model (IPM) for wolf (*Canis lupus*) in the western limit of its European distribution by incorporating a 13-year dataset (2007-2019) of population count, fecundity, and individual capture-recapture developed under a Bayesian framework to inform conservation actions.

We estimated population size, growth rate, number of adults and pups, and survival and emigration rates, the latter mostly unknown parameters for wolves in Iberia. This encroached wolf population showed a positive trend, with a growth rate of 1.06 ± 0.15. Estimated survival rates were 72% (95% BCI: 66-77%) for adults and 53% (95% BCI: 30-71%) for pups, and the emigration rate was 13% (95% BCI: 6-21%). Additionally, by forecasting population growth over ten years (2020-2029) upon different survival rate scenarios, we predict that a 10% decrease in adult survival leads to extinction and quasi-extinction (with a threshold of five wolves) probabilities of 1% and 6% by 2029. Conversely, a 20% decrease in adult survival increases these probabilities to 7% and 23%, respectively. These simulations emphasize that adult survival arises as a key factor shaping this population's growth and long-term survival. Conflicts around wolf depredations on livestock, boosting poaching rates, together with the impact of infrastructure development, could decrease wolf survival rates to an unknown extent. Thus, regularly estimating these vital rates for this and other wolf populations in human-dominated landscapes is highly relevant for better adaptation of wolf conservation actions.

Keywords: *Canis lupus,* capture-recapture, fecundity, demography, population viability analysis, survival

5.2. Introduction

Large carnivore (LC) management is one of the most challenging tasks in biodiversity and conservation since their predatory behaviour often results in real and perceived threats to human life, livelihoods, and property (Bautista et al., 2017; Bombieri et al., 2018). Several social, cultural, political and institutional issues make management even more challenging (Redpath et al., 2013), particularly in human-dominated landscapes, where LCs more frequently interfere with human activities, increasing human-wildlife conflicts even more (Carter and Linnell, 2016; Lamb et al., 2020; Treves and Karanth, 2003). Livestock depredation by LCs and competition for game species are the leading causes of such conflicts, although due to inadequate livestock protection measures or low density of wild prey due to poaching and habitat loss (Graham et al., 2005; Lute et al., 2018; Treves and Bruskotter, 2014; Woodroffe et al., 2005). The persistence of LCs in human-dominated landscapes is challenging due to the impact of humans on food availability, connectivity, productivity and social structure of LCs (Bassing et al., 2020; e.g., Cassidy et al., 2023; López-Bao et al., 2019). Consequently, it also impacts several demographic parameters, such as survival, which may be affected by conflicts and low tolerance due to the predatory behaviour of these species (Lute et al., 2018; Treves and Bruskotter, 2014).

In this context, assessing demographic parameters and understanding what limits the persistence of LC populations in human-dominated landscapes is a cornerstone to understanding how conflicts or infrastructure development may affect populations (Gantchoff et al., 2020; Lamb et al., 2020). Population monitoring is integral to species management by providing valuable information to ground management actions and devising conservation strategies (Bled et al., 2017). However, robust demographic estimates are seldom obtained for such elusive species since detecting groups, reproduction, mortality, and dispersers is challenging. LCs are usually nocturnal, wide-ranging, and occur in low densities, which makes it hard to observe or detect. Furthermore, the primary causes of mortality in LCs are anthropogenic, such as poaching, road kills or hunting (e.g., Lamb et al., 2023; Liberg et al., 2020), and a large proportion of this mortality is cryptic, making it even harder to obtain robust demographic estimates of LC populations (Heurich et al., 2018; Liberg et al., 2012).

Among LCs, wolves (*Canis lupus*) are one of the species showing more capabilities to adapt and persist in human-dominated landscapes (e.g., Chapron et al., 2014; Linnell et al., 2001). Several wolf populations have rapidly increased in human-dominated landscapes, with growth rates up to 36% in the case of Germany (Reinhardt et al., 2019), 27% in France (Marescot et al., 2011), and 24% in Russia (Kochetkov, 2015). Other populations have been relatively stationary with lower growth rates, either due to hunting or poaching, among other causes (Bassi et al., 2020; Bischof et al., 2020; Caniglia et al., 2012). Capture-recapture (CR) models based on non-invasive genetic sampling have increasingly been used to obtain wolf population estimates (Bischof et al., 2020; Hebblewhite and Whittington, 2020; López-Bao et al., 2018; Marucco et al., 2012, 2023).

In long-lived species, population growth is most sensitive to variation in adult survival (Heppell et al., 2000; Sæther and Bakke, 2000), and this information, coupled with abundance estimates, are relevant demographic indicators to assess the population status. In addition, other vital rates are also relevant for further management and conservation planning, such as fecundity, immigration or emigration. However, sensitivity analysis comparing the relevance of these vital rates remains to be developed. Due to conflicts around sharing the landscape with wolves, mainly due to livestock depredations (Graham et al., 2005; Olson et al., 2015), and subject to high cryptic poaching rates (Liberg et al., 2012; Treves et al., 2021), survival is probably one of the most important vital rates in large carnivore demography in human-dominated landscapes since high rates of human-caused mortality can limit or threaten LC populations due to the strong influence of adult survival on population growth and the potential impact on population connectivity (Cooley et al., 2009; Weaver et al., 1996).

Most estimates available on survival rates refer to wolf populations in North America, basically using CR models of non-invasive samples (0.74, Adams et al., 2011; e.g., 0.71, Marucco et al., 2012) and collared wolves (e.g., 0.73, Hebblewhite and Whittington, 2020; 0.82, Treves et al., 2017). However, equivalent estimates are scarcely available for European wolf populations. The overall survival rate for wolves was estimated at 0.84 based on a CR model in France between 1995 and 2003 (Marescot et al., 2011), and at 0.66 - 0.75 for weakly detectable individuals and at 0.75 - 0.90 for highly detectable individuals in Italy and France, respectively (Caniglia et al., 2012; Cubaynes et al., 2010). To the best of our knowledge, only two studies address wolf survival in Iberia. Blanco and Cortés (2007) estimated a 0.18 (95% CI: 0.059 – 0.285) mortality rate, based on 14 collared wolves between 1997 and 2004, with 44% of cases attributed to poaching (deaths associated with poisoning or illegal shooting); and Campos (2018) estimated a 0.62 (95% CI: 0.486-0.799) survival rate, based on 32 collared wolves between 1991 and 2017, with poaching as the leading cause of mortality in Portugal. Such variable mortality rates among human-dominated landscapes suggest that wolves are affected differently depending on the area and highlight the importance of obtaining robust estimates of vital rates to evaluate the viability of wolf populations in human-dominated landscapes.

Recently, integrated population models (IPMs) have been progressively developed for several species (Abadi et al., 2017; Bled et al., 2017; Paquet et al., 2021; Tempel et al., 2014). By formally describing the ecological processes, IPMs combine multiple data sets (e.g., population counts and fecundity estimates) into a single unified analytical framework to obtain higher accuracy and precision in estimating demographic parameters, including unknown parameters (Arnold et al., 2018; Besbeas et al., 2010; Schaub and Abadi, 2011). IPMs are useful for identifying population-limiting factors and informing conservation actions accordingly (Arnold et al., 2018). Although IPMs have been developed previously for wolves (Horne et al., 2019; Petracca et al., 2023; Sells et al., 2020; e.g., Stenglein et al., 2015), their application is still lacking in contexts of wolf populations in human-dominated landscapes, such as in the case of Europe.

Taking advantage of long-term wolf monitoring in Alto Minho – a fraction of the Portuguese part of the northwestern Iberian wolf population (Chapron et al., 2014) – we use this area as a case study for wolf population dynamics in a human-dominated and highly fragmented landscape. Here, we develop an IPM for wolf to estimate key population parameters (e.g., population size, growth rate, adult and pup survival rates) and to identify the main limiting demographic factors for the persistence of wolves in these human-dominated landscapes. We make population projections for ten years under different scenarios to identify the most critical parameters and thresholds for such persistence. We hypothesize that: H1) as a protected species, the trend of the population was positive over time and that it will keep increasing if the vital rates remain constant; H2) adult survival was higher than pup survival, as observed in other wolf populations (e.g., 0.63 vs 0.49, Potvin, 1988); H3) population growth is highly sensitive to both adult and pup survival rates (Chapron et al., 2003); H4) the population has a high probability of reaching near-extinction (i.e. population size of five wolves) by 2029 if survival rates decrease by 10% or 20%.

5.3. Material & Methods

5.3.1. Case study: Wolves in Alto Minho

We built our IPM around wolves in the genetic group of Alto Minho (Silva et al., 2018), northwest Portugal, located in Europe's westernmost limit of wolf range, over an area of approximately 2,200 km^2. The climate is temperate Atlantic with strong oceanic influence characterized by a marked seasonality (monthly average temperature range: 6.3ºC –21.7ºC, IPMA 2014; annual average precipitation: 1357 mm APA 2014). The altitude ranges between 180 m and 1416 m asl, and the landscape is very heterogeneous, with areas of low elevation and river valleys occupied by human settlements and agricultural land, whereas mountainous areas consist predominantly of scrublands, oak (*Quercus* spp.) forest patches, and forest plantations. Similar to most of the wolf range in northwestern Iberia (Dennehy et al., 2021; Lino et al., 2019; Sazatornil et al., 2016; Torres et al., 2015), wolves in the study area persist in a highly human-dominated landscape with a human population density of 107.7 inhabitants/km^2; (PORDATA 2014), dispersed human settlements, and high paved road densities (1.60 km/km^2; INE 2014). Wolves in Alto Minho form a distinct genetic cluster, with limited evidence of exchanging individuals with other neighbouring areas with wolf presence in Portugal and Spain (Silva et al., 2018). During the last two decades, the estimated number of packs ranged between 2 and 8, which declined between 1996 and 2005 and recovered between 2007 and 2016, with some recolonized areas (Casimiro et al., 2021; Nakamura et al., 2021).

5.3.2. Long-term wolf population monitoring in Alto Minho

Wolves in Alto Minho have been monitored annually between 2007 and 2019. The main goal of this monitoring has been to estimate the number of reproductions. To do this, during the wolf pup-rearing season (from late June to early November), transects for sign surveys covered on foot or by car (<10 km/h), simulated howling, observations (Harrington and Mech, 1982; Llaneza et al., 2014; Nakamura et al., 2023), camera trapping, Global Positioning System (GPS) telemetry of collared wolves, and individual molecular identification of invasive and non-invasive samples have been carried out (Nakamura et al., 2021, 2017; Rio-Maior et al., 2019). Overall, we performed 6,371 km of transects (average effort: 796 km/year), and 1,574 howling surveys complemented with 313 observation surveys. We carried out 1,559 nights of camera trapping and 3,501 trapping nights for capture for GPS-collaring (Table 5-1).

Table 5-1: Total effort and average effort per pack-year for each field methodology.

Survey method	**Total effort**	**Average effort per pack-year (range)**
Observation	313 surveys	6 surveys(1-24)
Howling	1,574 surveys	21 surveys (1-75)
Camera trapping	1,559 trapping nights	58 trapping nights (1-161)
Capture GPS-collaring	3,501 trapping nights	90 trapping nights (19-262)

For howling surveys, we considered a maximum of five adults and five pups joining a chorus in sequence or emitting from different directions because the precise count of wolves howling in a large group is difficult (Harrington and Mech, 1982; Joslin, 1967; Palacios et al., 2017). We used camera trapping (KG680V, Keepguard®, China) opportunistically, which complemented the remaining detection methods, mostly in packs, in which howling and visual observation surveys had low success. Wolves were captured using Belisle® leg-hold snares (size 8, Edouard Belisle, Saint Veronique, PQ, Canada), GPS collars (650g Model ProLight, Vectronic Aerospace GmBh, Berlin, Germany) weighted, on average, 1.96% of captured wolf body mass. Captured and GPS-collared wolves were evaluated as clinically healthy at the moment of capture, and they only had minor lesions associated with trapping (i.e., skin abrasions). All procedures were carried out under license from the national authority for Nature Conservation (Instituto da Conservação da Natureza e das Florestas; Licenses 326/2007/CAPT, 259/2008/CAPT, 261/2009/CAPT, 330/2010/CAPT, 20/2012/CAPT, 388/2013/CAPT, 73/2014/CAPT, 229/2015/CAPT and 126/2016/CAPT), and animal handling followed guidelines of the American Society of Mammologists (Sikes and Gannon, 2011). Detailed descriptions of field procedures are described in Nakamura et al. (2021) and Rio-Maior et al. (2019; 2018). GPS-collared wolves that died were retrieved and used as information on dead-recoveries for the survival estimation. Due to financial limitations, when the number of detected scats was high, we selected the less degraded scats for higher genetic individual identification success (Nakamura et al., 2017). We submitted all collected samples to molecular analysis for species and individual identification, which included blood, scats, urine, saliva and hair samples (see Appendix V-A for the description of the molecular procedures).

We confirmed the occurrence of a pack when we detected at least two resident wolves (Mech and Boitani, 2003) or whenever we confirmed a breeding occurrence within the territory of a pack. We joined adults and subadults in one group because we had very few individuals identified as subadults since this age is only distinguishable when individuals are live-trapped or when a genetically identified pup is detected in the following year. Thus, we considered individuals <1 year old as a pup and > 1 year old as a subadult/adult (hereafter named as 'adult'). The number of adults and pups in packs was obtained by observations, howling points, camera trapping, and captures for GPS-collaring. For molecularly identified individuals from non-invasive samples, we used pack assignment criteria applied in previous studies, which considered the individuals belonging to a pack when detected at least once in a homesite or when detected at least twice a year inside a pack territory (Nakamura et al., 2021; Stenglein et al., 2011).

5.3.3. Integrated population model components

We developed our IPM in a hierarchical Bayesian framework to join the data sources into a unified modelling framework that accounts for the observation process. This IPM incorporated three components into a post-breeding stage-structured population matrix model: i) population size, ii) fecundity, and iii) capture-recapture models (hereafter CR). We took into account the wolf social structure and annual biological cycle, considering the annual number of adults (Nad) and pups (Npup), survival of adults (Sad) and pups (Spup), the proportion of female breeders among adults (Prop_fb), and fecundity (F) (Fig. 5-1).

5.3.3.1. Population size and fecundity models

We combined the results of all the field and molecular methodologies to estimate the minimum annual values for the population size (Ntot), the number of adults (Nad) and pups (Npup), and the number of packs with and without reproduction. We obtained the annual population count (C) by the sum of the maximum number of individuals detected per pack. In years when the number of genetically identified individuals was higher than the previous sum, we considered the additional individuals for the final annual population count.

Although a pack could have multiple female breeders (Ausband, 2018), and we were aware of an event of multiple female breeders in a pack in our study area (Rio-Maior et al., 2018), due to the difficulties in detecting these events in Iberia, and for simplicity, we assumed only one female breed per pack-year (Mech and Boitani, 2003). Therefore, we considered the annual number of female breeders (Fb) equivalent to the annual number of packs with confirmed reproduction. Still, the inclusion of the proportion of female breeders among adults in our IPM gives some flexibility to the possibility of the existence of more female breeders in the population other than the ones detected, such as more packs reproducing or multiple breeding per pack.

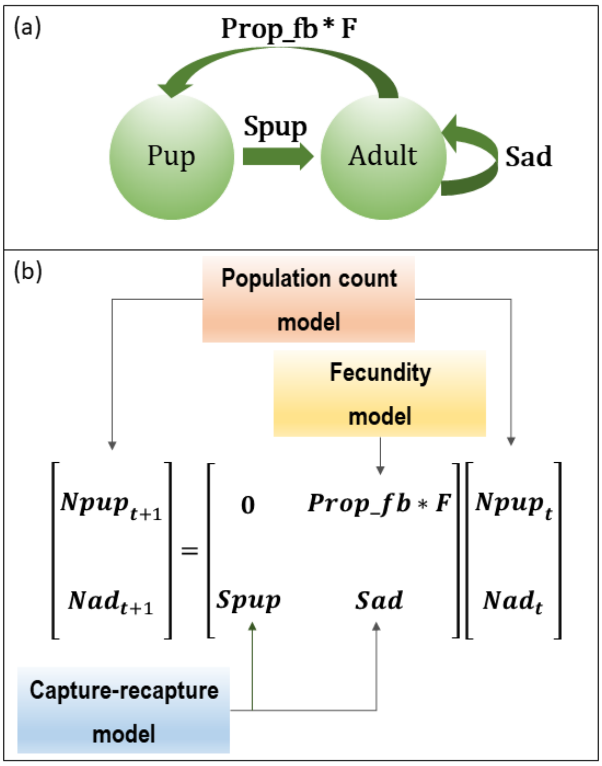


Fig. 5-1: **(a)** Scheme for building the age structured population matrix; **(b**) integrated population model components in a matrix model representation (for visual representation purposes only, since the matrix model is not implemented). **(**Prop_fb – proportion of female breeders among adults, F – fecundity, Spup – pup survival; Sad – adult survival; Nad – number of adults; Npup – number of pups)

The observation model included the data of annual population count and the annual number of female breeders:

$$C[t] \sim dnorm(Nad[t]\ +\ Npup[t], tau.obs)$$

$$Fb[t] \sim dbinom(Prop\_fb[t], Nad[t])$$

From this, we estimated the average annual proportion of female breeders among adults (Prop_fb). Furthermore, we obtained the average fecundity per pack (F) based on the average number of pups detected per pack-year by observation points. According to the field data collected, we generated priors with random time effects for the proportion of female breeders and fecundity to accommodate variations through sampling years:

$$mean.Prop\_fb \sim dbeta(4, 23)$$

$$mean.F \sim dlnorm\ (log(3.9), .2)$$

Due to scarce information on individuals of pup age (N=4) for the Alto Minho population, we decided to feed our model with a prior based on the average pup survival calculated by Ausband et al. (2015), which estimated lower and upper 95% CI's bounds of 0.28 and 0.72 the survival of pups with age between 3 and 15 months:

$$mean.Spup \sim dunif\ (0.28, 0.72)$$

5.3.3.2. Capture-recapture model

We initially organized the information on molecularly identified individuals as annual detection and non-detection (i.e. capture history) per individual. We ran goodness-of-fit tests on the capture history of individuals with R package R2ucare (Gimenez et al., 2018), which suggested a good fit of these data ($\chi^2 = 29.52$, df = 34, P = 0.69). We used a multistate formulation model to jointly analyze live-recapture and dead-recovery data (Kéry and Schaub, 2011; Lebreton et al., 1999). We organized the capture history and dead recoveries of molecularly identified individuals into three possible observation states: 1-‘seen alive', 2-‘recovered dead' or 3-‘neither seen nor recovered'; and four latent states: 1-‘alive in study area', 2-‘alive outside study area', 3-‘recently dead and recovered', and 4-‘recently dead but not recovered'. The parameters in the observation matrix were the probability of detecting an individual alive in the study area (recapture; pp) and the probability of recovering a dead individual (recovery; rr). The parameters in the transition matrix were true adult survival probability (Sad) and fidelity probability (Fp). Fidelity here is defined as the probability of remaining in the study area, given that an individual is alive (i.e. does not permanently emigrate). Taking into account the estimation of adult survival and fidelity probabilities, we also estimated the apparent survival rate (i.e. the probability that an individual is alive and present in the study area; Fp*Sad). We included random time effects on adult survival to accommodate variations in survival through the sampling years.

5.3.4. Model implementation and population viability analysis

We fit the IPM in JAGS (version 4.3.0) using the R package 'R2jags' (Su and Yajima, 2022) in RStudio (Posit team, 2022). We generated two chains for 200,000 iterations and 10,000 for burn-in. Code is provided in Appendix V-B. We assessed model convergence visually by inspecting the chains and by checking the Gelman-Rubin statistic (Rhat < 1.1) (Gelman et al., 2004).

On the other hand, we forecast the trajectory of the population over a ten-year period (2020-2029) following empirical dataset using the baseline parameters estimated from the IPM (control). Additionally, to evaluate to what extent adult and pup survival impact on the trajectory of this population, and its extinction probability, we projected the trajectory for the same period under eight different scenarios: increased and decreased adult or pup survival by 10% and 20%. We also evaluated how these variations in survival affect the number of adults and pups during the forecasted period. For all scenarios, we determined the cumulative probability of extinction (Ntot= 0; i.e. the probability of the population size becoming zero) and quasi-extinction with a threshold of Ntot = 5 individuals for the forecasting period.

We estimated the average annual proportion of female breeders, fecundity, pup and adult survival, fidelity, dead recovery, detection probabilities for the sampling period (2007-2009). We estimated annual population size (Ntot[t]), number of adults (Nad[t]) and pups (Npup[t]), annual growth rate (λ[t] = Ntot[t+1]/Ntot[t]), and adult survival (Sad.est[t]), for the sampling period, and predicted the same parameters for the forecast period (2020-2029).

5.4. Results

We obtained wolf-positive results (either the confirmation of reproduction or a positive detection of wolves) in 9% of howling surveys, 36% of observation surveys (note that observation surveys were carried out mainly to estimate the minimum number of individuals, and after the den or rendezvous site was located with other methods, such as howling surveys), and 5% of camera trapping nights (0.45% considering only pups).

During the study period, we fitted 18 individuals older than seven months old with GPS collars. We detected 4,525 putative wolf signs (348 ± 139 signs per year; ±SD; range:112-518). We collected noninvasively 31% of the detected signs (1,355 scat, 20 urine, 14 saliva, and 15 hair). Species identification was successful for 85% of the non-invasive samples. Of these, 92% were correctly identified as wolves and the remnant from dogs and red foxes (*Vulpes vulpes*). We achieved 709 individual genotypes among wolf samples during the study period, corresponding to 223 different wolves identified between 2007 and 2019.

Based on the naïve results from howling points, observations, camera trapping, captures for GPS-collaring, and molecular individual identification, the observed yearly minimum average population size was 34.8 ± 13.3 wolves in Alto Minho (range: 13-61), with an average naïve growth rate between 2007 and 2019 was 1.17 ± 0.40 (range: 0.63-1.85). The observed annual average number of adults and pups were 18.5 ± 7.2 (range: 6-31) and 12.4 ± 5.3 (range: 4-20), respectively. We detected an average of 4.8 ± 1.6 packs (range: 2-7) annually, of which we could confirm reproduction in 3.7 ± 1.4 of them (range: 1-6). The average annual proportion of female breeders among adults was 0.17 ± 0.06 (range: 0.09-0.29). We obtained an average fecundity of 3.4 ± 1.8 (N= 48; range: 1-9) when we considered all field methodologies, and of 3.9 ± 2.4 (N= 20; range: 1-9) when we considered only direct observations (Sup. Fig. 12 in Appendix V-C).

We identified a total of 222 different wolf genotypes, 3 of which were first captured and recovered dead in the same year (including one pup), 5 were pups, and 13 were first captured in the last year of sampling (average year of first capture: 7.6 ± 3.6). The final capture-recapture history for the CR model included 205 individuals, of which 144 were detected once, 30 twice, and the remnant were detected up to 7 times during the 13 years of sampling. We obtained eight dead-recoveries of GPS-collared wolves.

According to our IPM, the estimated average population size and annual growth rate were 35.8 ± 9.6 (range: 22-54) and 1.06 ± 0.15 (range: 0.87-1.29), respectively (Fig. 5-2A and B). The IPM estimated an annual average of 21.7 ± 5.7 (range: 14 – 32) adults and 13.6 ± 4.4 (range: 8-24) pups in the population (Fig. 5-2C), and an average survival rate of adults at 72% (95% Bayesian credible interval (95% BCI) 66-77%) and of pups at 53% (95% BCI 30-71%). The fidelity of adults to the study area was estimated to be 87% (95% BCI 79-94%), and apparent survival to be at 62% (95% BCI 52-72%). The probability of individual detection was 44%, and that of dead recovery was 6% (see details on parameter estimates in Table 5-2 and the model output with all estimates in Sup. Table 19 in Appendix V-C)

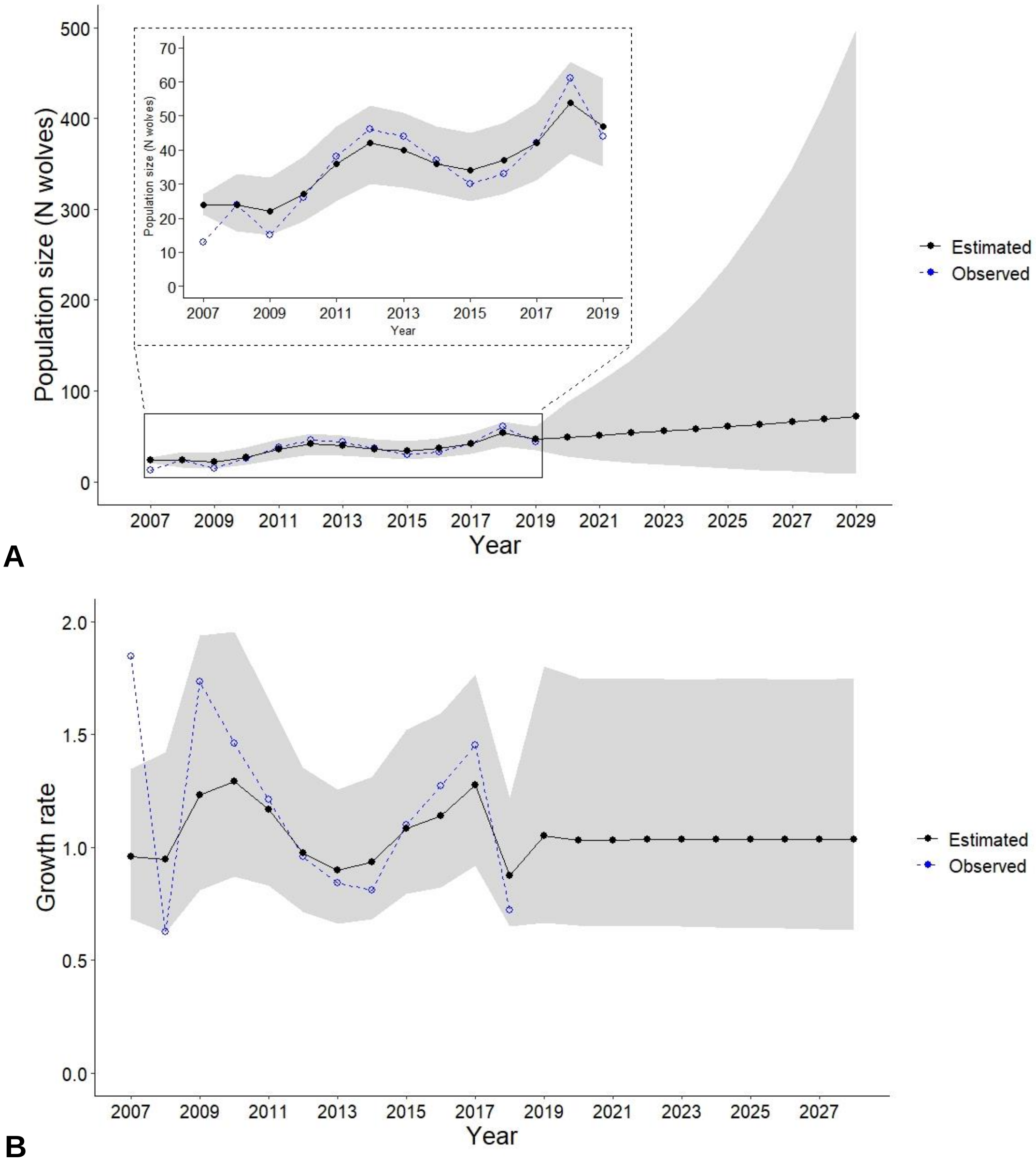


Fig. 5-2: Annual observed values and parameter median estimates obtained by the integrated population model for the sampling period (2007-2019) and the forecast period (2020-2029): A) population size; B) growth rate; C) number of adults and pups; and D) annual adult survival rate with overall average values of adult apparent survival rate (red dashed line) and of pup survival (orange line); shadow areas represent 95% Bayesian credible intervals.

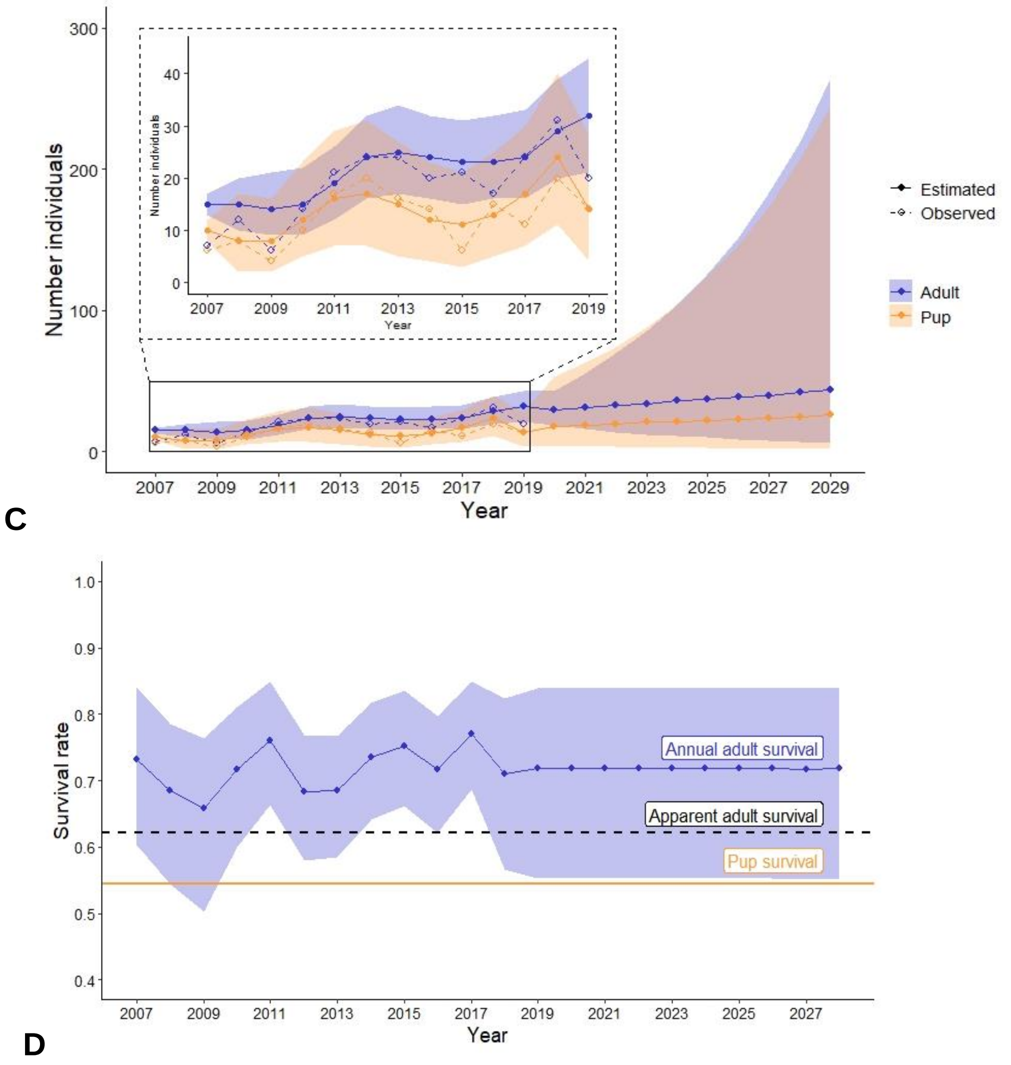


Fig. 5-2 (cont): Annual observed values and parameter median estimates obtained by the integrated population model for the sampling period (2007-2019) and the forecast period (2020-2029): A) population size; B) growth rate; C) number of adults and pups; and D) annual adult survival rate with overall average values of adult apparent survival rate (red dashed line) and of pup survival (orange line); shadow areas represent 95% Bayesian credible intervals.

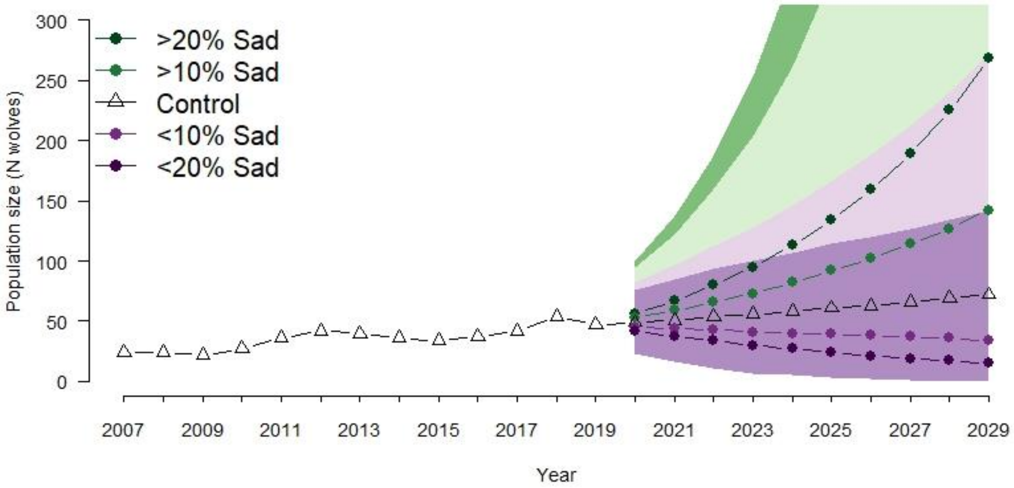
Table 5-2: Parameter estimates obtained by the integrated population model; posterior mean (Mean), standard deviation (SD), and 95% Bayesian credible interval (BCI).

Parameter	Mean ± SD	95% BCI
Proportion breeders (mean.Prop_fb)	0.16 ± 0.03	[0.11; 0.23]
Fecundity (mean.F)	3.89 ± 1.06	[2.20; 6.32]
Pup Survival (mean.Spup)	0.53 ± 0.12	[0.30; 0.71]
Adult Survival (Sad)	0.72 ± 0.03	[0.66; 0.77]
Fidelity (Fp)	0.87 ± 0.04	[0.79; 0.94]
Dead recovery (rr)	0.06 ± 0.02	[0.03; 0.10]
Detection (pp)	0.44 ± 0.04	[0.36; 0.52]

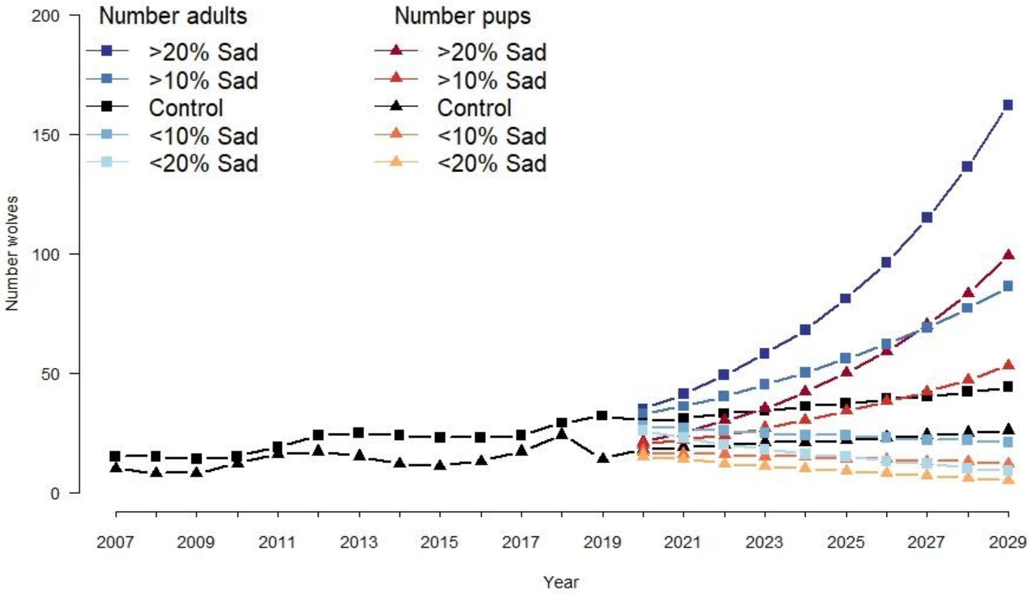
Our population viability analysis suggests that if adult survival maintains at 72% (i.e. the average estimated over the sampling period), the estimated average annual population size for the forecast period (2020-2029) would be 59.9 ± 7.7, with an average of 36.6 ± 4.7 adults and 21.9 ± 2.6 pups (Table 5-3 and Fig. 5-). By 2029, the extinction probability (Ntot = 0) of wolves in Alto Minho would be 0.1%, and the quasi-extinction probability (Ntot = 5) 1.2% (Table 5-3 and Fig. 5-4). Considering the PVA with different survival rate scenarios, if the adult survival rate decreases by 10%, the extinction and quasi-extinction probabilities increase to 1% and 7%, respectively. If the adult survival rate decreases by 20%, these probabilities increase to 6% and 23%, respectively (Table 5-3 and Fig. 5-4; see additional details in Sup. Fig. 13 and Sup. Fig. 14 in Appendix V-C). Varying pup survival rate for the forecast period had little effect on the population size, growth rate, number of adults and pups, and, consequently, on the extinction probabilities (Table 5-3 and Sup. Fig. 15 in Appendix V-C). Although both adult and pup survival affect population projections, growth rates are more strongly affected by adult survival variations than pup survival. However, this result should be interpreted with caution since pup survival was not estimated within the CR model due to a lack of data.

Table 5-3: Average annual estimates (mean ± SD; range) of population size, growth rate, and number of adults and pups for the forecast period (2020-2029) by maintaining (Control model) and increasing (+20%, +10%) or decreasing (-20%, -10%) adult survival (Sad) and pup survival (Spup) rates. Probability of extinction (population size = 0) and quasi-extinction (with a threshold of population size of 5 wolves) by 2029.

Model	Sad (%)		Spup (%)	Population size	Growth rate	N adult	N pup	Extinction (%)	Quasi - extinction (%)
	Real	Apparent							
+20% Sad	86	75	54	138.6 ± 70.81 (56-268)	1.18 ± 0.01 (1.17-1.21)	84.1 ± 42.57 (35-162)	51.4 ± 26.05 (21-99)	0	0.02
+10% Sad	79	69	54	91 ± 29.9 (53-142)	1.11 ± 0.01 (1.1-1.13)	55.4 ± 17.9 (33-86)	33.7 ± 11.13 (20-53)	0.01	0.14
+20% Spup	72	63	65	61 ± 8.42 (49-74)	1.04 ± 0 (1.03-1.04)	39 ± 5.08 (32-47)	20.6 ± 2.88 (17-25)	0.1	1.03
+10% Spup	72	63	60	60.4 ± 8.09 (49-73)	1.04 ± 0 (1.03-1.05)	37.9 ± 5.07 (31-46)	21.1 ± 2.77 (17-26)	0.13	1.09
Control	72	63	54	59.9 ± 7.67 (49-72)	1.04 ± 0 (1.03-1.05)	36.6 ± 4.67 (30-44)	21.9 ± 2.6 (18-26)	0.13	1.16
-10% Spup	72	63	49	59.2 ± 7.36 (49-71)	1.03 ± 0.01 (1.03-1.05)	34.8 ± 4.16 (29-41)	22.8 ± 2.66 (19-27)	0.15	1.27
-10% Spup	72	63	43	58.2 ± 6.36 (49-68)	1.03 ± 0.01 (1.03-1.06)	33.1 ± 3.67 (28-39)	23.8 ± 2.66 (20-28)	0.2	1.39
-10% Sad	65	56	54	39.7 ± 3.59 (34-45)	0.96 ± 0 (0.96-0.97)	24.2 ± 2.3 (21-28)	14.5 ± 1.58 (12-17)	1.1	6.68
-20% Sad	57	50	54	26.6 ± 9.03 (15-42)	0.89 ± 0 (0.88-0.89)	16.2 ± 5.57 (9-26)	9.7 ± 3.33 (5-15)	6.01	22.75



A



B

Fig. 5-3: Annual population size (A) and number of adults and pups (B) for the sampling (2007-2019) and forecast (2020-2029) period, based on the developed integrated population model. Predictions for the forecast period obtained without (control) and with variation (-10%, -20%, +10% and +20%) in adult survival rates (Sad); shadow areas represent 95% Bayesian credible intervals.

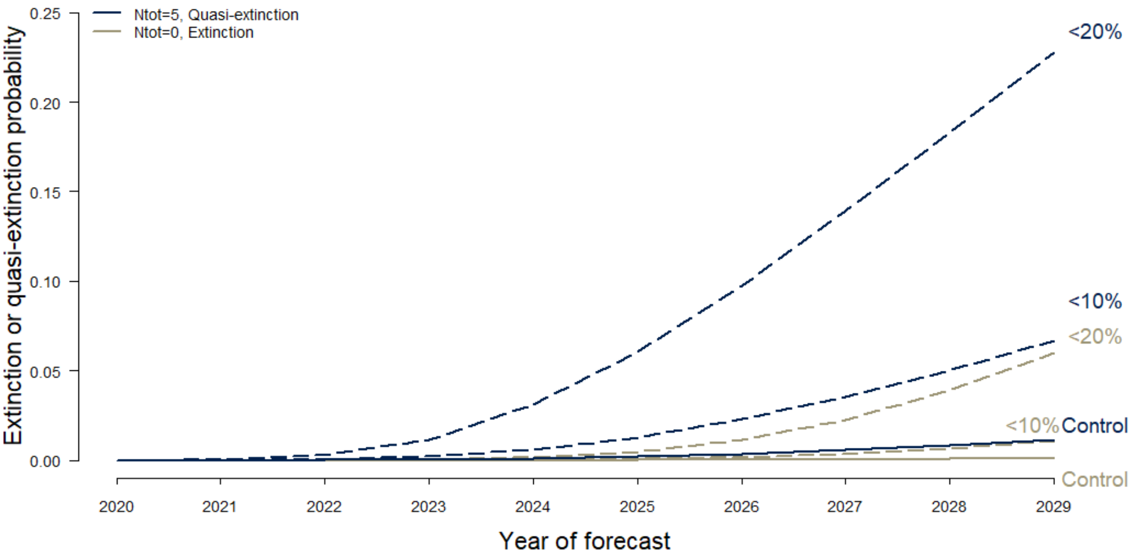


Fig. 5-4: Cumulative probability of extinction (Ntot= 0 individuals, blue lines) and of quasi-extinction (threshold of Ntot = 5 individuals, grey lines) for wolves in Alto Minho within 10 years for the forecast period (2020-2029). Probabilities obtained for the control (adult survival rate of 72%; solid lines) and for the model with a decrease of adult survival by 20% and 10% (dashed lines).

5.5. Discussion

Large carnivore population estimates, such as wolves, are usually obtained by population size estimates (e.g., using non-invasive genetics and spatial CR approaches; López-Bao et al., 2018; Marucco et al., 2023) or by monitoring a fraction of the population (e.g., family groups in brown bears or Eurasian lynx, Knight et al., 2019; Vogt et al., 2014; breeding packs in wolves, Prieto et al., 2022), applying conversion factors (Chapron et al., 2016), and extrapolating to broader areas (Blanco et al., 1992; Okarma, 1989). The application of IPM to assess wolf population trends and to make forecasts for the near future has been limited until recent times (Horne et al., 2019; Milleret, 2016; Paquet et al., 2021; Sells et al., 2022b, 2020). We estimated population size, growth rate, number of adults and pups, and survival and emigration rates, the latter mostly unknown parameters for wolves in Iberia. Here, we developed an IPM to assess the persistence of wolves in a highly human-dominated landscape in the westernmost limit of the wolf distribution in Europe, increasing the precision of population estimates and trends and estimating previously unknown demographic parameters. The population viability analysis showed the importance of adult survival for wolf persistence. Accordingly, we predict that the decrease of adult survival by 10%

until 2029 will result in a negative trend. Remarkably, estimates suggest that by decreasing adult survival by 20%, the population would decrease to only five individuals with a likelihood of 23%. These estimates and predictions are highly relevant for wolf conservation and management since they show that adult survival is crucial in sustaining wolves in human-dominated landscapes. Considering that conflicts with people often arise and result in the illegal killing of wolves to an unknown extent (e.g., Liberg et al., 2012; Musto et al., 2021; Santiago-Ávila et al., 2020; Suutarinen and Kojola, 2018), efforts are needed to decrease anthropogenic mortality, particularly illegal killing, to maintain or increase the adult survival rate.

Between 2007 and 2016, this population is reported to have been recovering and recolonizing previously occupied areas, with two to six packs detected annually (Nakamura et al., 2021). According to our results, the population kept expanding, with two additional packs detected since 2016. The growth rate based on field data (1.17) is similar to previous estimations (1.16; Nakamura et al., 2021). However, based on the IPM, we estimated a lower and more precise overall growth rate (1.06 ± 0.15). Despite some interannual fluctuations in population size, our findings suggest an overall positive trend in the population size between 2007 and 2019, confirming our hypothesis (H1). However, the results of the IPM also revealed some fluctuations in population size over time, though these seem to be less accentuated than previously thought (Nakamura et al., 2021). These fluctuations may be related to the annual variations observed in adult survival rates and the number of packs reproducing and pups. The annual variations in the number of pups were more accentuated than in the number of adults, which may suggest a demographic compensation mechanism when reproduction is less successful, such as lower dispersal rates of adults due to low social or intraspecific competition (Ballard et al., 1987; Sells et al., 2022a).

The estimated apparent survival rate of 63% was lower than in studies conducted in France (84%, Marescot et al., 2012) and Italy (66-75%, Caniglia et al., 2012). Our estimated apparent adult survival could be underestimated since some of the first captures in the CR model may include pups, which generally have a lower survival rate than adults (Marucco et al., 2009). Since we could not distinguish the age of individuals detected by non-invasive samples, it was not possible to proceed with a CR model with individual heterogeneity for age. Such a differentiation could distinguish part of the highly or weakly detectable individuals (Cubaynes et al., 2010). However, by including a prior for pup survival rate, we could estimate an average pup survival for this population (53%). Similarly to other studies (Marucco et al., 2009), we confirmed our expectations that adult survival is higher than pup survival (H2). Previous studies show that wolf populations decline when the mortality rate is above 32% or 35% (Chapron et al., 2003; Fuller, 1989; Marescot et al., 2012). Thus, according to the estimates of the adult mortality rate (28%; 95% BCI 23-34%) and the positive growth trend (1.04); 95% BCI 1.03-1.05), our estimated apparent adult mortality rate (38%; 95% BCI 28-48%) may be overestimated. Additionally, considering the wide credible intervals, the actual value of the apparent survival rate could be below the 32-35% threshold mentioned. Although we obtained robust survival estimates, methodological constraints, species' elusiveness or cryptic poaching may hinder the obtention of actual survival estimates.

The population projection based on the control model, although at a low rate, still shows a positive growth rate. However, in a scenario where the adult survival rate decreases by 10% (65%) between 2020 and 2029, the population declines at a rate of 0.96. In a more extreme scenario of a 20% decrease in adult survival, the growth rate reaches 0.89, with a risk of 23% probability that the population will decline to only five individuals by 2029. Despite the low quasi-extinction probabilities in such scenarios, the slight decrease in adult survival can significantly impact this population in a short time (H4). In fact, although wolves in Alto Minho have been increasing since 2007, the number of individuals suffered a steep decline between 1996 and 2007, possibly related to high levels of wolf persecution and decreased habitat connectivity (Nakamura et al., 2021). Our study shows the relevance of maintaining a high adult survival rate to ensure the persistence of wolves in Alto Minho since such a decline could occur until 2029, even with a slight increase in adult mortality.

The variation in survival rates between 2007 and 2019 likely resulted from natural or anthropogenic causes of wolf mortalities. A natural cause could be the prevalence of an infectious and contagious disease caused by *Sarcoptes scabiei* mite (Bornstein et al., 2001). Though it has been suggested that this disease has a limited demographic effect on wolf populations (Oleaga et al., 2011), wolf mortality from sarcoptic mange has been occasionally recorded in the Iberian Peninsula (Domínguez et al., 2008). In Alto Minho, several wolves were observed with skin lesions compatible with sarcoptic mange and peaks in the seroprevalence of this disease were detected in some years (Rousseau et al., 2021b, 2021a). Furthermore, concomitance and interactions between Canine Distemper Virus (Müller et al., 2011) and mite (Oleaga et al., 2015) may make individuals more susceptible. To some extent, these diseases could help explain the variations in wolf survival obtained in our study. Nevertheless, conflicts with people often occur due to the high frequency of livestock depredations by wolves (Pimenta et al., 2018), which suggests that a high proportion of wolf mortalities may be related to anthropogenic causes (Campos, 2018). To understand better the wolf mortality causes and to enhance the development of studies for wolf conservation, the Institute for Nature Conservation and Forests (ICNF) in Portugal has implemented the 'Monitoring System of Dead Wolves' since 1999, in which all wolf carcasses are registered in a national database, and samples are collected (Barroso and Pimenta, 2008). However, most dead wolves are opportunistically detected and do not reflect the actual rate of mortality causes, resulting in biased mortality causes toward more easily detectable deaths (e.g., road kills 35%), with lower detections of poaching (shot 20%, snared 12%, or poisoned, 3%) or natural causes (e.g., infectious diseases 6%)(Álvares et al., 2015). Nevertheless, poaching caused the deaths of 47% of GPS-collared wolves tracked in the study area during 2007-2017 (Rio-Maior et al., 2018). Thus, the prevalence of poaching may be the main limiting factor of wolves in this area and other regions of the range of the northwestern Iberian wolf population.

We estimated that only 13% of the wolves emigrate from the study area. Such remarkably high estimated fidelity was expected considering the cryptic genetic structuration of the northwestern Iberian wolf population (Silva et al., 2018). Previously, it has been estimated a very low proportion of dispersal individuals among genetic clusters in this population (4%; Silva et al., 2018) and even among packs in the same area (11%; Nakamura et al., 2021). The low emigration rate can be related to a high anthropogenic mortality rate (Campos, 2018), which can decrease pack sizes and resource competition in established populations (Jimenez et al., 2017; Treves et al., 2017; Webb et al., 2011). Additionally, since food is highly available and is not a limiting factor in the study area, the competition is lower and possibly delays the dispersal (Ballard et al., 1987; Sells et al., 2022a). Additionally, the mortality of pack members can increase the recruitment of new members into these vacant positions, reducing the need for inbreeding avoidance and dispersal since breeding opportunities arise within the pack (David E Ausband et al., 2017; Packard, 2003). The social dynamics of packs can be affected differently depending on the status of the individual lost. Breeder loss can lead to reproductive failure or pack instability that can result in the dissolvement of a pack, increasing the probability of dispersal of individuals (Borg et al., 2015; Brainerd et al., 2008; Cassidy et al., 2023; Mech, 2010). However, the low emigration rate in this population suggests a high mortality rate of young members since these are more likely to disperse (Gese and Mech, 1991).

The average litter size obtained by direct observations (3.9) was very similar to estimations in the Duero plateau and Montes de Léon (4.0, Fernández-Gil et al., 2020), but much lower than in the Cantabrian Mountains (5.15, Llaneza et al., 2023).

However, the number of pups detected in packs can be highly variable depending on the effort or difficulty of observation among packs (e.g., Barrientos, 2000). For instance, Barrientos (2000) observed 4.7 pups per pack, and Fernández-Gil (2020) observed 4.0 pups per pack in the same study area. Further studies are needed to assess differences in fecundity and pup survival within the wolf population across Iberia; although we can not rule out the influence of different methodological approaches on these figures (e.g., Palacios et al., 2017), as shown in our study (3.9 and 3.4 pups detected by direct observation and all field methods, respectively). Fecundity could vary with the age of the breeding females (Ferreras-Colino et al., 2021; Stahler et al., 2013) and food availability (Boertje and Stephenson, 1992). However, the high livestock densities suggest that food is not a limiting factor since it is the main component of the wolf diet in Alto Minho (Álvares et al., 2015). Our estimates of fecundity (3.9) could also be influenced by low pup survival in early months, which can be affected, for example, by diseases, the turnover of breeders (David E. Ausband et al., 2017; Brainerd et al., 2008; Johnson et al., 1994; Stahler et al., 2013), or inbreeding depression (Liberg et al., 2005). Indeed, the estimated average fecundity of female breeders in Iberia based on foetus and placental scar counts (5.5-6.0; Ferreras-Colino et al., 2021) was higher than the estimated fecundity in our study. Additionally, wolves in Alto Minho have shown a lower genetic diversity and higher relatedness than wolves in other areas of Iberia (Silva et al., 2018) and a frequent turnover of breeders (Pacheco et al., 2021), which could be affecting the reproductive success of packs. Lastly, in areas where people tolerate less wolf presence, pups might be more susceptible to being killed or poached than adults, particularly when homesites are located in more human-accessible areas with higher human disturbance (Sazatornil et al., 2016). Further studies are needed to study pup survival by tagging pups at densites, fitting pups with collars (e.g., Ballard et al., 1987; Fritts et al., 1981), or performing genealogy analysis from non-invasive samples to identify pups. However, the latter method requires a very intense sampling strategy, with most individuals genetically identified as in the Scandinavian wolf population (Liberg et al., 2005).

In 2018, the compensation program in Portugal suffered some changes, which resulted in a reduction in the number of attacks declared, according to Decree-Law nº. 54/2016 (ICNF). Before 2018, the government fully compensated wolf damages on livestock when livestock protection measures were implemented (namely, fences or livestock guarding). However, in 2018, the new compensation program limited compensation to 15 annual attacks per livestock owner and payment of animal value at 100% only for the first three declared animals (4th-7th attacks: 90%, 8th-11th attacks: 70%, and 12th-

15th attacks: 50%; according to the Portuguese legislation Decree-Law nº. 54/2016, published in Diário da República n.º 213/2017, Série I 2017-11-06). This change reduced the number of declared attacks because chronically affected farmers suffer more than 15 attacks/year that stopped being declared. In addition, the change in the submission system may also have contributed to the reduction in the number of officially declared attacks. Previously, attacks were declared through a call to the closest Protected Area. Since 2018, it has changed to an online form, which is not accessible to all communities, particularly those with no experience using such technology. Moreover changes in bureaucratic procedures have delayed compensation attribution decisions and payments. These changes likely impacted the tolerance towards the presence of wolves and the retaliatory killing of wolves. A follow-up on the compensation scheme adopted in Portugal is urgently needed to assess the impact of the implemented politics on wolf conservation and adapt accordingly.

Wolves in Alto Minho are recovering and recolonizing, although wolves in other areas of the northwestern Iberian wolf population may be incapable of maintaining or increasing population size under the estimated mortality rates observed here, mainly due to poaching. After the population bottleneck of 1970s (Lobo et al., 2023; Nores and López-Bao, 2022), the northwestern Iberian wolf population expanded in Spain (Blanco and Cortés, 2009; Chapron et al., 2014; López-Bao et al., 2018; Nores and López-Bao, 2022), but showed a remarkable regression pattern in Portugal, particularly south of Duero River (Monteiro, 2015). In the late 1990s, the species reached south of Castilla y León, north of Castilla-La Mancha (Guadalajara province) and Madrid (Blanco and Cortés, 2009, 2001). Genetic analyses have revealed that such expansion towards central Spain resulted from the expansion of wolves from the south-eastern Cantabrian Mountains (Silva et al., 2018). However, currently, the population appears to have stagnated in eastern Castilla y León, the Basque Country and north of Castilla-La Mancha (Guadalajara province) (Blanco and Cortés, 2009; López-Bao et al., 2018), and in Portugal (Monteiro, 2015); which might be as a consequence of high levels of poaching (associated to livestock depredation). Studies on wolf demography in other areas of this population are needed in order to detect demographic variations in space, particularly in conflict-prone areas, where wolves highly prey on livestock, in the expansion borders, and where wolf populations appear to be more fragile and at risk, such as in the small and isolated wolf areas, such as South of Douro River.

5.6. Acknowledgements

We thank lab assistance for genetic data D Castro and S Lopes. We are grateful to field assistants, colleagues and volunteers, particularly P Sierra, M Palma, C Barros, S Roque, V Palacios, R Campos, H Montenegro, J Pereira, P Alarcão, A Moedas. We thank the veterinaries N Santos, R Brandão, N Fandos, and C Ferreira for assisting wolf trapping and capture for GPS-telemetry. We are grateful to Instituto de Conservação da Natureza e Florestas (ICNF) and Serviço de Protecção da Natureza e do Ambiente – Guarda Nacional Republicana (SEPNA-GNR) for providing legal support for wolf monitoring in Portugal. Wolf trapping was conducted under permits from the national authority for Nature Conservation (ICNF).

5.7. Funding

MN worked under a PhD grant [SFRH/BD/144087/2019] and RG under research contracts [IF/564/2012 and DL57/2016] from Fundação para a Ciência e a Tecnologia. Field and lab work for wolf monitoring was supported by ACHLI (Associação de Conservação do Habitat do Lobo Ibérico) and Empreendimentos Eólicos do Vale do Minho S.A.. OG and MN were partly funded by the French National Research Agency [grant ANR-16-CE02-0007]. PM was supported by UID/BIA/50027/2021, with funding from FCT/MCTES through national funds. JVLB was supported by the Spanish Ministry of Economy, Industry and Competitiveness [CGL2017-87528-R AEI/FEDER EU], the Spanish Ministry of Science and Innovation [TED2021-132519B-I00], and by a GRUPIN research grant from the Regional Government of Asturias [AYUD/2021/51314].

5.8. Author contributions CRediT

Mónia Nakamura: Conceptualization, Data Curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing original draft, Writing review & editing. **Pedro Monterroso**: Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing review & editing, Supervision. **Helena Rio-Maior**: Conceptualization, Data Curation, Funding acquisition, Investigation, Methodology, Writing review & editing, Project administration, Supervision. **Raquel Godinho:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing review & editing. **Francisco Álvares**: Funding acquisition, Methodology, Writing review

& editing, Project administration, Supervision. **José Vicente López-Bao**: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Writing review & editing, Supervision. **Olivier Gimenez:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing review & editing, Supervision.

5.9. References

Abadi, F., Barbraud, C., Gimenez, O., 2017. Integrated population modeling reveals the impact of climate on the survival of juvenile emperor penguins. Glob. Chang. Biol. 23, 1353–1359. https://doi.org/10.1111/gcb.13538

Adams, J.R., Vucetich, L.M., Hedrick, P.W., Peterson, R.O., Vucetich, J.A., 2011. Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. Proc. R. Soc. B Biol. Sci. 278, 3336–3344. https://doi.org/10.1098/rspb.2011.0261

Álvares, F., Barroso, I., Costa, G.F. da, Espírito-Santo, C., Fonseca, C., Godinho, R., Nakamura, M., Petrucci-Fonseca, F., Pimenta, V., Rio-Maior, H., Santos, N., Torres, R.T., 2015. Situação de referência para o Plano de Ação para a Conservação do Lobo-ibérico em Portugal. Lisbon, Portugal.

APA, 2014. Sistema Nacional de Informação de Recursos Hídricos. Agência Portuguesa do Ambiente.

Arnold, T.W., Clark, R.G., Koons, D.N., Schaub, M., 2018. Integrated population models facilitate ecological understanding and improved management decisions. J. Wildl. Manage. 82, 266–274. https://doi.org/10.1002/jwmg.21404

Ausband, D.E., 2018. Multiple breeding individuals within groups in a social carnivore. J. Mammal. 99, 836–844. https://doi.org/10.1093/jmammal/gyy051

Ausband, David E., Mitchell, M.S., Stansbury, C.R., Stenglein, J.L., Waits, L.P., 2017. Harvest and group effects on pup survival in a cooperative breeder. Proc. R. Soc. B Biol. Sci. 284, 20170580. https://doi.org/10.1098/rspb.2017.0580

Ausband, David E, Mitchell, M.S., Waits, L.P., 2017. Effects of breeder turnover and harvest on group composition and recruitment in a social carnivore. J. Anim. Ecol., 86, 1094–1101. https://doi.org/10.1111/1365-2656.12707

Ausband, D.E., Stansbury, C.R., Stenglein, J.L., Struthers, J.L., Waits, L.P., 2015. Recruitment in a social carnivore before and after harvest. Anim. Conserv., 18, 415–423. https://doi.org/10.1111/acv.12187

Ballard, W.B., Whitman, J.S., Gardner, L, C., 1987. Ecology of an exploited wolf population in south-central Alaska. Wildl. Monogr. 98, 3–54.

Barrientos, L.M., 2000. Tamaño y composición de diferentes grupos de lobos en castilla y león. Galemys 12, 249–256.

Barroso, I., Pimenta, V., 2008. Sistema de Monitorização de Lobos Mortos: Relatório de Actividades 1999-2008.

Bassi, E., Gazzola, A., Bongi, P., Scandura, M., Apollonio, M., 2020. Relative impact of human harvest and wolf predation on two ungulate species in Central Italy. Ecol. Res. 35, 662–674. https://doi.org/10.1111/1440-1703.12130

Bassing, S.B., Ausband, D.E., Mitchell, M.S., Schwartz, M.K., Nowak, J.J., Hale, G.C., Waits, L.P., 2020. Immigration does not offset harvest mortality in groups of a cooperatively breeding carnivore. Anim. Conserv. 23, 750–761. https://doi.org/10.1111/acv.12593

Bautista, C., Naves, J., Revilla, E., Fernández, N., Albrecht, J., Scharf, A.K., Rigg, R., Karamanlidis, A.A., Jerina, K., Huber, D., Palazón, S., Kont, R., Ciucci, P., Groff, C., Dutsov, A., Seijas, J., Quenette, P.I., Olszańska, A., Shkvyria, M., Adamec, M., Ozolins, J., Jonozovič, M., Selva, N., 2017. Patterns and correlates of claims for brown bear damage on a continental scale. J. Appl. Ecol. 54, 282–292. https://doi.org/10.1111/1365-2664.12708

Besbeas, A.P., Freeman, S.N., Morgan, B.J.T., Catchpole, E.A., Besbeas, P., 2010. Integrating Mark-Recapture-Recovery and Census Data to Estimate Animal Abundance and Demographic Parameters. Society 58, 540–547. https://doi.org/10.1111/j.0006-341X.2002.00540.x

Bischof, R., Milleret, C., Dupont, P., Chipperfield, J., Tourani, M., Ordiz, A., de Valpine, P., Turek, D., Royle, J.A., Gimenez, O., Flagstad, Ø., Åkesson, M., Svensson, L., Brøseth, H., Kindberg, J., 2020. Estimating and forecasting spatial population dynamics of apex predators using transnational genetic monitoring. Proc. Natl. Acad. Sci. 117, 30531–30538. https://doi.org/10.1073/pnas.2011383117

Blanco, J.C., Cortés, Y., 2009. Ecological and social constraints of wolf recovery in Spain, in: Musiani, M., Boitani, L., Paquet, P.C. (Eds.), A New Era for Wolves and People: Wolf Recovery, Human Attitudes, and Policy. University of Calgary Press, Calgary, pp. 41–66.

Blanco, J.C., Cortés, Y., 2007. Dispersal patterns, social structure and mortality of wolves living in agricultural habitats in Spain. J. Zool. 273, 114–124. https://doi.org/10.1111/j.1469-7998.2007.00305.x

Blanco, J.C., Cortés, Y., 2001. Ecología, Censos, Percepción y Evolución del Lobo en España: Análisis de un Conflicto.

Blanco, J.C., Reig, S., De La Cuesta, L., 1992. Distribution, status and conservation problems of the wolf *Canis lupus* in Spain. Biol. Conserv. 60, 73–80. https://doi.org/10.1016/0006-3207(92)91157-N

Bled, F., Belant, J.L., Van Daele, L.J., Svoboda, N., Gustine, D., Hilderbrand, G., Barnes, V.G., 2017. Using multiple data types and integrated population models to improve our knowledge of apex predator population dynamics. Ecol. Evol. 7, 9531–9543. https://doi.org/10.1002/ece3.3469

Boertje, R.D., Stephenson, R.O., 1992. Effects of ungulate availability on wolf reproductive potential in Alaska. Can. J. Zool. 70, 2441–2443. https://doi.org/10.1139/z92-328

Bombieri, G., Delgado, M. del M., Russo, L.F., Garrote, P.J., López-Bao, J.V., Fedriani, J.M., Penteriani, V., 2018. Patterns of wild carnivore attacks on humans in urban areas. Sci. Rep. 8, 1–9. https://doi.org/10.1038/s41598-018-36034-7

Borg, B.L., Brainerd, S.M., Meier, T.J., Prugh, L.R., 2015. Impacts of breeder loss on social structure, reproduction and population growth in a social canid. J. Anim. Ecol. 84, 177–187. https://doi.org/10.1111/1365-2656.12256

Bornstein, S., Mörner, T., Samuel, W.M., 2001. Sarcoptes scabiei and Sarcoptic Mange, in: Parasitic Diseases of Wild Mammals. Wiley, pp. 107–119. https://doi.org/10.1002/9780470377000.ch5

Brainerd, S.M., Andrén, H., Bangs, E.E., Bradley, E.H., Fontaine, J.A., Hall, W., Iliopoulos, Y., Jimenez, M.D., Jozwiak, E.A., Liberg, O., others, 2008. The effects of breeder loss on wolves. J. Wildl. Manage. 72, 89–98. https://doi.org/10.2193/2006-305

Campos, R., 2018. Survival and Population Dynamics of the Iberian Wolf in Portugal.

MSc Thesis. Universidade do Porto.

Caniglia, R., Fabbri, E., Cubaynes, S., Gimenez, O., Lebreton, J.-D.D., Randi, E., 2012. An improved procedure to estimate wolf abundance using non-invasive genetic sampling and capture–recapture mixture models. Conserv. Genet. 13, 53–64. https://doi.org/10.1007/s10592-011-0266-1

Carter, N.H., Linnell, J.D.C., 2016. Co-Adaptation Is Key to Coexisting with Large Carnivores. Trends Ecol. Evol. 31, 575–578. https://doi.org/10.1016/j.tree.2016.05.006

Casimiro, J., Serronha, A., Rio-Maior, H., Nakamura, M., Godinho, R., Álvares, F., 2021. Investigação Aplicada à Conservação do Lobo no Noroeste de Portugal: Plano de Monitorização do lobo no âmbito do Sobreequipamento dos Parques Eólicos do Alto Minho I e de Arga (abril 2020-junho 2021 & análise global). Relatório Técnico.

Cassidy, K.A., Borg, B.L., Klauder, K.J., Sorum, M.S., Thomas-Kuzilik, R., Dewey, S.R., Stephenson, J.A., Stahler, D.R., Gable, T.D., Bump, J.K., Homkes, A.T., Windels, S.K., Smith, D.W., 2023. Human-caused mortality triggers pack instability in gray wolves. Front. Ecol. Environ. https://doi.org/10.1002/fee.2597

Chapron, G., Kaczensky, P., Linnell, J.D.C., von Arx, M., Huber, D., Andren, H., López-Bao, J.V., Adamec, M., Álvares, F., Anders, O., Balciauskas, L., Balys, V., Bed, P., Bego, F., Blanco, J.C., Breitenmoser, U., Broseth, H., Bufka, L., Bunikyte, R., Ciucci, P., Dutsov, A., Engleder, T., Fuxjager, C., Groff, C., Holmala, K., Hoxha, B., Iliopoulos, Y., Ionescu, O., Jeremi, J., Jerina, K., Kluth, G., Knauer, F., Kojola, I., Kos, I., Krofel, M., Kubala, J., Kunovac, S., Kusak, J., Kutal, M., Liberg, O., Maji, A., Mannil, P., Manz, R., Marboutin, E., Marucco, F., Melovski, D., Mersini, K., Mertzanis, Y., Mysłajek, R.W., Nowak, S., Odden, J., Ozolins, J., Palomero, G., Paunovi, M., Persson, J., Poto nik, H., Quenette, P.-Y., Rauer, G., Reinhardt, I., Rigg, R., Ryser, A., Salvatori, V., Skrbin ek, T., Stojanov, A., Swenson, J.E., Szemethy, L., Trajce, A., Tsingarska-Sedefcheva, E., Va a, M., Veeroja, R., Wabakken, P., Wolfl, M., Wolfl, S., Zimmermann, F., Zlatanova, D., Boitani, L., 2014. Recovery of large carnivores in Europe's modern human-dominated landscapes. Science (80-.). 346, 1517–1519. https://doi.org/10.1126/science.1257553

Chapron, G., Legendre, S., Ferrière, R., Clobert, J., Haight, R.G., 2003. Conservation and control strategies for the wolf (*Canis lupus*) in western Europe based on demographic models. C. R. Biol. 326, 575–587. https://doi.org/10.1016/S1631-0691(03)00148-3

Chapron, G., Wikenros, C., Liberg, O., Wabakken, P., Flagstad, Ø., Milleret, C., Månsson, J., Svensson, L., Zimmermann, B., Åkesson, M., Sand, H., 2016. Estimating wolf (*Canis lupus*) population size from number of packs and an individual based model. Ecol. Modell. 339, 33–44. https://doi.org/10.1016/j.ecolmodel.2016.08.012

Cooley, H.S., Wielgus, R.B., Koehler, G.M., Robinson, H.S., Maletzke, B.T., 2009. Does hunting regulate cougar populations? A test of the compensatory mortality hypothesis. Ecology 90, 2913–2921. https://doi.org/10.1890/08-1805.1

Cubaynes, S., Pradel, R., Choquet, R., Duchamp, C., Gaillard, J.-M., Lebreton, J.-D., Marboutin, E., Miquel, C., Reboulet, A.-M., Poillot, C., Taberlet, P., Gimenez, O., 2010. Importance of accounting for detection heterogeneity when estimating abundance: the case of French wolves. Conserv. Biol., 24, 621–626. https://doi.org/10.1111/j.1523-1739.2009.01431.x

Dennehy, E., Llaneza, L., López-Bao, J.V., 2021. Contrasting wolf responses to different paved roads and traffic volume levels. Biodivers. Conserv. 30, 3133–3150. https://doi.org/10.1007/s10531-021-02239-y

Domínguez, G., Espí, A., Prieto, J.M., Torre, J.A. De, 2008. Sarcoptic mange in Iberian wolves (*Canis lupus signatus*) in northern Spain. Vet. Rec.

Fernández-Gil, A., Quevedo, M., Barrientos, L.M., Nuño, A., Naves, J., de Gabriel, M., Ordiz, A., Revilla, E., 2020. Pack size in humanized landscapes: the Iberian wolf population. Wildlife Biol., 2020. https://doi.org/10.2981/wlb.00594

Ferreras-Colino, E., García-Garrigós, A., Gortázar, C., Llaneza, L., 2021. Wolf (*Canis lupus*) litter size in Spain. Eur. J. Wildl. Res. 67, 31. https://doi.org/10.1007/s10344-021-01473-7

Fritts, S.H., Mech, L.D., Roberts, K., 1981. Dynamics, movements and feeding ecology of a newly protected wolf population in northwestern Minnesota. Wildl. Monogr. 80, 3–79.

Fuller, T.K., 1989. Population dynamics of wolves in north-central Minnesota. Wildl. Monogr. 105, 1–41.

Gantchoff, M.G., Hill, J.E., Kellner, K.F., Fowler, N.L., Petroelje, T.R., Conlee, L., Beyer, D.E., Belant, J.L., 2020. Mortality of a large wide-ranging mammal largely caused by anthropogenic activities. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-65290-9

Gelman, A., Carlin, J.B., Stern, H.S., Rubin, D.B., 2004. Bayesian data analysis. Boca Raton FL: Chapman and Hall.

Gese, E.M., Mech, L.D., 1991. Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969–1989. Can. J. Zool. 69, 2946–2955. https://doi.org/10.1139/z91-415

Gimenez, O., Lebreton, J., Choquet, R., Pradel, R., 2018. R2ucare: An <scp>r</scp> package to perform goodness-of-fit tests for capture–recapture models. Methods Ecol. Evol. 9, 1749–1754. https://doi.org/10.1111/2041-210X.13014

Graham, K., Beckerman, A.P., Thirgood, S., 2005. Human–predator–prey conflicts: ecological correlates, prey losses and patterns of management. Biol. Conserv. 122, 159–171. https://doi.org/10.1016/j.biocon.2004.06.006

Harrington, F.H., Mech, L.D., 1982. An analysis of howling response parameters useful for wolf pack censusing. J. Wildl. Manage. 46, 686–693. https://doi.org/10.2307/3808560

Hebblewhite, M., Whittington, J., 2020. Wolves without borders: Transboundary survival of wolves in Banff National Park over three decades. Glob. Ecol. Conserv., 24, e01293. https://doi.org/10.1016/j.gecco.2020.e01293

Heppell, S.S., Caswell, H., Crowder, L.B., 2000. Life histories and elasticity patterns: Perturbation analysis for species with minimal demographic data. Ecology 81, 654–665. https://doi.org/10.1890/0012-9658(2000)081[0654:LHAEPP]2.0.CO;2

Heurich, M., Schultze-Naumburg, J., Piacenza, N., Magg, N., Červený, J., Engleder, T., Herdtfelder, M., Sladova, M., Kramer-Schadt, S., 2018. Illegal hunting as a major driver of the source-sink dynamics of a reintroduced lynx population in Central Europe. Biol. Conserv. 224, 355–365. https://doi.org/10.1016/j.biocon.2018.05.011

Horne, J.S., Ausband, D.E., Hurley, M.A., Struthers, J., Berg, J.E., Groth, K., 2019. Integrated population model to improve knowledge and management of Idaho wolves. J. Wildl. Manage. 83, 32–42. https://doi.org/10.1002/jwmg.21554

INE, 2014. Instituto Nacional de Estatística - Statistics Portugal - http://www.ine.pt/. Instituto Nacional de Estatística – Statistics Portugal.

IPMA, 2014. Normais climatológicas 1981-2010. Instituto Português do Mar e da Atmosfera http://www.ipma.pt/pt/oclima/normais.clima/.

Jimenez, M.D., Bangs, E.E., Boyd, D.K., Smith, D.W., Becker, S.A., Ausband, D.E., Woodruff, S.P., Bradley, E.H., Holyan, J., Laudon, K., 2017. Wolf dispersal in the Rocky Mountains, Western United States: 1993–2008. J. Wildl. Manage. 81, 581–592. https://doi.org/10.1002/jwmg.21238

Johnson, M.R., Boyd, D.K., Pletscher, D.H., 1994. Serologic Investigations of Canine Parvovirus and Canine Distemper in Relation to Wolf (*Canis lupus*) Pup Mortalities. J. Wildl. Dis. 30, 270–273. https://doi.org/10.7589/0090-3558-30.2.270

Kéry, M., Schaub, M., 2011. Bayesian population analysis using WinBUGS – a hierarchical perspective. Academic Press.

Knight, R.R., Blanchard, B.M., Eberhardt, L.L., Knight, R.R., Blanchard, B.M., Eberhardt, L.L., 2019. Appraising status of the Yellowstone grizzly bear population by counting females with cubs-of-the-year 23, 245–248. https://doi.org/http://www.jstor.org/stable/3782798

Kochetkov, V. V., 2015. Philopatry and dispersal in the wolf population (*Canis lupus* L.). Contemp. Probl. Ecol., 22, 388–397. https://doi.org/10.15372/sej20150306

Lamb, C.T., Ford, A.T., McLellan, B.N., Proctor, M.F., Mowat, G., Ciarniello, L., Nielsen, S.E., Boutin, S., 2020. The ecology of human–carnivore coexistence. Proc. Natl. Acad. Sci. U. S. A. 117, 17876–17883. https://doi.org/10.1073/pnas.1922097117

Lamb, C.T., Smit, L., Mowat, G., McLellan, B., Proctor, M., 2023. Unsecured attractants, collisions, and high mortality strain coexistence between grizzly bears and people in the Elk Valley, southeast British Columbia. Conserv. Sci. Pract. 5, 1–16. https://doi.org/10.1111/csp2.13012

Lebreton, J.D., Almeras, T., Pradel, R., 1999. Competing events, mixtures of information and multistratum recapture models. Bird Study 46, S39–S46. https://doi.org/10.1080/00063659909477230

Liberg, O., Andrén, H., Pedersen, H.-C., Sand, H., Sejberg, D., Wabakken, P., Åkesson, M., Bensch, S., 2005. Severe inbreeding depression in a wild wolf *Canis lupus* population. Biol. Lett. 1, 17–20. https://doi.org/10.1098/rsbl.2004.0266

Liberg, O., Chapron, G., Wabakken, P., Pedersen, H.C., Hobbs, N.T., Sand, H.H., Thompson Hobbs, N., Sand, H.H., 2012. Shoot, shovel and shut up: cryptic poaching slows restoration of a large carnivore in Europe. Proc. R. Soc. B-Biological Sci. 279, 910–915. https://doi.org/10.1098/rspb.2011.1275

Liberg, O., Suutarinen, J., Kesson, M., Andrn, H., Wabakken, P., Wikenros, C., Sand, H., Suutarinen J., kesson M., Andrn, H., Wabakken, P., Wikenros, C., Sand, H., Åkesson, M., Andrén, H., Wabakken, P., Wikenros, C., Sand, H., 2020. Poaching-related disappearance rate of wolves in Sweden was positively related to population size and negatively to legal culling. Biol. Conserv., 243, 108456. https://doi.org/10.1016/j.biocon.2020.108456

Linnell, J.D.C., Swenson, J.E., Andersen, R., 2001. Predators and people: conservation of large carnivores is possible at high human densities if management policy is favourable. Anim. Conserv. 4, 345–349. https://doi.org/10.1017/s1367943001001408

Lino, S., Sillero, N., Torres, J., Santos, X., Álvares, F., Alvares, F., 2019. The role of fire on wolf distribution and breeding-site selection: Insights from a generalist carnivore occurring in a fire-prone landscape. Landsc. Urban Plan. 183, 111–121. https://doi.org/10.1016/j.landurbplan.2018.12.003

Llaneza, L., García, E.J., López-Bao, J.V., 2014. Intensity of Territorial Marking Predicts Wolf Reproduction: Implications for Wolf Monitoring. PLoS One 9, e93015. https://doi.org/10.1371/journal.pone.0093015

Llaneza, L., Sazatornil, V., García, E.J., Palacios, V., López-Bao, J.V., 2023. Minimum average pack size in Iberian wolves. Eur. J. Wildl. Res. 69, 1–6. https://doi.org/10.1007/s10344-023-01695-x

Lobo, D., López-Bao, J.V., Godinho, R., 2023. The population bottleneck of the Iberian wolf impacted genetic diversity but not admixture with domestic dogs: A temporal genomic approach. Mol. Ecol. 32, 5986–5999. https://doi.org/10.1111/mec.17171

López-Bao, J.V., Aronsson, M., Linnell, J.D.C., Odden, J., Persson, J., Andrén, H., 2019. Eurasian lynx fitness shows little variation across Scandinavian human-dominated landscapes. Sci. Rep., 9, 8903. https://doi.org/10.1038/s41598-019-45569-2

López-Bao, J.V., Godinho, R., Pacheco, C., Lema, F.J., García, E., Llaneza, L., Palacios, V., Jiménez, J., 2018. Toward reliable population estimates of wolves by combining spatial capture-recapture models and non-invasive DNA monitoring. Sci. Rep. 8, 2177. https://doi.org/10.1038/s41598-018-20675-9

Lute, M.L., Carter, N.H., López-Bao, J.V., Linnell, J.D.C., 2018. Conservation professionals agree on challenges to coexisting with large carnivores but not on solutions. Biol. Conserv., 218, 223–232. https://doi.org/10.1016/j.biocon.2017.12.035

Marescot, L., Gimenez, O., Duchamp, C., Marboutin, E., Chapron, G., 2012. Reducing matrix population models with application to social animal species. Ecol. Modell. 232, 91–96. https://doi.org/10.1016/j.ecolmodel.2012.02.017

Marescot, L., Pradel, R., Duchamp, C., Cubaynes, S., Marboutin, E., Choquet, R., Miquel, C., Gimenez, O., 2011. Capture-recapture population growth rate as a robust tool against detection heterogeneity for population management. Ecol. Appl. 21, 2898–2907. https://doi.org/10.1890/10-2321.1

Marucco, F., Boiani, M. V., Dupont, P., Milleret, C., Avanzinelli, E., Pilgrim, K., Schwartz, A.M.K., von Hardenberg, A., Perrone, S., Friard, O., Menzano, A., Bisi, F., Fattori, U., Tomasella, M., Calderola, S., Carolfi, S., Ferrari, P., Chioso, C., Truc, F., Bombieri, G., Pedrotti, L., Righetti, D., Acutis, P.L., Guglielmo, F., Hauffe, H.C., Rossi, C., Caniglia, R., Aragno, P., La Morgia, V., Genovesi, P., Bischof, R., 2023. A multidisciplinary approach to estimating wolf population size for long-term conservation. Conserv. Biol. https://doi.org/10.1111/cobi.14132

Marucco, F., Pletscher, D.H., Boitani, L., Schwartz, M.K., Pilgrim, K.L., Lebreton, J.-D.D., 2009. Wolf survival and population trend using non-invasive capture-recapture techniques in the Western Alps. J. Appl. Ecol. 46, 1003–1010. https://doi.org/10.1111/j.1365-2664.2009.01696.x

Marucco, F., Vucetich, L.M., Peterson, R.O., Adams, J.R., Vucetich, J.A., 2012. Evaluating the efficacy of non-invasive genetic methods and estimating wolf survival during a ten-year period. Conserv. Genet. 13, 1611–1622. https://doi.org/10.1007/s10592-012-0412-4

Mech, L.D., 2010. Considerations for Developing Wolf Harvesting Regulations in the Contiguous United States. J. Wildl. Manage. 74, 1421–1424.

https://doi.org/10.2193/2009-540

Mech, L.D., Boitani, L., 2003. Wolves: Behavior, Ecology, and Conservation. The University of Chicago Press.

Milleret, C., 2016. Spatial ecology of wolves in Scandinavia: From spatio-temporal dynamics of wolf pairs to wolf population dynamics. PhD Thesis. Hedmark Univ. Appl. Sci.

Monteiro, R., 2015. Demographic history of the isolated and endangered wolf population in the south of the Douro river. MSc thesis. University of Porto, Portugal.

Müller, A., Silva, E., Santos, N., Thompson, G., 2011. Domestic Dog Origin of Canine Distemper Virus in Free-ranging Wolves in Portugal as Revealed by Hemagglutinin Gene Characterization. J. Wildl. Dis. 47, 725–729. https://doi.org/10.7589/0090-3558-47.3.725

Musto, C., Cerri, J., Galaverni, M., Caniglia, R., Fabbri, E., Mucci, N., Bonilauri, P., Maioli, G., Fontana, M.C., Prosperi, A., Rossi, A., Garbarino, C., Fiorentini, L., Ciuti, F., Berzi, D., Merialdi, G., Delogu, M., Apollonio, M., Mucci, N., Bonilauri, P., Maioli, G., Fontana, M.C., Gelmini, L., Prosperi, A., Rossi, A., Garbarino, C., Fiorentini, L., Ciuti, F., Berzi, D., Merialdi, G., Delogu, M., 2021. Men and wolves: Anthropogenic causes are an important driver of wolf mortality in human-dominated landscapes in Italy. Glob. Ecol. Conserv. 32, e01892. https://doi.org/10.1016/j.gecco.2021.e01892

Nakamura, M., Godinho, R., Rio-Maior, H., Roque, S., Kaliontzopoulou, A., Bernardo, J., Castro, D., Lopes, S., Petrucci-Fonseca, F., Álvares, F., 2017. Evaluating the predictive power of field variables for species and individual molecular identification on wolf noninvasive samples. Eur. J. Wildl. Res. 63, 53. https://doi.org/10.1007/s10344-017-1112-7

Nakamura, M., López-Bao, J.V., Rio-Maior, H., Roque, S., Gil, P., Serronha, A., García, E., Hernández Palacios, O., Ferrão da Costa, G., Álvares, F., Petrucci-Fonseca, F., Gimenez, O., Monterroso, P., 2023. Insights into the dynamics of wolf occupancy in human-dominated landscapes. Biol. Conserv. 286. https://doi.org/10.1016/j.biocon.2023.110316

Nakamura, M., Rio-Maior, H., Godinho, R., Petrucci-Fonseca, F., Álvares, F., 2021. Source-sink dynamics promote wolf persistence in human-modified landscapes: Insights from long-term monitoring. Biol. Conserv. 256, 109075. https://doi.org/10.1016/j.biocon.2021.109075

Nores, C., López-Bao, J.V., 2022. Historical data to inform the legal status of species in Europe: An example with wolves. Biol. Conserv. 272. https://doi.org/10.1016/j.biocon.2022.109639

Okarma, H., 1989. Distribution and numbers of wolves in Poland. Acta Theriol. (Warsz)., 34, 497–503.

Oleaga, A., Casais, R., Balseiro, A., Espi, A., Llaneza, L., Hartasanchez, A., Gortazar, C., Oleaga, Á., Casais, R., Balseiro, A., Espí, A., Llaneza, L., Hartasánchez, A., Gortázar, C., 2011. New techniques for an old disease: Sarcoptic mange in the Iberian wolf. Vet. Parasitol. 181, 255–266. https://doi.org/10.1016/j.vetpar.2011.04.036

Oleaga, A., Vicente, J., Ferroglio, E., Pegoraro de Macedo, M.R., Casais, R., del Cerro, A., Espí, A., García, E.J., Gortázar, C., 2015. Concomitance and interactions of pathogens in the Iberian wolf (*Canis lupus*). Res. Vet. Sci. 101, 22–27. https://doi.org/10.1016/j.rvsc.2015.05.010

Olson, E.R., Van Deelen, T.R., Wydeven, A.P., Ventura, S.J., Macfarland, D.M., 2015. Characterizing wolf-human conflicts in Wisconsin, USA. Wildl. Soc. Bull., 39, 676–688. https://doi.org/10.1002/wsb.606

Pacheco, C., Nakamura, M., Rio-Maior, H., Álvares, F., Godinho, R., 2021. Reproductive strategies of a social carnivore in a highly humanized landscape, in: Animal Behavior Society 2021 Virtual Meeting.

Packard, J.M., 2003. Wolf Behavior: Reproductive, Social, and Intelligent, in: Mech, L.D., Boitani, L. (Eds.), Wolf: Behavior, Ecology and Conservation. Chicago, IL, pp. 35–65.

Palacios, V., Font, E., García, E.J., Svensson, L., Llaneza, L., Frank, J., López-Bao, J.V., 2017. Reliability of human estimates of the presence of pups and the number of wolves vocalizing in chorus howls: implications for decision-making processes. Eur. J. Wildl. Res. 63, 1–8. https://doi.org/10.1007/s10344-017-1115-4

Paquet, M., Knape, J., Arlt, D., Forslund, P., Pärt, T., Flagstad, Ø., Jones, C.G., Nicoll, M.A.C., Norris, K., Pemberton, J.M., Sand, H., Svensson, L., Tatayah, V., Wabakken, P., Wikenros, C., Åkesson, M., Low, M., 2021. Integrated population models poorly estimate the demographic contribution of immigration. METHODS Ecol. Evol. 12, 1899–1910. https://doi.org/10.1111/2041-210X.13667

Petracca, L.S., Gardner, B., Meletzke, B.T., Converse, S.J., 2023. Merging integrated population models and individual-based models to project population dynamics of recolonizing species 29, 1–19. https://doi.org/https://www.biorxiv.org/content/10.1101/2023.03.14.532675v1

Pimenta, V., Barroso, I., Boitani, L., Beja, P., 2018. Risks a la carte: Modelling the occurrence and intensity of wolf predation on multiple livestock species. Biol. Conserv. 228, 331–342. https://doi.org/10.1016/j.biocon.2018.11.008

PORDATA, 2014. Bases de dados Portugal Contemporâneo. http://www.pordata.pt/Portugal.

Posit team, 2022. RStudio: Integrated Development Environment for R. Posit Software, PBC.

Potvin, F., 1988. Wolf movements and population dynamics in Papineau-Labelle reserve, Quebec. Can. J. Zool. 66, 1266–1273. https://doi.org/10.1139/z88-185

Prieto, Abraham, Gonzalez, Victoria, Barrios, Laura, Palacios, Fernando, Peninsula, I., 2022. Field work effort to evaluate biological parameters of interest for decision-making on the wolf (*Canis lupus*). HYSTRIX-ITALIAN J. Mammal. 33, 65–72. https://doi.org/10.4404/hystrix-00414-2021

Redpath, S.M., Young, J., Evely, A., Adams, W.M., Sutherland, W.J., Whitehouse, A., Amar, A., Lambert, R.A., Linnell, J.D.C., Watt, A., Gutiérrez, R.J., 2013. Understanding and managing conservation conflicts. Trends Ecol. Evol. 28, 100–109. https://doi.org/10.1016/j.tree.2012.08.021

Reinhardt, I., Kluth, G., Nowak, C., Szentiks, C.A., Krone, O., Ansorge, H., Müller, T., Mueller, T., 2019. Military training areas facilitate the recolonization of wolves in Germany. Conserv. Lett., 12. https://doi.org/10.1111/conl.12635

Rio-Maior, H., Beja, P., Nakamura, M., Álvares, F., 2018. Use of space and homesite attendance by Iberian wolves during the breeding season. Mamm. Biol. 92, 1–10. https://doi.org/10.1016/j.mambio.2018.03.014

Rio-Maior, H., Nakamura, M., Álvares, F., Beja, P., 2019. Designing the landscape of coexistence: Integrating risk avoidance, habitat selection and functional connectivity to inform large carnivore conservation. Biol. Conserv. 235, 178–188.

https://doi.org/10.1016/j.biocon.2019.04.021

Rousseau, J., Costa, J., Nakamura, M., Rio-maior, H., Álvares, F., Santos, N., Madeira de carvalho, L., 2021a. Monitoring of alopecia in wild carnivores using camera-trapping : insights for the study of sarcoptic mange, in: 69th Wildlife Disease Association / 14th European Wildlife Disease Association – Joint Virtual Conference. Cuenca, Spain.

Rousseau, J., Nakamura, M., Rio-Maior, H., Álvares, F., Choquet, R., Madeira de Carvalho, L., Godinho, R., Santos, N., Rousseau, Julieta, Nakamura, Monia, Rio-Maior, Helena, Alvares, Francisco, Choquet, Remi, Carvalho, M. de, Luis, Godinho, Raquel, Santos, Nuno, Rousseau, J., Nakamura, M., Rio-Maior, H., Álvares, F., Choquet, R., Madeira de Carvalho, L., Godinho, R., Santos, N., 2021b. Non-Invasive Molecular Survey of Sarcoptic Mange in Wildlife: Diagnostic Performance in Wolf Faecal Samples Evaluated by Multi-Event Capture-Recapture Models. Pathogens 10, 1–15. https://doi.org/10.3390/pathogens10020243

Sæther, B.E., Bakke, Ø., 2000. Avian life history variation and contribution of demographic traits to the population growth rate. Ecology 81, 642–653. https://doi.org/10.1890/0012-9658(2000)081[0642:alhvac]2.0.co;2

Santiago-Ávila, F.J., Chappell, R.J., Treves, A., 2020. Liberalizing the killing of endangered wolves was associated with more disappearances of collared individuals in Wisconsin, USA. Sci. Rep. 10, 13881. https://doi.org/10.1038/s41598-020-70837-x

Sazatornil, V., Rodríguez, A., Klaczek, M., Ahmadi, M., Álvares, F., Arthur, S., Blanco, J.C., Borg, B.L., Cluff, D., Cortés, Y., García, E.J., Geffen, E., Habib, B., Iliopoulos, Y., Kaboli, M., Krofel, M., Llaneza, L., Marucco, F., Oakleaf, J.K., Person, D.K., Potočnik, H., Ražen, N., Rio-Maior, H., Sand, H., Unger, D., Wabakken, P., López-Bao, J.V., 2016. The role of human-related risk in breeding site selection by wolves. Biol. Conserv. 201, 103–110. https://doi.org/10.1016/j.biocon.2016.06.022

Schaub, M., Abadi, F., 2011. Integrated population models: A novel analysis framework for deeper insights into population dynamics. J. Ornithol. 152, S227–S237. https://doi.org/10.1007/s10336-010-0632-7

Sells, S.N., Keever, A.C., Mitchell, M.S., Gude, J.A., Podruzny, K.M., Inman, R.M., 2020. Improving estimation of wolf recruitment and abundance, and development of an adaptive harvest management program for wolves in Montana. Final Report for Federal Aid in Wildlife Restoration Grant W-161-R-1 124.

Sells, S.N., Mitchell, M.S., Podruzny, K.M., Ausband, D.E., Emlen, D.J., Gude, J.A., Smucker, T.D., Boyd, D.K., Loonam, K.E., 2022a. Competition, prey, and mortalities influence gray wolf group size. J. Wildl. Manage. 86, 1–17. https://doi.org/10.1002/jwmg.22193

Sells, S.N., Podruzny, K.M., Nowak, J.J., Smucker, T.D., Parks, T.W., Boyd, D.K., Nelson, A.A., Lance, N.J., Inman, R.M., Gude, J.A., Bassing, S.B., Loonam, K.E., Mitchell, M.S., 2022b. Integrating basic and applied research to estimate carnivore abundance. Ecol. Appl. 32. https://doi.org/10.1002/eap.2714

Sikes, R.S., Gannon, W.L., 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92, 235–253. https://doi.org/10.1644/10-MAMM-F-355.1

Silva, P., López-Bao, J.V., Llaneza, L., Álvares, F., Lopes, S., Blanco, J.C., Cortés, Y., García, E., Palacios, V., Rio-Maior, H., Ferrand, N., Godinho, R., 2018. Cryptic population structure reveals low dispersal in Iberian wolves. Sci. Rep. 8, 14108.

https://doi.org/10.1038/s41598-018-32369-3

Stahler, D.R., MacNulty, D.R., Wayne, R.K., VonHoldt, B., Smith, D.W., 2013. The adaptive value of morphological, behavioural and life-history traits in reproductive female wolves. J. Anim. Ecol. 82, 222–234. https://doi.org/10.1111/j.1365-2656.2012.02039.x

Stenglein, J.L., Waits, L.P., Ausband, D.E., Zager, P., Mack, C.M., 2011. Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. J. Mammal. 92, 784–795. https://doi.org/10.1644/10-MAMM-A-200.1

Stenglein, J.L., Zhu, J., Clayton, M.K., Van Deelen, T.R., 2015. Are the numbers adding up? Exploiting discrepancies among complementary population models. Ecol. Evol. 5, 368–376. https://doi.org/10.1002/ece3.1365

Su, Y.S., Yajima, M., 2022. R2jags: using R to Run ‘JAGS’. R package version 0.7-1.

Suutarinen, J., Kojola, I., 2018. One way or another: predictors of wolf poaching in a legally harvested wolf population. Anim. Conserv. 21, 414–422. https://doi.org/10.1111/acv.12409

Tempel, D.J., Peery, M.Z., Gutiérrez, R.J., 2014. Using integrated population models to improve conservation monitoring: California spotted owls as a case study. Ecol. Modell. 289, 86–95. https://doi.org/10.1016/j.ecolmodel.2014.07.005

Torres, R.T., Silva, N., Brotas, G., Fonseca, C., 2015. To Eat or Not To Eat? The Diet of the Endangered Iberian Wolf (*Canis lupus signatus*) in a Human- Dominated Landscape in Central Portugal. PLoS One 10, e0129379. https://doi.org/10.1371/journal.pone.0129379

Treves, A., Bruskotter, J., 2014. Tolerance for Predatory Wildlife. Science (80-.). 344, 476–477. https://doi.org/10.1126/science.1252690

Treves, A., Karanth, K.U., 2003. Human-carnivore conflict and perspectives on carnivore management worldwide. Conserv. Biol. 17, 1491–1499. https://doi.org/10.1111/j.1523-1739.2003.00059.x

Treves, A., Langenberg, J.A., López-Bao, J. V., Rabenhorst, M.F., 2017. Gray wolf mortality patterns in Wisconsin from 1979 to 2012. J. Mammal., 98, 17–32. https://doi.org/10.1093/jmammal/gyw145

Treves, A., Santiago-Ávila, F.J., Putrevu, K., 2021. Quantifying the effects of delisting wolves after the first state began lethal management. PeerJ 9, e11666. https://doi.org/10.7717/peerj.11666

Vogt, K., Zimmermann, F., Kölliker, M., Breitenmoser, U., 2014. Scent-marking behaviour and social dynamics in a wild population of Eurasian lynx Lynx lynx. Behav. Processes 106, 98–106. https://doi.org/10.1016/j.beproc.2014.04.017

Weaver, J.L., Paquet, P.C., Ruggiero, L.F., 1996. Resilience and Conservation of Large Carnivores in the Rocky Mountains. Conserv. Biol. 10, 964–976. https://doi.org/10.1046/j.1523-1739.1996.10040964.x

Webb, N.F., Allen, J.R., Merrill, E.H., 2011. Demography of a harvested population of wolves (*Canis lupus*) in west-central Alberta, Canada. Can. J. Zool. 89, 744–752. https://doi.org/10.1139/z11-043

Woodroffe, R., Lindsey, P., Romanach, S., Stein, A., Oleranah, S., ole Ranah, S.M.K., 2005. Livestock predation by endangered African wild dogs (*Lycaon pictus*) in northern Kenya. Biol. Conserv. 124, 225–234. https://doi.org/10.1016/j.biocon.2005.01.028

Chapter VI

General discussion

6. General discussion

In this thesis, I provide relevant results for a better understanding of large carnivore persistence in human-dominated landscapes. I assessed environmental and anthropogenic factors affecting wolf spatial ecology and pack structure. Focusing on wolves in northwestern Portugal (Alto Minho), I further assessed the population dynamics and demographic parameters of a wolf population in a human-dominated landscape, which are mostly unknown for wolves in Iberia. To accomplish this, I took advantage of a long-term wolf monitoring in Alto Minho, in which I have been involved since 2007, implementing several field methodologies, including transects of sign surveys, observation and howling surveys, camera trapping, capture for GPS-collaring, and collection of non-invasive genetic samples. In my thesis, I have expanded my network of collaborators, working with other wolf researchers in Iberia, to cover a more extensive sampling area and temporal span for my work. Thus, I gathered more information on wolf occupancy and the number of individuals at homesites across several areas in Portugal and Spain, allowing me to obtain more robust results. As a territorial and social species, scent marking can reflect wolves' spatial use and point out areas of importance for wolves. During the breeding season, wolves use homesites with high frequency for pup raising and protection. Thus, homesites and areas with high scent marking frequency are expected to be important places for wolves within territories.

The habitat characteristics for improving wolf persistence in human-dominated landscapes were revealed by identifying environmental, anthropogenic, or intraspecific factors affecting wolf spatial selection in territories (dynamic occupancy) and the number of individuals observed at homesites as a proxy for the impact of exposure risk **(Chapters II and III).** Furthermore, to appoint priority areas for implementing conservation and mitigation measures in Iberia, wolf occupancy, colonization, and extinction probabilities were estimated for the current wolf range and potentially recolonizing areas of Iberia (**Chapter II**).

Demographic parameters were assessed to understand wolves' spatial and temporal dynamics in Alto Minho, as an example of wolves persisting in human-dominated landscapes, including population size, growth rate, and the number of packs and dispersers (**Chapter IV**). Furthermore, additional vital rates (fecundity, survival, and migration rates) were estimated through an integrated population model (IPM), and the viability of wolves in Alto Minho was assessed until 2029, with the estimated vital rates and in different scenarios of adult and pup survival rates (**Chapter V**).

I identified limiting factors for wolves in human-dominated landscapes at the spatial scale and demographic level. The main findings of this thesis are described and discussed in this chapter. Based on these findings, I propose conservation actions, mitigation measures, and future research prospects.

6.1. Anthropogenic and environmental factors affecting wolf persistence in human-dominated landscapes

Based on the surveys conducted in human-dominated landscapes of Iberia over several years, I developed a dynamic occupancy model to identify anthropogenic and environmental factors affecting wolf spatial use (Chapter II). Considering the field methodology used (transects and territorial marks), ruggedness, unpaved road density, and transect effort affected the detection of the species. Taking this into account, the model predicted that wolf occupancy in human-dominated landscapes is more likely in areas with higher altitude, livestock density, and unpaved and local road densities. Conversely, increased highway and national-regional road densities and the proportion of burned areas had a negative effect on wolf occupancy and colonization probabilities.

The factors affecting wolf occupancy identified in this thesis are in line with the remarkable adaptation capabilities and opportunistic behaviour expected from a generalist species, such as wolves (Mech and Boitani, 2003). Wolves take advantage of linear infrastructures with lower human presence and more accessible and available prey (i.e. livestock) to occupy and colonize areas (DeCesare et al., 2018; Dennehy et al., 2021; Zimmermann et al., 2014)**.** Factors influencing wolf extinction probability were not as clear, possibly due to wolves' high resilience and dynamic recoveries that allow them to persist in human-dominated landscapes. Wolves have shown high social dynamics and adaptation when disturbed (Ausband et al., 2017; Borg et al., 2015). Through dispersal, wolves can rapidly compensate, recover, and recolonize areas if allowed. However, persisting under high human-related mortality rates can be challenging even for such a resilient species.

By comparing the colonization and occupancy probabilities in areas of expansion, persistence, regression, and potential recolonization of this species in the last twenty years, I mapped priority areas for implementing conservation actions and mitigation measures. As expected, the occupancy and colonization probabilities were higher in areas where the species persisted or expanded than in areas where the species went extinct in the last twenty years.

The areas where the species got extinct in the last twenty years or where the occupancy probabilities are high in the surroundings of the species range can be potential indicators of higher conflicts around the presence of wolves and poaching pressure. Results indicate that other factors not considered in this study may be operating, including other environmental and anthropogenic factors and social factors related to wolf-human conflicts (Bhatia et al., 2020; Redpath et al., 2013). Wolf range has been expanding in most European countries (Boitani et al., 2022). However, the expansion has stagnated in several suitable areas of Iberia, identified in this thesis, seemingly caused by wolf persecution triggered by high livestock damage (Blanco and Cortés, 2009); although further investigation is needed to asses other potential causes (e.g., diseases). Conversely, expansion fronts have occurred in areas with lower suitability (Blanco and Cortés, 2009). These unexpected expansion fronts and stagnations emphasize the effect and the need to assess human-related social factors on the recovery and persistence of wolf populations in human-dominated landscapes (Arbieu et al., 2019; Behr et al., 2017; Ditmer et al., 2023; Llaneza et al., 2012). By making available the mapped output of the occupancy model, with the identified priority areas, I provide a tool for stakeholders to direct future actions and mitigation measures on the ground.

Identifying environmental and anthropogenic factors affecting wolves' presence and spatial use is challenging for a generalist and highly resilient species (Llaneza et al., 2012). Assuming that variations in the number of observed individuals per pack can shed some light on how environmental, anthropogenic, and intraspecific factors influence wolf persistence, I used an additional approach to identify such factors based on observations at homesites and characterizing the surrounding habitat (Chapter III). The models showed some evidence of refuge availability and unpaved road density affecting the number of observed pups in packs, though they explained little of the variation in my data. This suggests that other factors may influence the number of pups, such as mortality in the early months of life (Johnson et al., 1994).

Refuge availability at homesites (1 km and 2 km buffer) and within the remnant territory (5 km buffer) emerged as a crucial factor positively influencing the number of adults observed in packs in human-dominated landscapes, aligning with previous studies on homesite selection (Llaneza et al., 2018; Sazatornil et al., 2016). Food availability was not a limiting factor since wolves mostly feed on livestock in the context of the study areas considered. Human population density, paved road density, livestock density, the proportion of burned areas, ruggedness, and pack density negatively affected the number of adults at homesites. These results reveal the impact of anthropogenic disturbance on pack structure in the form of infrastructures and human presence and activities. Previous studies have shown that wolves select breeding sites with lower human disturbance and higher refuge availability (e.g., Sazatornil et al., 2016), though the impact on wolf demographic parameters was yet to be assessed. The impact of homesite selection on the number of adults detected in this thesis supports the importance of decreasing human disturbance and increasing refuge availability in human-dominated landscapes (Llaneza et al., 2016). Thus, efforts are needed to increase the availability of suitable and undisturbed habitats, particularly for homesite use, since wolves can be particularly exposed and susceptible during the breeding season (Linnell et al., 2017; Ruprecht et al., 2012).

Although livestock density was positively correlated with wolf colonization and occupancy, the number of adults at homesites was negatively affected. These results suggest that wolves opportunistically select areas with higher livestock density and can persist, though the number of adults is somehow reduced, possibly due to conflicts with humans and retaliatory killing due to livestock depredation.

6.2. Wolf population dynamics and demographic parameters in human-dominated landscapes

The twenty-year monitoring in northwestern Portugal (Alto Minho) allowed me to gain unprecedented insights into wolf population dynamics in a human-dominated landscape. As an initial approach, I estimated population size, pack persistence, and breeding rates between 1996 and 2016 based on pack and breeding detection and naïve wolf counts obtained through different available methodologies. This allowed me to detect fluctuations in population size and growth rates, which are challenging to detect in one-off studies (Chapter IV; Nakamura et al., 2021).

The number of wolves in Alto Minho significantly decreased between 1996 and 2005, with only two of the six packs remaining at the end of this period. This decline was possibly related to the combined effect of high direct wolf persecution through poisoning or shooting and the low habitat connectivity due to the construction of two highways crossing the study area. However, wolves in Alto Minho have been recovering since 2007, recolonizing the western part of the study area and recovering the number of packs. Overall, the two packs that resisted such decline (i.e. core packs) showed higher values of group persistence, breeding success, and number of individuals compared to packs that went extinct for several years (i.e. sink packs). GPS-collared wolves and non-invasive sampling showed that core packs acted as sources of dispersers to recover sink packs and that the recolonization was through a stepping-stone dispersal strategy (Chapter IV; Nakamura et al., 2021). Detecting and understanding the process of wolf population dynamics is challenging since several factors related to anthropogenic disturbance, human activities, wolf social dynamics, and intraspecific competition may be influencing concurrently (e.g., Sells et al., 2022). This thesis provides evidence that in human-dominated landscapes, a few core packs can play a crucial role in maintaining and recovering several neighbouring sink packs, while sink packs may act as stepping-stones to facilitate dispersal.

To further understand the population dynamics of wolves in human-dominated landscapes, I used an additional approach to obtain demographic parameters relevant to wolf persistence. With the integrated population model developed for wolves in Alto Minho between 2007 and 2019, the population size and growth rate estimates were more precise than the results obtained from the naïve counts. Although some interannual fluctuations in population size were observed, the overall growth rate (1.06 ± 0.15) suggests a positive trend during this period. Furthermore, I estimated the number of adults and pups and survival and emigration rates, the latter utterly unknown for most wolf populations in Iberia (Chapter V). Adult and pup mortality rates were estimated at 28% (95% BCI 23-34%) and 47% (95% BCI 29-70%), respectively. Previous studies show that wolf populations decline when the apparent mortality rate is above 32% or 35% (Chapron et al., 2003; Fuller, 1989; Marescot et al., 2012). According to the estimated emigration rate (13%), the apparent adult mortality rate was above the mentioned threshold (38%; 95% BCI 28-48%). Although survival estimates were robust, methodological constraints, species elusiveness or cryptic poaching may hinder the obtention of actual survival estimates. Additionally, the wide credible intervals call for further investigation to improve the estimation of some parameters, such as survival rates for pups.

This thesis highlights the relevance of adult survival in sustaining the persistence of wolf populations in human-dominated landscapes and underscores the need to address wolf mortality rates and causes. Even though a small portion of wolf mortality may be due to accidental causes (e.g., road kills) or diseases (e.g., canine distemper or sarcoptic mange), the mortalities detected by GPS-collared wolves in Alto Minho suggest that the majority are illegal killing through shooting, snaring, and poisoning, and recent studies conducted in Spain show that 26% of the 23 GPS-collared wolves were illegally killed (Campos, 2018; Rio-Maior et al., 2019; López-Bao pers. comm.). In this thesis, I used several approaches to obtain different demographic parameters for wolf pups. However, the difficulties in detecting reproduction sites and all pups in packs, particularly until two months of age, and knowing the age of individuals detected through non-invasive sampling precluded the obtention of precise estimates for fecundity and survival. Thus, obtaining robust estimates on pup-related parameters, assessing litter sizes through foetus counting or placental scar counting (e.g., Ferreras-Colino et al., 2021; Rausch, 1967), assessing the number of pups during the first two months of life and making a higher effort to detect all the pups in packs through observation surveys is urged.

According to the population viability analysis I performed, decreasing the adult survival rate could lead to a significant population decline by 2029, similar to what occurred between 1996 and 2006 in Alto Minho. Although population fluctuations can be caused naturally in wolves (Solomon, 1949), abrupt fluctuations as observed in this population, apparently caused by anthropogenic factors related to poaching and human-disturbed areas with low habitat connectivity (Rio-Maior et al., 2019), could be addressed by implementing adequate conservation actions.

The limited dispersal observed in wolves of Alto Minho, with low estimated dispersal rates among detected individuals (11%) and low estimated emigration rates from the study area (13%), is in concordance with results found by Silva et al.(2018). Dispersal needs may be reduced in this study area since food availability is not a limiting factor and large packs are sustainable. However, considering the importance of dispersal in wolf population dynamics and the limited dispersal rates in Iberia, these results emphasize the need for conservation efforts focused on improving connectivity and reducing human-caused mortality to increase dispersal success and guarantee the maintenance and recovery of packs.

Identifying core and sink packs, delineating conservation priority areas, and understanding whether the observed number of wolves at homesites are correlated with habitat characteristics and which vital rates limit wolf persistence helps to select where and which conservation actions and mitigation measures are needed. As a generalist species, wolves can be highly dynamic and adaptable to different conditions. Thus, such actions are context-dependent and should be implemented case-by-case.

6.3. Conservation actions and mitigation measures in human-dominated landscapes

All approaches used in this thesis – wolf spatial use, homesite selection, population dynamics and demographic parameters – suggest that wolves are subject to high anthropogenic pressure through highly human-disturbed landscapes and possible direct persecution. This urges the need to include parameters related to social conflict or management to understand wolf persistence in human-dominated landscapes better (Behr et al., 2017; Hull et al., 2023; Vogel et al., 2023). Therefore, according to the results obtained in this thesis, the conservation actions, mitigation measures, and future research I propose for human-dominated landscapes mostly aim to reduce exposure risk for wolves and reduce anthropogenic disturbance, human-wildlife conflicts and human-caused mortality, particularly illegal killing. Efforts are needed to maintain or increase the adult survival rate and ensure the persistence of wolf populations.

Based on the findings of this thesis, the implementation of the following conservation actions and mitigation measures are proposed:

- Promote the use of livestock damage preventive measures and develop awareness campaigns about available funding and compensation schemes. The implementation of such actions should be prioritized in areas with a higher frequency of wolf damage on livestock, recently extinct or recolonized areas, and probable areas of wolf recolonization;
- Establish an optimal composite system of depredation prevention and damages mitigation using economic incentives, including EU subsidies and insurance for livestock farms supporting coexistence with predators.
- The breeders suffering the most significant losses should receive priority attention for the deployment of prevention measures. The preventive measures should be monitored to evaluate their efficacy in loss prevention.
- Review the compensation programs to reduce or avoid the increase of conflicts. Changes in legislation and compensation conditions should be evaluated through social studies to understand how such changes impacted human attitudes towards wolves. Compensation programs need to be compiled and improved by informing the owners of the outcome of the evaluation, meeting the deadlines of payments, and evaluating the financial loss for the owner, not only the value of the depredated animal but also other associated costs;

- Improve management involving all stakeholders, including livestock owners, hunters, public and private sectors, academic and research institutions, and politicians. Social science research is needed to assess conflicts and improve management through stakeholder engagement (Hovardas, 2020).
- Promote awareness campaigns and workshops in close cooperation between forest rangers, nature protection inspectors and the national or regional authorities to collect and compile information on the use of poison, snares and other traps.
- Reinforce patrolling activities to detect and prevent wildlife poaching, including illegal shooting, snares, and poisoning. Patrolling should also be conducted in organized hunting activities to prevent direct persecution and illegal killing. Field surveys should be conducted among local hunters and livestock farmers to better understand the causes, frequency, and consequences of the illegal use of poison baits and snares.
- Increase and improve law enforcement against poaching;
- The location of homesites should be reported in real-time to the responsible nature and conservation institutions to implement mitigation and conservation measures onsite and avoid disturbance around homesites (1-2 km buffer) (e.g., construction of infrastructures, leisure and hunting activities).
- Wolf requirements for persistence should be integrated into landscape management approaches in order to ensure refuge availability for wolves, particularly for reproduction site use, by increasing areas of shrublands and autochthonous forests and implementing fire prevention measures;
- Promote awareness campaigns for local communities about habitat and fire management and its impact on wildlife and landscape quality to promote habitat improvement and increase the habitat suitability for wolves and avoid disturbance;
- Raise awareness campaigns about human-wolf coexistence in areas with low occupancy probability within the wolf range. Additionally, considering packs identified as source and sink, promote connectivity and dispersal movements between them through ecological corridors, permeability of barriers, and reduction of human-caused mortality and disturbance;
- Implement road-kill preventive measures, particularly on local and national-regional roads, frequently used passages by large carnivores (e.g., around reproduction sites). Road-kill preventive measures could include road fencing, wildlife crossings, and speed limit traffic signs.

6.4. Final considerations and future research

This thesis offers insights into large carnivore spatial use by identifying factors affecting wolf occupancy, colonization, and extinction and further bridges the gap between suitable habitat at reproduction sites and population demographic parameters. This study makes available a valuable tool that identifies priority areas for implementing conservation actions and mitigation measures within the wolf range in Iberia, and in colonization areas for the near future. Lastly, this thesis reveals the population dynamics and demographic parameter estimates of previously unknown vital rates for wolves in the human-dominated landscapes of Iberia.

Although this thesis filled in some of the knowledge gaps identified in the introduction, large carnivore persistence can be context-dependent, particularly in generalist species. Thus, targeting heterogeneous landscapes with variable demographic parameters and ecological conditions is highly relevant to understanding population dynamics and identifying the limiting factors for large carnivore (wolf) persistence. In the light of the conclusions of this thesis, I propose several lines of future research studies, including methodological procedures and assessment of demographic parameters of large carnivores in human-dominated landscapes:

Future studies of large carnivore monitoring in human-dominated landscapes should ideally be long-term, using a homogenous and comparable approach over time (e.g., Ausband et al., 2014; Jiménez et al., 2016; López-Bao and Blanco, 2023; Marucco et al., 2023) to detect spatiotemporal variations and use a multimethod approach to combine several complementary information on large carnivore populations (Jiménez et al., 2016). Besides visual and acoustic detections that provide population estimates and breeding rates (Palacios et al., 2016), large carnivore monitoring should include the combination of GPS-collaring and non-invasive sampling (Barber-Meyer, 2022; Kojola et al., 2018). Compared to any other methodology, GPS-collaring offers the most detailed and reliable information on fine-scale spatial use and, most importantly, on mortality causes and rates. By combining this information with individual genetic identification of non-invasive sampling (e.g., Marucco et al., 2012; Stenglein et al., 2011) throughout several years, it is possible to have insights into more accurate mortality rates, including cryptic mortality, and additional demographic parameters, such as sex ratio and dispersal rates, as obtained in this thesis.

GPS-collaring, non-invasive sampling, and camera trapping in the limits of species range would allow the detection of population expansions and dispersal events.

However, an evaluation of the non-invasive sampling effort is needed to get the best possible estimates with a balance between the effort, budget, and results. Furthermore, studies including genealogies should also provide insights into large carnivore social dynamics to detect dispersal, multiple-breeding and even breeder turnover.

The main factors affecting large carnivore persistence in human-dominated landscapes were related to refuge availability, anthropogenic activities and disturbance. However, approaches used in this thesis suggest that other factors may be influencing wolf persistence in human-dominated landscapes, which are probably social factors related to human-wildlife conflicts. Hence, incorporating socio-ecological factors into occupancy or demographic models can provide a more comprehensive understanding of large carnivore persistence in these landscapes (e.g., Behr et al., 2017; Vogel et al., 2023). Additionally, spatially explicit models could help understand the slow recovery and recolonization process observed in the wolf population in Iberia. Studies on wolf demography in several areas within the wolf population in Iberia are needed in order to detect demographic variations in space, particularly in conflict-prone areas, where wolves highly prey on livestock, in the expansion borders, and where wolf populations appear to be more fragile and at risk, such as in the small and isolated wolf areas, such as South of Douro River.

Density estimates of wild ungulate populations in Iberia are lacking. Thus, studies should be promoted to estimate local wild ungulate abundance, detect illegal killings, and better understand the relation between wolf spatial use and predatory behaviour on wild prey and livestock. Additional research is needed to better understand how and why increased livestock densities are correlated to a lower number of observed adult wolves in packs. Although it is possibly related to conflicts due to livestock depredation by wolves and retaliatory killing, further studies are needed to confirm this relation.

An assessment of the age of breeding females, food availability, or intra- and interspecific competition can explain the observed differences in fecundity and number of pups within the wolf population across Iberia. These studies should consider the methodological approaches since the implemented methods can influence the fecundity and pup number estimates. Furthermore, efforts are needed to detect and understand the particular conditions in which multiple breeding in packs occurs, either due to intrinsic social wolf behaviour or for compensation for human-caused mortalities in human-dominated landscapes. Further studies are needed to assess annual pup survival and pup survival at early stages of life (< 2 months of age) through complementary methods, such as ear-tagging pups at den sites, fitting pups with temporary GPS collars, den site non-invasive sampling, or performing genealogy analysis from non-invasive samples to identify pups. Additionally, with non-invasive sampling at homesites and year-round sampling throughout the territories, pup and adult mortality rates could be determined by spatial capture-recapture models.

Spatial aggregations of wolf road kill and frequently used passages (e.g., around reproduction sites or based on the movement of GPS-collared wolves) should be identified to implement mitigation measures for this cause of mortality.

In the case of wolves in Portugal, an impact assessment of the modifications in the legislation of livestock depredation compensation schemes, implemented since 2018, is urgently needed. Such modifications might have decreased the tolerance of local communities towards the presence of wolves and induced retaliatory killing of wolves. For the wolves in Alto Minho, in particular, by continuing the long-term monitoring and implementing the integrated population model for the following years, it will be possible to assess variations in survival before and after the changes in the legislation. Thus, research on the variation in the number of declared livestock depredations and wolf mortality causes and rates before and after 2018 should shed light on the impact of changes in the legislation.

Finally, conducting national and transboundary studies with systematic and synchronized sampling methods is desirable for comparable results and to use as guidance for reaching compatible management policies throughout the wolf range and across political borders. However, conservation actions and mitigation measures must also be adapted or selected since requirements can be context-dependent.

Conflicts between large carnivores and humans often arise in human-dominated landscapes, resulting in retaliatory killing of large carnivores. The way for humans and large carnivores to successfully coexist in human-dominated landscapes is through co-adaptation (Carter and Linnell, 2016; Chapron and López-Bao, 2016). Hence, the involvement of all stakeholders, including researchers, livestock owners, hunters, public and private sectors, non-governmental institutions, and politicians, is crucial. According to the studies conducted in the present thesis, results highlight the need to assess the causes and rates of anthropogenic large carnivore mortality and include factors related to social conflicts and human dimensions in similar research.

6.5. References

Arbieu, U., Mehring, M., Bunnefeld, N., Kaczensky, P., Reinhardt, I., Ansorge, H., Böhning-Gaese, K., Glikman, J.A., Kluth, G., Nowak, C., Müller, T., 2019. Attitudes towards returning wolves (*Canis lupus*) in Germany: Exposure, information sources and trust matter. Biol. Conserv. 234, 202–210. https://doi.org/10.1016/j.biocon.2019.03.027

Ausband, D.E., Mitchell, M.S., Waits, L.P., 2017. Effects of breeder turnover and harvest on group composition and recruitment in a social carnivore. J. Anim. Ecol. 86, 1094–1101. https://doi.org/10.1111/1365-2656.12707

Ausband, D.E., Rich, L.N., Glenn, E.M., Mitchell, M.S., Zager, P., Miller, D.A.W., Waits, L.P., Ackerman, B.B., Mack, C.M., 2014. Monitoring Gray Wolf Populations Using Multiple Survey Methods. J. Wildl. Manage. 78, 335–346. https://doi.org/10.1002/jwmg.654

Barber-Meyer, S.M., 2022. Can non-invasive methods replace radiocollar-based winter counts in a 50-year wolf study? Lessons learned from a three-winter trial. Wildl. Res. https://doi.org/10.1071/WR22001

Behr, D.M., Ozgul, A., Cozzi, G., 2017. Combining human acceptance and habitat suitability in a unified socio-ecological suitability model: a case study of the wolf in Switzerland. J. Appl. Ecol., 54, 1919–1929. https://doi.org/10.1111/1365-2664.12880

Bhatia, S., Redpath, S.M., Suryawanshi, K., Mishra, C., 2020. Beyond conflict: exploring the spectrum of human–wildlife interactions and their underlying mechanisms. Oryx, 54, 621–628. https://doi.org/10.1017/S003060531800159X

Blanco, J.C., Cortés, Y., 2009. Ecological and social constraints of wolf recovery in Spain, in: Musiani, M., Boitani, L., Paquet, P.C. (Eds.), A New Era for Wolves and People: Wolf Recovery, Human Attitudes, and Policy. University of Calgary Press, Calgary, pp. 41–66.

Boitani, L., Kaczensky, P., Alvares, F., Andrén, H., Balys, V., Blanco, J.C., Chapron, G., Chiriac, S., Cirovic, D., Drouet-Houguet, N., Groff, C., Huber, D., Iliopoulos, Y., Ionescu, O., Kojola, I., Krofel, M., Kutal, M., Linnell, J., Majic, A., Mannil, P., Marucco, F., D. Melovski, Mengüllüoğlu, D., Mergeay, J., Nowak, S., Ozolins, J., Perovic, A., Rauer, G., Reinhardt, I., Rigg, R., Salvatori, V., Sanaja, B., Schley, L., Shkvyria, M., Sunde, P., Tirronen, K., Trajce, A., Trbojevic, I., Trouwborst, A., Arx, M. von, Wolfl, M., Zlatanova, D., Patkó, L., 2022. Assessment of the conservation status of the Wolf (*Canis lupus*) in Europe.

Borg, B.L., Brainerd, S.M., Meier, T.J., Prugh, L.R., 2015. Impacts of breeder loss on social structure, reproduction and population growth in a social canid. J. Anim. Ecol. 84, 177–187. https://doi.org/10.1111/1365-2656.12256

Campos, R., 2018. Survival and Population Dynamics of the Iberian Wolf in Portugal. MSc Thesis. Universidade do Porto.

Carter, N.H., Linnell, J.D.C., 2016. Co-Adaptation Is Key to Coexisting with Large Carnivores. Trends Ecol. Evol. 31, 575–578. https://doi.org/10.1016/j.tree.2016.05.006

Chapron, G., Legendre, S., Ferrière, R., Clobert, J., Haight, R.G., 2003. Conservation and control strategies for the wolf (*Canis lupus*) in western Europe based on demographic models. C. R. Biol. 326, 575–587. https://doi.org/10.1016/S1631-0691(03)00148-3

Chapron, G., López-Bao, J.V., 2016. Coexistence with Large Carnivores Informed by

Community Ecology. Trends Ecol. Evol. 31, 578–580. https://doi.org/10.1016/j.tree.2016.06.003

DeCesare, N.J., Wilson, S.M., Bradley, E.H., Gude, J.A., Inman, R.M., Lance, N.J., Laudon, K., Nelson, A.A., Ross, M.S., Smucker, T.D., 2018. Wolf-livestock conflict and the effects of wolf management. J. Wildl. Manage. 82, 711–722. https://doi.org/10.1002/jwmg.21419

Dennehy, E., Llaneza, L., López-Bao, J.V., 2021. Contrasting wolf responses to different paved roads and traffic volume levels. Biodivers. Conserv. 30, 3133–3150. https://doi.org/10.1007/s10531-021-02239-y

Ditmer, M.A., Wittemyer, G., Zeller, K.A., Breck, S.W., Fletcher, R.J., Crooks, K.R., 2023. Predicting dispersal and conflict risk for wolf recolonization in Colorado. J. Appl. Ecol. 1–13. https://doi.org/10.1111/1365-2664.14504

Ferreras-Colino, E., García-Garrigós, A., Gortázar, C., Llaneza, L., 2021. Wolf (*Canis lupus*) litter size in Spain. Eur. J. Wildl. Res. 67, 31. https://doi.org/10.1007/s10344-021-01473-7

Fuller, T.K., 1989. Population dynamics of wolves in north-central Minnesota. Wildl. Monogr. 105, 1–41.

Hovardas, T., 2020. A Social Learning Approach for Stakeholder Engagement in Large Carnivore Conservation and Management. Front. Ecol. Evol. 8, 1–19. https://doi.org/10.3389/fevo.2020.525278

Hull, V., Bian, X., Episcopio-Sturgeon, D.J., Rivera, C.J., Rojas-Bonzi, V., Morzillo, A.T., 2023. Living with wildlife: a review of advances in social-ecological analysis across landscapes. Landsc. Ecol. 38, 4385–4402. https://doi.org/10.1007/s10980-023-01778-9

Jiménez, J., García, E.J., Llaneza, L., Palacios, V., González, L.M., García-Domínguez, F., Múñoz-Igualada, J., López-Bao, J.V., 2016. Multimethod, multistate Bayesian hierarchical modeling approach for use in regional monitoring of wolves. Conserv. Biol. 30, 883–893. https://doi.org/10.1111/cobi.12685

Johnson, M.R., Boyd, D.K., Pletscher, D.H., 1994. Serologic Investigations of Canine Parvovirus and Canine Distemper in Relation to Wolf (*Canis lupus*) Pup Mortalities. J. Wildl. Dis., 30, 270–273. https://doi.org/10.7589/0090-3558-30.2.270

Kojola, I., Heikkinen, S., Holmala, K., 2018. Balancing costs and confidence: volunteer-provided point observations, GPS telemetry and the genetic monitoring of Finland’s wolves. Mammal Res., 63, 415–423. https://doi.org/10.1007/s13364-018-0371-3

Linnell, J.D.C., Swenson, J.E., Andersen, R., Barnes, B., Linnell, J.D.C., Swenson, J.E., Barnes, B., 2017. How vulnerable are denning bears to disturbance ? 28, 400–413. https://doi.org/10.2307/3783698

Llaneza, L., García, E.J., Palacios, V., Sazatornil, V., López-Bao, J.V., 2016. Resting in risky environments: the importance of cover for wolves to cope with exposure risk in human-dominated landscapes. Biodivers. Conserv. 25, 1515–1528. https://doi.org/10.1007/s10531-016-1134-6

Llaneza, L., López-Bao, J.V., Sazatornil, V., 2012. Insights into wolf presence in human-dominated landscapes: the relative role of food availability, humans and landscape attributes. Divers. Distrib. 18, 459–469. https://doi.org/10.1111/j.1472-4642.2011.00869.x

Llaneza, L., Sazatornil, V., López-Bao, J.V., 2018. The importance of fine-scale breeding site selection patterns under a landscape-sharing approach for wolf conservation. Biodivers. Conserv. 27, 1239–1256. https://doi.org/10.1007/s10531-017-1491-9

López-Bao, J.V., Blanco, J.C., 2023. Large scale wolf monitoring without snow and genetics, and in a transboundary context. Some insights and challenges from Spain, in: Fifth LIFE WolfAlps EU Phygital Conference, Turin - 19th September 2023 "Towards Large-Scale Wolf Population Monitoring-Challenges and Perspectives", Book of Abstracts.

Marescot, L., Gimenez, O., Duchamp, C., Marboutin, E., Chapron, G., 2012. Reducing matrix population models with application to social animal species. Ecol. Modell. 232, 91–96. https://doi.org/10.1016/j.ecolmodel.2012.02.017

Marucco, F., Boiani, M. V., Dupont, P., Milleret, C., Avanzinelli, E., Pilgrim, K., Schwartz, A.M.K., von Hardenberg, A., Perrone, S., Friard, O., Menzano, A., Bisi, F., Fattori, U., Tomasella, M., Calderola, S., Carolfi, S., Ferrari, P., Chioso, C., Truc, F., Bombieri, G., Pedrotti, L., Righetti, D., Acutis, P.L., Guglielmo, F., Hauffe, H.C., Rossi, C., Caniglia, R., Aragno, P., La Morgia, V., Genovesi, P., Bischof, R., 2023. A multidisciplinary approach to estimating wolf population size for long-term conservation. Conserv. Biol. https://doi.org/10.1111/cobi.14132

Marucco, F., Vucetich, L.M., Peterson, R.O., Adams, J.R., Vucetich, J.A., 2012. Evaluating the efficacy of non-invasive genetic methods and estimating wolf survival during a ten-year period. Conserv. Genet. 13, 1611–1622. https://doi.org/10.1007/s10592-012-0412-4

Mech, L.D., Boitani, L., 2003. Wolves: Behavior, Ecology, and Conservation. The University of Chicago Press.

Nakamura, M., Rio-Maior, H., Godinho, R., Petrucci-Fonseca, F., Álvares, F., 2021. Source-sink dynamics promote wolf persistence in human-modified landscapes: Insights from long-term monitoring. Biol. Conserv. 256, 109075. https://doi.org/10.1016/j.biocon.2021.109075

Palacios, V., López-Bao, J.V., Llaneza, L., Fernández, C., Font, E., 2016. Decoding Group Vocalizations: The Acoustic Energy Distribution of Chorus Howls Is Useful to Determine Wolf Reproduction. PLoS One 11, e0153858. https://doi.org/10.1371/journal.pone.0153858

Rausch, R.A., 1967. Some Aspects of the Population Ecology of Wolves, Alaska. Am. Zool., 7, 253–265.

Redpath, S.M., Young, J., Evely, A., Adams, W.M., Sutherland, W.J., Whitehouse, A., Amar, A., Lambert, R.A., Linnell, J.D.C., Watt, A., Gutiérrez, R.J., 2013. Understanding and managing conservation conflicts. Trends Ecol. Evol. 28, 100–109. https://doi.org/10.1016/j.tree.2012.08.021

Rio-Maior, H., Nakamura, M., Álvares, F., Beja, P., 2019. Designing the landscape of coexistence: Integrating risk avoidance, habitat selection and functional connectivity to inform large carnivore conservation. Biol. Conserv. 235, 178–188. https://doi.org/10.1016/j.biocon.2019.04.021

Ruprecht, J.S., Ausband, D.E., Mitchell, M.S., Garton, E.O., Zager, P., 2012. Homesite attendance based on sex, breeding status, and number of helpers in gray wolf packs. J. Mammal., 93, 1001–1005. https://doi.org/10.1644/11-MAMM-A-330.1

Sazatornil, V., Rodríguez, A., Klaczek, M., Ahmadi, M., Álvares, F., Arthur, S., Blanco, J.C., Borg, B.L., Cluff, D., Cortés, Y., García, E.J., Geffen, E., Habib, B., Iliopoulos, Y., Kaboli, M., Krofel, M., Llaneza, L., Marucco, F., Oakleaf, J.K., Person, D.K., Potočnik, H., Ražen, N., Rio-Maior, H., Sand, H., Unger, D., Wabakken, P., López-Bao, J.V., 2016. The role of human-related risk in breeding site selection by wolves. Biol. Conserv. 201, 103–110. https://doi.org/10.1016/j.biocon.2016.06.022

Sells, S.N., Mitchell, M.S., Podruzny, K.M., Ausband, D.E., Emlen, D.J., Gude, J.A., Smucker, T.D., Boyd, D.K., Loonam, K.E., 2022. Competition, prey, and mortalities influence gray wolf group size. J. Wildl. Manage. 86, 1–17. https://doi.org/10.1002/jwmg.22193

Silva, P., López-Bao, J.V., Llaneza, L., Álvares, F., Lopes, S., Blanco, J.C., Cortés, Y., García, E., Palacios, V., Rio-Maior, H., Ferrand, N., Godinho, R., 2018. Cryptic population structure reveals low dispersal in Iberian wolves. Sci. Rep. 8, 14108. https://doi.org/10.1038/s41598-018-32369-3

Solomon, M.E., 1949. The Natural Control of Animal Populations. J. Anim. Ecol. 18, 1. https://doi.org/10.2307/1578

Stenglein, J.L., Waits, L.P., Ausband, D.E., Zager, P., Mack, C.M., 2011. Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. J. Mammal. 92, 784–795. https://doi.org/10.1644/10-MAMM-A-200.1

Vogel, S.M., Vasudev, D., Ogutu, J.O., Taek, P., Berti, E., Goswami, V.R., Kaelo, M., Buitenwerf, R., Munk, M., Li, W., Wall, J., Chala, D., Amoke, I., Odingo, A., Svenning, J.C., 2023. Identifying sustainable coexistence potential by integrating willingness-to-coexist with habitat suitability assessments. Biol. Conserv. 279, 109935. https://doi.org/10.1016/j.biocon.2023.109935

Zimmermann, B., Nelson, L., Wabakken, P., Sand, H., Liberg, O., 2014. Behavioral responses of wolves to roads: scale-dependent ambivalence. Behav. Ecol. 25, 1353–1364. https://doi.org/10.1093/beheco/aru134

Chapter VII

Synthèse

7.1. Contexte et objectifs

Les populations de grands carnivores se sont reconstituées et ont recolonisé plusieurs régions en Europe au cours des dernières décennies. Cependant, leur comportement prédateur entraîne souvent des conflits avec l'homme, ce qui se traduit par l'abattage préventif ou en représailles des grands carnivores. La persécution directe et d'autres perturbations anthropogéniques rendent la conservation et la gestion des grands carnivores dans les paysages dominés par l'homme particulièrement difficiles. Parmi tous les grands carnivores, les loups (*Canis lupus*) sont remarquablement résistants et capables de s'adapter à diverses conditions d'habitat. Les facteurs anthropiques et environnementaux affectant la persistance des loups ont souvent été évalués en Amérique du Nord et, de plus en plus, en Europe. Néanmoins, des études à long terme dans des paysages dominés par l'homme et des évaluations de la manière dont ces facteurs affectent le nombre d'individus font encore défaut. En outre, des recherches sur la dynamique des populations de loups dans les paysages dominés par l'homme sont nécessaires pour estimer les paramètres démographiques, pour la plupart inconnus, qui sont importants pour la conservation des loups.

Cette thèse vise à contribuer à la compréhension de l'impact des facteurs anthropogéniques et environnementaux sur la persistance des grands carnivores dans les paysages dominés par l'homme. Le loup est utilisé comme espèce modèle pour étudier comment différents niveaux d’anthropisation peuvent influencer la sélection de l'habitat du loup et finalement affecter sa démographie, son statut et sa viabilité. En étudiant la répartition spatiale des loups, la sélection des sites de reproduction, la dynamique de la population et la démographie, cette thèse comble certaines des lacunes de connaissances identifiées dans l'analyse de la littérature.

Cette recherche contribue à la conservation des grands carnivores en apportant des connaissances supplémentaires sur les populations et en proposant des mesures de gestion et de conservation pour les zones présentant différents niveaux d'adéquation pour les loups.

Les principaux objectifs de recherche de cette thèse sont les suivants :

1. Estimer la probabilité d'occupation des espèces afin de mieux comprendre la structure spatiale des populations de loups et d'identifier les zones potentielles de recolonisation dans les paysages dominés par l'homme ;
2. Évaluer la sélection des sites de reproduction dans des zones présentant différents niveaux de perturbation humaine et détecter les effets sur le nombre d'individus dans les meutes ;
3. Fournir des informations sur la dynamique d'une population de loups dans des paysages dominés par l'homme ;
4. Estimer les paramètres démographiques sur la base d'un modèle de population intégré et étudier la viabilité d'une population de loups dans un paysage dominé par l'homme.

Pour ce faire, des facteurs anthropogéniques et environnementaux ont été inclus dans un modèle d'occupation dynamique afin d'estimer les probabilités de colonisation, d'extinction et d'occupation des loups en Ibérie et dans des modèles mixtes linéaires généralisés afin de détecter leurs effets sur le nombre d'adultes et de petits détectés sur les sites de reproduction. La dynamique des loups au niveau de la population, de la meute et des individus a été évaluée dans le nord-ouest du Portugal à l'aide d'une approche de suivi multi-méthodes, comprenant des transects de récolte des signes de présence, des études de hurlements provoqués et d'observation directe, du piégeage photographique, du suivi par GPS et de l'identification moléculaire individuelle d'échantillons non invasifs. En outre, en incorporant toutes les méthodes, un modèle de population intégré a été construit pour estimer les paramètres démographiques (taille de la population, nombre de meutes et nombre d'adultes et de petits) et les taux vitaux (taux de croissance, fécondité, survie et taux d'émigration) et une analyse de viabilité de la population sur dix ans a été réalisée selon différents scénarios de taux de survie des loups.

7.2. Principaux résultats

Les résultats sont présentés pour les quatre objectifs de recherche mentionnés ci-dessus.

Occupation des loups dans les paysages dominés par l'homme

Selon le modèle d'occupation dynamique basé sur les transects dans quatre zones d'étude en Ibérie, la probabilité de détection des loups a augmenté avec l'effort (β = 0,89 ± 0,11), la rugosité (β = 0,89 ± 0,14) et la densité des routes non asphaltées (β = 0,20 ± 0,14). En ce qui concerne le processus dynamique d'occupation des loups dans ces paysages dominés par l'homme, la probabilité de colonisation a augmenté avec l'altitude (β = 3,36 ± 2,14), la densité du bétail (β = 3,01 ± 1,64) et la densité des routes non asphaltées (β = 1,86 ± 1,62). En revanche, le développement des infrastructures linéaires (routes nationales/régionales : β = -1,49 ± 1,99, routes locales : β = -1,01 ± 1,48) et la proportion de zones brûlées (β = -1,26 ± 2,12) ont influencé négativement la probabilité de colonisation. La probabilité d'extinction augmente avec la proportion de zones brûlées (β = 0,09 ± 0,15) et les grandes infrastructures linéaires (routes nationales-régionales : β = 0,09 ± 0,23, autoroutes : β = 0,08 ± 0,27). Au contraire, la probabilité d'extinction diminue avec la densité des routes non asphaltées (β = -0,25 ± 0,42 ; 50% BCI) et, dans une moindre mesure, avec des densités de population humaine plus élevées (β = -0,10 ± 0,30), l'altitude (β = -0,06 ± 0,31) et la densité des routes locales (β = -0,09 ± 0,25).

Les probabilités annuelles moyennes de colonisation et d'extinction étaient respectivement de 63,1 % et de 5,6 %. La probabilité d'occupation moyenne pour l'ensemble des zones échantillonnées était de 85,2 ± 0,2 % (81,0-89,5 %) et elle est restée constante de 2005 à 2022. Selon les prévisions obtenues pour l'ensemble de la période d’étude, au cours de la dernière année (2020-2021), 49 % de la péninsule ibérique avaient une probabilité de colonisation supérieure à 50 % (0-100 %), 90 % avaient une probabilité d'extinction supérieure à 10 % (3-58 %) et 89 % avaient une probabilité d'occupation supérieure à 50 % (23-97 %).

La probabilité de colonisation annuelle moyenne était relativement élevée dans les zones d'expansion et de persistance des loups ($\gamma_{\text{expansion}}$ = 76,2 ± 0,3% ; $\gamma_{\text{persistance}}$ = 66,9 ± 0,1%) mais inférieure à 50% dans la zone de régression ($\gamma_{\text{régression}}$ = 43,1 ± 0,0%). La probabilité d'extinction annuelle moyenne était généralement faible et similaire pour tous les types de zones ($\varepsilon_{\text{expansion}}$ = 5,8 ± 1,2 % ; $\varepsilon_{\text{persistance}}$ = 5,8 ± 0,2 % ; $\varepsilon_{\text{potentiel}}$ = 6,9 ± 0,1 % ; $\varepsilon_{\text{régression}}$ = 6,4 ± 0,0 %). La probabilité d'occupation annuelle moyenne était toujours supérieure à 80% quel que soit le type de zone ($\psi_{\text{expansion}}$ = 90,0 ± 3,6% ; $\psi_{\text{persistance}}$ = 88,3 ± 3,4% ; $\psi_{\text{régression}}$ = 81,0 ± 4,5%). Les probabilités de colonisation et d'occupation pour la zone de recolonisation potentielle ($\gamma_{\text{potentiel}}$ = 49,5 ± 0,1% ; $\psi_{\text{potentiel}}$ = 82,1 ± 4,7%) étaient relativement plus élevées que les valeurs obtenues pour la zone de régression. En outre, 48 % (103 141 km^2) de la zone de recolonisation potentielle considérée a une probabilité de plus de 50 % d'être colonisée.

Choix du site de reproduction et influence sur le nombre d'individus

Le nombre d'adultes et de jeunes a été obtenu par des observations directes sur 144 sites de reproduction pour 63 meutes, entre 1986 et 2021. La taille minimale observée des meutes était en moyenne de 7,9 ± 2,4 loups, dont 4,6 ± 1,6 petits et 3,1 ± 1,6 adultes.

Pour les modèles sur les jeunes, les modèles nuls pour tous les tampons se sont classés en premier, et les modèles construits ont eu des résultats similaires entre les tampons de 1 et 2 km. Parmi les modèles incluant des covariables, les meilleurs modèles expliquant le nombre de jeunes étaient principalement liés à la disponibilité des refuges. Bien que non significatives, les distributions postérieures des covariables dans ces modèles étaient très similaires, avec une tendance à un effet positif sur le nombre de jeunes : rugosité (β = 0,04 ± 0,05), proportion de refuge (β = 0,04 ± 0,05), et densité de la rivière (β = 0,02 ± 0,04). Les modèles de disponibilité de la nourriture ont été classés ensuite avec des résultats similaires, bien que les densités de bétail et de proies sauvages n'aient pas affecté le nombre de jeunes (β = 0,00 ± 0,04). Parmi tous les modèles, la densité des routes non alphaltées était la seule covariable ayant une influence significative (par exemple, β = -0,11 ± 0,05). Lorsque les covariables de perturbation humaine ont été incluses, bien que non significatives, la proportion d'établissements humains (β = -0,07 ± 0,05) et la densité de proies sauvages (β = -0,04 ± 0,05) ont eu tendance à avoir un effet négatif, et la densité du bétail un effet positif (β = 0,04 ± 0,05).

Les deux meilleurs modèles expliquant le nombre d'adultes étaient pour les tampons de 2 et 5 km liés à la vulnérabilité anthropique. La rugosité (β = -0,20 ± 0,06) et le refuge (β = 0,19 ± 0,07) ont influencé de manière significative le nombre d'adultes pendant la saison de reproduction. Le modèle complet et le modèle global de vulnérabilité anthropique dans la zone tampon de 1 km étaient les troisième et quatrième modèles qui expliquaient le mieux le nombre d'adultes. Ces modèles ont donné des résultats similaires, les mêmes covariables influençant le nombre d'adultes sur les sites de reproduction, avec un effet négatif pour la densité de la population humaine (β = -0,14 ± 0,08), la rugosité (β = -0.12 ± 0,07), la densité des meutes (β = -0,12 ± 0,06), la densité des routes asphaltées (β = -0,11 ± 0,06) et les zones brûlées (β = -0,10 ± 0,07) ; et un effet positif pour la proportion de refuges (β= 0,13 ± 0,08).

Dynamique de la population dans le nord-ouest du Portugal (1996 - 2016)

La taille de la population estimée à partir des comptages minimaux était en moyenne de 27,0 ± 2,1 (SE) loups par an pendant l'été-automne entre 1996 et 2016, ce qui correspond à une densité moyenne globale de 1,7 ± 0,1 loups/100km^2 . Le taux de croissance global de cette population de loups était de 1,04 ± 0,76 entre 2006 et 2016, avec une tendance à la baisse entre 1996 et 2005 (taux de croissance : 0,92 ± 0,09) et une tendance à la hausse entre 2007 et 2016 (taux de croissance : 1,16 ± 0,11). Le sex-ratio global de la population était de 1,1:1,0 (M:F). Parmi les individus identifiés génétiquement, 60 % ont été considérés comme des résidents d'une seule meute, 11 % comme des disperseurs et 29 % comme des individus non catégorisés.

Sur l'ensemble de la période d'échantillonnage (1996-2016), deux meutes ‘source’ ont été détectées chaque année (taux de persistance du groupe : 100 % ; n = 20 ans), tandis que quatre meutes ‘puits’ n'ont pas été détectées pendant 6 à 12 ans, ce qui a donné des taux de persistance du groupe de 70 %, 70 %, 40 % et 50 %. Le succès de la reproduction pour chaque meute a varié de 36% à 95%, avec une moyenne globale de 75,9 ± 9,8%, en considérant un total de 86 meutes-années sur 20 ans. La taille moyenne des meutes en été-automne était de 6,2 ± 0,3 loups, et la taille moyenne des meutes sans reproduction était de 2,7 ± 0,2 individus. Si l'on considère uniquement les meutes avec reproduction, la taille moyenne de la meute était de 7,2 ± 0,3 loups, dont 3,8 ± 0,2 adultes et 3,3 ± 0,2 petits. La plus grande taille de meute de 16 individus (8 adultes et 8 chiots) a été détectée dans une meute ‘source’.

Au total, 18 événements de dispersion ont été détectés entre les meutes surveillées, impliquant 17 individus, dont 9 mâles et 8 femelles (sex-ratio : 1,1:1,0). Cinq disperseurs ont été détectés à la fois par analyse moléculaire et par télémétrie GPS, et les 12 restants uniquement par analyse moléculaire non invasive. La distance minimale estimée parcourue par les disperseurs était en moyenne de 24,8 ± 1,2 km. Les valeurs du taux de persistance du groupe, du succès de la reproduction, de la taille de la meute, du nombre d'adultes et du nombre de petits étaient significativement différentes entre les meutes ‘source’ et les meutes ‘puits’, les meutes ‘puits’ présentant un sex-ratio moyen biaisé par les mâles (1,5:1).

Un modèle de population intégré pour les loups du nord-ouest du Portugal (2007-2019)

Sur la base des résultats naïfs des points de hurlement, des observations, du piégeage photographique, des captures pour le marquage GPS et de l'identification moléculaire des individus, la taille moyenne minimale annuelle observée de la population était de 34,8 ± 13,3 loups (13-61) dans le nord-ouest du Portugal (Alto Minho), avec un taux de croissance naïf moyen entre 2007 et 2019 de 1,17 ± 0,40 (0,63-1,85). Le nombre annuel moyen observé d'adultes et de jeunes était respectivement de 18,5 ± 7,2 (6-31) et de 12,4 ± 5,3 (4-20). Une moyenne de 4,8 ± 1,6 meutes (2-7) a été détectée chaque année, dont la reproduction a été confirmée dans 3,7 ± 1,4 (1-6). La proportion annuelle moyenne de femelles reproductrices parmi les adultes était de 0,17 ± 0,06 (0,09-0,29). La fécondité moyenne était de 3,4 ± 1,8 jeunes lorsque toutes les méthodologies de terrain étaient prises en compte et de 3,9 ± 2,4 uniquement avec les observations directes.

Le jeu de données pour le modèle de capture-recapture comprenait 205 individus, dont 144 ont été détectés une fois, 30 deux fois, et le reste a été détecté jusqu'à 7 fois au cours des 13 années d'échantillonnage. Nous avons obtenu huit reprises de loups marqués au GPS qui étaient morts. Selon le modèle de population intégré, la taille moyenne estimée de la population et le taux de croissance annuel étaient respectivement de 35,8 ± 9,6 (22-54) et de 1,06 ± 0,15 (0,87-1,29). Le modèle a estimé une moyenne annuelle de 21,7 ± 5,7 (14 - 32) adultes et 13,6 ± 4,4 (8-24) jeunes dans la population, et un taux de survie moyen des adultes de 72% (95% BCI : 66-77%) et des jeunes de 53% (95% BCI : 30-71%). La fidélité des adultes à la zone d'étude a été estimée à 87% (95% BCI : 79-94%) et la survie apparente des adultes à 62% (95% BCI : 52-72%). La probabilité de détection d'un individu était de 44% et celle de reprise d'un individu mort était de 6%.

L'analyse de la viabilité de la population suggère que si la survie des adultes se maintient à 72% (c'est-à-dire la moyenne estimée sur la période d'échantillonnage), la taille moyenne annuelle estimée de la population pour la période de prévision (2020-

2029) serait de 59,9 ± 7,7, avec une moyenne de 36,6 ± 4,7 adultes et 21,9 ± 2,6 jeunes. D'ici 2029, la probabilité d'extinction des loups dans l'Alto Minho serait de 0,1 % et la probabilité de quasi-extinction (c'est-à-dire une population de cinq loups) de 1,2 %. Si l'on considère l'analyse de la viabilité de la population avec différents scénarios de taux de survie, si le taux de survie des adultes diminue de 10 %, les probabilités d'extinction et de quasi-extinction augmentent respectivement de 1 % et de 7 %. Si le taux de survie des adultes diminue de 20 %, ces probabilités passent à 6 % et 23 %, respectivement. La variation du taux de survie des petits pour la période de prévision a eu peu d'effet sur la taille de la population, le taux de croissance, le nombre d'adultes et de jeunes et, par conséquent, sur les probabilités d'extinction.

7.3. Discussion

Le modèle d'occupation dynamique a identifié la rugosité, la densité des routes non asphaltées et l'effort influençant la probabilité de détection des fécès. Ainsi, la probabilité de colonisation augmente avec l'altitude, la densité du bétail et la densité des routes non asphaltées, et avec la diminution de la proportion de zones brûlées et de la densité des routes nationales/régionales et locales. La probabilité d'extinction diminue avec l'augmentation de la densité des routes non asphaltées. En évaluant la dynamique de l'aire de répartition du loup au cours des vingt dernières années en Ibérie, les probabilités d'occupation et de colonisation étaient plus élevées dans les zones où l'espèce a persisté ou s'est étendue que dans les zones où l'espèce s'est éteinte ou dans les environs de l'aire de répartition de l'espèce. Cependant, les faibles probabilités d'occupation et de colonisation dans certaines zones où l'espèce est présente suggèrent que des efforts sont nécessaires pour améliorer l'habitat et réduire les perturbations anthropogéniques. Les zones où l'espèce s'est éteinte au cours des vingt dernières années ou celles où la probabilité d'occupation est élevée dans les environs de l'aire de répartition de l'espèce peuvent être des indicateurs potentiels de conflits plus importants liés à la présence de loups et au braconnage. Ces résultats indiquent que d'autres facteurs non pris en compte dans cette étude peuvent intervenir, y compris d'autres facteurs environnementaux et anthropogéniques et des facteurs sociaux liés aux conflits entre le loup et l'homme. Sur la base des probabilités de colonisation et d'occupation, une carte d'adéquation de l'habitat a été élaborée avec les zones prioritaires identifiées pour la mise en œuvre d'actions de conservation et de mesures d'atténuation.

En évaluant les caractéristiques autour des sites de reproduction et les variations du nombre de loups, les résultats montrent que la disponibilité des refuges influence positivement le nombre d'adultes à toutes les tailles de zones tampons (1, 2 et 5 km). La densité de la population humaine, la densité des routes asphaltées, la proportion de zones brûlées, la rugosité et la densité des meutes influencent négativement le nombre d'adultes, en particulier dans les zones tampons les plus petites. Bien que les résultats indiquent qu'une plus grande disponibilité de refuges et une plus faible densité de routes non asphaltées augmentent le nombre de jeunes, des recherches complémentaires sont nécessaires pour évaluer les facteurs qui influencent le nombre de jeunes. Cette étude révèle l'importance des zones avec suffisamment de refuges et peu de perturbations humaines pour la persistance des loups, en particulier sur les sites de reproduction, car les loups peuvent être particulièrement exposés et sensibles pendant la saison de reproduction.

Selon les résultats d'une étude de suivi des loups à long terme dans le nord-ouest du Portugal, le nombre de loups dans cette zone d'étude a diminué entre 1996 et 2005, avec seulement deux des six meutes restantes à la fin de cette période. Ce déclin était probablement lié à la persécution directe des loups et à la faible connectivité de l'habitat entre les meutes. Depuis 2007, les loups se rétablissent et recolonisent la zone d'étude, deux meutes ‘source’ jouant un rôle crucial dans le rétablissement et le maintien de plusieurs meutes voisines. Par la suite, les meutes ont facilité la dispersion par le biais d'un processus en pas japonais (‘stepping-stone’). Les meutes ‘source’ ont montré des valeurs plus élevées de persistance de groupe, de succès de reproduction et de taille de meute par rapport aux meutes ‘puits’, qui se sont éteintes au cours de plusieurs années. En accord avec les études précédentes en Ibérie, les résultats montrent une dispersion limitée dans la zone d'étude, avec une faible distance moyenne de dispersion (24,8 km), de faibles taux de dispersion parmi les individus détectés (11 %), et de faibles taux d'émigration hors de la zone d'étude (13 %), soulignant l'impact probable des paysages dominés par l'homme sur la dispersion des loups et la structure de la population.

Le modèle de population intégré a montré quelques fluctuations dans la taille de la population entre 2007 et 2019, avec un taux de croissance global de (1,06 ± 0,15), suggérant une tendance positive globale. En outre, le modèle a estimé un taux de mortalité des adultes de 28 %, un taux de mortalité apparent des adultes de 38 % et un taux de mortalité des petits de 47 %. Bien que la taille de la population ait montré une tendance positive, les taux de mortalité apparente des adultes obtenus ici étaient plus élevés que ceux d'autres populations ayant une tendance négative. En prévoyant la croissance de la population sur dix ans (2020-2029) en fonction de différents scénarios de taux de survie, les résultats soulignent que la survie des adultes est un facteur clé de la croissance et de la survie à long terme de cette population.

Les approches utilisées dans cette thèse suggèrent que les loups dans les paysages dominés par l'homme sont soumis à une forte pression anthropogénique. L'exposition constante aux humains a un impact évident sur la persistance des loups en raison de la faible disponibilité des refuges, des fortes densités d'infrastructures linéaires et des activités humaines constantes. L'influence de la densité du bétail sur la dynamique de l'occupation des loups et le nombre d'individus dans les meutes souligne la nécessité d'évaluer les facteurs sociaux et les dimensions humaines, ainsi que les causes et les taux de mortalité illégale liés aux conflits associés à la déprédation du bétail.

Cette thèse souligne l'importance de comprendre la dynamique des meutes et d'identifier les meutes ‘source’ et secondaires dans les zones d'étude, car la persistance des groupes et les taux de reproduction varient d'une meute à l'autre. Cette étude a montré que la dispersion joue un rôle vital dans la persistance et le rétablissement des populations de loups. Cependant, la dispersion en Ibérie est limitée, probablement en raison des caractéristiques particulières des zones perturbées par l'homme qui empêchent une dispersion réussie. En outre, la forte influence des taux de mortalité des loups sur la viabilité d'une population, en particulier des taux de mortalité des adultes, souligne également la nécessité d'évaluer davantage les causes et les taux de mortalité des loups. Bien qu'une partie de la mortalité des loups puisse être due à des accidents de la route ou à des maladies (par exemple la gale sarcoptique ou le virus de la maladie de Carré), la plupart des causes de mortalité sont probablement des persécutions directes et des morts en représailles de la part des humains en raison de conflits résultant de déprédations sur le bétail. Il est donc nécessaire d'évaluer la mortalité des loups causée par l'homme et sa relation avec les conflits sociaux. Enfin, des recherches supplémentaires sont nécessaires pour évaluer et estimer la fécondité, la taille des portées et la survie des jeunes dans les paysages dominés par l'homme afin de mieux comprendre la dynamique des populations et d'obtenir des paramètres démographiques plus précis. Bien que les estimations de survie soient robustes, les contraintes méthodologiques, le caractère insaisissable des espèces ou le braconnage cryptique peuvent entraver l'obtention d'estimations de survie.

7.4. Conclusion générale

Des actions de conservation des grands carnivores et des mesures d'atténuation sont proposées sur la base des résultats de cette thèse. L'identification des meutes ‘source’ et des meutes ‘puits’, la cartographie des zones prioritaires de conservation et la connaissance des caractéristiques de l'habitat sur les sites d'origine qui augmentent le nombre d'adultes et des taux vitaux qui limitent la persistance des loups aident à sélectionner où et quelles actions de conservation et mesures d'atténuation sont nécessaires. En tant qu'espèce généraliste, le loup peut être très dynamique et s'adapter à différentes conditions. Ces mesures dépendent donc du contexte et doivent être mises en œuvre au cas par cas. Toutefois, une stratégie consensuelle et collaborative est nécessaire car les loups peuvent couvrir de vastes zones avec des stratégies de gestion différentes en fonction des frontières politiques.

Les priorités pour la conservation des loups dans les paysages dominés par l'homme suggérées dans cette thèse comprennent principalement l'amélioration de la gestion du paysage (par exemple, en augmentant la disponibilité des refuges, en améliorant la connectivité de l'habitat entre les meutes et les autres populations de loups, et en mettant en œuvre des mesures d'atténuation et de conservation pour éviter les perturbations humaines) et la réduction des conflits entre l'homme et les grands carnivores (par exemple, en menant des campagnes de sensibilisation parmi les communautés locales sur la coexistence et en promouvant des mesures de prévention des dommages causés par le bétail). L'implication de toutes les parties prenantes, y compris les chercheurs, les propriétaires de bétail, les chasseurs, les secteurs public et privé, les institutions non gouvernementales et les politiciens, est cruciale.

Cette thèse fournit des connaissances et des outils pertinents pour la conservation des grands carnivores dans les paysages dominés par l'homme. Elle offre un aperçu de l'utilisation de l’espace par les grands carnivores en identifiant les facteurs affectant l'occupation, la colonisation et l'extinction des loups et en comblant le fossé entre l'habitat favorable sur les sites de reproduction et les paramètres démographiques de la population. Cette thèse met à disposition un outil précieux qui identifie les zones prioritaires pour la mise en œuvre d'actions de conservation et de mesures d'atténuation dans l'aire de répartition de l'espèce et dans les zones de colonisation dans un avenir proche. Enfin, cette thèse révèle la dynamique de la population et les estimations des paramètres démographiques des taux vitaux précédemment inconnus pour un grand carnivore dans les paysages dominés par l'homme en Ibérie.

Bien que cette thèse ait comblé certaines lacunes, la persistance des grands carnivores peut dépendre du contexte, en particulier chez les espèces généralistes. Ainsi, cibler des paysages hétérogènes avec des paramètres démographiques et des conditions écologiques variables est très pertinent pour comprendre la dynamique des populations et identifier les facteurs limitant la persistance des grands carnivores. À la lumière des conclusions de cette thèse, plusieurs lignes de recherche futures sont proposées, y compris des procédures méthodologiques et l'évaluation de paramètres pour améliorer la conservation et la gestion des grands carnivores dans les paysages dominés par l'homme.

Les conflits entre les grands carnivores et les humains surviennent souvent dans les paysages dominés par l'homme, ce qui entraîne des représailles contre les grands carnivores. Pour que les humains et les grands carnivores puissent coexister avec succès dans les paysages dominés par l'homme, il faut qu'ils s'adaptent les uns aux autres. L'implication de toutes les parties prenantes est donc cruciale. Les études menées dans le cadre de la présente thèse soulignent la nécessité d'évaluer les causes et les taux de mortalité anthropique des grands carnivores et d'inclure dans la recherche les facteurs liés aux conflits sociaux et aux dimensions humaines. Ainsi, une meilleure connaissance de l'écologie des grands carnivores, de leur comportement, de la génétique des populations, de la dynamique des populations et de la démographie en tenant compte des facteurs sociaux est nécessaire pour améliorer la coexistence dans les paysages dominés par l'homme.

Appendix

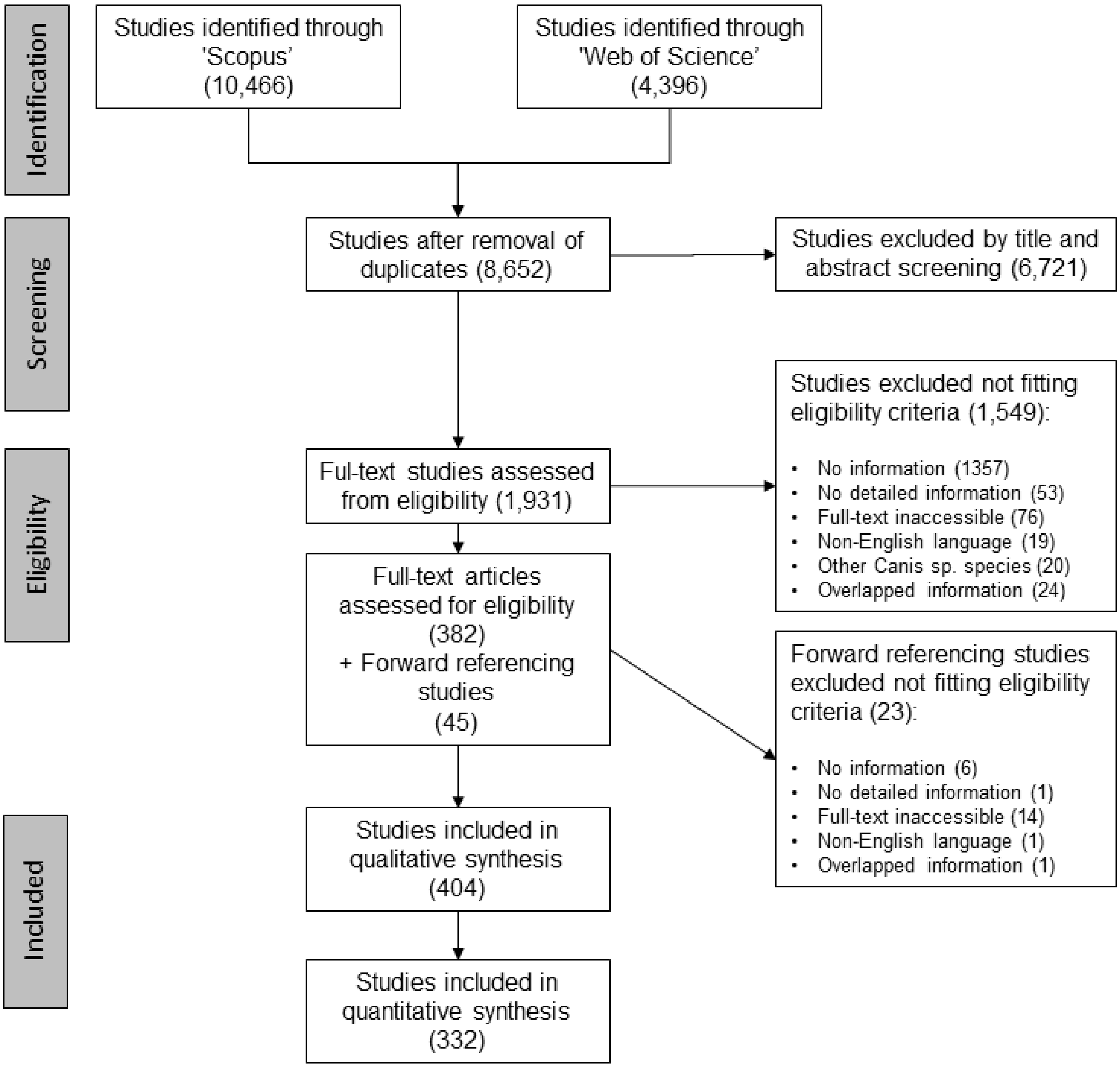
Appendix I – Supplementary data for Chapter I

Sup. Table 1: Literature review search on demographic parameters in wolf studies, performed in SCO (Scopus; (https://www.scopus.com) and WOS (Web of Science; (https://www.webofknowledge.com) on the 31st of December 2020, keyword search and the number of studies detected by search ('N. studies') and by demographic parameter after the removal of duplicates ('Final'). After the removal of all duplicates, 8,652 studies were screened for title and abstract subjects using 'revtools' R Package (Martin et al., 2019).

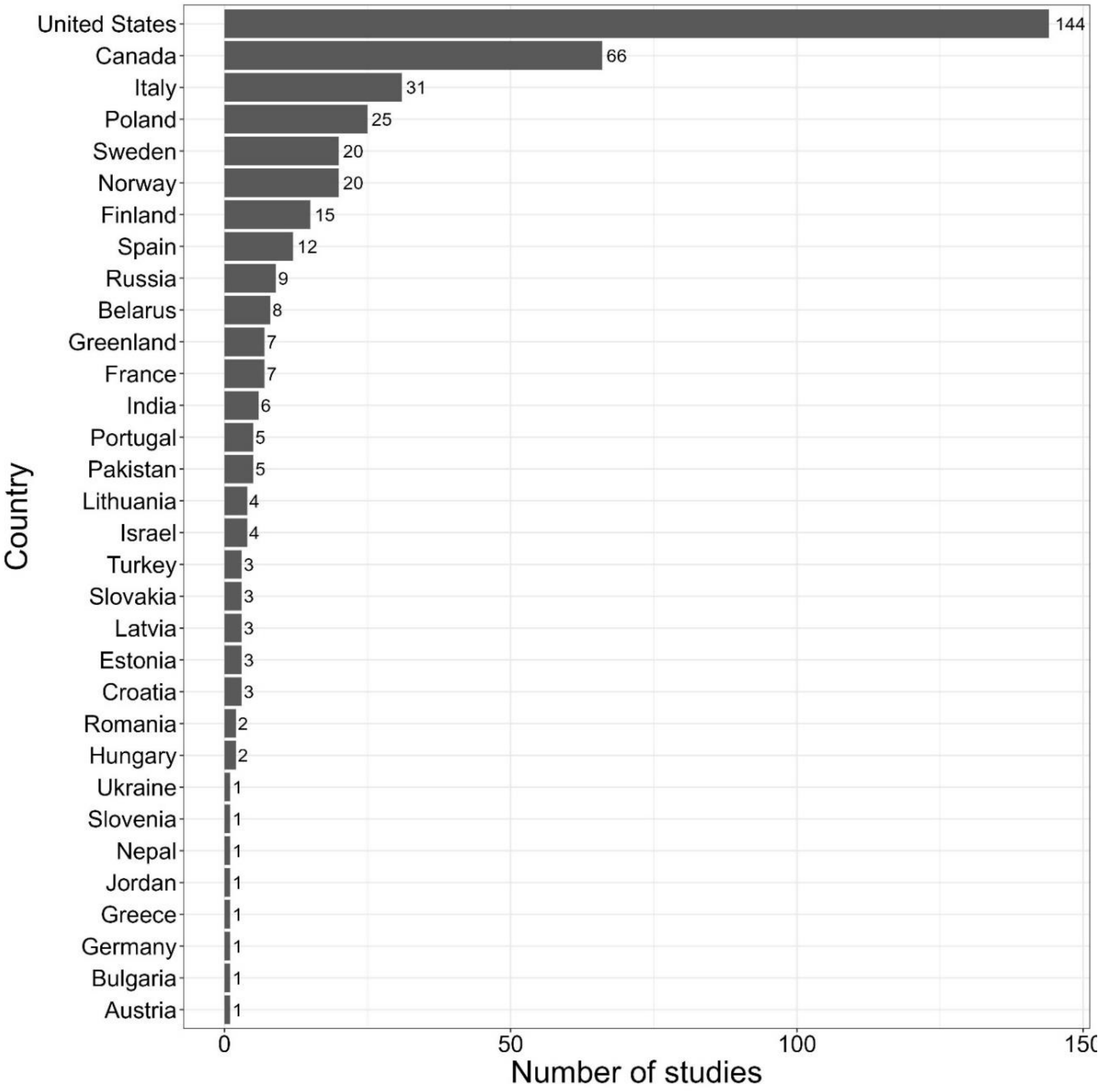
Parameters	**Search fields**	**Search engine**	**Keyword search** [all searches included: AND "Canis lupus" OR "Grey Wolf" OR "Gray Wolf"]	**N. studies**	**Final**
• Density	All manuscript	SCO	(wolf W/3 density) OR (population W/3 density)	2219	2764
	All manuscript	WOS	(density OR "wolf density" OR "Population density")	674	
	Title, abstract, keywords	WOS	((wolf NEAR/3 density) OR (population NEAR/3 density))	701	
• Litter Size / Fecundity • Number pups • Number adults • Pack size • Sex ratio	All manuscript	SCO	"litter size" OR "fecundity" OR (number W/3 pup) OR "number of pup" OR (number W/3 adult) OR "number of adults" OR (wolf W/3 number) OR "number of individual" OR (pack W/3 size) OR (group W/3 size) OR "Sex ratio"	2752	2921
	All manuscript	WOS	("litter size" OR "fecundity" OR "pup number" OR "number of pup" OR "number of adults" OR "wolf number" OR "number of wolf" OR "number of individual" OR "pack size" OR "size of pack" OR "group size" OR "size of group" OR "Sex ratio")	215	
	Title, abstract, keywords	WOS	("litter size" OR "fecundity" OR (number NEAR/3 pup) OR "number of pup" OR (number NEAR/3 adult) OR "number of adults" OR (wolf NEAR/3 number) OR "number of individual" OR (pack NEAR/3 size) OR (group NEAR/3 size) OR "Sex ratio")	517	
• Mortality/survival rate • Dispersal • Migrants • Migration ratio • Proportion emigrants • Proportion Immigrants	All manuscript	SCO	(mortality W/3 rate) OR (surviv* W/3 rate) OR (surviv* W/3 probability) OR dispers* OR (dispers* W/3 rate) OR (dispers* W/3 probabilit*) OR "migrant" OR migrati* W/3 rate OR migrati* OR immigra* OR emigra*	3599	4425
	All manuscript	WOS	("mortality rate" OR "rate of mortality" OR "surviv* rate" OR "surviv* probability" OR "probability of surviv*" OR surviv* OR mortality OR dispers* OR "dispers* rate" OR dispers* probability OR probability of dispers* OR "migrant" OR "migrat* rate" OR migrat* OR immigra* OR emigra*)	976	
	Title, abstract, keywords	WOS	((mortality NEAR/3 rate) OR (surviv* NEAR/3 rate) OR (surviv* NEAR/3 probability) OR dispers* OR (dispers* NEAR/3 rate) OR (dispers* NEAR/3 probabilit*) OR "migrant" OR (migrati* NEAR/3 rate) OR migrati* OR immigra* OR emigra*)	823	
• Probability adoption • Probability budding • Carrying capacity	All manuscript	SCO	adopt* OR (adoption W/3 probability) OR budd* OR (budd* W/3 probabilit*) OR "Carrying capacity"	1896	1969
	All manuscript	WOS	(adopt* OR "adopt* probability" OR "probability of adopt*" OR budd* OR "budd* probability" OR "probability of budd*" OR "Carrying capacity")	228	
	Title, abstract, keywords	WOS	(adopt* OR (adoption NEAR/3 probability) OR budd* OR (budd* NEAR/3 probabilit*) OR "Carrying capacity")	262	

Sup. Table 2: List and description of the 37 parameters on wolf population dynamics and demography extracted from the systematic literature review. Ages were defined as: pup (≤1 year old), subadult (1-2 year old), and adult (≥ 2 year old; or ≥ 1 year old when undistinguished).

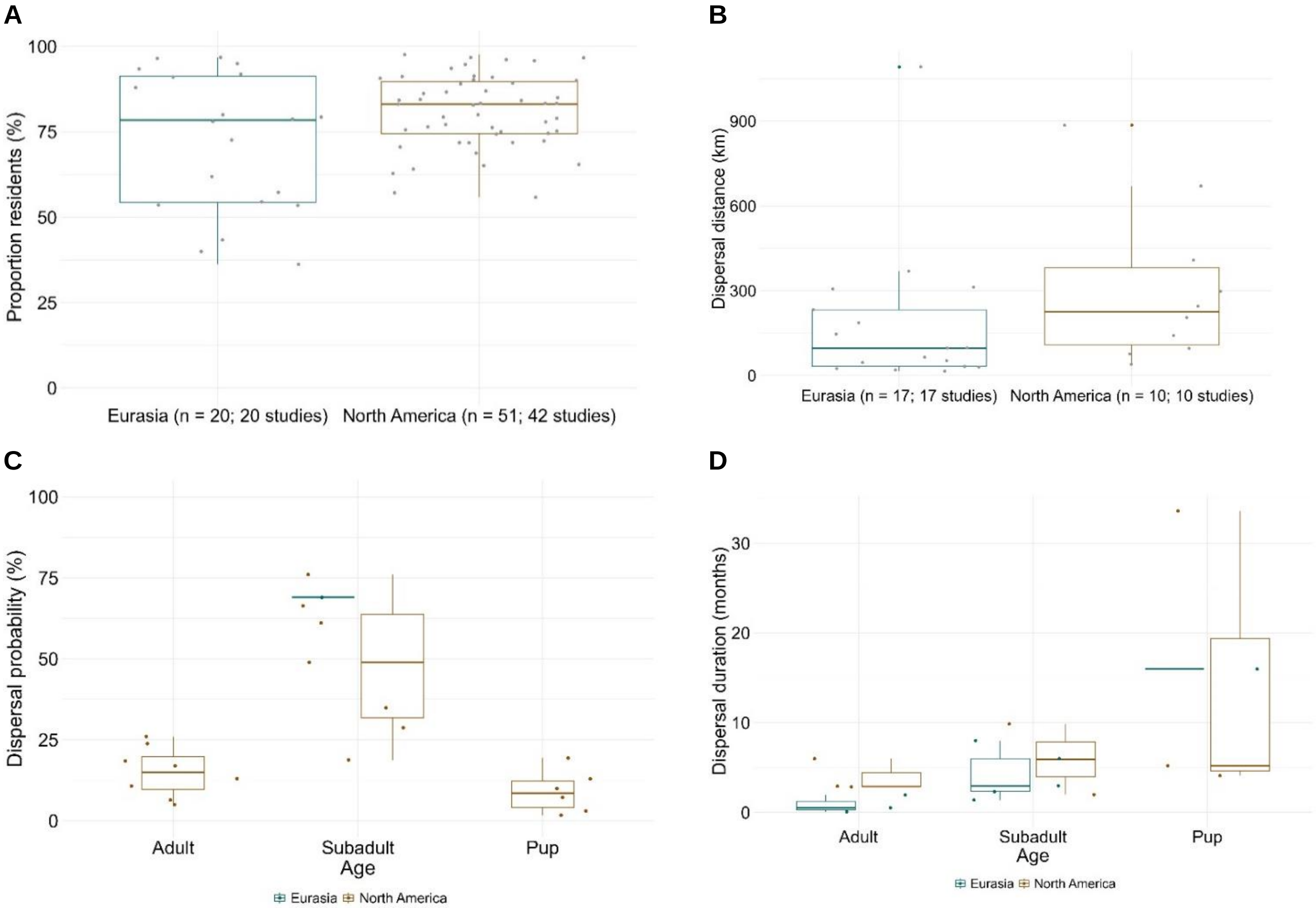
Parameter level	Parameter group	Parameter (units)	Description
Population	Density	Density (wolves/1000 km^2)	Number of wolves estimated per 1000 km^2
	Growth rate	Growth rate (%)	Annual population growth rate
	Carrying capacity	Carrying capacity (wolves)	Maximum number of wolves that the study area can support
	Proportion residents	Proportion of residents (%)	Proportion of residents in the population
	Age structure	Age structure (%)	Proportion of pups, subadults and adults in the population
	Sex ratio	SR population (M:F)	Sex ratio of the overall population
	Mortality rate	MR population (%)	Overall mortality rate of the population
	Migration	Migration, Immigration and Emigration rates (%)	Proportion of detected individuals that migrate, immigrate and emigrate
		N immigrant or emigrant per generation (wolves)	Number of immigrants or emigrants per generation
Pack	Pack size	Pack size (wolves)	Number of individuals detected per pack
	Sex ratio	SR pack (M:F)	Sex ratio of packs
	Recruitment	Recruitment rate (%)	Annual rate of individuals recruited
Age /pack	N Individuals	Litter size (wolves)	Number of pups detected per pack (<2 months old)
		N pup (wolves)	Number of pups per pack
		N subadult (wolves)	Number of subadults per pack
		N adult (wolves)	Number of adults per pack
Age	Sex ratio (SR)	SR pup (M:F)	Sex ratio of litters/pups
		SR subadult (M:F)	Sex ratio of subadults
		SR adult (M:F)	Sex ratio of adults
	Mortality rate (MR)	MR pup 0-8 month (%)	Mortality rate of pups until the first 8 months of age
		MR pup 8-12 month (%)	Mortality rate of pups between 8-12 months of age
		MR pup (%)	Annual mortality rate of pups
		MR subadult (%)	Annual mortality rate of subadults
		MR adult (%)	Annual mortality rate of adults
Disperser /age	Dispersal duration	D duration pup (km)	Dispersal distance of pups
		D duration subadult (km)	Dispersal distance of subadults
		D duration adult (km)	Dispersal distance of adults
	Dispersal probability	D probability pup (%)	Dispersal probability of pups
		D probability subadult (%)	Dispersal probability of subadults
		D probability adult (%)	Dispersal probability of adults
Disperser /Lone	Sex ratio	SR disperser (M:F)	Sex ratio of dispersers
	Mortality rate	MR disperser (%)	Annual mortality rate of dispersers
		MR lone (%)	Annual mortality rate of lone wolves
	Dispersal distance	D distance (km)	Overall dispersal distance



Sup. Fig. 1**:** PRISMA flow chart showing the exclusion process and the number of studies included in the review. Eligibility criteria: Full text available; English language; Peer reviewed (e.g., excluded thesis, reports or conference proceedings); *Canis lupus* species (e.g., excluded *C. lycaon* or *C. rufus*); With information on the population dynamic and demographic parameters or with information to obtain it, and with detailed information on the study area location and methods; In case of overlapped information between studies, we included the most recent or the one with more data available. Only studies with average values were included in the quantitative synthesis.



Sup. Fig. 2**:** Number of studies per country with information on the parameters searched in the literature review.



Sup. Fig. 3: Boxplots for: A) the proportion of residents in the population: B) dispersal distance per continent, C) dispersal probability by age, and C) dispersal duration by age. Ages are defined as: ‘pup’ ≤1 year old; ‘subadult’ 1–2 years old; and ‘adult’ ≥ 2 years old. Boxplots for Eurasia (green) and North America (brown).

Appendix II – Supplementary data for Chapter II

The supplementary data to the article can be found online at:
https://doi.org/10.1016/j.biocon.2023.110316

Supplementary Material

Manuscript Title: Insights into the dynamics of wolf occupancy in human-dominated landscapes

Appendix A1 – Methodological details on the dynamic occupancy model

According to the available information and the variation of the covariates between 2005 and 2022, we used human population density and the proportion of agricultural lands, refuge, and burned areas as yearly site covariates (i.e. vary through the years). We included the remnant covariates as site covariates (i.e. constant through the years). We used RStudio (Posit team, 2022) to manage the datasets of covariates and obtain the values for each site.

Before the analysis, we assessed pairwise covariate relationships using Spearman's correlation coefficient. The covariates of ruggedness, human settlements, and refuge were highly correlated with each other and with agricultural lands. The covariate of human settlements was also highly correlated with annual population density. As our main interest was to identify anthropogenic covariates affecting wolf spatial use, ruggedness, human settlements, and refuge covariates were excluded from the ecological submodel (see below).

We defined $y_{i,j,t}$ as the observed state of site *i* during season *j* in the year *t*, which was set to 1 when wolf presence was confirmed (regardless of the number of wolf signs detected), and 0 otherwise. We considered a state-space formulation of the dynamic occupancy model (Royle and Kéry, 2007), which incorporates an ecological submodel describing the latent ecological state of a site (i.e. occupied or not) and an observation submodel describing the probability of detection process. Thus, the colonization probability $\gamma_{i,t}$ is defined as the probability that an unoccupied site *i* during year *t* becomes occupied during year *t+1*, while the extinction probability $\varepsilon_{i,t}$ refers to the probability that an occupied site *i* during year *t* becomes unoccupied during year *t+1*.

We were interested in the dynamics of wolf occurrence in a given area. To do this, we assume that: i) the detection of scat markings confirms the presence of the species and reflects sites that wolves preferentially use or scent mark, and ii) the non-detection of scat markings reflects unused or non-preferentially used nor scent marked sites. Thus, we highlight that the meaning of the terms' colonization' and 'extinction' used for interpreting the occupancy model results are not actual colonization and extinction of the species but instead refer to a probability of a site becoming, respectively, used and unused from one year to another.

We defined $z_{i,1}$ as the initial latent state of site *i* as being drawn from a Bernoulli distribution with the success probability being $\psi_{i,1}$:

$$z_{i,1} \sim Bernoulli\ (\psi_{i,1}) \qquad \text{Eq. (A1)}$$

All the other latent states $z_{i,t}$ for $t > 1$ are drawn from a Bernoulli distribution as:

$$z_{i,t} \mid z_{i,t-1} \sim Bernoulli\ (z_{i,t-1}\left(1-\varepsilon_{i,t-1}\right) + \left(1-z_{i,t-1}\right)\gamma_{i,t-1}) \qquad \text{Eq. (A2)}$$

Environmental and anthropogenic covariates were not included for the initial occupancy parameter since we only sampled 68 sites (out of 395) in the first year of the study. We used the same static site covariates and yearly site covariates for colonization and extinction parameters.

For the observation process, specified conditional on the latent process $z_{i,t}$, the detection/non-detections are drawn from a Bernoulli distribution:

$$y_{i,j,t} \mid z_{i,t} \sim Bernoulli\ (z_{i,k}\, p_{i,j,t}) \qquad \text{Eq. (A3)}$$

where $p_{i,j,t}$ is the probability that the species is detected at a site *i* for a season *j* during year *t*.

Priors for the parameters ψ, γ, ε, and *p* included: for intercepts (α) normal distribution with a mean of 0 and variance (σ^2) 0.1, and random effects normal distribution with a mean of 0 and σ^2 with a uniform distribution between 0 and 25.

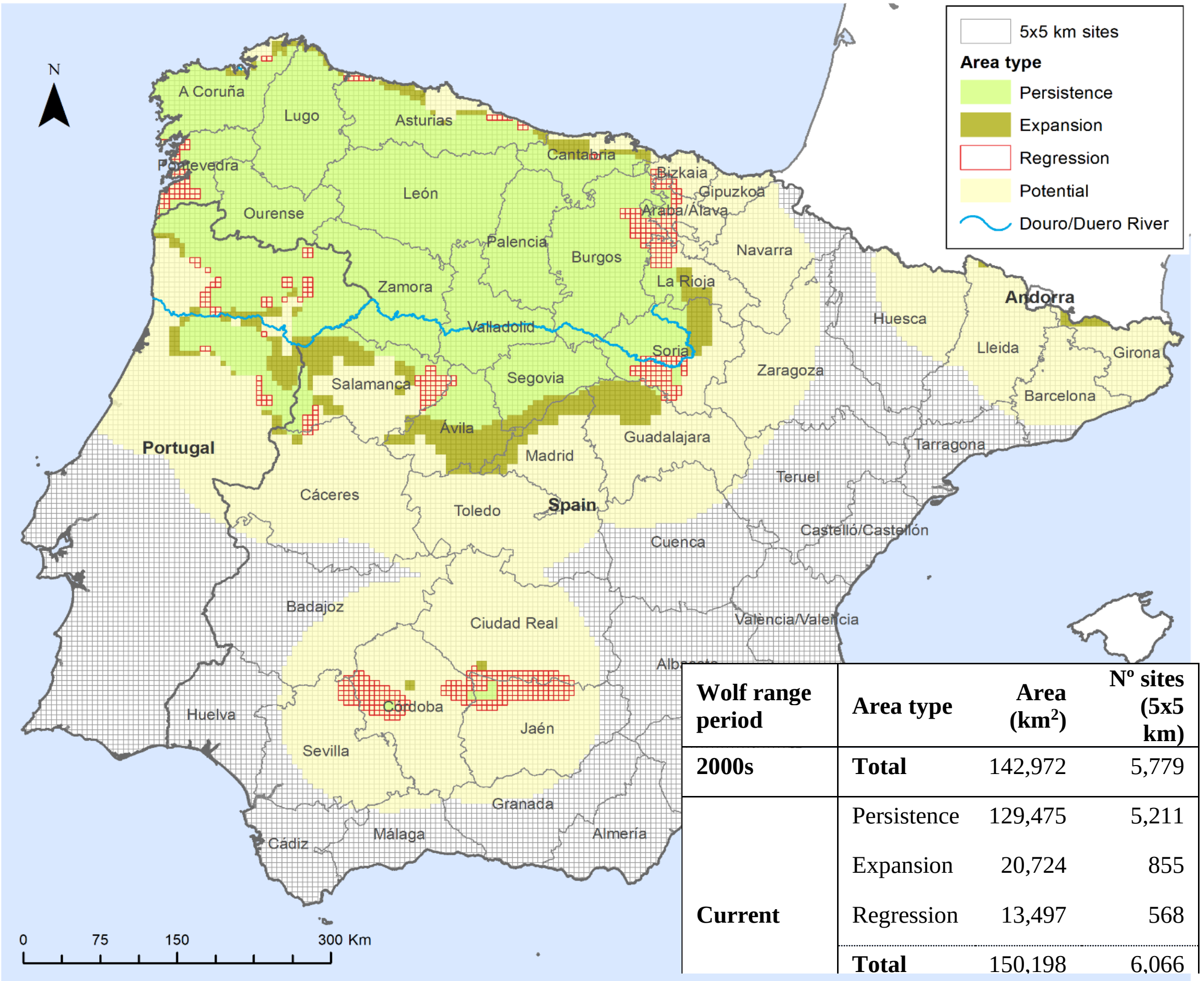
For the colonization and extinction parameters, we applied shrinkage on regression coefficients (β_i) using Laplace priors (i.e. double-exponential or L1 regularisation; van

Erp et al., 2019). This penalization method offers better model performance and allows us to get better parameter estimates (Hooten and Hobbs, 2015).

Considering the sampling methodology and wolf scent-marking behaviour, we included effort (transect length), unpaved road density, and average ruggedness as covariates for the detection parameter. We included the effort as an observation site covariate (i.e. varies per season) and unpaved road density and ruggedness as static site covariates.

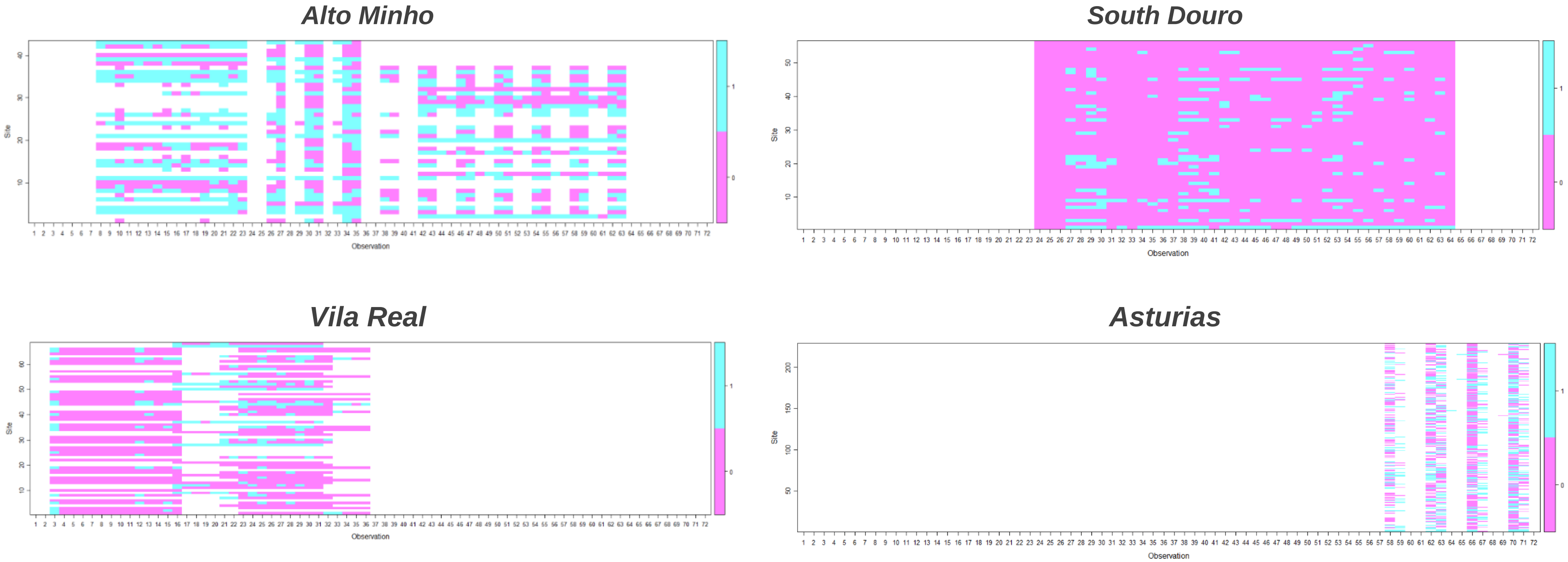
Appendix A2 – Methodological details on the detection rate evaluation

To further evaluate the detectability of the species by transects, considering that a GPS-collared wolf location confirms the presence of the species at a site, we took advantage of the available locations of 15 GPS-collared wolves between December 2007 and February 2017 in Alto Minho study area (Rio-Maior et al., 2019). Capturing and tagging wolves with GPS collars is the most accurate method to study wolf spatial use. However, continuous monitoring is rarely possible due to the high costs, high capture effort, and low capture or recapture success. Although transects of sign survey detect spatial use of several wolves, we performed this additional evaluation because results may be biased due to different marking behaviour between wolves with different social status. For instance, dispersal individuals may be more challenging to detect since these are usually a small part of the population, do not have a defined territory, and may not mark as often as residents (Marucco et al., 2009). Therefore, we obtained the seasonal proportion of sites with presence confirmed by GPS-collared wolves that had successful detection by transects. Thus, a high proportion of sites per season with species detected by trasects, allows us to assume that the methodology of transects of sign survey reflects wolf spatial use and supports the approach used in our study.



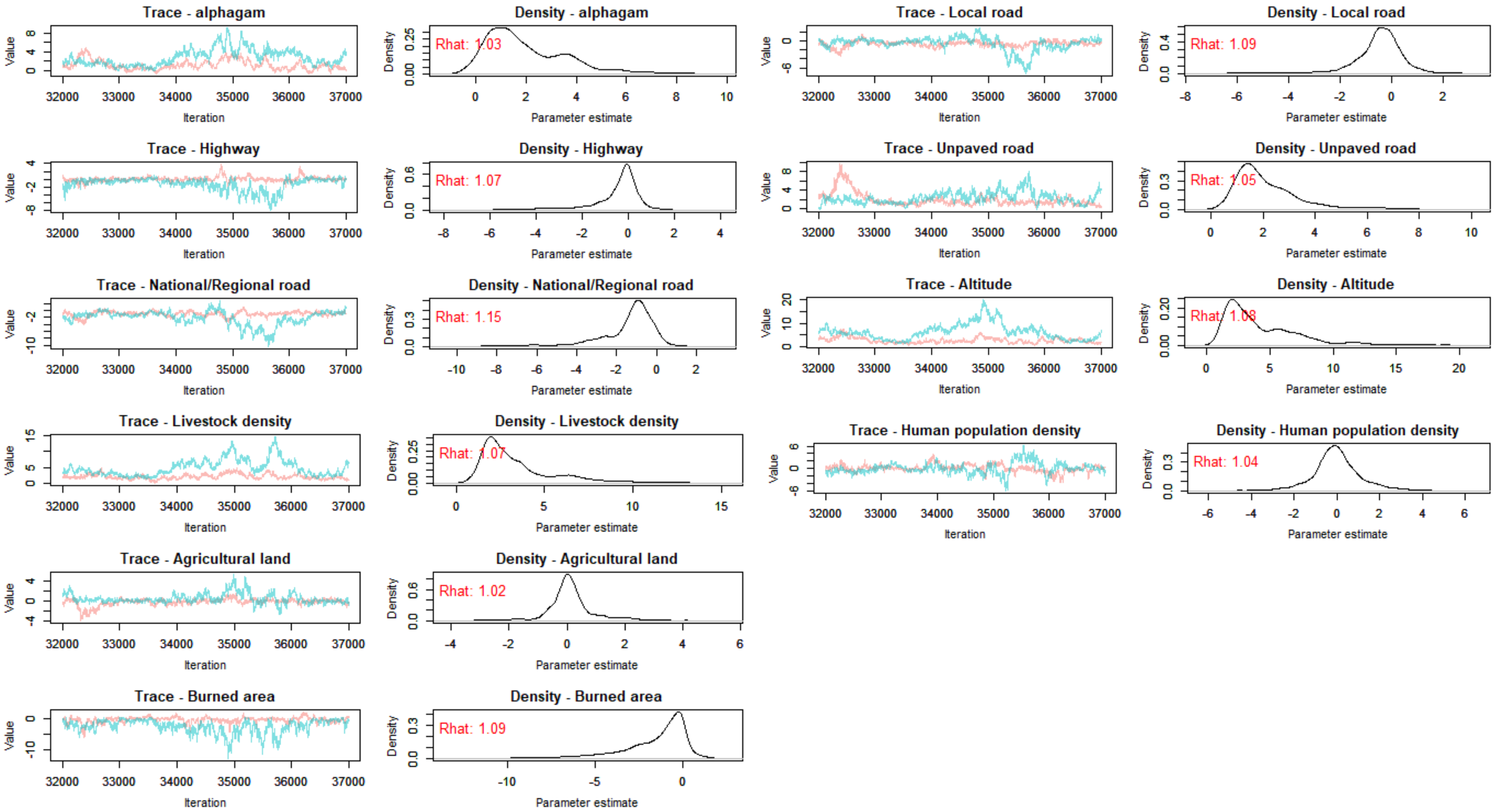
Wolf range period	Area type	Area (km^2)	Nº sites (5x5 km)
2000s	Total	142,972	5,779
Current	Persistence	129,475	5,211
	Expansion	20,724	855
	Regression	13,497	568
	Total	150,198	6,066

Sup. Fig. 4: Wolf range change in the Iberian Peninsula per 5x5 km site: obtained from the differences between the wolf range estimated at the beginning of the 2000s (adapted from Álvares et al., 2005) and the current range (adapted from Kaczensky, 2018). The four situations (i.e. area types) are presented: persistence, expansion, regression, and potential recolonization (i.e. 100 km buffer of the current range).



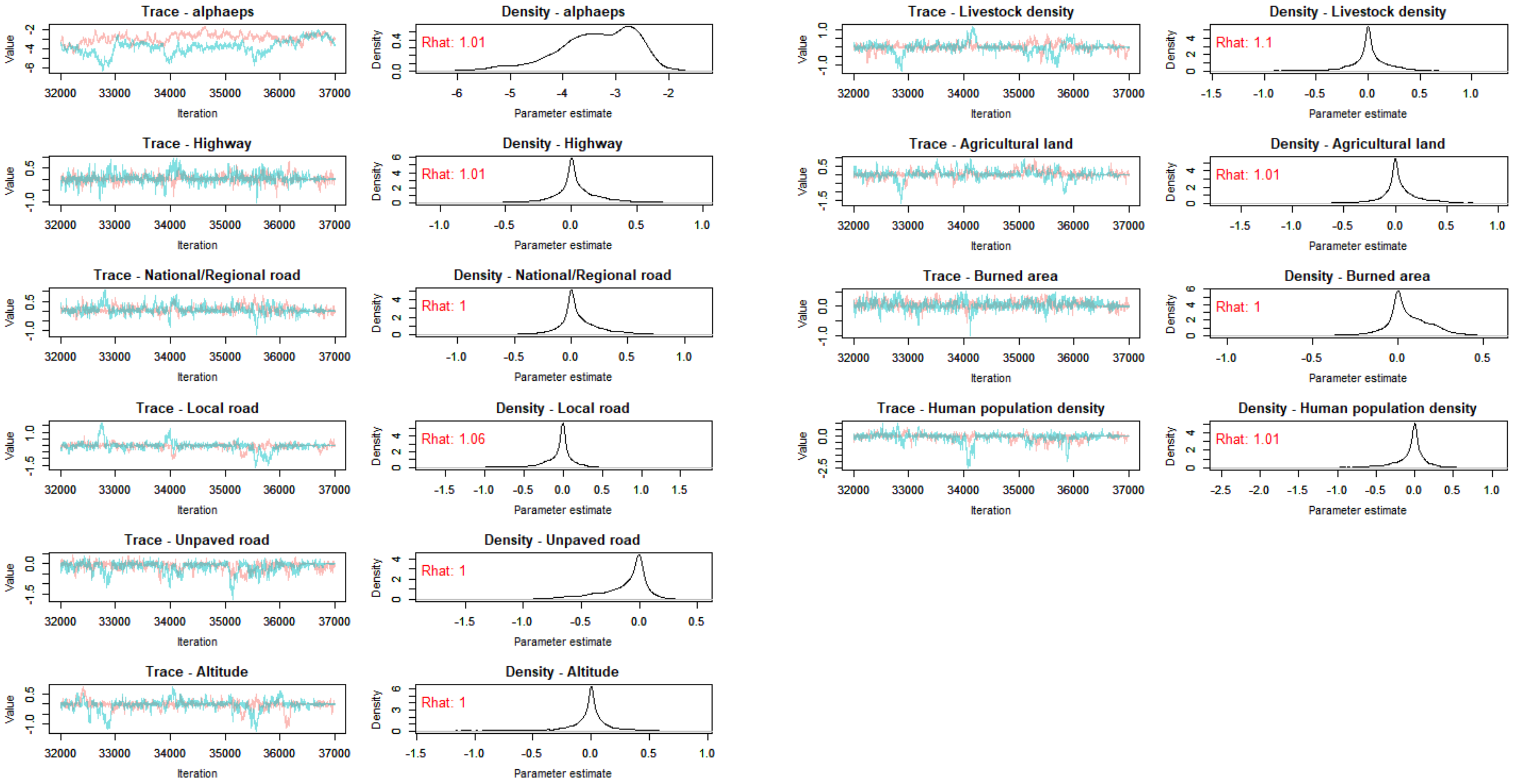
Sup. Fig. 5: Visualization of data used for the wolf dynamic occupancy model: detection (1-blue) and non-detection (0-pink) of wolf scats by transects per site-survey for each study area from 2005 to 2022 (395 sites; 72 surveys = 18 years*4 seasons).

Colonization



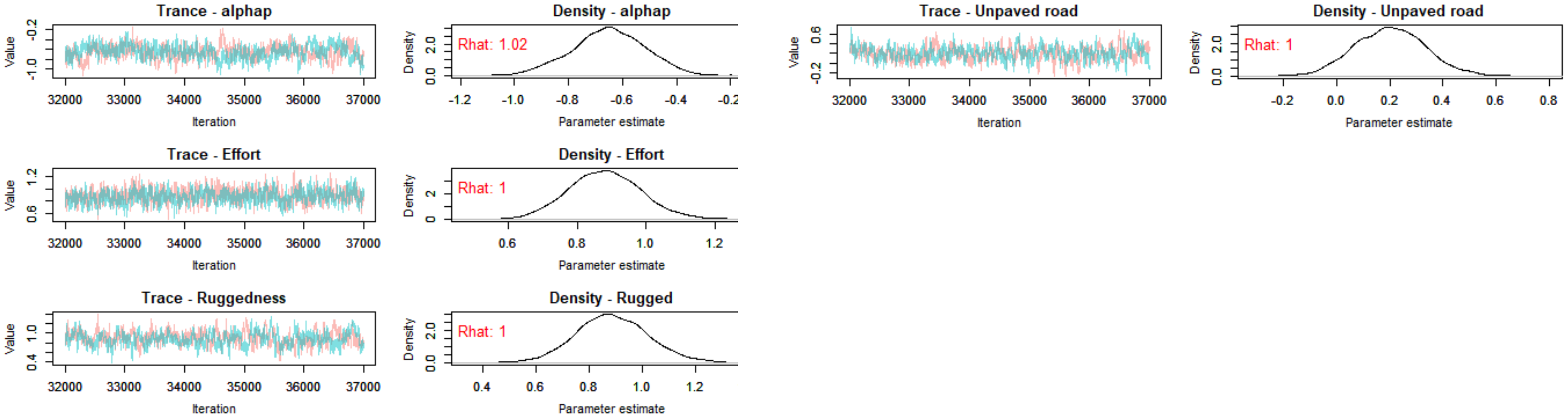
Sup. Fig. 6: Results of the wolf dynamic occupancy model : MCMC traceplots and density plots of the estimated covariates for the parameters of colonization, extinction, initial occupancy, and detection.

Extinction

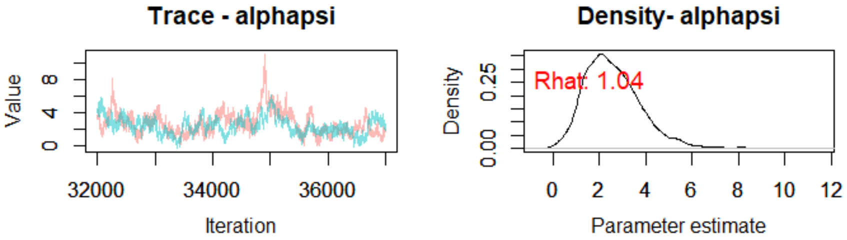


Sup Fig. 6: (cont.) Results of the wolf dynamic occupancy model : MCMC traceplots and density plots of the estimated covariates for the parameters of colonization, extinction, initial occupancy, and detection.

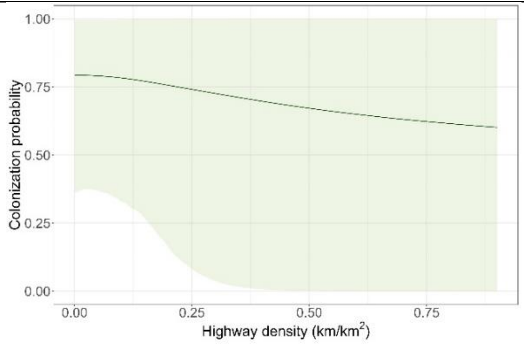
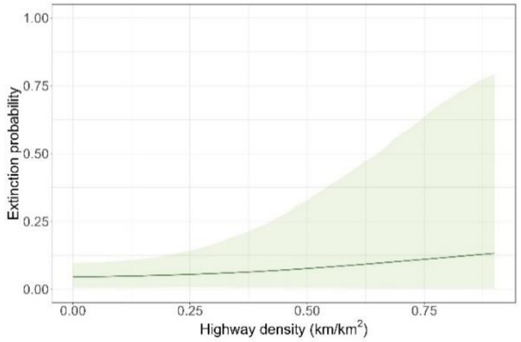
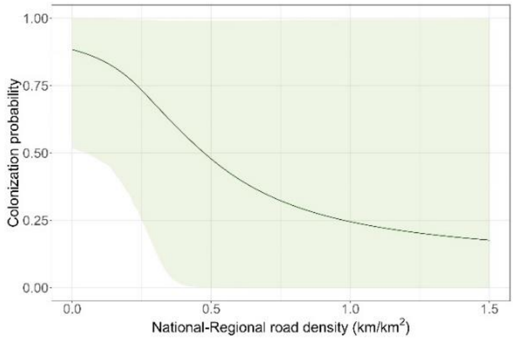
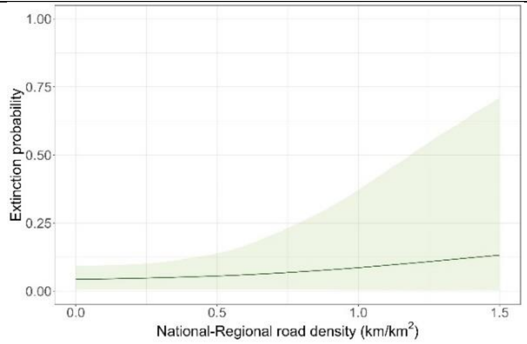
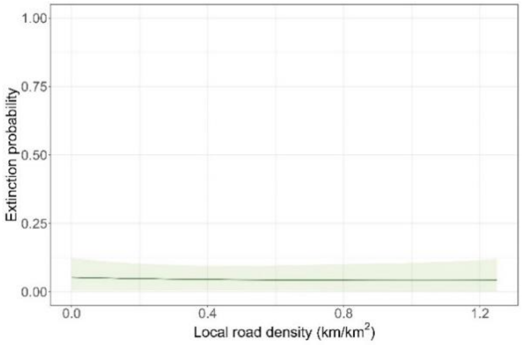
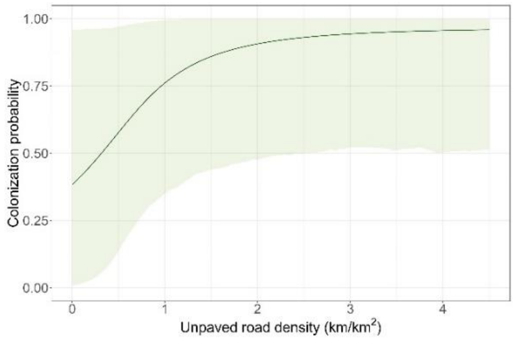
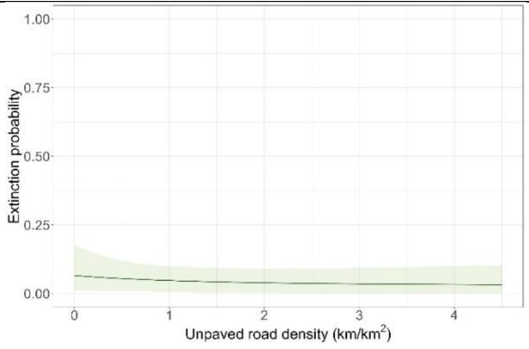
Detection



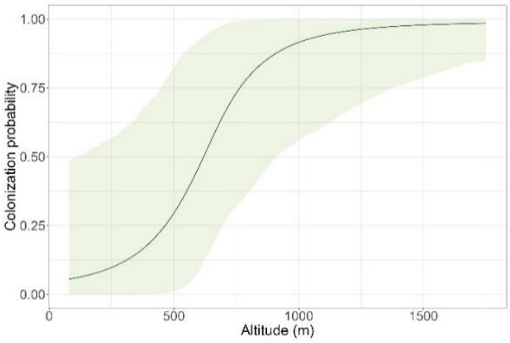
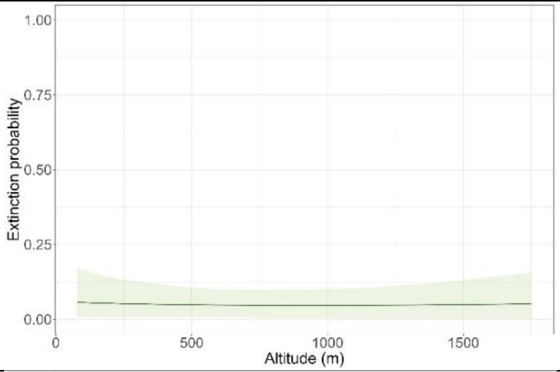
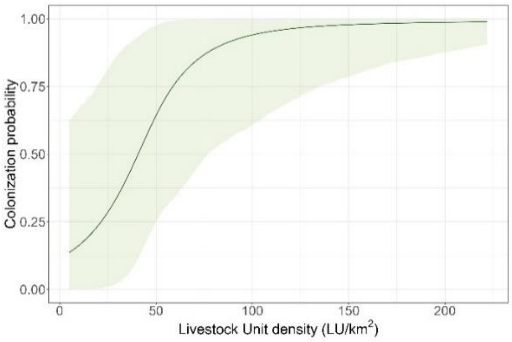
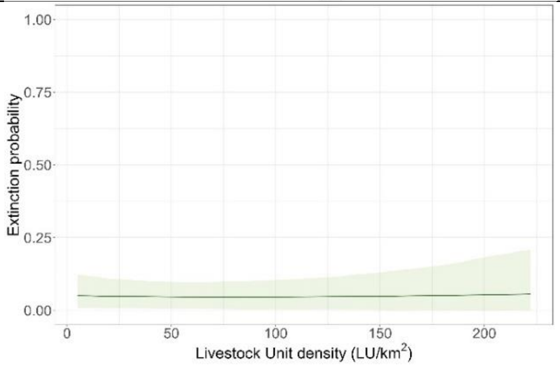
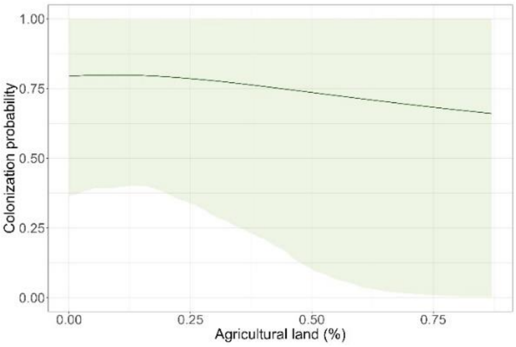
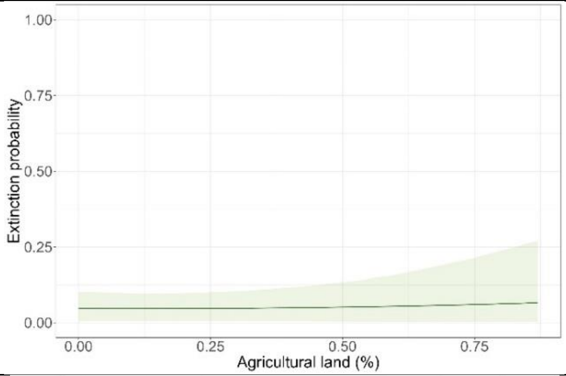
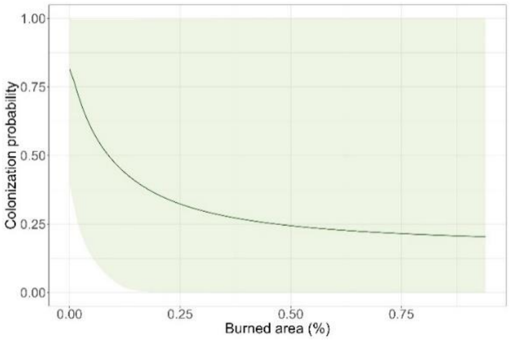
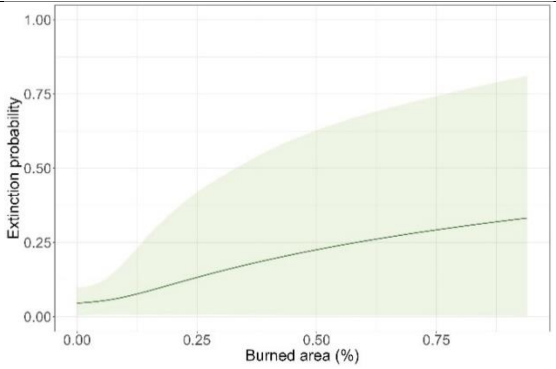
Initial occupancy



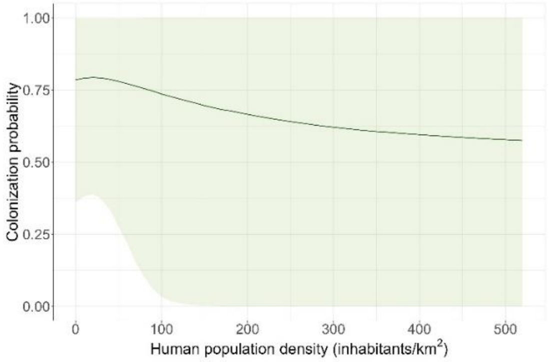
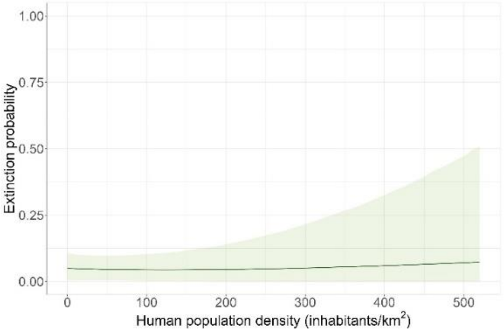
Sup. Fig. 6: (cont.) Results of the wolf dynamic occupancy model : MCMC traceplots and density plots of the estimated covariates for the parameters of colonization, extinction, initial occupancy, and detection.

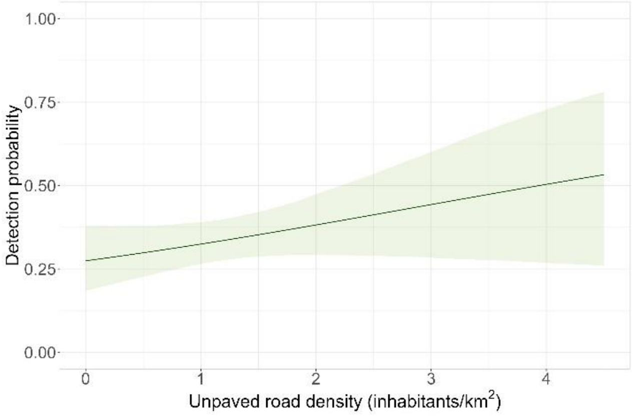
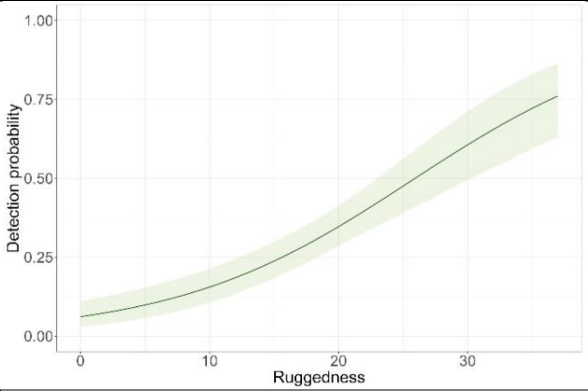
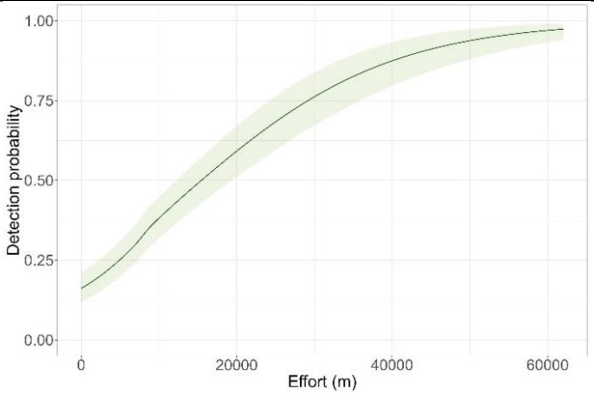
	Colonization	**Extinction**
Highway density		
National-Regional road desnity		
Local road density		
Unpaved road density		

Sup. Fig. 7: Results of the wolf dynamic occupancy model: estimated relationships between each covariate and colonization, extinction, and detection probabilities.

	Colonization	Extinction
Altitude		
Livestock density		
Agricultural lands		
Burned areas		

Sup. Fig. 7 (cont.): Results of the wolf dynamic occupancy model: estimated relationships between each covariate and colonization, extinction, and detection probabilities.

	Colonization	**Extinction**
Human population density		

	Detection
Track	
Ruggedness	
Effort	

Sup. Fig. 7 (cont.): Results of the wolf dynamic occupancy model: estimated relationships between each covariate and colonization, extinction, and detection probabilities.

Sup. Table 3: Anthropogenic and environmental covariates considered for the wolf dynamic occupancy model from 2005 to 2022. Description with units, source of information, covariates used (+) and excluded due to high correlation (x) for each model parameter; Col – colonization; Ext – extinction; Det – detection), and summary statistics for sampled and predicted 5x5 km sites. We used the R packages *sf*, *raster*, and *terra* (Hijmans, 2022, 2021; Pebesma, 2018) to manage the datasets of covariates and obtain the values for each site.

Type of covariate	**Covariate**	**Description (units)**	**Source**	**Parameters**			**Sampled sites (N=395)**		**Predicted sites [h] (N=23,204)**	
				Col (γ)	**Ext (ε)**	**Det (p)**	**Mean±SD**	**Range**	**Mean±SD**	**Range**
Observation covariates										
-	Effort	Sum of transect lengths per season (km)	-			+	8.75± 6.85	0.05-55.00	-	-
Environmental	Rugged	Terrain Ruggedness Index (TRI, Riley et al., 1999)	Average TRI from DEM raster [a]	x	x	+	19.4 ± 8.3	3.7-36.8	8.40 ± 6.57	0.48-48.40
Site covariates										
Anthropogenic	High	Paved road - Highway density (km/km^2)	OpenStreetMap® [b] (‘Motorway’)	+	+		0.04 ± 0.13	0.00-0.90	0.08 ± 0.22	0.00-3.38
	NatioRegio	Paved road – National and regional road density (km/km^2)	OpenStreetMap® [b] (‘Primary’+‘Secondary’ roads)	+	+		0.19 ± 0.21	0.00-1.44	0.18 ± 0.27	0.00-6.08
	Local	Paved road - Local road density (km/km^2)	OpenStreetMap® [b] (‘Tertiary’ roads)	+	+		0.31 ± 0.27	0.00-1.25	0.32 ± 0.43	0.00-7.79
	Unpaved	Unpaved road density (km/km^2)	OpenStreetMap® [b] (‘Tracks’)	+	+	+	1.12 ± 0.80	0.00-4.41	1.35 ± 0.96	0.00-9.01
	LUden	Livestock Unit density (LU/km^2)	Density of livestock (1LU of cattle and horse for 0.150 LU of sheep and goat) from Gridded Livestock of the World 2010 [c]	+	+		63.2 ± 41.1	5.1-221.0	50.5 ± 48.3	0.0-741.0
	Sett	Proportion of human settlements (%)	European Settlement Map 2015 [c]	x	x		1.4 ± 1.9	0.0-3.8	-	-
Environmental	Altitude	Altitude a.s.l. (m)	Average altitude from DEM raster [a]	+	+		800 ± 330	85-1749	636 ± 393	-2-2,834
Yearly site covariates										
Anthropogenic	Agric	Annual proportion of agricultural lands (%)	Corine Land Cover (CLC) 2006, 2012, 2018 [e]	+	+		20.0 ± 16.6	0.0-87.0	48.3 ± 32.6	0.00-100
	Burn	Annual proportion of burned areas (%)	MCD64A1 - Combined Level 3 Direct Broadcast Burned Area Monthly Global 500m SIN Grid [f]	+	+		1.6 ± 7.1	0.0-94.6	0.30 ± 0.04	0.00-100
	PopDen	Annual human population density (nº inhabitants/km^2)	Gridded Population of the World (GPWv4) 2005, 2010, 2015, 2020 [g]	+	+		28.73 ± 45.69	0.032-522.77	86 ± 385	0-17,000
Environmental	Refuge	Annual proportion of forest, shrubland and bare rocks (%)	Corine Land Cover (CLC) 2006, 2012, 2018 [e]	x	x		78.2 ± 17.5	13.0-100	-	-

[a] https://land.copernicus.eu/imagery-in-situ/eu-dem/eu-dem-v1.1; Riley, S.J., DeGloria, S.D., Elliot, R., 1999. A Terrain Ruggedness Index that Qauntifies Topographic Heterogeneity. Intermt. J. Sci. 5, 23–27; [b] https://download.geofabrik.de/; [c] Livestock unit determined based on Portuguese legislation Decree-Law nº 81/2013 Annex II; https://land.copernicus.eu/pan-european/GHSL/european-settlement-map/esm-2015-release-2019?tab=download; [d] https://www.fao.org/livestock-systems/global-distributions/en/; [e] https://land.copernicus.eu/pan-european/corine-land-cover/; to attribute the annual value, we considered the temporal extent of the metadata: CLC 2006 [2005-2010], CLC 2012 [2011-2016], CLC 2018 [2017-2022]; [f] https://ladsweb.modaps.eosdis.nasa.gov/missions-and-measurements/products/MCD64A1; [g] https://sedac.ciesin.columbia.edu/data/set/gpw-v4-population-density-adjusted-to-2015-unwpp-country-totals-rev11/data-download#close; [h] The grids smaller than 20km^2 (n=933) were excluded from parameter prediction.

Sup. Table 4: Results of the wolf dynamic occupancy model: parameter (α, β) estimates for initial occupancy, colonization, extinction, and detection (mean, standard deviation, confidence intervals, potential scale reduction factor, and number of effective samples).

Model Parameter	**Estimate/Covariate**	**Parameter (α, β)**	**Mean**	**SD**	**2.5%**	**50%**	**97.5%**	**Rhat**	**n.eff**
Extinction	Intercept (α_ε)	alphaeps	-3.21	0.75	-5.31	-3.06	-2.22	1.01	119
Colonization	Intercept (α_γ)	alphagam	1.87	1.58	-0.48	1.58	5.81	1.03	136
Detection	Intercept (α_p)	alphap	-0.70	0.14	-0.98	-0.70	-0.42	1.02	1,017
Initial occupancy	Intercept (α_ψ)	alphapsi	2.64	1.34	0.77	2.39	6.01	1.04	230
Extinction (ε)	Highway	betaeps[1]	0.08	0.27	-0.37	0.03	0.74	1.01	583
	National-Regional road	betaeps[2]	0.09	0.23	-0.31	0.04	0.67	1.00	942
	Local road	betaeps[3]	-0.09	0.25	-0.76	-0.03	0.29	1.06	718
	Unpaved road	betaeps[4]	-0.25	0.42	-1.38	-0.10	0.15	1.00	231
	Altitude mean	betaeps[5]	-0.06	0.31	-0.93	-0.01	0.44	1.00	472
	Livestock density	betaeps[6]	-0.02	0.40	-1.12	0.01	0.68	1.10	355
	Agricultural land	betaepsyear[1]	0.02	0.24	-0.51	0.01	0.52	1.01	819
	Burned area	betaepsyear[2]	0.09	0.15	-0.19	0.06	0.41	1.00	1,092
	Human population density	betaepsyear[3]	-0.10	0.30	-0.93	-0.02	0.31	1.01	604
Colonization (γ)	Highway	betagam[1]	-0.09	1.19	-2.69	-0.05	2.35	1.07	403
	National-Regional road	betagam[2]	-1.49	1.99	-6.67	-1.02	0.45	1.15	97
	Local road	betagam[3]	-1.01	1.48	-5.22	-0.65	0.65	1.09	99
	Unpaved road	betagam[4]	1.86	1.62	-0.11	1.55	6.42	1.05	153
	Altitude mean	betagam[5]	3.36	2.14	0.54	2.91	8.62	1.08	146
	Livestock density	betagam[6]	3.01	1.64	0.60	2.72	7.10	1.07	148
	Agricultural land	betagamyear[1]	-0.08	0.85	-1.87	-0.06	1.59	1.02	340
	Burned area	betagamyear[2]	-1.26	2.12	-6.29	-0.77	1.35	1.09	273
	Human population density	betagamyear[3]	0.04	1.29	-2.73	0.02	2.85	1.04	317
Detection (p)	Effort	betap[1]	0.89	0.11	0.68	0.88	1.10	1.00	2,496
	Ruggedness	betap[2]	0.89	0.14	0.62	0.89	1.18	1.00	1,612
	Unpaved road	betap[3]	0.20	0.14	-0.07	0.20	0.48	1.00	1,843

Sup. Table 5: Proportion of mass of posterior distribution on the negative and positive side per covariate estimate for colonization and extinction parameters of the dynamic occupancy model. Covariates: highway, national-regional, local and unpaved road densities; altitude; livestock unit density; proportion of agricultural land; proportion of burned area; and human population density. Values with a high (> 0.6) tendency to negative or positive side are in bold.

Covariate	**Colonization**		**Extinction**	
	Negative	Positive	Negative	Positive
Highway	0.536	0.464	0.374	**0.626**
Natio-Regio	**0.914**	0.086	0.343	**0.657**
Local	**0.849**	0.151	**0.635**	0.365
Unpaved	0.038	**0.962**	**0.770**	0.230
Altitude	0.006	**0.994**	0.538	0.462
Livestock	0.004	**0.996**	0.463	0.537
Agric	0.547	0.453	0.453	0.547
Burn	**0.844**	0.156	0.276	**0.724**
PopDen	0.486	0.514	0.598	0.402

Sup. Table 6: Estimated annual wolf productivity for the Iberian Peninsula, based on the most recently known number of packs and pups per pack in Portugal and Spain.

Area	**Nº packs** [a]	**Nº pups/pack** [b]	**Estimated annual productivity** [c]
Portugal	48	3.1	148.8
Spain	297	4.8	1,425.6
TOTAL	**345**	**-**	**1,574**

[a] Minimum number of packs detected in Portugal (Álvares et al., 2015) and Spain (MAGRAMA, 2016);

[b] Minimum average number of pups detected per pack in Portugal (Álvares et al., 2015) and Spain (Llaneza et al., 2012);

[c] Estimated annual number of pups born, assuming all detected packs reproduce.

References

Álvares, F., Barroso, I., Blanco, J.C., Correia, J., Cortés, Y., Costa, G., Llaneza, L., Moreira, L., Nascimento, J., Palacios, V., Petrucci-Fonseca, F., Pimenta, V., Roque, S., Santos, E., 2005. Wolf status and conservation in the Iberian Peninsula, in: Conference Frontiers of Wolf Recovery: Southern US and the World. Colorado, pp. 76–77.

Álvares, F., Barroso, I., Costa, G.F. da, Espírito-Santo, C., Fonseca, C., Godinho, R., Nakamura, M., Petrucci-Fonseca, F., Pimenta, V., Rio-Maior, H., Santos, N., Torres, R.T., 2015. Situação de referência para o Plano de Ação para a Conservação do Lobo-ibérico em Portugal. Lisbon, Portugal.

Hijmans, R.J., 2022. terra: Spatial Data Analysis. R package version 1.5-21.

Hijmans, R.J., 2021. raster: Geographic Data Analysis and Modeling. R package version 3.4-13.

Hooten, M.B., Hobbs, N., 2015. A guide to Bayesian model selection for ecologists. Ecol. Monogr. 85, 3–28.

Kaczensky, P., 2018. IUCN Red List Mapping for the regional assessment of the Wolf (*Canis lupus*) in Europe.

Llaneza, L., García, E.J., Palacios, V., Sazatornil, V., López-Bao, J.V., 2012. Pack size in the Iberian wolf (*Canis lupus signatus*). Oral Commun. Abstr. III Iber. Wolf Congr.

MAGRAMA, 2016. Spanish Ministry of Agriculture, Food and Environment. Censo Nacional Lobo ibérico 2012-2014 (*Canis lupus*, Linnaeus, 1758) en España.

Marucco, F., Pletscher, D.H., Boitani, L., Schwartz, M.K., Pilgrim, K.L., Lebreton, J.-D., 2009. Wolf survival and population trend using non-invasive capture-recapture techniques in the Western Alps. J. Appl. Ecol. 46, 1003–1010. https://doi.org/10.1111/j.1365-2664.2009.01696.x

Pebesma, E., 2018. Simple Features for R: Standardized Support for Spatial Vector Data. R J. 10, 439. https://doi.org/10.32614/RJ-2018-009

Posit team, 2022. RStudio: Integrated Development Environment for R. Posit Software, PBC.

Riley, S.J., DeGloria, S.D., Elliot, R., 1999. A Terrain Ruggedness Index that Qauntifies Topographic Heterogeneity. Intermt. J. Sci. 5, 23–27.

Rio-Maior, H., Nakamura, M., Álvares, F., Beja, P., 2019. Designing the landscape of coexistence: Integrating risk avoidance, habitat selection and functional connectivity to inform large carnivore conservation. Biol. Conserv. 235, 178–188. https://doi.org/10.1016/j.biocon.2019.04.021

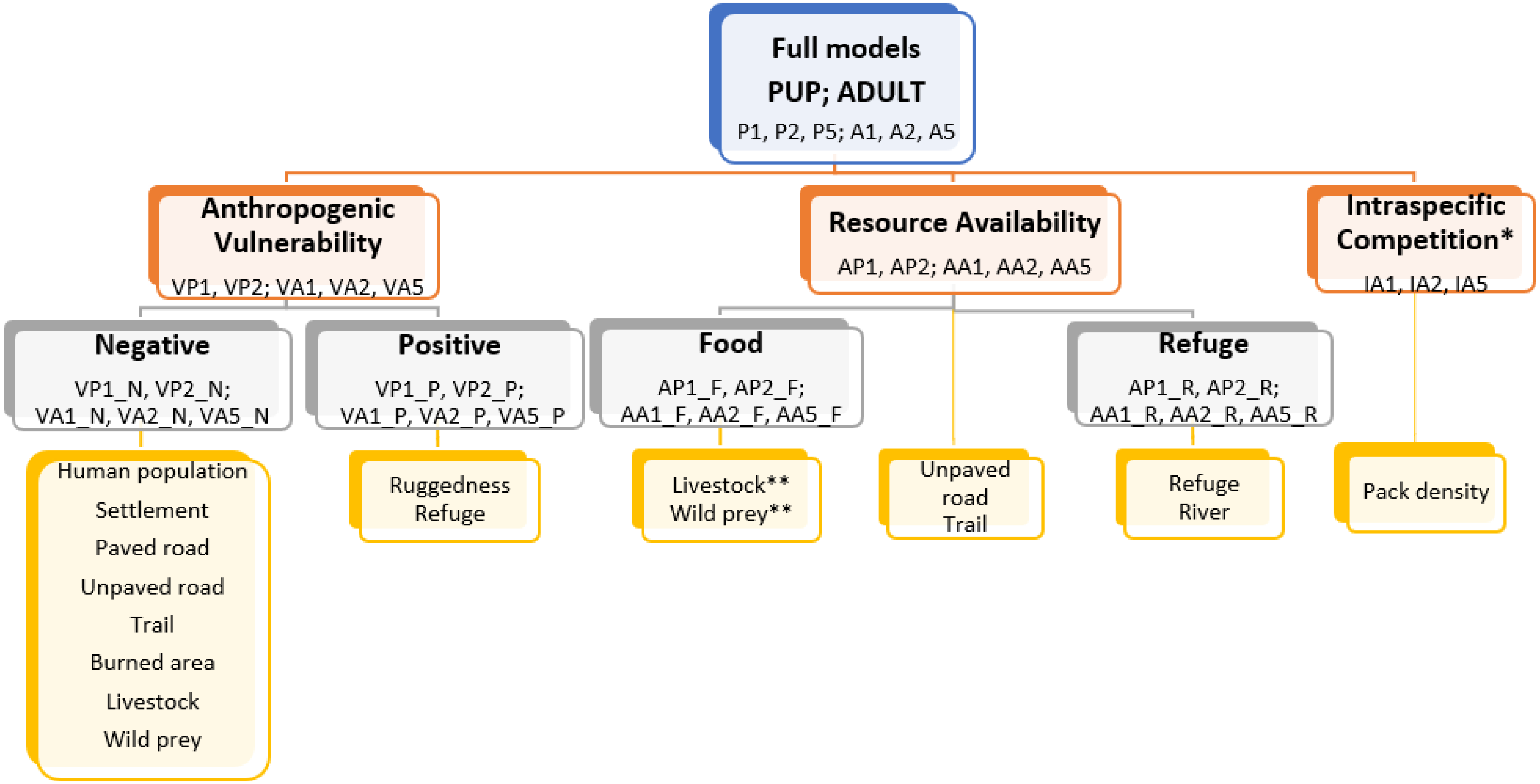
Royle, J.A., Kéry, M., 2007. A Bayesian State-space Formulation of Dynamic Occupancy Models. Ecol. Soc. Am. 88, 1813–1823.

van Erp, S., Oberski, D.L., Mulder, J., 2019. Shrinkage priors for Bayesian penalized regression. J. Math. Psychol. 89, 31–50. https://doi.org/10.1016/j.jmp.2018.12.004

Appendix III – Supplementary data for Chapter III

Sup. Table 7**:** Covariates considered for the models. Description with units, source of information, and summary statistics for detected homesite buffers. We used the R packages *sf*, *raster*, and *terra* (Hijmans, 2022, 2021; Pebesma, 2018) to manage the datasets of covariates and obtain the values for each site.

Type of covariate	**Covariate**	**Description (units)**	**Source**
Anthropogenic	humanpop	Human population density (inhabitants/km^2)	Gridded Population of the World (GPWv4) 2005, 2010, 2015, 2020; https://sedac.ciesin.columbia.edu/data/set/gpw-v4-population-density-adjusted-to-2015-unwpp-country-totals-rev11/data-download#close
	sett	Proportion of human settlements (%)	European Settlement Map 2015; https://land.copernicus.eu/pan-european/GHSL/european-settlement-map/esm-2015-release-2019?tab=download
	pavedroad	Paved road density (km/km^2)	OpenStreetMap® ^{c} ('Motorway', 'Primary', 'Secondary' and 'Tertiary' roads); https://download.geofabrik.de/
	unpavedroad	Unpaved road density (km/km^2)	OpenStreetMap® ^{c} ('Tracks'); https://download.geofabrik.de/
	trail	Trail density (km/km^2)	OpenStreetMap® ^{c} ('Trails); https://download.geofabrik.de/
	burn	Annual proportion of burned areas (%)	1986-2001: The European Space Agency Fire Disturbance Climate Change Initiative (1986-2001), from Otón et al. (2021); https://data.ceda.ac.uk/neodc/esacci/fire/data/burned_area/AVHRR-LTDR/pixel/v1.1 2002-2021: MCD64A1 - Combined Level 3 Direct Broadcast Burned Area Monthly Global 500m SIN Grid
	livestock	Livestock Unit density (LU/km^2)	Density of livestock (1LU of cattle and horse for 0.150 LU of sheep and goat; Livestock unit determined based on Portuguese legislation Decree-Law nº 81/2013 Annex II); Gridded Livestock of the World 2010; https://www.fao.org/livestock-systems/global-distributions/en
Anthropogenic/ Environmental	wildprey	Average wild prey density (individuals/ km^2)	Average ungulate (Isard, Red deer, Roe deer, Wild boar and Fallow deer) densities from ENETWILD-consortium, Illanas et al. (2022), clipped with species distribution by Linnell et al. (2020)
Environmental	river	Waterline density (km/km^2)	https://www.miteco.gob.es/es/cartografia-y-sig/ide/descargas/agua/masas-de-agua-phc-2015-2021.html; https://sniamb.apambiente.pt/content/cat%C3%A1logo
	rugged	Terrain Ruggedness Index (TRI)	Average TRI (Riley et al., 1999) from DEM raster; https://land.copernicus.eu/imagery-in-situ/eu-dem/eu-dem-v1.1
	refuge	Annual proportion of forest, shrubland and bare rocks (%)	Corine Land Cover (CLC) 2000, 2006, 2012, 2018; to attribute the annual value, we considered the temporal extent of the metadata: CLC2000 [1986-2004], CLC 2006 [2005-2010], CLC 2012 [2011-2016], CLC 2018 [2017-2022]
Intraspecific Competition	packdensity	Pack density (pack/100km^2)	Average pack density from raster (number of overlapping packs with 20 km buffer of the Iberian Peninsula); pack locations from Álvares et al. (2005)



Sup. Fig. 8: Diagram with the rationale for constructing the models for the number of pups or adults. The top three levels of the diagram represent the models, which include the covariates in the lowest level (yellow).* Intraspecific competition was only considered for models for the number of adults; ** The model for the number of pups in the 5 km buffer (P5) only included these covariates.

Sup. Table 8: List of constructed models for the **number of pups** with three buffer levels around homesites (1, 2 and 5km), evaluated for anthropogenic vulnerability (Vul), and resource availability (Ava).

Model	Buffer (km)	Type Covariates	Covariates
P1_null	1	Null model	(1 | Year)
P2_null	2	Null model	(1 | Year)
P5_null	5	Null model	(1 | Year)
P1	1	Vul+Ava (full model)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + (1 | Year)
P2	2	Vul+Ava (full model)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + (1 | Year)
P5	5	Vul+Ava (full model)	livestock + wildprey + (1 | Year)
VP1	1	Vul	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
VP2	2	Vul	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
VP1_N	1	Vul (Negative)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VP2_N	2	Vul (Negative)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VP1_P	1	Vul (Positive)	rugged + refuge + (1 | Year)
VP2_P	2	Vul (Positive)	rugged + refuge + (1 | Year)
AP1	1	Ava	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)
AP2	2	Ava	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)
AP1_F	1	Ava (Food)	livestock + wildprey + (1 | Year)
AP2_F	2	Ava (Food)	livestock + wildprey + (1 | Year)
AP1_R	1	Ava (Refuge)	river + refuge + (1 | Year)
AP2_R	2	Ava (Refuge)	river + refuge + (1 | Year)

Sup. Table 9: List of constructed models for the **number of adults** with three buffer levels around homesites (1, 2 and 5km), evaluated for anthropogenic vulnerability (Vul), resource availability (Ava), and Intraspecific competition (Int).

Model	**Buffer**	**Type covariates**	**Covariates**
A1_null	1	Null model	(1 | Year)
A2_null	2	Null model	(1 | Year)
A5_null	5	Null model	(1 | Year)
A1	1	Vul+Ava+Int (full model)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + packdensity + (1 | Year)
A2	2	Vul+Ava+Int (full model)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + packdensity + (1 | Year)
A5	5	Vul+Ava+Int (full model)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + packdensity + (1 | Year)
VA1	1	Vul	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
VA2	2	Vul	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
VA5	5	Vul	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
VA1_N	1	Vul (Negative)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VA2_N	2	Vul (Negative)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VA5_N	5	Vul (Negative)	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VA1_P	1	Vul (Positive)	rugged + refuge + (1 | Year)
VA2_P	2	Vul (Positive)	rugged + refuge + (1 | Year)
VA5_P	5	Vul (Positive)	rugged + refuge + (1 | Year)
AA1	1	Ava	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)
AA2	2	Ava	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)
AA5	5	Ava	unpavedroad + trail + livestock + wildprey + refuge + (1 | Year)
AA1_F	1	Ava (Food)	livestock + wildprey + (1 | Year)
AA2_F	2	Ava (Food)	livestock + wildprey + (1 | Year)
AA5_F	5	Ava (Food)	livestock + wildprey + (1 | Year)
AA1_R	1	Ava (Refuge)	river + refuge + (1 | Year)
AA2_R	2	Ava (Refuge)	river + refuge + (1 | Year)
AA5_R	5	Ava (Refuge)	refuge + (1 | Year)
IA1	1	Instraspecific	packdensity + (1 | Year)
IA2	2	Instraspecific	packdensity + (1 | Year)
IA5	5	Instraspecific	packdensity + (1 | Year)

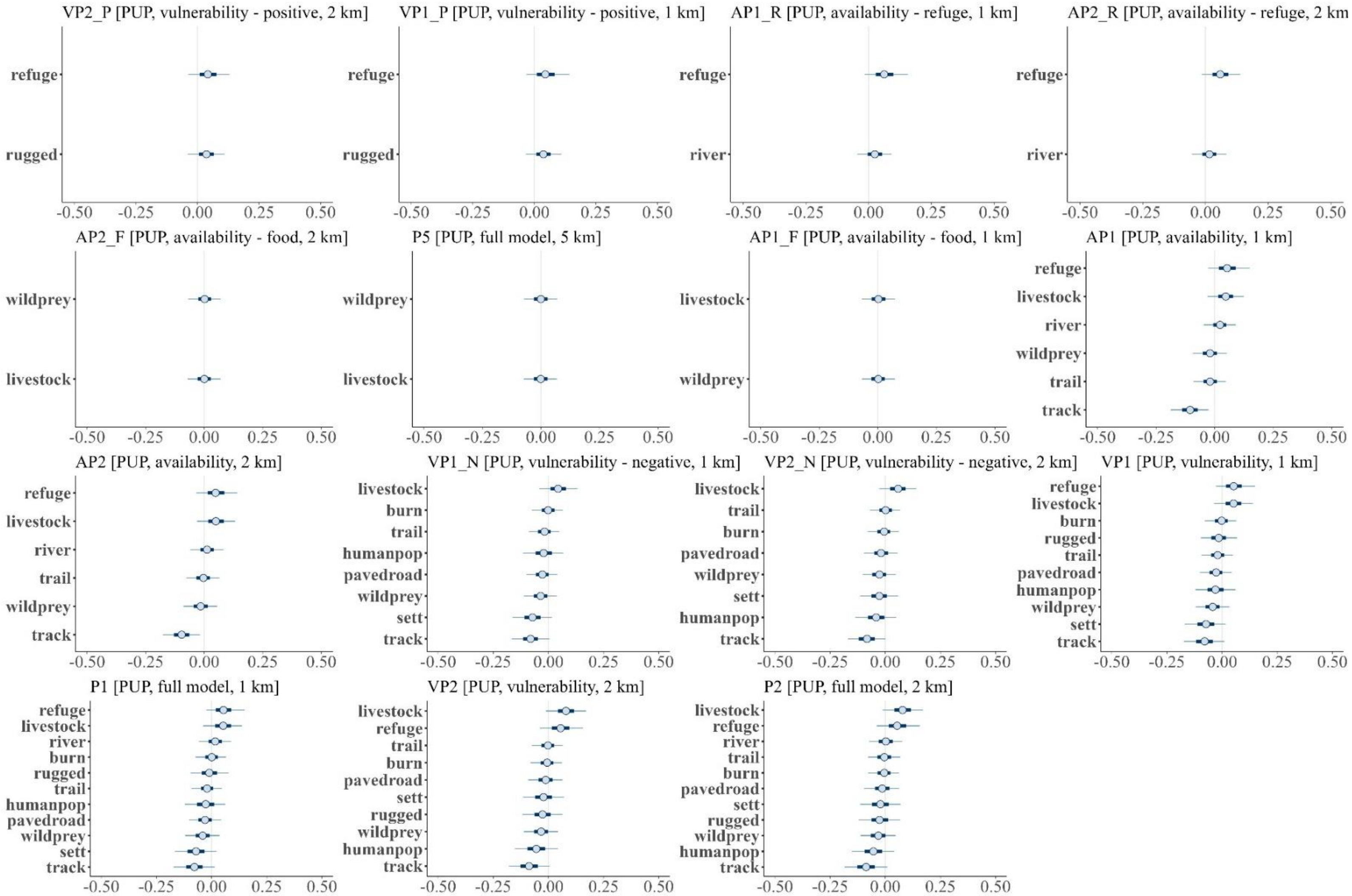
Sup. Table 10: Models ranked by lowest |ELPD| or WAIC, with outputs obtained from the comparison of all models for the number of pups, using 'loo_compare' function from the 'brms' R package (Bürkner, 2017). The models above the dashed line have a difference of WAIC < 2 or of ELPD < 1, compared to the best model).

Model	**elpd_diff**	**se_diff**	**elpd_waic**	**se_elpd_**	**p_waic**	**se_p_wai**	**waic**	**se_waic**	**weight**	**Covariates**
P5_null	0.00	0.00	-270.95	4.19	1.42	0.16	541.91	8.38	0.17	(1 | Year)
P2_null	-0.03	0.02	-270.98	4.18	1.44	0.17	541.96	8.37	0.17	(1 | Year)
P1_null	-0.05	0.02	-271.00	4.20	1.46	0.17	542.00	8.39	0.17	(1 | Year)
VP2_P	-0.61	1.18	-271.56	4.21	2.54	0.41	543.12	8.41	0.09	rugged + refuge + (1 | Year)
VP1_P	-0.61	0.79	-271.56	4.15	2.29	0.36	543.12	8.31	0.09	rugged + refuge + (1 | Year)
AP1_R	-0.88	0.67	-271.84	4.29	2.42	0.40	543.68	8.58	0.07	river + refuge + (1 | Year)
AP2_R	-1.01	1.15	-271.96	4.38	2.88	0.48	543.92	8.76	0.06	river + refuge + (1 | Year)
AP2_F	-1.72	0.16	-272.67	4.27	2.76	0.36	545.35	8.55	0.03	livestock + wildprey + (1 | Year)
P5	-1.76	0.15	-272.71	4.27	2.70	0.34	545.43	8.54	0.03	livestock + wildprey + (1 | Year)
AP1_F	-1.77	0.18	-272.72	4.28	2.77	0.37	545.44	8.57	0.03	livestock + wildprey + (1 | Year)
AP1	-1.79	2.16	-272.75	4.11	4.88	0.67	545.49	8.21	0.03	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)
AP2	-2.26	2.11	-273.22	4.34	5.28	0.74	546.43	8.69	0.02	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)
VP1_N	-2.54	2.56	-273.50	4.18	5.91	0.81	547.00	8.35	0.01	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VP2_N	-3.37	2.25	-274.32	4.17	5.83	0.76	548.64	8.35	0.01	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VP1	-3.78	2.64	-274.74	4.23	6.87	0.91	549.48	8.46	0.00	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
P1	-4.32	2.63	-275.28	4.32	7.17	0.92	550.55	8.64	0.00	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + (1 | Year)
VP2	-4.68	2.37	-275.64	4.20	7.00	0.89	551.27	8.41	0.00	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
P2	-5.61	2.40	-276.57	4.29	7.68	0.96	553.14	8.58	0.00	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + (1 | Year)

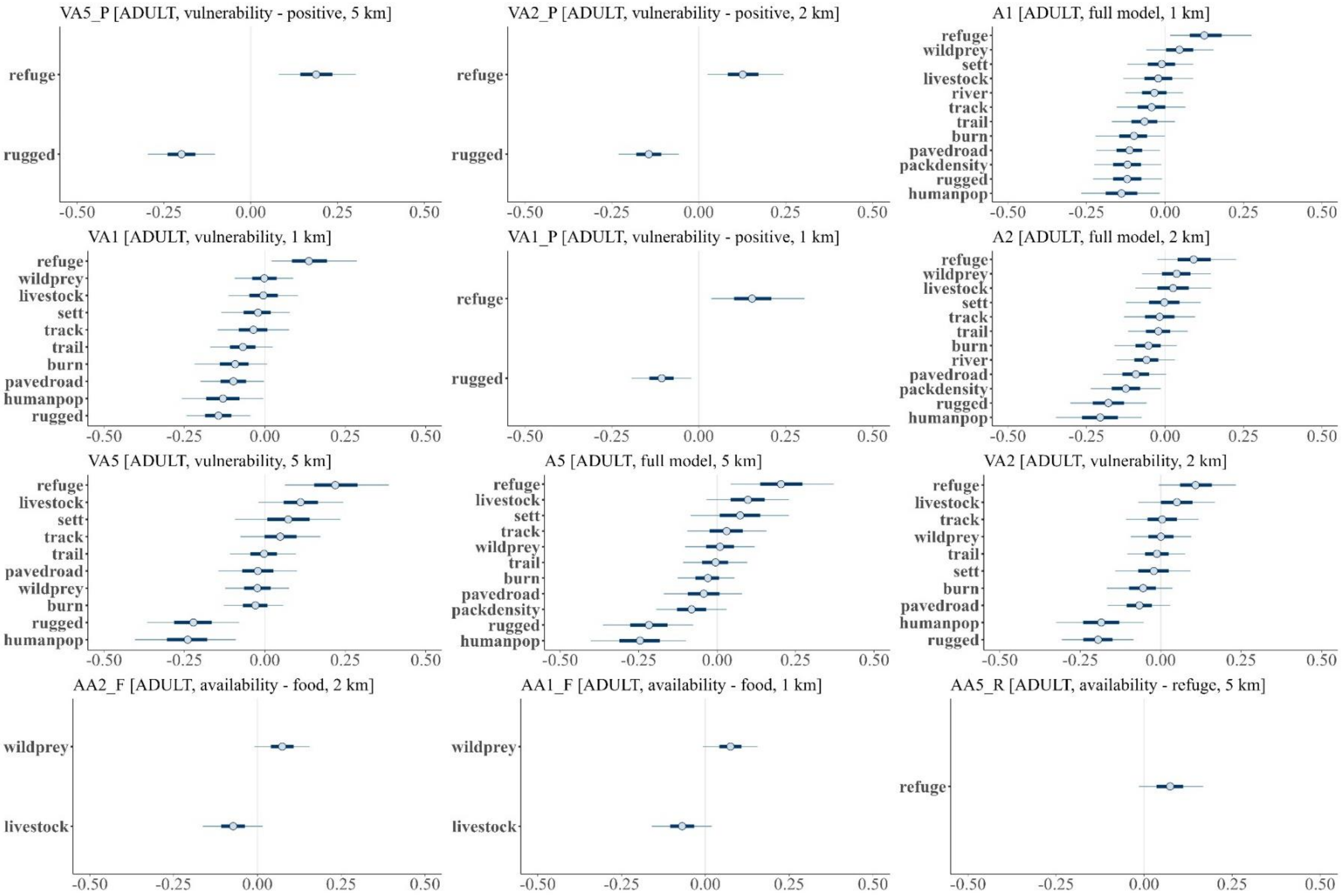
Sup. Table 11: Models ranked by lowest |ELPD| or WAIC, with outputs obtained from the comparison of all models for the number of adults, using 'loo_compare' and 'model_weights' functions from the 'brms' R package (Bürkner, 2017). The models above the dashed line have a difference of WAIC < 2 or of ELPD < 1, compared to the best model).

Model	**elpd_diff**	**se_diff**	**elpd_waic**	**se_elp**	**p_waic**	**se_p_w**	**waic**	**se_waic**	**weight**	**Covariates**
VA5_P	0.00	0.00	-256.31	5.80	3.77	0.52	512.61	11.61	0.68	rugged + refuge + (1 | Year)
VA2_P	-2.08	1.29	-258.38	5.64	3.69	0.46	516.77	11.28	0.08	rugged + refuge + (1 | Year)
A1	-2.63	4.02	-258.94	5.97	10.74	1.48	517.87	11.94	0.05	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + packdensity + (1 | Year)
VA1	-2.73	3.40	-259.04	5.71	9.42	1.19	518.08	11.41	0.04	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
VA1_P	-2.97	2.05	-259.28	5.58	3.63	0.39	518.55	11.15	0.03	rugged + refuge + (1 | Year)
A2	-3.78	4.19	-260.08	6.38	11.68	1.80	520.17	12.77	0.02	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + river + rugged + refuge + packdensity + (1 | Year)
VA5	-3.81	2.64	-260.12	5.66	10.43	1.41	520.24	11.33	0.01	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
A5	-3.98	2.93	-260.28	5.79	11.04	1.52	520.57	11.57	0.01	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + packdensity + (1 | Year)
VA2	-4.13	3.15	-260.43	5.79	10.18	1.35	520.86	11.58	0.01	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + rugged + refuge + (1 | Year)
AA2_F	-4.52	3.04	-260.83	6.04	4.60	0.79	521.66	12.08	0.01	livestock + wildprey + (1 | Year)
AA1_F	-4.64	3.02	-260.95	5.99	4.55	0.77	521.90	11.99	0.01	livestock + wildprey + (1 | Year)
A1_null	-4.87	3.02	-261.17	5.60	2.74	0.27	522.35	11.21	0.01	(1 | Year)
A2_null	-4.91	3.01	-261.22	5.62	2.76	0.28	522.44	11.24	0.00	(1 | Year)

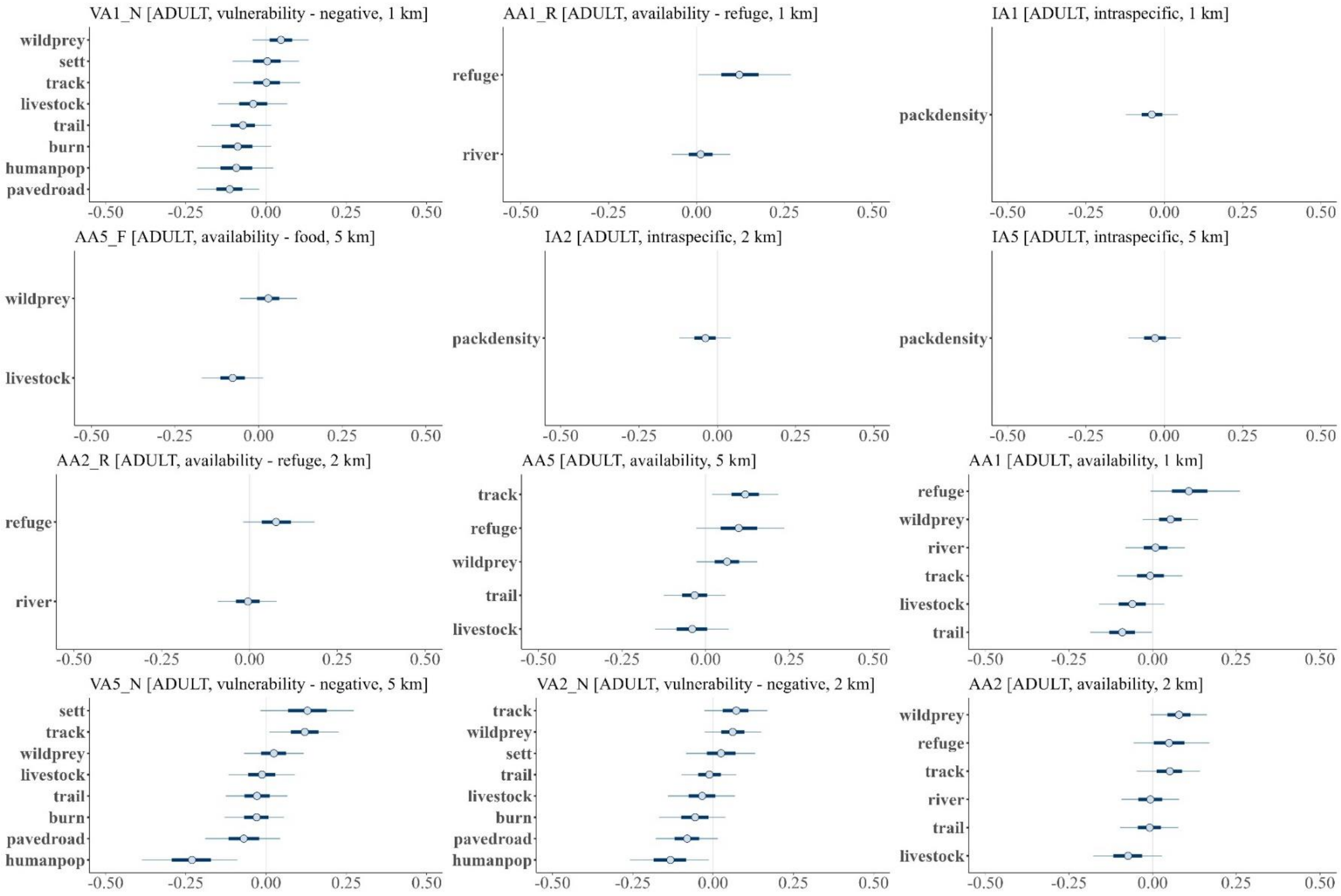
Model	elpd_diff	se_diff	elpd_waic	se_elp	p_waic	se_p_w	waic	se_waic	weight	Covariates
A5_null	-4.92	3.01	-261.22	5.62	2.74	0.28	522.44	11.23	0.00	(1 | Year)
AA5_R	-4.96	2.65	-261.27	5.73	3.45	0.40	522.53	11.45	0.00	refuge + (1 | Year)
VA1_N	-5.11	4.03	-261.41	5.83	8.49	1.16	522.83	11.66	0.00	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
AA1_R	-5.36	2.69	-261.67	5.62	3.93	0.44	523.33	11.23	0.00	river + refuge + (1 | Year)
IA1	-5.51	3.11	-261.82	5.70	3.49	0.35	523.64	11.40	0.00	packdensity + (1 | Year)
AA5_F	-5.55	2.78	-261.86	6.09	4.61	0.75	523.72	12.18	0.00	livestock + wildprey + (1 | Year)
IA2	-5.56	3.12	-261.87	5.72	3.46	0.35	523.73	11.44	0.00	packdensity + (1 | Year)
IA5	-5.74	3.12	-262.05	5.74	3.58	0.37	524.10	11.47	0.00	packdensity + (1 | Year)
AA2_R	-6.18	2.65	-262.49	5.79	4.44	0.62	524.98	11.57	0.00	river + refuge + (1 | Year)
AA5	-6.29	2.08	-262.60	6.00	6.86	0.95	525.19	12.00	0.00	unpavedroad + trail + livestock + wildprey + refuge + (1 | Year)
AA1	-6.40	3.09	-262.71	6.20	7.65	1.24	525.41	12.40	0.00	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)
VA5_N	-6.48	3.35	-262.79	5.67	9.51	1.34	525.57	11.35	0.00	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
VA2_N	-6.78	3.59	-263.08	5.72	8.86	1.13	526.17	11.43	0.00	humanpop + sett + pavedroad + unpavedroad + trail + burn + livestock + wildprey + (1 | Year)
AA2	-8.37	3.01	-264.68	6.28	8.46	1.48	529.36	12.56	0.00	unpavedroad + trail + livestock + wildprey + river + refuge + (1 | Year)



Sup. Fig. 9: Plots with posterior distributions of the models of the number of pups, ranked by lowest |ELPD| or WAIC (by rows), with the average β estimates (point), standard deviation (± SD; thick bars), and 95% Bayesian Credible Intervals (± 95% BCI; thin bars) for each covariate.



Sup. Fig. 10: Plots with posterior distributions of the models of the number of adults, ranked by lowest |ELPD| or WAIC (by rows), with the average β estimates (point), standard deviation (± SD; thick bars), and 95% Bayesian Credible Intervals (± 95% BCI; thin bars) for each covariate. [1]



VA1_N [ADULT, vulnerability - negative, 1 km]

AA1_R [ADULT, availability - refuge, 1 km]

IA1 [ADULT, intraspecific, 1 km]

AA5_F [ADULT, availability - food, 5 km]

IA2 [ADULT, intraspecific, 2 km]

IA5 [ADULT, intraspecific, 5 km]

AA2_R [ADULT, availability - refuge, 2 km]

AA5 [ADULT, availability, 5 km]

AA1 [ADULT, availability, 1 km]

VA5_N [ADULT, vulnerability - negative, 5 km]

VA2_N [ADULT, vulnerability - negative, 2 km]

AA2 [ADULT, availability, 2 km]

Sup. Fig. 10 (cont.) Plots with posterior distributions of the models of the number of adults ranked by lowest |ELPD| or WAIC (by rows). , with the average β estimates (point), standard deviation (± SD; thick bars), and 95% Bayesian Credible Intervals (± 95% BCI; thin bars) for each covariate [2]

References

Álvares, F., Barroso, I., Blanco, J.C., Correia, J., Cortés, Y., Costa, G., Llaneza, L., Moreira, L., Nascimento, J., Palacios, V., Petrucci-Fonseca, F., Pimenta, V., Roque, S., Santos, E., 2005. Wolf status and conservation in the Iberian Peninsula, in: Conference Frontiers of Wolf Recovery: Southern US and the World. Colorado, pp. 76–77.

Bürkner, P.-C., 2017. brms : An R Package for Bayesian Multilevel Models Using Stan. J. Stat. Softw. 80. https://doi.org/10.18637/jss.v080.i01

Illanas, S., Croft, S., Smith, G.C., López-Padilla, S., Vicente, J., Blanco-Aguiar, J.A., Scandura, M., Apollonio, M., Ferroglio, E., Zanet, S., Vada, R., Keuling, O., Plis, K., Podgorski, T., Brivio, F., Fernández-López, J., Ruiz-Rodríguez, C., Soriguer, R.C., Acevedo, P., 2022. New models for wild ungulates occurrence and hunting yield abundance at European scale. EFSA Support. Publ. 19. https://doi.org/10.2903/sp.efsa.2022.EN-7631

Linnell, J.D.C., Cretois, B., Nilsen, E.B., Rolandsen, C.M., Solberg, E.J., Veiberg, V., Kaczensky, P., Van Moorter, B., Panzacchi, M., Rauset, G.R., Kaltenborn, B., 2020. The challenges and opportunities of coexisting with wild ungulates in the human-dominated landscapes of Europe's Anthropocene. Biol. Conserv. 244, 108500. https://doi.org/10.1016/j.biocon.2020.108500

Otón, G., Lizundia-Loiola, J., Pettinari, M.L., Chuvieco, E., 2021. Development of a consistent global long-term burned area product (1982–2018) based on AVHRR-LTDR data. Int. J. Appl. Earth Obs. Geoinf. 103. https://doi.org/10.1016/j.jag.2021.102473

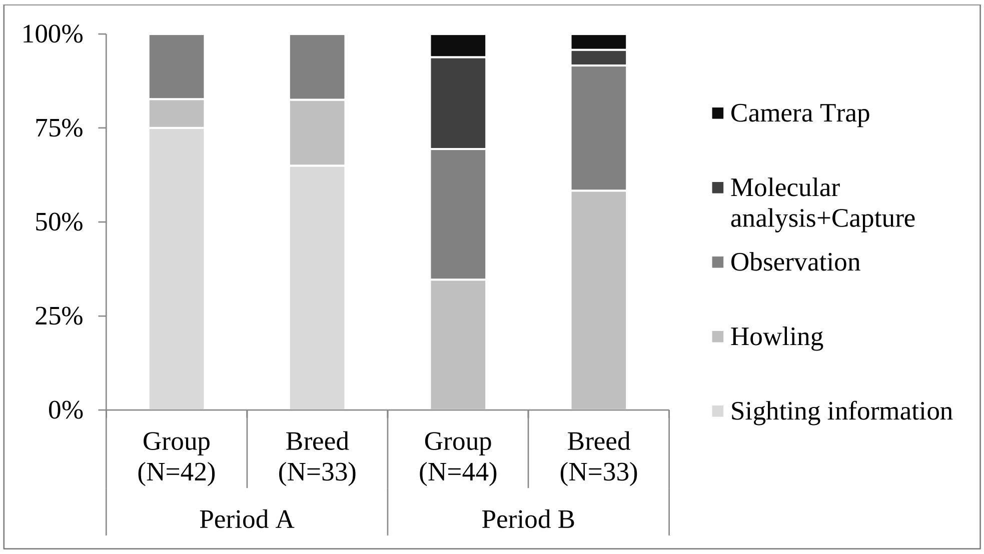
Riley, S.J., DeGloria, S.D., Elliot, R., 1999. A Terrain Ruggedness Index that Qauntifies Topographic Heterogeneity. Intermt. J. Sci. 5, 23–27.

Appendix IV – Supplementary data for Chapter IV

The supplementary data to the article can be found online at:
https://doi. org/10.1016/j.biocon.2021.109075,

Manuscript Title: Source-sink dynamics promote wolf persistence in human-modified landscapes: insights from long-term monitoring

Appendix IV-A. Proportion of methodological approaches used for wolf monitoring in northwest Portugal between 1996 and 2016.



Sup. Fig. 11: Proportion of each methodological approach that contributed to group (Group) and breeding (Breed) detections of wolves in northwest Portugal during period A (1996-2005) and period B (2007-2016).

Sup. Table 12: Proportion of each method and combined methods used for group and breeding detections of wolves in northwest Portugal during period A (1996-2005) and period B (2007-2016).

Period	**Methods***	**%**	**%**
A	Sight	76.2	81.0
	Obs	2.4	
	How	2.4	
	Sight + Obs	4.8	9.5
	Sight + How	2.4	
	How + Obs	2.4	
	Sight + How + Obs	9.5	9.5
B	Obs	2.3	36.4
	How	25.0	
	Mol	6.8	
	Cam	2.3	
	How + Obs	9.1	22.7
	How + Cap	2.3	
	How + Mol	4.5	
	Mol + Cap	2.3	
	Mol + Cam	4.5	
	How + Obs + Cap	9.1	20.5
	How + Obs + Mol	4.5	
	How + Mol + Cap	2.3	
	How + Obs + Cam	2.3	
	How + Mol + Cam	2.3	
	How + Obs + Mol + Cap	13.6	13.6
	How + Obs + Mol + Cap + Cam	6.8	6.8

* Sight – Sighting Information; Obs – Observation Survey; How – Howling Survey; Mol – Molecular Analysis; Cap – Capture/GPS Collaring; Cam – Camera Trapping

Appendix IV-B. Additional methodological details regarding genetic analysis of wolf non-invasive samples for species and individual identification

We assessed species identification through the amplification of a ≈420bp sized fragment of the mtDNA control region I using universal primers ThrL-15926 and DLH-16340 (Vilà et al. 1999). Successful amplifications were sequenced for both strands following the BigDye chemistry (Applied Biosystems) and sequencing products were separated in an ABI3130xl DNA analyzer. Sequences were aligned and compared using SeqScape 2.0 (Applied Biosystems). Samples with wolf mtDNA were genotyped for a set of 19 microsatellites amplified in four multiplex reactions, and for the DBX/DBY sex identification system (Seddon 2005) using the QIAGEN Multiplex PCR Kit and following the manufacturer's instructions (Table B1). Fluorescence labeling was achieved following (Blacket et al., 2012).

Sup. Table 13: Nuclear markers, multiplex, dye, and PCR conditions for amplification of the 19 loci genotyped for non-invasive samples from wolves in northwest Portugal.

Nuclear	Multiplex	Dye	Annealing	Extension	N cycles
AHT111 **AHT121** **C04.140** **C09.173** **C20.253** **CPH9** **FH2001**	MP 1	VIC VIC FAM NED PET NED PET	56°C/45 sec	30 sec	45
AHT137 **C22.279** **INRA21** **INU55**	MP 2	VIC FAM FAM FAM	58°C/45 sec	30 sec	45
FH2054 **FH2161** **INU30** **REN169O18**	MP 3	VIC NED NED FAM	58°C/45 sec	30 sec	45
CPH14 **PEZ3** **PEZ5** **REN247M23**	MP 4	FAM NED VIC PET	56°C/45 sec	30 sec	45

Error rates were estimated for allele dropout (ADO) and the presence of false alleles (FA). Average values for the whole dataset were 10.6% (range 28.1 – 1.3) for ADO and 0.7% (range 3.4 – 0.0) for FA (see Table B2 for individual values per locus). The Probability of Identity for this dataset was PID = 2.33 x 10^{-13} and PIDsib = 3.39x 10^{-6} (see Table B2 for individual values per locus).

Sup. Table 14: Error rates (allele dropout and false alleles) and Probability of Identity among all individuals (PID) and among siblings (PIDsibs) for each locus used in this study.

Locus	Allele dropout	False alleles	PID	PIDsibs
AHT111	0.013	0.000	0.142	0.443
AHT121	0.048	0.000	0.231	0.532
AHT137	0.139	0.000	0.251	0.533
C04.140	0.071	0.005	0.179	0.465
C09.173	0.022	0.000	0.549	0.747
C20.253	0.023	0.000	0.113	0.414
C22.279	0.038	0.023	0.149	0.445
CPH9	0.076	0.007	0.410	0.653
CPH14	0.206	0.034	0.179	0.484
FH2001	0.078	0.011	0.281	0.564
FH2054	0.156	0.007	0.403	0.636
FH2161	0.215	0.025	0.133	0.430
INRA21	0.020	0.000	0.128	0.431
INU030	0.151	0.005	0.335	0.616
INU055	0.260	0.000	0.108	0.408
Pez3	0.086	0.005	0.142	0.439
Pez5	0.020	0.000	0.157	0.454
REN169O18	0.107	0.012	0.390	0.648
REN247M23	0.281	0.008	0.387	0.620

References (Appendix IV-B)

Blacket MJ, Robin C, Good RT, et al. (2012) Universal primers for fluorescent labeling of PCR fragments--an efficient and cost-effective approach to genotyping by fluorescence. Mol Ecol Resour 12:456–63. doi: 10.1111/j.1755-0998.2011.03104.x

Seddon JM (2005) Canid-specific primers for molecular sexing using tissue or non-invasive samples. Conservation Genetics, 6, 147–149.

Vilà C, Amorim I, Leonard J, et al. (1999) Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. Mol Ecol 8:2089–103.

Appendix IV-C. Additional information and results regarding wolf monitoring in northwest Portugal between 1996 and 2016: wolf population size estimation based on a capture-recapture model; number of wolves, average sex ratios, group confirmation, and breeding success; dispersal events; and pack migrant ratios.

Sup. Table 15 – Input data and results of wolf population size estimation based on a capture-recapture model for period B (2007-2016) in northwest Portugal. Number of times an individual was captured and genetically identified from noninvasive samples per year (*Class*), average number of captures per individual per year, annual population size Maximum Likelihood Estimates (*PS_MLE*) with 95% confidence intervals (*95% CI*), and p-value of Likelihood ratio test (*LRT p-value*). See the Methods section for details.

Class	**Year**									
	2007	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
1	6	8	9	10	14	15	17	6	12	17
2	3	2	3	9	3	12	11	3	6	10
3	2	2	1	3	2	3	7	3	3	2
4	0	0	1	0	3	0	2	1	2	2
5	0	0	0	0	1	0	1	1	2	0
6	0	0	0	0	0	0	0	0	0	1
7	0	0	0	0	0	1	0	0	0	0
Average captures per individual	1.64	1.50	1.50	1.64	1.87	1.77	1.92	2.14	2.04	1.78
PS_MLE **(95% CI)**	17 (12-25)	19 (12-32)	21 (14-39)	31 (22-43)	44 (33-69)	42 (33-55)	48 (38-58)	16 (14-20)	30 (25-37)	43 (34-56)
LRT p-value	0.30	0.29	0.19	0.87	0.01	0.42	0.32	0.23	0.12	0.20

Sup. Table 16 – Annual number of wolves detected in northwest Portugal during period A (1996-2005) and period B (2007-2016), based on minimum counts from all methodologies. The number of wolves detected per year is presented for each pack (pack size), including the number of residents, dispersers, and uncategorized individuals, and for all the study area (Population size). It is also presented the annual percentage of the population detected by field methods or genetically identified, average sex ratios M:F (only for Period B; see Methods section for details), group persistence rate (GroupPR), and breeding success rate (BreedS) for each pack. Annual pack sizes are highlighted considering group detection without reproduction (light grey) or group detection with reproduction (dark grey).

		Period A										**Period B**										**Average sex ratio** (Period B)		**Group PR**	**Breed S**
	Year	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**				
Pack Type	**Pack size**																								
Sink	Arga	6	6	4	4	4	4	0	0	0	0	0	0	0	0	0	0	3	11	9	7	2.1:1	1.5:1	50.0	90.0
	Boulhosa	5	6	2	4	2	2	0	0	2	0	0	0	2	0	4	3	3	2	2	5	0.9:1		70.0	35.7
	Cruz Vermelha	4	0	0	0	0	0	0	0	0	0	0	0	0	6	6	6	5	6	3	6	1.0:1		40.0	87.5
	Peneda	10	5	2	3	3	7	6	2	0	0	0	6	2	2	6	7	6	0	0	0	1.9:1		70.0	57.1
Core	Soajo	7	8	9	6	4	6	4	5	10	8	6	7	3	10	6	12	9	10	6	5	1.2:1	0.9:1	100	90.0
	Vez	8	8	10	9	6	9	14	9	8	6	6	6	7	7	16	11	15	6	7	9	0.8:1		100	95.0
N residents		40	33	27	26	19	28	24	16	20	14	12	19	14	25	38	39	41	35	27	32	1.3:1			
N dispersers and uncategorized individual		0	0	1	0	0	0	1	1	2	0	0	0	2	0	0	0	2	0	0	1				
Population size		40	33	28	26	19	28	25	17	22	14	12	19	16	25	38	39	43	35	27	33	1.1:1			
% population detected by field methods		100	100	100	100	100	100	100	100	100	100	100	100	88	100	97	100	93	89	96	100				
% population genetically identified		-	-	-	-	-	-	-	-	-	-	92	74	100	88	66	85	100	46	89	100				

Sup. Table 17 – Description of the 17 individuals involved in dispersal events between packs in northwest Portugal, during each sub-period 2007-2010, 2011-2013, and 2014-2016 (highlighted in grey), based on GPS telemetry of collared wolves ("GPS") and noninvasive genetic sampling ("gNIS"), with reference to sex, the total number of detections in the noninvasive genetic sampling, dispersal distances, and packs where annual detections were obtained. Pack ID: A- Arga, Cv-Cruz Vermelha, B-Boulhosa, P-Peneda, S-Soajo, V-Vez; packs where the wolf was confirmed as a resident are highlighted in bold. * Wolf ID8 was involved in two different dispersal events.

Wolf ID	Sex	N detections gNIS	Dispersal distance (km)	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Method
1	F	8	28.8		V/B	**B**		**B**						gNIS
2	F	8	5.5	V	V	**P**								gNIS
3	M	7	36.1				**V**	**V**		A		**A**		GPS+gNIS
4	F	3	25.0				**S**		**S/B**	**B**				GPS+gNIS
5	F	12	42.2				**S**			**A**	**A**	**A**	**A**	gNIS
6	F	2	15.1						V/P					gNIS
7	M	8	59.6						**P**	**A**	**A**		**A**	GPS+gNIS
8*	M	5	32.2; 12.6					P	**P**	Cv			**B**	GPS+gNIS
9	M	6	7.4						S			**V**		gNIS
10	F	4	35.8						Cv	**Cv**			P	gNIS
11	M	6	7.4						**S**				**V**	gNIS
12	F	6	13.0							Cv	**B**	**B**	B	gNIS
13	M	5	22.2							**Cv**		**A**	A	GPS+gNIS
14	F	5	7.6							**S**			**V**	gNIS
15	M	9	22.0							**S**		**Cv**	**Cv**	gNIS
16	M	8	22.0								**A**	**A**	B	gNIS
17	M	10	52.6								**A**	**A**	**P**	gNIS

Sup. Table 18 – Values of Migrant Ratio (MR) used to identify pack type as either Core packs (MR≤0) or Sink packs (MR>0) in northwest Portugal, considering each sub-period included in period B (2007-2016), and for all period B. See the Methods section for details.

Pack type	Pack	Sub-period			Average period B 2007-2016	
		2007-2010	2011-2013	2014-2016		
Sink	Arga	-	1.0	-0.3	0.3	0.50
	Cruz Vermelha	-	1.0	-0.6	0.2	
	Boulhosa	1.0	1.0	1.0	1.0	
	Peneda	1.0	-0.3	1.0	0.6	
Core	Soajo	-	-1.0	-1.0	-1.0	-0.43
	Vez	-1.0	-1.0	1.0	-0.3	

Appendix V – Supplementary data for Chapter V

Appendix V-A. Brief description of the procedures for species and individual molecular identification:

DNA from the blood of the captured wolves was extracted using the commercial DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's instructions, and samples were genotyped for a total of 50 microsatellites following Godinho et al. (2015). Non-invasive samples were extracted and PCR amplified following the procedures of Boom et al. (1990) and Frantz et al. (2003) for scats preserved in 96% ethanol and for urine, saliva, and hair samples preserved in silica-gel, whereas urine and saliva samples preserved in 96% ethanol were extracted using the commercial QIAamp DNA Micro Kit (QIAGEN). Negative controls were included throughout the entire process to monitor for potential DNA contamination. All pre-PCR procedures were performed in dedicated laboratories used exclusively for low-quality DNA samples. Species identification was performed through mitochondrial (mtDNA) control region sequencing, and samples exhibiting wolf mtDNA were genotyped for a set of 19 microsatellites selected among the most variable in Iberian wolves (Godinho et al., 2015, 2011) and for a sex identification marker (DBX/DBY, Seddon, 2005). Microsatellites were amplified in four multiplex reactions and separated by size on an ABI3130xl DNA analyzer. Alleles were scored using GENEMAPPER 5.0 (Applied Biosystems) and checked manually. Quality control for non-invasive DNA was assessed by PCR replication as described in Nakamura et al. (2017), and consensus genotype over four replicas for each sample was achieved following rules defined in Godinho et al. (2015). Error rates and the probability of identity for our dataset were calculated using the software GIMLET 1.3.3 (Valière, 2002). The same software was used to identify multiple samples of the same individual.

References (Appendix V-A)

Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Noordaa, J. Van Der, 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28, 495–503.

Frantz, A.C., Pope, L.C., Carpenter, P.J., Roper, T.J., Wilson, G.J., Delahay, R.J., Burke, T., 2003. Reliable microsatellite genotyping of the Eurasian badger (Meles meles) using faecal DNA. Mol. Ecol. 12, 1649–1661. https://doi.org/10.1046/j.1365-294X.2003.01848.x

Godinho, R., Llaneza, L., Blanco, J.C., Lopes, S., Álvares, F., García, E.J., Palacios, V., Cortés, Y., Talegón, J., Ferrand, N., 2011. Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. Mol. Ecol. 20, 5154–5166. https://doi.org/10.1111/j.1365-294X.2011.05345.x

Godinho, R., López-Bao, J.V., Castro, D., Llaneza, L., Lopes, S., Silva, P., Ferrand, N., 2015. Real-time assessment of hybridization between wolves and dogs: combining noninvasive samples with ancestry informative markers. Mol. Ecol. Resour., 15, 317–328. https://doi.org/10.1111/1755-0998.12313

Nakamura, M., Godinho, R., Rio-Maior, H., Roque, S., Kaliontzopoulou, A., Bernardo, J., Castro, D., Lopes, S., Petrucci-Fonseca, F., Álvares, F., 2017. Evaluating the predictive power of field variables for species and individual molecular identification on wolf noninvasive samples. Eur. J. Wildl. Res. 63, 53. https://doi.org/10.1007/s10344-017-1112-7

Valière, N., 2002. GIMLET: a computer program for analysing genetic individual identification data. Mol. Ecol. Notes 2, 377–379. https://doi.org/10.1046/j.1471-8278

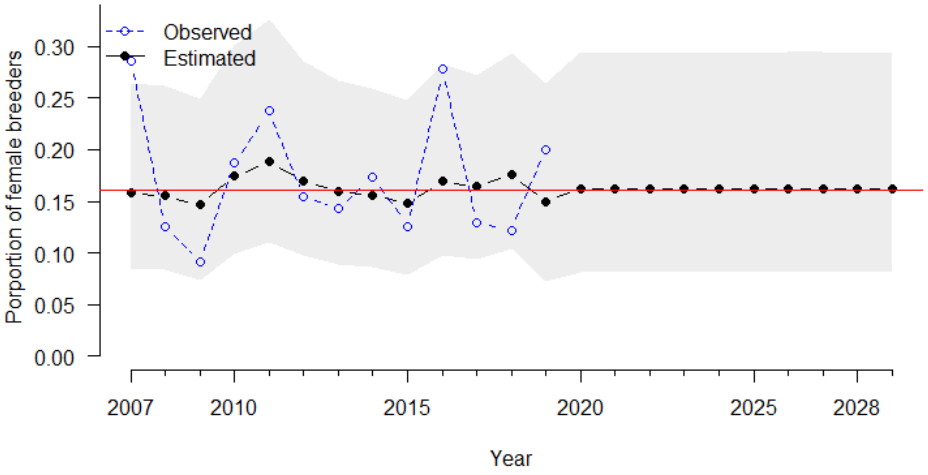
Appendix V-B: R code of the integrated population model developed for the sampling period (2007-2019) with a population viability analysis for the forecasting period (2020-2029, K=10 years)

```
# Specify model in BUGS language
cat(file = "model_IPM_FINAL_PVA_Control.jags", "
model {

# Priors and linear models
  # Proportion of female breeders
    mean.Prop_fb ~ dbeta(4, 23)
    mu.Prop_fb <- logit(mean.Prop_fb)
    sigma.Prop_fb ~ dunif(0, 5)
    sqrt.tau.Prop_fb <- 1/sigma.Prop_fb
    sigma.Prop_fb2 <- pow(sigma.Prop_fb, 2)

  # Fecundity (litter size)
    mean.F ~ dlnorm(log(3.9), .2)
    mu.F <- log(mean.f)
    sigma.F ~ dunif(0, 5)
    sqrt.tau.F <- 1/sigma.F
    sigma.F2 <- pow(sigma.F, 2)

  # Observation
    sigma.obs ~ dunif(0.5, 100)
    tau.obs <- pow(sigma.obs, -2)

  # Population Count
    Nprior[1,1] ~ dnorm(10, 1)
    Nprior[2,1] ~ dnorm(15, 1)

  # Pup survival
    mean.Spup ~ dunif(0.28, 0.72)

  # Capture-Recapture
# -------------------------------------------------
# Parameters:
# Sad: true survival probability
# Fd: fidelity probability
# rr: recovery probability
# pp: recapture/resighting probability
# -------------------------------------------------
# States (S):
# 1 alive in study area
# 2 alive outside study area
# 3 recently dead and recovered
# 4 recently dead, but not recovered, or dead (absorbing)
# Observations (O):
# 1 seen alive
# 2 recovered dead
# 3 neither seen nor recovered
# -------------------------------------------------
    Sad ~ dunif(0, 1)
    mu.Sad <- logit(Sad)
    sigma.Sad ~ dunif(0, 5)
    sqrt.tau.Sad <- 1/sigma.Sad
    sigma.Sad2 <- pow(sigma.Sad, 2)

    Fd ~ dunif(0, 1)
    rr ~ dunif(0, 1)
    pp ~ dunif(0, 1)
```

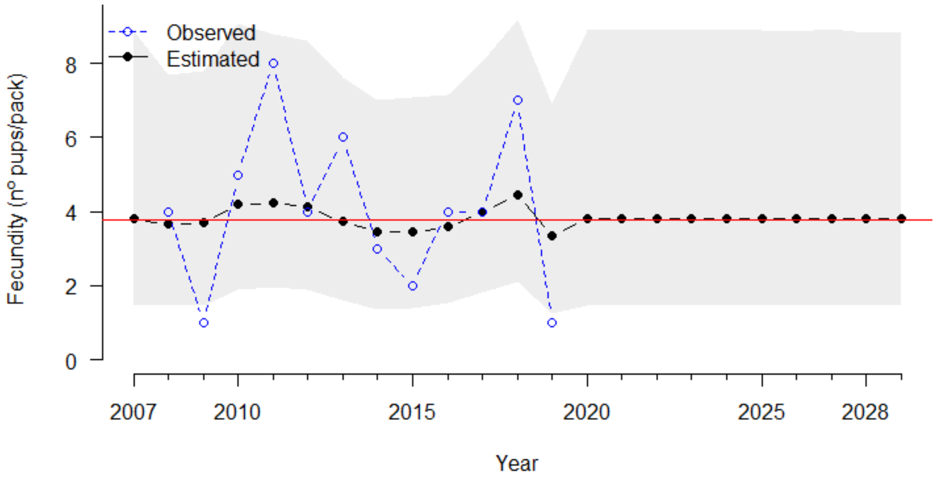
```
# Constraints Capture-recapture, Proportion Breeders and Fecundity
 for (t in 1:(n.occasions+K)){
    epsilon.Prop_fb[t] ~ dnorm(0, 1)
    epsilon.F[t] ~ dnorm (0, 1)

    # calculate annual estimates of proportion breeders and fecundity
    logit(Prop_fb[t])  <-  mu.Prop_fb  +  sqrt.tau.Prop_fb  *  epsilon.
Prop_fb[t]
    log(F[t]) <- mu.F +  sqrt.tau.F * epsilon.F[t]
}

 for (t in 1:(n.occasions-1+K)){
    epsilon.Sad[t] ~ dnorm(0, 1)

    # calculate annual estimates of survival, fidelity, recovery and
recapture
    logit(Sad.est[t]) <- mu.Sad + sqrt.tau.Sad * epsilon.Sad[t]

    # Define state-transition and observation matrices
    # Define probabilities of state S(t+1) given S(t)
      ps[1,1,t] <- Sad.est[t] * ff
      ps[1,2,t] <- Sad.est[t] * (1 - ff)
      ps[1,3,t] <- 1 - Sad.est[t]
      ps[1,4,t] <- 0
      ps[2,1,t] <- 0
      ps[2,2,t] <- Sad.est[t]
      ps[2,3,t] <- 1 - Sad.est[t]
      ps[2,4,t] <- 0
      ps[3,1,t] <- 0
      ps[3,2,t] <- 0
      ps[3,3,t] <- 0
      ps[3,4,t] <- 1
      ps[4,1,t] <- 0
      ps[4,2,t] <- 0
      ps[4,3,t] <- 0
      ps[4,4,t] <- 1
  } #t

    # Define probabilities of O(t) given S(t)
      po[1,1] <- pp
      po[1,2] <- 0
      po[1,3] <- 1 - pp
      po[2,1] <- 0
      po[2,2] <- 0
      po[2,3] <- 1
      po[3,1] <- 0
      po[3,2] <- rr
      po[3,3] <- 1 - rr
      po[4,1] <- 0
      po[4,2] <- 0
      po[4,3] <- 1

# Capture-Recapture data
    # Likelihood
      for (i in 1:nind){
         # Define latent state at first capture
         z[i,first[i]] <- y[i,first[i]]

         for (t in (first[i]+1):n.occasions){

            # State process: draw S(t) given S(t-1)
```

```
      z[i,t] ~ dcat(ps[z[i,t-1], 1:4, t-1])

      # Observation process: draw O(t) given S(t)
      y[i,t] ~ dcat(po[z[i,t], 1:3])

      } #t
    } #i

# Population count data (state-space model)
# Model for the initial stage-specific population sizes
  N[1,1] <- round(Nprior[1,1])
  N[2,1] <- round(Nprior[2,1])

 # Loop over time
 for (t in 1:(n.occasions-1+K)){

  # For Spup and Sad.est
    # Population projection
  N1p[t+1] ~ dbinom(mean.Spup, N[1,t])    # number of pups born in
year t that survive and become an adult in t+1

  N2a[t+1] ~ dbinom(Sad.est[t], N[2,t])    # number of adults that
survive from t to t+1

  N[2,t+1] <- sum(N1p[t+1], N2a[t+1])    # number of adults

  N[1,t+1] ~ dpois(N[2,t+1] * Prop_fb[t+1] * F[t+1]) # number of
pups
 }

# Observation model
   # Productivity data: Nº females (proportion of female breeders
   within adults)
    for (t in 1:(n.occasions-1)){
    y_female[t] ~ dbinom(Prop_fb[t], N[2,t])
    }

   # Population count data
   for (t in 1:n.occasions){
     C[t] ~ dnorm(N[1,t] + N[2,t], tau.obs)
   }

# Derived parameters
 # Annual population growth rate
 for (t in 1:(n.occasions-1+K)){
 lambda[t] <- (N[1,t+1] + N[2,t+1]) / (N[1,t] + N[2,t] + 0.001)
 }

 # Total population size
 for (t in 1:(n.occasions+K)){
  Ntot[t] <- N[1,t] + N[2,t]
 }

 # Check whether the population is extinct in the future
  for (t in 1:K) {
 extinct[t] <- equals(Ntot[n.occasions+t], 0) # Determines whether
population is still thriving (extinct = 0) or went extinct (extinct =
1)
  }
}
", fill = TRUE)
```

Appendix V-C

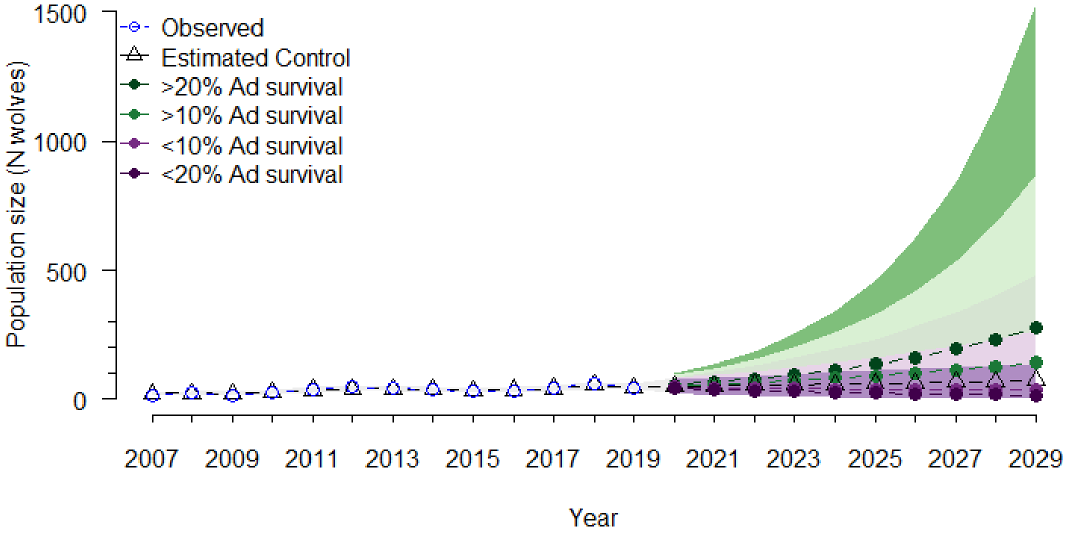


A

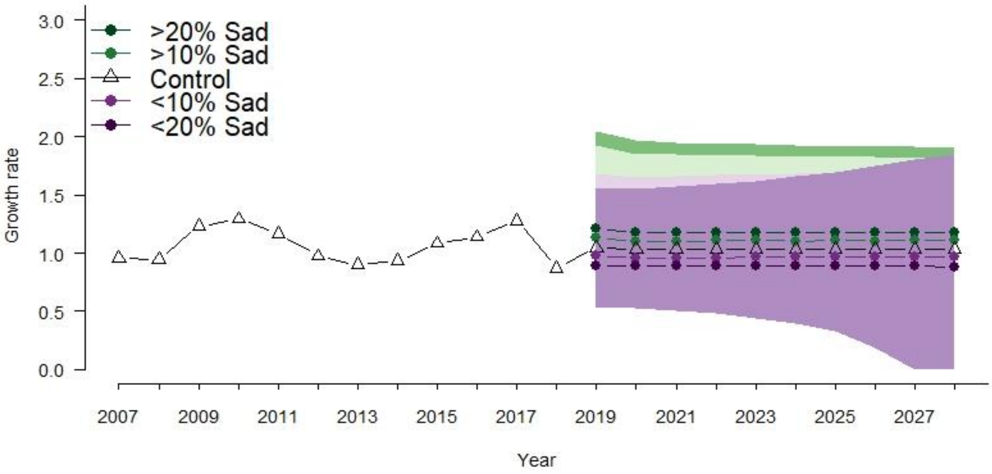


B

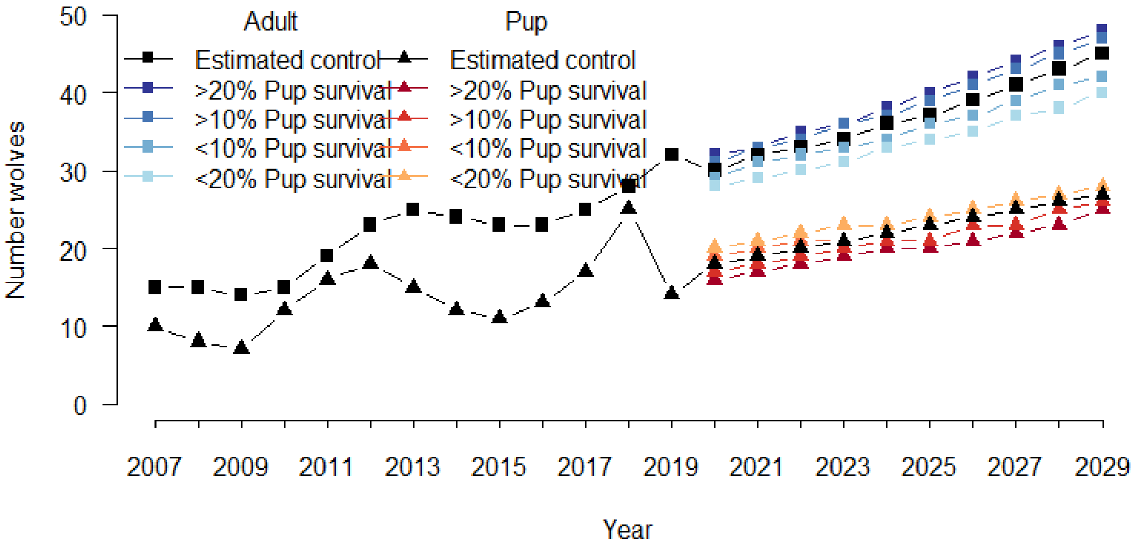
Sup. Fig. 12: Annual observed values and parameter estimates obtained by the integrated population model for the sampling period (2007-2019) and the forecast period (2020-2029): A) proportion of female breeders among adults; and B) fecundity. (red horizontal lines represent average values of annual estimates, and shadow areas represent 95% confidence intervals).



Sup. Fig. 13**:** Annual observed values and estimates for population size obtained by the integrated population model for the sampling period (2007-2019) and the forecast period (2020-2029). Predictions for the forecast period obtained without (control) and with variation (-10%, -20%, +10% and +20%) in adult survival rates; shadow areas represent 95% Bayesian credible intervals.



Sup. Fig. 14: Annual growth rate estimated by the integrated population model for the sampling period (2007-2019) and the forecast period (2020-2029). Predictions for the forecast period obtained without (control) and with variation (-10%, -20%, +10% and +20%) in adult survival rates (Sad); shadow areas represent 95% Bayesian credible intervals.



Sup. Fig. 15**:** Annual estimated number of adults and pups obtained by the integrated population model for the sampling (2007-2019) and forecast (2020-2029) periods. Predictions for the forecast period obtained without (control) and with variation (-10%, -20%, +10% and +20%) in pup survival rates.

Sup. Table 19 – Integrated population model output for sampling (t=1 to t=13; 2007-2019) and forecast (t=14 to t=23; 2020-2029) periods.

Parameters:

N[1,t] - Number of pups
N[2,t] - Number of adults
Ntot[t] - Population size
λ[t] – Growth rate
F [t] – fecundity;
Prop_fb[t] – proportion of female breeders
Sad.est[t] – annual adult survival
mean.F – mean fecundity
mean.Prop_fb – mean proportion of breeders
mean.Spup – mean pup survival
Sad – adult survival
Fd – fidelity
pp – detection probability
rr – dead recovery probability

parameter	mean	sd	X2.5.	X25.	X50.	X75.	X97.5.	Rhat	n.eff
N[1,1]	9.68	1.04	8.00	9.00	10.00	10.00	12.00	1.00	380.00
N[2,1]	14.61	1.03	13.00	14.00	15.00	15.00	17.00	1.00	73,000
N[1,2]	8.38	3.87	2.00	6.00	8.00	11.00	17.00	1.00	380,00
N[2,2]	15.38	2.61	10.00	14.00	15.00	17.00	20.00	1.00	140,00
N[1,3]	7.96	3.73	2.00	5.00	8.00	10.00	16.00	1.00	110,00
N[2,3]	14.45	3.08	9.00	12.00	14.00	16.00	21.00	1.00	84,000
N[1,4]	12.68	4.60	5.00	9.00	12.00	15.00	23.00	1.00	130,00
N[2,4]	14.89	3.24	9.00	13.00	15.00	17.00	22.00	1.00	120,00
N[1,5]	16.81	5.61	7.00	13.00	16.00	20.00	29.00	1.00	28,000
N[2,5]	18.78	3.66	12.00	16.00	19.00	21.00	26.00	1.00	380,00
N[1,6]	17.75	6.10	7.00	13.00	17.00	22.00	31.00	1.00	380,00
N[2,6]	23.70	4.35	16.00	21.00	24.00	27.00	32.00	1.00	12,000
N[1,7]	15.12	5.63	5.00	11.00	15.00	19.00	27.00	1.00	160,00
N[2,7]	25.17	4.55	17.00	22.00	25.00	28.00	34.00	1.00	17,000
N[1,8]	12.29	5.02	4.00	9.00	12.00	15.00	23.00	1.00	15,000
N[2,8]	23.98	4.24	16.00	21.00	24.00	27.00	32.00	1.00	13,000
N[1,9]	10.99	4.66	3.00	8.00	11.00	14.00	21.00	1.00	29,000
N[2,9]	22.98	4.03	15.00	20.00	23.00	26.00	31.00	1.00	53,000
N[1,10]	13.75	5.09	5.00	10.00	13.00	17.00	25.00	1.00	34,000
N[2,10]	23.16	4.06	16.00	20.00	23.00	26.00	32.00	1.00	380,00
N[1,11]	17.52	5.81	7.00	13.00	17.00	21.00	30.00	1.00	110,00
N[2,11]	24.47	4.31	16.00	22.00	24.00	27.00	33.00	1.00	380,00
N[1,12]	24.64	7.70	11.00	19.00	24.00	30.00	40.00	1.00	4,800
N[2,12]	28.85	4.86	20.00	25.00	29.00	32.00	39.00	1.00	380,00
N[1,13]	14.99	6.18	4.00	11.00	14.00	19.00	28.00	1.00	12,000
N[2,13]	31.95	5.48	21.00	28.00	32.00	36.00	43.00	1.00	5,900
N[1,14]	20.99	16.08	4.00	12.00	18.00	26.00	53.00	1.01	380,00
N[2,14]	30.50	6.24	19.00	26.00	30.00	35.00	43.00	1.00	23,000
N[1,15]	22.89	72.96	4.00	12.00	19.00	29.00	63.00	1.26	240,00
N[2,15]	32.67	11.78	16.00	26.00	31.00	38.00	56.00	1.00	50,000
N[1,16]	24.92	67.75	4.00	12.00	20.00	31.00	74.00	1.20	150,00
N[2,16]	35.26	50.24	14.00	25.00	33.00	42.00	70.00	1.00	290,00
N[1,17]	27.38	58.00	3.00	12.00	21.00	33.00	88.00	1.05	170,00
N[2,17]	38.18	52.50	12.00	25.00	34.00	46.00	86.00	1.18	360,00
N[1,18]	30.44	108.75	3.00	12.00	21.00	36.00	104.00	1.04	150,00
N[2,18]	41.63	63.66	11.00	25.00	36.00	50.00	104.00	1.01	300,00

parameter	mean	sd	X2.5.	X25.	X50.	X75.	X97.5.	Rhat	n.eff
N[1,19]	34.89	537.28	3.00	12.00	22.00	39.00	124.00	1.06	380,00
N[2,19]	45.76	103.95	10.00	25.00	37.00	55.00	126.00	1.01	240,00
N[1,20]	41.44	1,748.4	2.00	12.00	23.00	43.00	147.00	1.27	380,00
N[2,20]	51.22	370.03	9.00	24.00	39.00	60.00	152.00	1.03	380,00
N[1,21]	44.25	498.87	2.00	12.00	24.00	46.00	174.00	1.02	380,00
N[2,21]	58.82	1,265.3	8.00	24.00	40.00	66.00	184.00	1.26	380,00
N[1,22]	58.88	4,149.0	2.00	12.00	25.00	50.00	208.00	1.29	150,00
N[2,22]	65.68	1,201.0	7.00	24.00	42.00	72.00	220.00	1.23	380,00
N[1,23]	62.78	1,651.2	2.00	12.00	26.00	55.00	245.00	1.27	55,000
N[2,23]	77.74	2,109.9	6.00	24.00	44.00	78.00	265.00	1.13	240,00
Ntot[1]	24.29	1.46	21.00	23.00	24.00	25.00	27.00	1.00	110,00
Ntot[2]	23.76	4.13	16.00	21.00	24.00	26.00	33.00	1.00	220,00
Ntot[3]	22.40	4.49	15.00	19.00	22.00	25.00	32.00	1.00	380,00
Ntot[4]	27.57	4.87	19.00	24.00	27.00	31.00	38.00	1.00	380,00
Ntot[5]	35.59	5.44	25.00	32.00	36.00	39.00	47.00	1.00	26,000
Ntot[6]	41.45	5.87	30.00	38.00	42.00	45.00	53.00	1.00	27,000
Ntot[7]	40.29	5.51	29.00	37.00	40.00	44.00	51.00	1.00	64,000
Ntot[8]	36.28	5.05	27.00	33.00	36.00	39.00	47.00	1.00	380,00
Ntot[9]	33.98	5.00	25.00	31.00	34.00	37.00	45.00	1.00	220,00
Ntot[10]	36.90	5.33	27.00	33.00	37.00	40.00	48.00	1.00	67,000
Ntot[11]	41.98	5.58	31.00	38.00	42.00	45.00	54.00	1.00	85,000
Ntot[12]	53.49	6.86	39.00	49.00	54.00	58.00	66.00	1.00	3,900
Ntot[13]	46.94	6.51	35.00	43.00	47.00	51.00	61.00	1.00	150,00
Ntot[14]	51.49	18.12	28.00	41.00	49.00	59.00	88.00	1.00	110,00
Ntot[15]	55.56	78.63	24.00	40.00	51.00	65.00	110.00	1.00	380,00
Ntot[16]	60.18	94.62	21.00	40.00	54.00	72.00	134.00	1.00	380,00
Ntot[17]	65.57	91.65	19.00	39.00	56.00	79.00	164.00	1.00	160,00
Ntot[18]	72.07	161.24	17.00	39.00	58.00	86.00	198.00	1.02	160,00
Ntot[19]	80.65	594.89	15.00	39.00	61.00	94.00	240.00	1.04	380,00
Ntot[20]	92.66	2,052.5	13.00	39.00	63.00	103.00	289.00	1.26	380,00
Ntot[21]	103.08	1,610.0	12.00	39.00	66.00	112.00	346.00	1.19	380,00
Ntot[22]	124.56	4,493.8	10.00	38.00	69.00	123.00	417.00	1.22	220,00
Ntot[23]	140.52	3,328.7	9.00	38.00	72.00	134.00	499.00	1.20	85,000
λ[1]	0.98	0.17	0.68	0.87	0.96	1.08	1.35	1.00	100,00
λ[2]	0.96	0.21	0.62	0.82	0.94	1.08	1.42	1.00	380,00
λ[3]	1.27	0.29	0.81	1.07	1.23	1.42	1.94	1.00	380,00
λ[4]	1.32	0.28	0.87	1.13	1.29	1.48	1.95	1.00	28,000
λ[5]	1.18	0.21	0.83	1.05	1.17	1.30	1.66	1.00	380,00
λ[6]	0.99	0.16	0.71	0.87	0.97	1.08	1.35	1.00	220,00
λ [7]	0.91	0.15	0.66	0.81	0.90	1.00	1.26	1.00	78,000
λ [8]	0.95	0.16	0.68	0.84	0.93	1.03	1.31	1.00	330,00
λ [9]	1.10	0.18	0.79	0.97	1.08	1.21	1.52	1.00	380,00
λ [10]	1.16	0.20	0.82	1.02	1.14	1.27	1.59	1.00	380,00
λ [11]	1.29	0.22	0.92	1.15	1.27	1.42	1.76	1.00	4,000
λ [12]	0.89	0.15	0.65	0.79	0.87	0.97	1.22	1.00	8,000
λ [13]	1.10	0.36	0.67	0.90	1.05	1.23	1.80	1.00	250,00
λ [14]	1.08	0.33	0.65	0.89	1.03	1.21	1.75	1.00	140,00

parameter	mean	sd	X2.5.	X25.	X50.	X75.	X97.5.	Rhat	n.eff
λ[15]	1.08	0.42	0.65	0.89	1.03	1.21	1.74	1.00	380,00
λ[16]	1.08	0.32	0.65	0.89	1.03	1.21	1.75	1.01	110,00
λ[17]	1.08	0.36	0.65	0.89	1.03	1.21	1.74	1.04	380,00
λ[18]	1.08	0.32	0.65	0.89	1.03	1.21	1.74	1.00	210,00
λ[19]	1.08	0.33	0.64	0.89	1.03	1.21	1.75	1.01	380,00
λ[20]	1.08	0.31	0.64	0.89	1.04	1.21	1.74	1.00	270,00
λ[21]	1.07	0.32	0.64	0.89	1.03	1.21	1.74	1.01	380,00
λ[22]	1.08	0.32	0.63	0.89	1.04	1.21	1.75	1.00	340,00
F[1]	4.18	2.71	1.46	2.87	3.80	4.99	8.96	1.00	380,00
F[2]	3.85	1.59	1.41	2.77	3.62	4.67	7.58	1.00	380,00
F[3]	3.97	1.66	1.51	2.86	3.72	4.79	7.89	1.00	200,00
F[4]	4.60	1.99	1.92	3.29	4.24	5.48	9.40	1.00	63,000
F[5]	4.55	1.81	1.96	3.31	4.24	5.43	8.93	1.00	29,000
F[6]	4.39	1.73	1.87	3.20	4.10	5.26	8.54	1.00	380,00
F[7]	3.95	1.55	1.60	2.88	3.72	4.76	7.63	1.00	380,00
F[8]	3.66	1.45	1.38	2.66	3.46	4.44	7.05	1.00	29,000
F[9]	3.64	1.44	1.35	2.65	3.44	4.41	7.01	1.00	380,00
F[10]	3.78	1.45	1.52	2.78	3.58	4.55	7.16	1.00	65,000
F[11]	4.30	1.66	1.86	3.16	4.04	5.15	8.28	1.00	90,000
F[12]	4.73	1.82	2.11	3.47	4.43	5.64	9.13	1.00	15,000
F[13]	3.52	1.43	1.23	2.54	3.33	4.29	6.86	1.00	59,000
F[14]	4.18	3.03	1.45	2.87	3.79	5.00	8.97	1.00	380,00
F[15]	4.18	2.61	1.46	2.87	3.80	4.99	8.96	1.00	380,00
F[16]	4.17	3.05	1.45	2.87	3.79	4.99	8.94	1.00	380,00
F[17]	4.17	2.36	1.45	2.87	3.80	4.99	8.92	1.00	150,00
F[18]	4.17	2.52	1.46	2.86	3.79	4.99	8.93	1.00	380,00
F[19]	4.17	2.28	1.45	2.87	3.79	4.99	8.92	1.00	380,00
F[20]	4.17	2.41	1.45	2.87	3.80	5.00	8.95	1.00	380,00
F[21]	4.17	2.26	1.46	2.87	3.79	5.00	8.91	1.00	140,00
F[22]	4.16	2.29	1.45	2.87	3.79	4.99	8.93	1.00	380,00
F[23]	4.17	2.45	1.46	2.87	3.79	4.99	8.95	1.00	380,00
Fd	0.87	0.04	0.79	0.84	0.87	0.89	0.94	1.00	380,00
mean.F	3.89	1.06	2.20	3.14	3.76	4.50	6.32	1.00	380,00
mean.Prop_fb	0.16	0.03	0.11	0.14	0.16	0.18	0.23	1.00	25,000
mean.Spup	0.53	0.12	0.30	0.44	0.54	0.63	0.71	1.00	17,000
pp	0.44	0.04	0.36	0.41	0.44	0.47	0.52	1.00	380,00
Prop_fb[1]	0.16	0.05	0.09	0.13	0.16	0.19	0.26	1.00	120,00
Prop_fb[2]	0.16	0.04	0.08	0.13	0.15	0.18	0.26	1.00	270,00
Prop_fb[3]	0.15	0.04	0.07	0.12	0.15	0.18	0.25	1.00	28,000
Prop_fb[4]	0.18	0.05	0.10	0.15	0.18	0.21	0.31	1.00	380,00
Prop_fb[5]	0.20	0.06	0.11	0.16	0.19	0.23	0.33	1.00	190,00
Prop_fb[6]	0.17	0.05	0.10	0.14	0.17	0.20	0.28	1.00	87,000
Prop_fb[7]	0.16	0.05	0.09	0.13	0.16	0.19	0.27	1.00	50,000
Prop_fb[8]	0.16	0.04	0.09	0.13	0.16	0.19	0.26	1.00	88,000
Prop_fb[9]	0.15	0.04	0.08	0.12	0.15	0.18	0.25	1.00	41,000
Prop_fb[10]	0.17	0.05	0.10	0.14	0.17	0.20	0.28	1.00	310,00
Prop_fb[11]	0.17	0.05	0.10	0.14	0.17	0.20	0.28	1.00	380,00

parameter	mean	sd	X2.5.	X25.	X50.	X75.	X97.5.	Rhat	n.eff
Prop_fb[12]	0.18	0.05	0.10	0.15	0.18	0.21	0.29	1.00	21,000
Prop_fb[13]	0.16	0.05	0.07	0.12	0.15	0.18	0.26	1.00	11,000
Prop_fb[14]	0.17	0.06	0.08	0.13	0.16	0.20	0.29	1.00	220,00
Prop_fb[15]	0.17	0.05	0.08	0.13	0.16	0.20	0.29	1.00	49,000
Prop_fb[16]	0.17	0.06	0.08	0.13	0.16	0.20	0.30	1.00	50,000
Prop_fb[17]	0.17	0.06	0.08	0.13	0.16	0.20	0.29	1.00	57,000
Prop_fb[18]	0.17	0.06	0.08	0.13	0.16	0.20	0.30	1.00	71,000
Prop_fb[19]	0.17	0.06	0.08	0.13	0.16	0.20	0.29	1.00	52,000
Prop_fb[20]	0.17	0.06	0.08	0.13	0.16	0.20	0.29	1.00	36,000
Prop_fb[21]	0.17	0.05	0.08	0.13	0.16	0.20	0.30	1.00	79,000
Prop_fb[22]	0.17	0.05	0.08	0.13	0.16	0.20	0.29	1.00	110,00
Prop_fb[23]	0.17	0.05	0.08	0.13	0.16	0.20	0.29	1.00	28,000
rr	0.06	0.02	0.03	0.05	0.06	0.07	0.10	1.00	23,000
sigma.F	3.37	1.04	1.29	2.57	3.47	4.26	4.93	1.00	140,00
sigma.Prop_fb	3.59	0.94	1.64	2.90	3.71	4.38	4.94	1.00	6,400
sigma.Sad	3.48	0.92	1.70	2.78	3.53	4.25	4.92	1.00	380,00
Sad	0.72	0.03	0.66	0.70	0.72	0.74	0.77	1.00	190,00
Sad.est[1]	0.73	0.06	0.60	0.69	0.73	0.77	0.84	1.00	380,00
Sad.est[2]	0.68	0.06	0.55	0.64	0.68	0.72	0.79	1.00	30,000
Sad.est[3]	0.65	0.07	0.50	0.61	0.66	0.70	0.76	1.00	380,00
Sad.est[4]	0.71	0.05	0.60	0.68	0.72	0.75	0.81	1.00	280,00
Sad.est[5]	0.76	0.05	0.66	0.73	0.76	0.79	0.85	1.00	380,00
Sad.est[6]	0.68	0.05	0.58	0.65	0.68	0.72	0.77	1.00	380,00
Sad.est[7]	0.68	0.05	0.59	0.65	0.68	0.71	0.77	1.00	240,00
Sad.est[8]	0.73	0.04	0.64	0.70	0.74	0.76	0.82	1.00	380,00
Sad.est[9]	0.75	0.04	0.66	0.72	0.75	0.78	0.84	1.00	36,000
Sad.est[10]	0.71	0.04	0.62	0.69	0.72	0.75	0.80	1.00	380,00
Sad.est[11]	0.77	0.04	0.69	0.74	0.77	0.80	0.85	1.00	51,000
Sad.est[12]	0.71	0.07	0.57	0.67	0.71	0.75	0.82	1.00	26,000
Sad.est[13]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	380,00
Sad.est[14]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	110,00
Sad.est[15]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	200,00
Sad.est[16]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	330,00
Sad.est[17]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	380,00
Sad.est[18]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	380,00
Sad.est[19]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	380,00
Sad.est[20]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	380,00
Sad.est[21]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	380,00
Sad.est[22]	0.71	0.07	0.55	0.67	0.72	0.76	0.84	1.00	310,00

Appendix VI – Additional papers published during the PhD

Rocha J.L., Silva P., Santos N., **Nakamura M.**, Afonso S., Qninba A., Boratynski Z., Sudmant P.H., Brito J.C., Nielsen R. & Godinho R. (2023) North-African foxes genomes show signatures of repeated introgression and adaptation to life in deserts. *Nature Ecology and Evolution*. 1-20, 10.1038/s41559-023-02094-w

Pereira P., Esteruelas N.F., **Nakamura M.,** Rio-Maior H., Krofel M., Blasio A.D., Zoppi S. Robett S., Llaneza L., García E., Oleaga A., López-Bao J.V., Martinez M.F., Stavenow J., Ågren E.O., Álvares F. & Santos N. (2022) Hair cortisol concentration reflects the life cycle and management of grey wolves across four European populations. Scientific Reports, 12(1), 1-10. https://doi.org/10.1038/s41598-022-09711-x

Grilo *et al*. (2022) MAMMALS IN PORTUGAL: A data set of terrestrial, volant, and marine mammal occurrences in Portugal. Ecology. https://doi.org/10.1002/ecy.3654

Rousseau J., **Nakamura M.**, Rio-Maior H., Álvares F., Choquet R., Carvalho L. M., Godinho R., Santos N. (2021) Non-invasive molecular survey of sarcoptic mange in wildlife: diagnostic performance in wolf faecal samples evaluated by multievent capture-recapture models. *Pathogens*, 10 (2), 243-259. https://doi.org/10.3390/pathogens10020243

Santos N., **Nakamura M**, Rio-Maior H., Álvares F., Barasona, J.A., Rosalino L.M., Santos M., Santos-Reis M., Ferreras P., Díaz-Ruíz F. & Monterroso P. (2020) Protein metabolism and physical fitness are physiological determinants of body condition in Southern European carnivores. *Scientific Reports* 10, 15755. https://doi.org/10.1038/s41598-020-72761-6