

Survival and Population Dynamics of the Iberian Wolf in Portugal

Rafael de Faria Campos

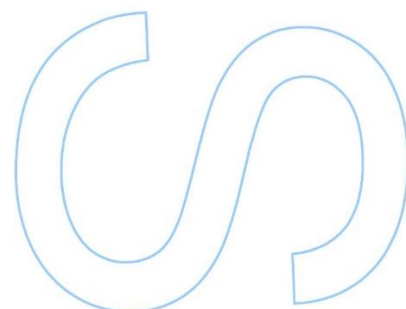
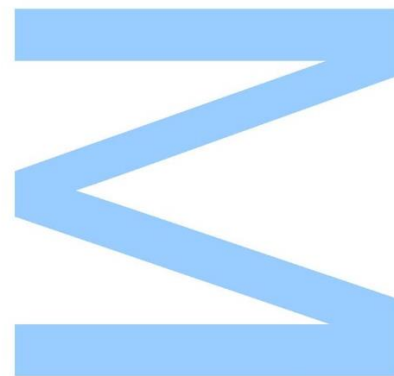
Biodiversidade, Genética e Evolução
Departamento de Biologia
2018

Orientador principal

Francisco Alvares, PhD, CIBIO-InBIO

Co-orientador

John Benson, PhD, University of Nebraska

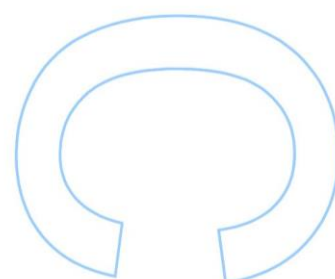
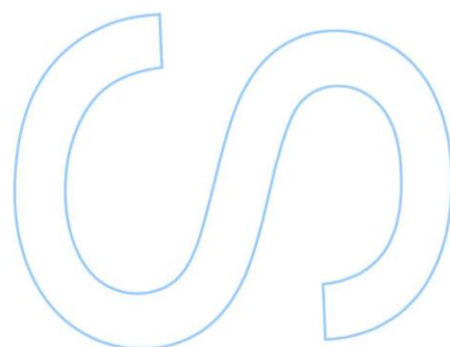
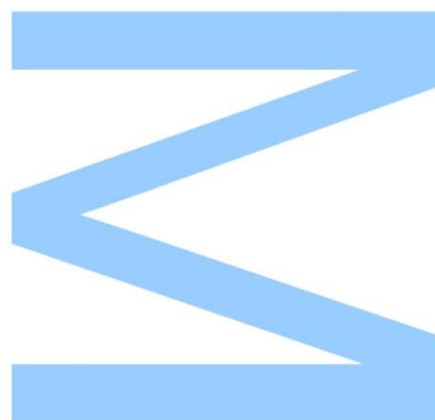




Todas as correções determinadas
pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ____/____/____



Acknowledgments

Ao Francisco Álvares agradeço tudo o que de si entregou à realização desta tese. O seu empenho foi insuperável e indispensável ao trabalho que aqui apresento. Aproveito também para lhe dar o longo e devido obrigado por me ter recebido em 2013 no mundo do Lobo.

I thank John Benson for agreeing to be my co-advisor from across the Atlantic. He too gave so much of his time so that my words would make sense. His guidance and contribution were fundamental to the methodological structure of this study and his vision indispensable to its execution.

À Mónia Nakamura agradeço por me ter ensinado grande parte dos fundamentais da ecologia do Lobo, foi ela que verdadeiramente me introduziu à disciplina e guiou através dos seus conceitos e problemáticas. Durante esse tempo que me emprestaste como professora, tive também o espetacular prazer de te ter como amiga. Finalmente, o teu papel na minha ida para a Roménia não pode ser esquecido, foste tu que mostraste e, consequentemente, tornaste possível uma das experiências mais espetaculares que tive. Obrigado por tudo e em particular por me deixares ser um ajudante num trabalho onde não precisavas de ajuda.

À Helena Rio-Maior agradeço por me ter confiado a oportunidade de trabalhar ao seu lado. Obrigado por me receberes e também por tudo o que me ensinaste.

A Francisco Petrucci-Fonseca (Grupo Lobo), Sara Roque (Grupo Lobo), Virgínia Pimenta (ICNF), Inês Barroso (ICNF) e a Luís Miguel Moreira (ICNF) agradeço pela cedência de dados de seguimento de lobos por telemetria, permitindo dessa forma a elaboração desta tese. Agradeço em particular à Sara Roque pela sua simpatia e disposição para responder às minhas perguntas nos momentos em que tive o prazer da sua companhia.

À minha família tenho a agradecer o apoio incondicional que me deram. À minha avó pela vontade constante de atender ao que me falta, ao João por ser o pai imparcial que tantas vezes precisei (e preciso), a à minha mãe não consigo agradecer (não seria capaz de fazer justiça), és tu.

Ao Pissarra, à Dri e ao Fred agradeço pelos momentos de descontração. Dou um agradecimento especial ao Pissarra pelo companheirismo indiscutível.

À Teresa agradeço pela sua presença e companheirismo neste percurso que temos a percorrer.

Ao Simão e ao Chico agradeço por serem quem são e particularmente por serem os irmãos que nunca tive.

To the guys from Romania I thank for the profound impact you had on my life. To Gazzola for failing to kill me multiple times, for sharing its infinite knowledge on wolf and for having the biggest heart I have seen in my life, to Teo I thank for always being available to help and for always making me feel at home. To Corradini I thank for being an inspiration and an impulsive force; the field work with you was always as much of a challenge as it was fulfilling, without a doubt a memory I'll hold for as long time allows me. The three of you are friends I wish to keep for the rest of my life.

E à Eva tudo, porque é tudo o que tens sido.

Abstract

Estimates of survival and accurate knowledge on demographic traits that influence population dynamics are fundamental to large carnivore conservation. Despite their recent demographic expansion and their need for efficient management, comprehensive survivorship analysis based on telemetry data are relatively rare for populations of large carnivores, particularly in Europe. This type of analysis is particularly relevant when these populations occur in human-dominated landscapes where the risk of human-caused mortality is high, such as the case of the Iberian wolf population (SW Europe). However, although several small-scale telemetry-based studies have been conducted in the Iberian Peninsula, only a single estimate of survival has been obtained from a small number of individuals tracked in Spain. In this context, my goals were to: i) estimate annual survival of Iberian wolves in Portugal, by compiling data of all collared wolves in Portugal until the present day, ii) evaluate the impact of several biological and environmental factors on mortality risk at a national level and iii) investigate the population dynamics of wolves in Alto Minho subpopulation to understand the demographic impact of the estimated survival, based on available monitoring data between 2007 and 2016. Despite full legal protection, the annual survival of radio collared wolves in Portugal, was low (0.623 ± 0.0791 SE, 95% CI [0.486-0.799], $n = 32$), similar to that observed in harvested wolf populations. All confirmed mortality events of collared wolves were caused by humans ($n = 14$) with poaching being the most common (50%) cause of death. Other mortality events included wolves that died accidentally in illegal snares (36%) or resulted from collisions with vehicles (14%). The seasonal pattern of mortality suggested a high occurrence of mortality during the breeding season, which raises concerns regarding the possible impact of breeder loss on pack stability and reproductive success. No significant results were obtained from the mortality risk models. Between 2007 and 2016, the Alto Minho wolf subpopulation displayed strong demographic growth and a marked East-to-West geographic expansion. The recovery of 3 packs that were absent from the study area, the high reproductive output of two packs and a relatively high breeding frequency were the main contributors for the observed trend.

Keywords: Kaplan-Meier, Cox Proportional Hazard Models, Population Growth, Mortality, Poaching, *Canis lupus signatus*, Large Carnivore, Europe, Alto Minho.

Resumo

Estimativas de taxas de sobrevivência e o conhecimento detalhado sobre os fatores demográficos que influenciam a dinâmica populacional, é fundamental para a conservação de grandes carnívoros. Apesar da sua recente expansão demográfica e a necessidade de serem geridos através de medidas de conservação eficazes, análises à sua sobrevivência com base em dados de telemetria são relativamente raras, particularmente na Europa. Estes tipos de estudos são particularmente relevantes quando estas populações habitam paisagens altamente modificadas onde o risco de mortalidade causada pelo homem é elevada, como no caso do Lobo-Ibérico. No entanto, embora vários estudos de telemetria em lobos tenham sido realizados até hoje na Península Ibérica, a única taxa de sobrevivência disponível foi obtida com base num pequeno número de animais seguidos em Espanha. Neste contexto, os meus objetivos foram: i) compilar todos os registos de lobos seguidos por telemetria em Portugal e estimar a sua taxa de sobrevivência, ii) estudar o impacto de várias variáveis biológicas e ambientais no risco de mortalidade do Lobo em Portugal e iii) investigar a dinâmica populacional do lobo na subpopulação do Alto Minho de forma a entender o impacto demográfico da taxa de sobrevivência obtida, com base em dados de monitorização populacional entre 2007 e 2016. Apesar de ser uma espécie protegida por lei, a taxa anual de sobrevivência obtida através dos lobos seguidos por telemetria em Portugal foi reduzida (0.623 ± 0.0791 SE, 95% CI [0.486-0.799], $n = 32$) e comparável à observada em populações que são caçadas. Todas as mortalidades foram causadas, directa ou indirectamente, pelo homem ($n=14$), sendo que a perseguição ilegal por tiro e veneno foi a causa de morte mais comum (50%). Os restantes lobos morreram acidentalmente em laços (36%) e atropelados (14%). A sazonalidade de mortalidade foi particularmente incisiva na época de reprodução, o que pode ter graves consequências na estabilidade das alcateias e no seu sucesso reprodutor devido à possível perda de um dos elementos do par reprodutor. Nenhum dos modelos de risco de mortalidade identificou impactos significativos das variáveis escolhidas. Entre 2007 e 2016 a subpopulação do Alto Minho demonstrou um forte crescimento assim como uma clara expansão geográfica de Este para Oeste. Os principais fatores que contribuíram para os padrões observados foi o reaparecimento de 3 alcateias que estavam ausentes da área de estudo, o elevado sucesso reprodutor de duas alcateias e a uma frequência de reprodução elevada.

Palavras-chave: Kaplan-Meier, Cox Proportional Hazard Models, Crescimento populacional, Mortalidade, Caça furtiva, *Canis lupus signatus*, Carnívoros de grande porte, Europa, Alto Minho.

Table of Contents

ACKNOWLEDGMENTS.....	V
ABSTRACT.....	VII
RESUMO.....	IX
TABLE OF CONTENTS	XI
INDEX OF FIGURES.....	XIII
INDEX OF TABLES	XV
ABBREVIATION LIST.....	XVII
INTRODUCTION	1
SURVIVAL AS A VITAL RATE	1
THE WOLF IN PORTUGAL.....	3
GOALS	5
CHAPTER 1 - SURVIVAL AND MORTALITY RISK OF WOLVES ACROSS PORTUGAL	7
1.1 STUDY AREA DESCRIPTION AT NATIONAL LEVEL	7
1.2 METHODS	9
1.2.1 Data collection	9
1.2.2 Environmental variables.....	11
1.2.3 Annual survival	12
1.2.4 Mortality risk.....	14
1.3 RESULTS.....	17
1.3.1 Causes of death.....	17
1.3.2 Annual survival	19
1.3.3 Mortality risk.....	19
CHAPTER 2 - WOLF POPULATION DYNAMICS IN ALTO MINHO	21
2.1 STUDY AREA DESCRIPTION AT REGIONAL LEVEL	21
2.2 METHODS	25
2.2.1 Data collection	25
2.2.2 Field effort for pack and breeding detection	25
2.2.3 Pack and population size	26
2.2.4 Population growth	27
2.2.5 Count-based model.....	27

2.2.6 <i>Breeding frequency and litter size</i>	29
2.2.7 <i>Annual survival and opportunistic mortality events</i>	29
2.3 RESULTS	31
2.3.1. <i>Pack detection, breeding frequency and litter size</i>	31
2.3.2 <i>Pack and population size</i>	32
2.3.3 <i>Population growth</i>	34
2.3.4 <i>Count-based model</i>	34
2.3.5 <i>Annual survival and mortality events</i>	35
DISCUSSION	37
SURVIVAL AND MORTALITY RISK OF WOLVES IN PORTUGAL	37
WOLF POPULATION DYNAMICS IN ALTO MINHO	39
POSSIBLE EFFECTS ON DISPERSAL AND BREEDER LOSS	42
CONSERVATION IMPLICATIONS	43
REFERENCES	47
ANNEX	57
A.1 DESCRIPTION OF THE SOCIAL STATUS INFERRED FOR THE COMPILED COLLARED WOLVES	57
A.2 SUPPLEMENTARY TABLES	59
A.3 SUPPLEMENTARY FIGURES	62

Index of Figures

Figure 1 - Geographical distribution of home ranges (MCP100) from all documented wolves collared in Portugal, according to each subpopulation (A: Alto Minho; B: Montalegre; C: Bragança; D: Sul do Douro). Wolf permanent and sporadic distribution were obtained from Chapron et al. 2014.....	8
Figure 2 - A) Individual tracking records (line) and respective mortality events (red dot). (B) Variation in the representativeness of each subpopulation on recorded wolf-years across the studied period (1990 – 2017).	17
Figure 3 - Causes of death of individual collared wolves (n = 15) documented in Portugal during the 1990 – 2017 period, considering each subpopulation.	18
Figure 4 - Estimated Kaplan-Meier survival curve and 95% confidence interval for the 32 wolves radio-collared across Portugal between 1990 and 2017. Vertical drops correspond to mortality events.	19
Figure 5 - Study area in Alto Minho and location of packs (red circles) and turbines from wind farms (black triangles). Pack location is based on breeding sites detected in 2011 and 2014 (Álvares, unpublished data). From the right to the left: A: Peneda pack, B: Soajo pack, C: Vez pack, D: Boulhosa pack, E: Cruz vermelha pack and F: Arga pack.....	22
Figure 6 - Estimated Kaplan-Meier survival curve and 95% confidence interval for the 17 wolves radio-collared in Alto Minho between 2007 and 2016.....	35
 Figure S1 - Relationship between mortality risk and each of the environmental predictors for 29 collared wolves in Portugal.....	62
Figure S2 - Tracking periods for all radio-collared wolves in Portugal by subpopulation (this study) and for wolves radio-collared in two different areas of Spain (Picos de Europa according to Fdez et al. 2014; Llana & Fdez 2016; and central Spain according to Blanco & Cortés 2007). Red dots represent tracking period that ended with the death of the collared individual.	63
Figure S3 - Relationship between the annual logarithmic growth rates and annual population size of Alto Minho wolf subpopulation between 2007 and 2016.....	63

Index of Tables

Table 1 - Variables tested in univariate model in the mortality risk analysis, the number of records to which each model was fitted, cohort that was held as the reference group and the fraction of individual days of tracking data it represents of the whole.	15
Table 2 - Causes of death of collared wolves (n = 20) documented in Portugal since early 1980s until 2017, by decade. Numbers between parentheses correspond to the total number of collared wolves in each decade.	18
Table 3 - Univariate models of mortality risk of all 32 radio collared wolves considered in the survival analysis (model 1), 29 radio collared wolves with available information on residency status (model 2) and 18 radio collared resident wolves (model 3), respectively, and corresponding null model. The hazard ration is the multiplicative change in mortality risk and “95% CI” is its 95% confidence interval. Ph is the p-value of the proportional hazards assumption test, significant for $\alpha = 0.05$ indicates deviation from the critical assumption.	20
Table 4 - Number of pups and reproductions detected in Alto Minho between 2007 and 2016.	31
Table 5 - Detection of packs and reproduction between 2007 and 2016 in Alto Minho wolf subpopulation. Green cells represent years of pack presence and red cells represent year of pack absence.	32
Table 6 - Demographic characteristics of the wolf subpopulation in Alto Minho during summer, between 2007 and 2016.	33
Table 7 - Mean number of adults, pups and pack size, with respective minimums and maximums observed per pack in Alto Minho between 2007 and 2016.	34
Table 8 - Parameter estimates and AICc values for the density independent model (exponential) and the Ricker model applied to Alto Minho wolf subpopulation.	35
 Table S1 - Compiled information for all collared wolves in Portugal, including gender, age (A for Adults, Y for yearlings and P for pups), tracking period (Capture and End date), available home range (MCP100), fate and cause of mortality. The Subpopulation of collared wolves is implicit in each ID, AM for Alto Minho, MA to Montalegre, BG to Bragança and SD to SDR.	59
Table S2 - Reclassification criteria used to obtain the land cover variables and respective Corine Land Cover (CLC) grid code.	60
Table S3 - Association criteria used for each collared wolf in Portugal between its tracking period and variable year for Corine Land cover (CLC) and Nightlight. “End year”	

corresponds to the year when contact with the collared animal was lost, either due to death or other causes mentioned in the methods. The CLC was only available for years 1990, 2000, 2006, and 2012. Nightlight was available for the period 1992 – 2013. 61

Abbreviation List

% - Percentage

€ - Euro

A - Adult

AIC - Akaike Information Criterion

CI - Confidence Interval

CIBIO - Research Center in Biodiversity and Genetic Resources

CLC - CORINE land cover

e.g. - *exempli gratia* (LATIM)

F - Female

GPS - Global Positioning System

ICNF - Instituto da Conservação da Natureza e das Florestas

ID - Individual Identifier

K - Carrying Capacity

km - Kilometer

M - Male

m - Meters

MCP - Minimum Convex Polygon

n - Number

N - Population size

Nt - Abundance at time t

Nt+1 - Abundance at time t+1

P - Pup

r - Maximum Intrinsic Growth Rate

SD - Standard Deviation

SDouro - South of the Douro

SE - Standard Error

SW - South-west

VHF - Very High Frequency

Y - Yearling

ε - Process Error

λ - Lambda (finite rate of population growth)

Introduction

Survival as a vital rate

Estimating survival and understanding the problematic of mortality is crucial for the proper management of wild populations (Morris and Doak 2002). Survival rates are a key indicator of demographic vigor, being particularly relevant for long lived species with long generation times (Pianka 1970, Southwood et al. 1974, Heppell et al. 2000), such as large carnivores. The pivotal role of survival on the demography of large carnivores is supported by the sensitivity of demographic growth (λ) to the survival of specific cohorts such as breeding females (*Ursos arctos*: Knight & Eberhardt 1985; Chapron et al. 2003; *Puma concolor*: Lambert et al. 2006; Benson et al. 2016), dominant adults (*Lynx pardinus*: Gaona et al. 1998; *Canis lupus*: Chapron, Legendre, et al. 2003), adults (*Lycaon pictus*: Cross & Beissinger 2001; *Lynx lynx*: Nilsson 2013) or pups and yearlings (*Lycaon pictus*: Creel et al. 2004).

Understanding survival is particularly relevant for large carnivore populations occurring in human-dominated landscapes, where their predatory behavior often stems discontent in local communities (Treves and Bruskotter 2014). Overall, human-carnivore coexistence has been difficult to achieve and appropriate solutions are often frustrated by complex sociopolitical dilemmas (Treves and Karanth 2003, Treves 2009). In fact, large carnivore conservation is one of the most challenging tasks in wildlife management (Mech 1995, Treves and Karanth 2003), but also one of the most important given that carnivores are believed to play an important role in maintaining stable and functioning ecosystems (Berger et al. 2001, Terborgh et al. 2001, Ripple et al. 2014). Nevertheless, experience has shown us that coexistence is possible under properly enforced conservation measures (Linnell et al. 2001); measures that are most effective when based upon scientific and evidenced-based frameworks (Pullin and Knight 2001).

Telemetry-based survivorship analysis is a good example of how reliable data can help guide conservation efforts in a wide range of iconic large carnivore species (e.g. *Canis spp.*: Mills et al. 2008; Murray et al. 2015; *Gulo gulo*: Persson et al. 2009; *Lynx spp.*: Fuller et al. 1985; Fuller et al. 1995; Ferreras et al. 1992; Andrén et al. 2006; *Lycaon pictus*: Woodroffe et al. 2007; *Panthera tigris*: Goodrich et al. 2008; Robinson et al. 2015; *Puma concolor*: Lindzey et al. 1988; Vickers et al. 2015; *Ursos arctos*: Knick & Kasworm 1989; McLellan et al. 1999). Furthermore, comprehensive survivorship data at the population level allows the scientific community to inform stakeholders about factors affecting the mortality risk of individuals (e.g Riley et al. 2003; Benson et al. 2014; Suutarinen & Kojola 2018) and to

understand how life-history processes may be disrupted by ill-timed mortality or indiscriminate harvest (e.g. Wielgus & Bunnell 2000; Brainerd et al. 2008; Wielgus et al. 2013; Borg et al. 2015; Loveridge et al. 2016; Balme et al. 2017).

The importance of survivorship data is well illustrated in wolf conservation, as human-caused mortality is known to induce strong responses on the species' demographic dynamics (e.g. Rutledge et al. 2010; Liberg et al. 2011; Milleret et al. 2017). After making a remarkable demographic recovery in the last 50 years (Pereira and Navarro 2015), the wolf is now the second most abundant large carnivore in Europe (Chapron et al. 2014b) and is once again the focus of debate on coexistence and conservation (Boitani 2000, Linnell and Boitani 2012, Chapron et al. 2014b). Two of the more concerning threats to the viability of European wolf populations are thought to be direct persecution (Kaczensky et al. 2012), widely motivated by the conflict between the livelihoods of rural communities and the carnivorous behavior of the species (Gangaas et al. 2013, Treves and Bruskotter 2014, Suutarinen and Kojola 2018), and accidental human-caused mortality (Kaczensky et al. 2012). The particular impact of direct persecution through poaching, has been studied in the Scandinavian and Karelian wolf populations (Liberg et al. 2007, 2011, Suutarinen and Kojola 2017). Surprisingly, survivorship data on the remainder of European populations is scarce or non-accessible through published literature, with exception of estimates of apparent survival in Italian and Alpine wolf populations obtained from non-invasive genetic sampling capture-recapture frameworks (Marucco et al. 2009, Caniglia et al. 2012).

In the Iberian Peninsula, a single estimate of annual survival has been obtained from a sample of 14 wolves collared in Spain (Blanco and Cortés 2007), while most studies have investigated the problematic of mortality through opportunistic collection of dead animals (Petrucci-Fonseca 1990, Blanco et al. 1992, Llanaez et al. 2004, Llanaez and Blanco 2005, Pimenta et al. 2005). These later efforts, although extremely valuable, are not as informative as those based on known-fate survival models, the principal tool for estimating survival (Murray 2006; Ciucci et al. 2007). In Portugal, several small-scale telemetry studies have been conducted since early 1980s, the majority involving a relatively small numbers of animals (< 5) which were collared to study spatial ecology and individual activity patterns (van Haaften 1982, Pereira et al. 1985, Moreira 1992, Moreira et al. 1997, Pimenta 1998, Grilo 2002, Álvares 2011, Roque et al. 2011, Rio-Maior et al. 2018). In addition to the intended spatial data, these studies collected valuable information on the fate of each collared individual that, to this day, have not been explored under a survival framework.

Despite the relative high importance of survival for conservation of large carnivore populations, there seems to be a wide lack of knowledge on wolf survival in Europe. This is particularly concerning for the Iberian population provided that it inhabits a highly disturbed

and human-dominated landscape (Blanco et al. 1992, Eggermann et al. 2011, Llaneza et al. 2012), where wild prey is scarce and wolves prey mainly on livestock (Cuesta et al. 1991, Vos 2000, Álvares 2011, Torres et al. 2015b). This trophic dependency appears to motivate wolves to occupy areas closer to rural settlements, with little regard for human density (Eggermann et al., 2010; Llaneza et al. 2012). This centuries-old conflictual coexistence between humans and wolves is deeply rooted in the traditions and culture of rural communities and remains unresolved (Álvares et al. 2011).

The wolf in Portugal

In the mid-19th century, wolf distribution spanned north to south across mainland Portugal (Petrucchi-Fonseca 1990). Around the beginning of the 20th century it suffered a drastic contraction after complete extirpation from the southern and coastal areas (Petrucchi-Fonseca 1990), a population decline that was simultaneous to that observed throughout Europe (Boitani 2003). The species received legal protection at the national level in 1988 and since the late 1990s its range has been stable at around 20% of its original extension (Petrucchi-Fonseca 1990, Pimenta et al. 2005, Álvares et al. 2015). The Douro river, one of the largest in Portugal, disrupts the continuity of the wolf's range in the peninsula and is thought to constrain dispersion across much of its extension (Grilo et al. 2002, Pimenta et al. 2005). In Portugal, the fraction of the population located in the northern bank of the Douro river, estimated to be composed by approximately 55 packs (data from 2002/2003), is contiguous with the remaining Iberian wolf range that spans across the north of Spain (Pimenta et al. 2005), and holds 3 distinct genetic clusters (hereafter, subpopulations; Alto Minho, Montalegre and Bragança; Silva et al. 2018). The single subpopulation located south of the Douro river (hereafter, SDouro) shows strong evidences of breeding instability, isolation and fragmentation, and is currently estimated to be composed by less than 10 packs (Pimenta et al. 2005). The high levels of genetic structure, observed not only in Portugal but also in Spain, revealed an unexpected low rate of gene flow for wolves in Iberia (Silva et al. 2018). This finding is nevertheless congruent with the low mean dispersal distances of collared wolves from north-central Spain observed by Blanco & Cortés (2007), compared to other populations of wolves in North America and in Europe. In Portugal, these recently identified genetic clusters had already been described as distinct demographic nucleus of uniform pack presence, limited by areas of sporadic pack formations (Pimenta et al. 2005), albeit with some local exceptions.

The ecological context of each of the 4 identified subpopulations is likely unique, as factors that are known to affect the dynamics of wolf populations, such as human presence

and food availability (Fuller et al. 2003), have marked gradients across the Portuguese landscape. Specifically, the SDouro subpopulation is one of the few in Europe considered to be on the verge of extinction (Boitani and Ciucci 2009). The packs that compose it ($n = \sim 9$) occupy a fragmented habitat with low prey availability in geographical isolation from the remaining Iberian population, which has resulted in a low genetic diversity (Grilo et al. 2002, Pimenta et al. 2005, Hindrikson et al. 2017), the lowest observed in the Iberian Peninsula (Silva et al. 2018). The ecological contexts of Alto Minho (~ 6 packs) and Montalegre (~ 23 packs), both located in the Northwest of Portugal, are thought to be very similar, although anthropogenic pressure is likely higher in Alto Minho, a highly humanized region, adjacent to the heavily urbanized sea coast. In 2013, these two subpopulations were responsible for 50% of all reported livestock predations even though they comprise 25% of all known packs. On the other hand, Bragança subpopulation (~ 24 packs) is the only where the number of wolf attacks on livestock has been in a steady decline over the last years, a trend that is mostly attributed to good practices in livestock herding and increasing abundance of a rich community of wild prey (Álvares et al. 2015). However, given the lack of recent population census, it is impossible to rule out the possibility that Bragança wolf subpopulation is in demographic decline.

Unfortunately, demographic data on most of these subpopulations, the cornerstone of management actions, is scarce or largely outdated as the last census spanned from 2002 to 2003, and for the last 15 years no data on wolf abundance or distribution has been collected at the national level. This happens in part because obtaining demographic data on wolves is a difficult task due to their elusive behavior and wide movements. Snow tracking, both terrestrial or aerial, plays a vital role on wolf monitoring in northern and high altitude populations (Wabakken et al. 2001, Duchamp et al. 2012, Marucco et al. 2012). Besides facilitating aerial sightings and preserving animal tracks, severe conditions of snow depth and compaction induces pack members to move together more often than in mild winter to summer conditions (Fuller 1991). These conditions allow researchers to collect non-invasive genetic samples as well as count and track animals and packs more efficiently. However, in southern and low-altitude areas within wolf range, where snow cover is scarce or even absent, snow tracking is impossible (Blanco and Yolanda 2012) or limited to opportunistic sampling (Reinhardt et al. 2015). This is the case of the Iberian Peninsula, where field methods employed to monitor wolves are mostly based on the detection of signs along snowless roads and acoustic/visual detection of packs (Llaneza et al. 2005, 2014) during their period of lowest mobility, the pup rearing season (Packard 2003). Acoustic and visual detection of packs allow researchers to count individuals and obtain estimates of population size. These methods, although well adapted to the conditions of the

population, require continuous effort and funding to produce management-relevant knowledge, which has not happened in Portugal over the past years.

Goals

This study aims to assess survival and population dynamics of wolves in Portugal by focusing two different scales: a national level approach focused on survival based on the compilation of all collared wolves in Portugal (Chapter 1) and a regional approach focused on population dynamics and based on available monitoring data from the Alto Minho subpopulation, obtained between 2007 and 2016 (Chapter 2). For the national-level approach in Chapter 1, I have compiled data on all wolves captured and collared in Portugal since the early 1980s until the present day ($n = 49$) in order to: i) estimate annual survival; and ii) investigate the relationship between mortality risk and several environmental and biological predictors. I hypothesize that the survival of wolves in Portugal would be low given the human-dominated landscape the species occupies, although not low enough to imply severe population decline as there have not been indications of critical range contractions in the past decades. Furthermore, by investigating the relationship between mortality risk and several environmental and biological predictors I wanted to test the hypothesis that human pressure is associated with a highest mortality risk for wolves in Portugal. Finally, for the regional level approach in Chapter 2, I analyzed 10 years of wolf monitoring data collected in the Alto Minho between 2007 and 2016 in order to i) estimate pack and population sizes, ii) build a count-based model to assess population growth and iii) estimate litter sizes and breeding frequency to explore the relationship between the estimated annual survival and the demographic dynamics of the population. I expect that this more in-depth analysis in Alto Minho region will allow me to understand the true impact of the observed survival and the underlying mechanisms of wolf demography in the subpopulation. The Alto Minho subpopulation is a local exception to the general lack of information of wolf abundance in Portugal and therefore my findings should shed some light on the condition of wolves across the country. Furthermore, I hope to provide a valuable insight on the efficacy of the instated legal protection and contribute towards the quantification of the influence of human pressure on wolves across the Portuguese landscape under a survival framework. My work also highlights the value of telemetry data and its contribution to our understanding of wildlife populations, which hopefully may encourage similar research with wolves and other carnivores, in Portugal and across Europe.

Chapter 1 - Survival and Mortality Risk of Wolves Across Portugal

1.1 | Study area description at national level

The study area for Chapter 1 was by definition the entire Iberian wolf range in Portugal (SW Europe) (**Figure 1**). Data compilation from collared wolves spawns across multiple regions of distinct environmental profiles while being mostly restricted to mountainous ranges above the 400 meters of altitude (Pimenta et al. 2005). Human density is overall moderate (less than 50 inhabitants per km²); Human settlements are denser along the coast while rural exodus has been felt in most of the central and eastern mountain ranges of the country (INE 2011). The landscape is homogeneous and mostly characterized by a matrix of rural and natural areas, where villages and agricultural land are predominantly located along river valleys, whereas scrublands, oak forest patches and forest plantations are more common in the mountains. Human activities are present throughout the entire wolf's distribution as extensive livestock grazing, tourism, hunting, and infrastructure development (e.g. wind farms and roads) are common even in the most remote mountainous ranges (Pimenta et al. 2005). Livestock is the primary food source of wolf in Portugal (Vos 2000, Torres et al. 2015b) with the exception being Montesinho Natural Park, located in north-eastern Portugal where wild ungulates constituted a larger proportion of the species' diet (Álvares et al. 2015). Wolf attacks on livestock peaked in 2012-2013 with a total of 2,725 wolf attacks reported across the country (Pimenta et al. 2017). Most of these events were reported in the regions occupied by the Alto Minho and Montalegre subpopulations (78.9%) while only 8.1% were reported in Sul do Douro and 0.2% in Bragança (Pimenta et al. 2017).

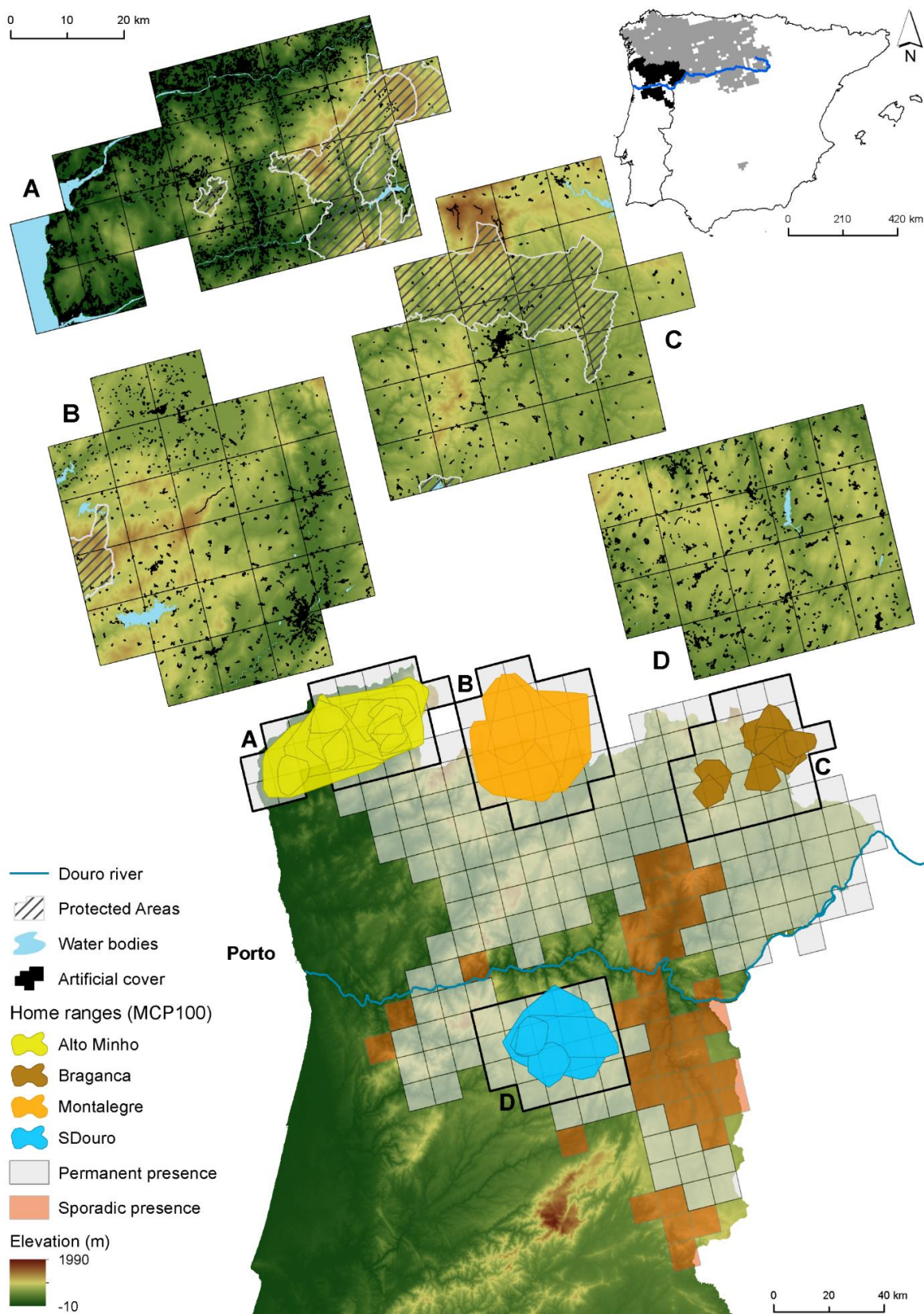


Figure 1 - Geographical distribution of home ranges (MCP100) from all documented wolves collared in Portugal, according to each subpopulation (A: Alto Minho; B: Montalegre; C: Bragança; D: Sul do Douro). Wolf permanent and sporadic distribution were obtained from Chapron et al. 2014.

1.2 | Methods

1.2.1 Data collection

I compiled data on capture and fate of radio-collared wolves in Portugal from several technical reports (van Haaften 1982, Pereira et al. 1985, Moreira 1992, Moreira et al. 1997, Pimenta 1998, Grilo 2002, Álvares 2011, Roque et al. 2011), one peer-reviewed article (Rio-Maior et al. 2018) and from additional unpublished data collected in on-going projects carried out by CIBIO (Research Centre in Biodiversity and Genetic Resources from Porto University) in the Alto Minho region. These records span across the entire extent of the Portuguese wolf distribution (**Figure 1**) and contain animals captured in all 4 identified subpopulations. Most animals from the Montalegre subpopulation had transboundary home ranges with Spain. To my knowledge, the compiled records (n=49) include every wolf that has been captured for radio-collaring in Portugal until the present day. I identified all captured animals with subpopulation-specific codes (AM for Alto Minho, MA for Montalegre, BA for Bragança and SD for SDouro) and a serial number according to the capture order (e.g. AM1, BG1...).

The earliest capture dates back to 1982 and the last tracking period ended in 2017, with 10 wolves collared in the 1980s (between 1980 and 1989), 14 in the 1990s, 6 in the 2000s and 19 in 2010s (until 2017). The large number of animals collared during the last decade is the result of the continuous trapping effort of ongoing CIBIO's projects in Alto Minho region. Trapping effort was not constant over the entire period. All trapping and handling of wolves conducted in Portugal was carried out under licenses from the National Authority for Nature Conservation (currently, ICNF) which, since 2007, imposes a limit on the number of captures per year in each project (n = 3) as well as a halt on trapping during wolf breeding season (from February to July).

The studies conducted in the 1980s report the use of feeding sites to lure and trap wolves (n = 10 captures; van Haaften 1982; Pereira et al. 1985) while more recent trapping efforts (n = 39 captures) trapped exclusively along trails and roads using several types of olfactory lures such as rotting meat or fish, scats, and commercial hormone based solutions (Moreira 1992, Moreira et al. 1997, Pimenta 1998, Grilo 2002, Álvares 2011, Roque et al. 2011). Three different types of traps were reported: snares (n = 6 captures), steel traps (n = 18) and foothold snares (n = 23). Two wolves were collared after being rescued from illegal snares (Grilo 2002, Rio-Maior et al. 2016). The compiled dataset of captured wolves (n=49) includes six pups captured in early stages of growth (< 6 months) that were not collared (Moreira et al. 1997; Roque et al. 2011, Rio-Maior, personal communication) due to animal welfare concerns related to their small size, as advised in other studies (van Ballenberghe

and Mech 1975, Mech 1977) and one individual that died during capture, and therefore was also not collared (Pereira et al. 1985). Consequently, only 42 of the 49 captured animals were eligible to be collared. The youngest collared animal was estimated to be around 8 months old (Roque et al. 2011). From the 42 collared wolves, 22 were equipped with very high frequency (VHF) telemetry collars and 20 were equipped with global positioning system (GPS) telemetry collars. Three animals were not released immediately after capture: one captured male in Bragança (ID = BG1) spent 1 week in captivity before being released because the collar was not ready by the time of capture (van Haaften 1982); a second individual (AM8), a female from Alto Minho, suffered a fracture on the right front limb during capture, was submitted to surgery and spent 65 days in captivity before being collared and released (Rio-Maior et al. 2016); and a third wolf (MAE4) rescued from an illegal snare, a yearling male from Montalegre, was collared and released, after spending 84 days in captivity, with a surgically amputated right hind limb (Rio-Maior et al. 2016). These last two animals (AM8 and MAE4), although having suffered severe traumatic injuries, displayed normal movement patterns and use of space during the post-release period (Rio-Maior et al. 2016). One adult female (BG9) was recaptured shortly before dying (Moreira 1992) and the initial cause of death was attributed to the recapture event. However, a toxicological report latter revealed that the cause of death was not recapture related as the animal contained large quantities of rodent poison (dicoumarol) in its system (Moreira 1992). One female collared in Alto Minho (AM4) died shortly after release due to an infection sustained during capture (Rio-Maior, personal communication). The inclusion in the models of the collared wolves with captivity periods before release ($n=3$; BG1, AM8 and MAE4) and of AM4, that died due to capture related causes, is discussed below.

All telemetry collars were programed to emit a mortality signal; mortality events were confirmed in the field and the cause of death was investigated. In two cases, following detection of a mortality signal, only the collar was found at the site with no evidences of the dead animal but with clear signs of human interaction (Rio-Maior, personal communication, 2018). In both cases I considered the animals to have been poached, although the exact causes of death were undetermined. All other carcasses were collected, necropsied and cause of death was determined or confirmed through veterinary assessment. Detailed information on capture and handling procedures can be found in each of the technical reports cited above and in Rio-Maior et al. (2018) for the unpublished records.

Some records ($n = 6$) were lacking key details, such as the end of tracking period or fate of the animal and were consequently not included in the analysis. For all other collared wolves ($n = 36$), I obtained the date of capture, end of tracking period, fate, sex, and age class of each animal (**Table S1**). I classified age as follows: pups (<1 year), yearlings (1-2

years) or adults (>2 years). Wolf locations collected during individual tracking periods were not available for most animals. Instead, I was provided with 100% minimum convex polygon (MCP) home ranges that had been estimated previously, as well as the residency status (resident or nonresident) for 29 of the 36 collared wolves (**Table S1**), as this data had been collected in the scope of a previous work (Álvares et al. 2015). The method used to estimate individual home ranges, the MCP of all observed locations, is known to produce unreliable estimates because they often include large areas that were never actually used by the animal (Börger et al. 2006). However, given the nature of the data, these home ranges estimates were, for most of the wolves, the only spatial data that was available. Additional notes on data compilation regarding residency status can be found in the Annex A.1 | Description of the social status inferred for the compiled collared wolves.

1.2.2 Environmental variables

I characterized the obtained individual home ranges ($n = 29$) with respect to several environmental variables. Humans are thought to be one of the main factors influencing the survival of Iberian wolves (Blanco and Cortés 2007). Unfortunately, fine-scale spatial data on human presence and activity are difficult to obtain or not available, so instead, I used indirect data, such as the presence of infrastructures (e.g. road density and artificial sources of light) and land uses associated with human activities (e.g. agricultural land), to estimate the level of human presence within home ranges. I calculated road density (km/km²) inside each home range considering 3 classes: “all roads”, “paved roads”, and “unpaved roads”. I obtained all the information on roads from GEOFABRIK (OpenStreetMap contributors 2018). I averaged relative light intensity values from Nightlight (DMSP_OLS) satellite data obtained from the National Centers for Environmental Information (NOAA 2018), that had been post-processed to eliminate natural sources of light such as the sun, the moon, and ephemeral events (e.g. fires), while preserving persistent artificial sources such as public illumination and others associated with socioeconomic activities. I estimated the percentage of each home range covered by areas associated with agricultural practice and livestock grazing by reclassifying several types of available land cover data from the CORINE land cover (CLC) inventory, that was collected by the European Union’s Earth Observation Programme, *Copernicus* (Copernicus 2018). Criteria used for these reclassifications are displayed in **Table S2**. Livestock presence is both associated with higher food availability for wolves as well as conflicts that arise from livestock depredation. I also calculated the percentage of forest cover as a proxy for wild prey availability, which is likely the most adequate habitat for species such as red deer (*Cervus elaphus*) and roe deer (*Capreolus*

capreolus). Finally, I calculated the proportion of each home range encompassed by the two main protected areas located within wolf range: Peneda-Gerês National Park and Montesinho Natural Park (**Figure 1**) from spatial data made available by the ICNF (ICNF 2018). Although these protected areas do not provide additional legal protection from poaching (as the species is fully protected at the national level), the protection of the landscape might influence survival by dissuading poachers, decreasing the level of human activities and providing more suitable habitat for refuge. These last variables, forest cover and protected area, should work as indicators of habitat with high suitability for wolves.

Nightlight and Land cover data were available from different time periods which allowed me to extract the variables for years that best matched the tracking period of each individual wolf (**Table S3**). I conducted all spatial processing and analysis using QGIS version 3.2.0 (QGIS Development Team 2018).

In summary, I characterized the obtained individual home ranges with respect to three groups of variables, Human presence (i.e. Road density, Nightlight intensity and percentage of agricultural lands), Livestock presence (percentage of open areas) and Habitat suitability (percentage of protected area and forest cover).

1.2.3 Annual survival

I pooled survival data from all collared wolves across Portugal with enough available information and estimated a single annual survival rate using the nonparametric Kaplan-Meier (product-limit) procedure extended to allow for staggered entry of animals (Kaplan and Meier 1958, Pollock et al. 1989) and estimated the variance using the Greenwood formula (Pollock et al. 1989, Therneau and Grambsch 2000). I used an annual recurrent time scale (Fieberg and Delgiudice 2009) standardized to a biological year beginning on 25 May (mean day for parturition reported for the Alto Minho wolf population; Rio-Maior et al. 2018) and ending on 24 May the following year. Animals entered the model on the day of their release with a working telemetry collar (any day of the annual time-scale) and exited after mortality (coded 1; $n=14$) or censoring (coded 0). I right-censored animals whose monitoring period ended before detection of a mortality event, either due to collar failure ($n = 8$), the end of the study ($n = 8$), loss of signal caused by unknown causes ($n = 2$), scheduled collar drop-off ($n = 2$) or dispersal outside of the study area ($n = 1$). Additionally, I right-censored animals on the last day of a biological year (24 May) and re-entered them into the model on the first day of the following year (25 May). Animals whose tracking period started at the 24th of May were only entered on the 25th of the following biological year. The annual recurrent time-scale pools data across years under the assumption that variation in

seasonality of mortality between years is minimal (Fieberg and Delgiudice 2009). Inspecting dates of mortality across years as well as the plots of survival suggested that this assumption was met. To be conservative, I excluded 3 records of wolves captured before 1988 (BG1, BG2 and BG3), the year when legal protection of the Iberian wolf was implemented in Portugal, due to the likelihood that mortality differed following protection. I excluded AM4, the female from Alto Minho that died 27 days after release due to capture-related causes, from analysis to meet the assumption that capture does not influence future survival of animals (Pollock et al. 1989). Based on the results in Rio-Maior et al. (2016) I considered the probability of survival of the two wolves that underwent surgery before release (AM8 and MAE4) to have been unaffected by the injuries or period of captivity. Similarly, I also considered the period of captivity of an additional wolf (BG1) to have not influenced the probability of survival of the animal, although this individual was excluded from the analysis as it was captured before 1988. During its tracking period, one collared wolf (SD3) was rescued from an illegal snare by local people (Grilo 2002). Given that rescue events like this one are most likely rare, and that the presence of the collar might have influenced the decision to rescue this animal, I treated it as a mortality caused by illegal snaring, as SD3 certainly would have died if not rescued.

In summary, from the 49 records I had initially compiled on wolves trapped in Portugal, 6 were relative to captured pups that were not collared, 6 were incomplete regarding the fate of the individual and 2 described events of wolves that died due to capture related incidents. Out of the 35 remaining records I choose to exclude those that were relative to wolves captured before legal protection was legislated, i.e. 1988 ($n = 3$). This left me with 32 collared wolves captured after 1988, including 14 mortality events that were eligible to be included in the survival analysis. The sex ratio in the resulting sample was balanced (17 males, 15 females) and the distribution of age classes is as follows: 22 adults, 7 yearlings and 3 pups (all older than 8 months).

To facilitate the discussion of the results I made use of 3 fundamental phases of the wolf's reproductive cycle: mating season, gestation and pup rearing season. I have defined mating season as the combinations of the *proestrus* and *estrus* phases described in Packard (2003), in which *Proestrus* marks the beginning of courtship behaviors in females; and in *estrus* females are receptive to copulating. All phases were standardized in an annual scale relative to the mean day for parturition reported for the Alto Minho wolf population (25 of May; Rio-Maior et al. 2018) with durations of 62 and 75 days (Kreeger 2003) (gestation and mating season, respectively). Pup rearing season was defined as the 20 weeks (approx. 5 months) after births corresponding to pup dependency to home sites (Packard 2003).

1.2.4 Mortality risk

I investigated the relationship between mortality risk of wolves across Portugal and several intrinsic and environmental variables with semiparametric Cox proportional hazard models (Therneau and Grambsch 2000). Specifically, I investigated the impact of age-class and sex on the mortality risk of all 32 collared wolves (**Table 1**). Age-class was included as a time-varying covariate and was updated on an annual basis. Thus, animals transitioned from pups to yearlings after their first biological year and from yearlings to adults after their second biological year. I also investigated the possibility that mortality risk varied across subpopulations by comparing the mortality risk of individuals belonging to a given subpopulation (coded = 1) against the remainder (coded = 0) (**Table 1**). I treated “Subpopulation” as a non-time varying covariate given that none of the collared animals dispersed from one subpopulation to another. Due to the small sample size obtained for SDouro, this subpopulation was not tested individually against the remainder. Finally, I investigated the influence of the environmental variables mentioned above (**Table 1**) on mortality risk of resident individuals ($n = 18$ resident wolves). I left nonresidents out of the models considering environmental variables due to their erratic use of space and because they may not have been exhibiting home range behavior in a manner consistent with residents.

The time of origin and censoring approach in the Cox models were the same as for the Kaplan-Meier procedure described above. I dummy-coded categorical variables by coding each row of data ‘1’ for the correct level of the variable and coding all other levels ‘0’. Then I included dummy-coded variables in the Cox models and withheld at least one level as the reference category to test specific hypotheses of interest. I was not able to investigate within-year (e.g. seasonal) effects on mortality risk parametrically, but seasonality in mortality risk is accounted for nonparametrically in the baseline hazard in models with the annual recurrent timescale (Fieberg and Delgiudice 2009). Furthermore, I present survival curves across the biological year which are effective for visualizing the seasonality of mortality risk. Multiple records from the same individuals are introduced in the model due to changes in time-varying covariates within a year (i.e. shift from resident to non-resident) and because many individuals were tracked across several biological years. To account for the lack of independence between the multiple observations from the same individual, I clustered entries by individual and estimated robust (“sandwich”) standard errors and p -values (Therneau and Grambsch 2000). I investigated the relationship between each predictor variable and mortality risk separately with univariate models to avoiding overfitting, given the relatively small sample size (32 collared wolves and 14 mortalities, Harrell et al. 1996; Hosmer et al. 2013) and compared the relative fit of models with identical datasets

using Akaike's Information Criterion corrected for small samples (AICc; Burnham & Anderson 2002). I compared candidate models to the null model using AICc differences ($\Delta AICc$). I only made inference on models that were substantially superior to the null model ($\Delta AICc > 2$; Benson et al. 2018), indicating that the variable of interest contributed substantial information to the model (Burnham and Anderson 2002). I conducted all survival and mortality risk analyses using the "survival" (Therneau and Grambsch 2015) and "AICcmodavg" (Mazerolle 2017) packages in R version 3.5.0 (R Core Team 2018). I tested the proportional hazard assumption of cox proportional hazards models by examining the distribution of Schoenfeld residuals (Therneau and Grambsch 2000) and found that the "unpaved roads" model showed strong evidence for nonproportionality as p-value of the Chi test was significative for $\alpha = 0.05$. This model was therefore excluded.

Table 1 - Variables tested in univariate model in the mortality risk analysis, the number of records to which each model was fitted, cohort that was held as the reference group and the fraction of individual days of tracking data it represents of the whole.

Variable	n	Reference	
		group	fraction
Discrete variables			
Constant			
Sex	32	females	0.63
Alto Minho	32	non-Alto Minho	1.45
Montalegre	32	non-Montalegre	3.62
Bragança	32	non-Bragança	3.98
Residency status	29	nonresidents	0.24
Time varying			
Age class	32	yearlings and pups	0.29
Continuous variables			
Constant			
Paved roads	18	NA	-
Unpaved roads	18	NA	-
All roads	18	NA	-
Agriculture	18	NA	-
Forest	18	NA	-
Open Areas	18	NA	-
Protected Areas	18	NA	-
Nightlight	18	NA	-

1.3 | Results

A total of 20 mortality events were documented for the 49 wolves trapped in Portugal between 1982 and 2017, of which 3 were capture-related. The 32 wolves with complete tracking records considered for the annual survival estimate (see 1.2.3 Annual survival) spawned from 1990 to 2017 and added to a total of 28.9 wolf-years, averaging 0.9 ± 0.6 wolf-years per individual. Wolves were not tracked equally across time nor across subpopulations (**Figure 2**), with Alto-Minho representing roughly 40% of the total tracking time by the end of the compiled period.

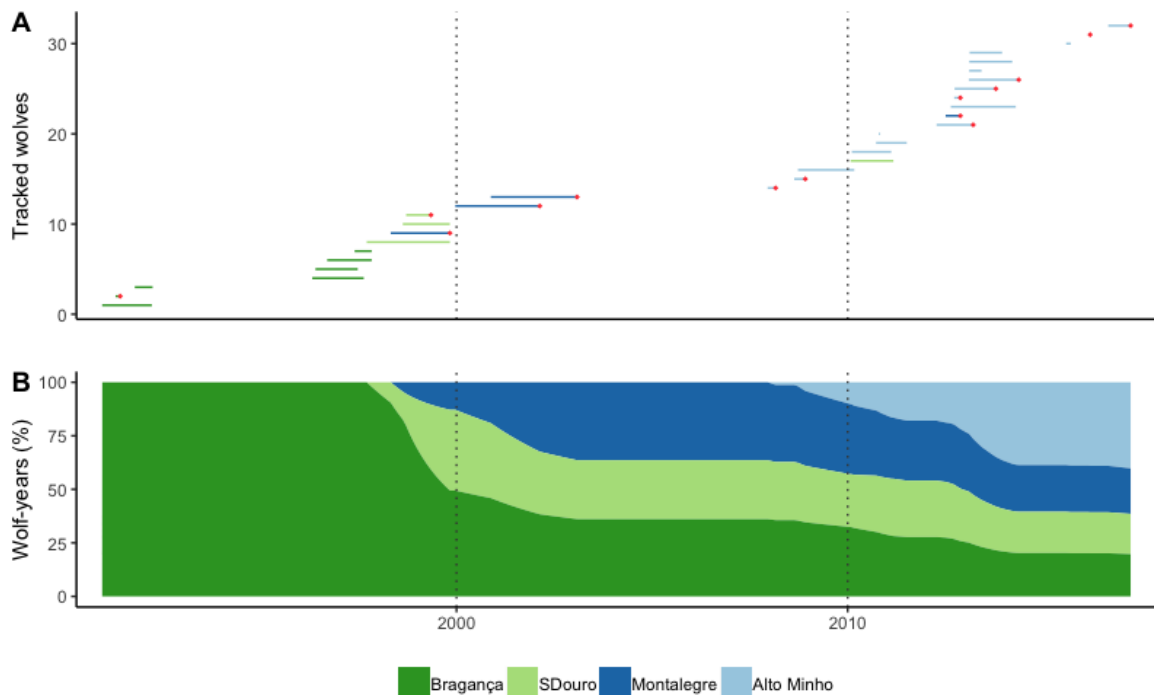


Figure 2 - A) Individual tracking records (line) and respective mortality events (red dot). (B) Variation in the representativeness of each subpopulation on recorded wolf-years across the studied period (1990 – 2017).

1.3.1 Causes of death

Before legal protection, all collared wolves ($n=3$) were killed by gunshot during a hunting season. After 1988, human related incidents continued to be the only source of mortality of collared wolves in Portugal, despite the species being now fully protected by law. The most common cause of death during the period of 1990 - 2017 was snaring ($n = 5$, 36%). The remaining animals were poisoned ($n = 3$, 21%), road-killed ($n = 2$, 14%), shot ($n = 2$, 14%), or poached through unknown means ($n = 2$, 14%) (**Figure 3**). Capture related mortalities occurred both before ($n = 2$) and after ($n = 1$) 1988.

Table 2 - Causes of death of collared wolves (n = 20) documented in Portugal since early 1980s until 2017, by decade. Numbers between parentheses correspond to the total number of collared wolves in each decade.

Cause	1982-89	1990-99	2000-09	2010-17
Snare	-	2	-	3
Gunshot	3	-	-	2
Poison	-	1	2	-
Road-kill	-	-	1	1
Undetermined causes ¹	-	-	1	1
Capture	2	-	1	-
	5 (10)	3 (11)	5 (6)	7 (17)

¹Human-related

The spatial distribution of mortality causes seems to suggest a high incidence of snare mortality in Alto Minho (**Figure 3**). Alto Minho was the only subpopulation where mortality by gunshot was observed after legal protection. Causes of death related to direct persecution (e.g. gunshot, poison or unknown human-related causes) were detected in all subpopulations except SDouro (**Figure 3**).

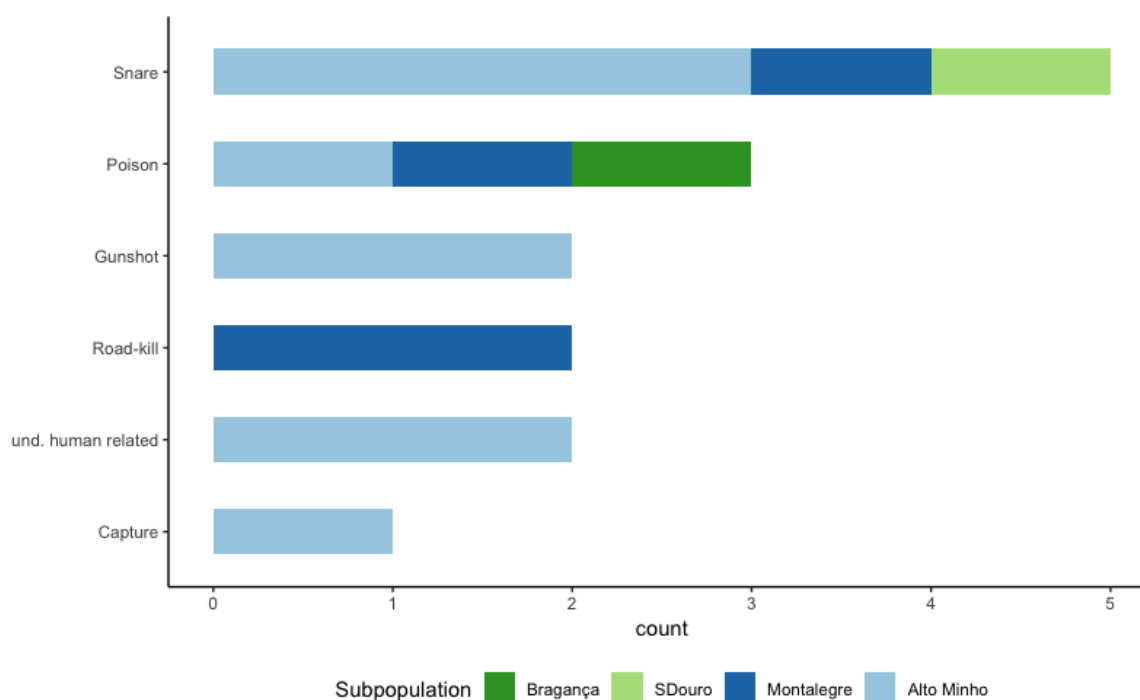


Figure 3 - Causes of death of individual collared wolves (n = 15) documented in Portugal during the 1990 – 2017 period, considering each subpopulation.

On the temporal scale, the occurrence of death by poison was limited to the turn of the 20th century (1991-2008) and road-kills were only detected in the last two decades (2000, 2010; **Table 2**). However, the small sample size, and particularly its irregular distribution in

space and time (**Table 2**) advises caution when it comes to inference on the spatial or temporal trends of death causes as it is impossible to attribute the effect to either scale (**Table 2**). Additionally, the tracking periods of the 32 collared animals are not uniformly distributed across years. Instead, there are two periods when no wolf was tracked: 1992 - 1996 and 2003 – 2007 (**Figure 2, A**).

1.3.2 Annual survival

Annual survival of the 32 animals captured and collared during the period of legal protection (1991-2017) was 0.623 (SE = 0.0791, 95% CI [0.486-0.799], n = 32, **Figure 4**). On the Kaplan-Meier survival plot it is possible to see that all but one mortality happened outside the pup-rearing season (from late-May to mid-October) and that a large portion of the mortalities (n = 6) occurred during mating season (proestrus + estrus) and early gestation periods (**Figure 4**).

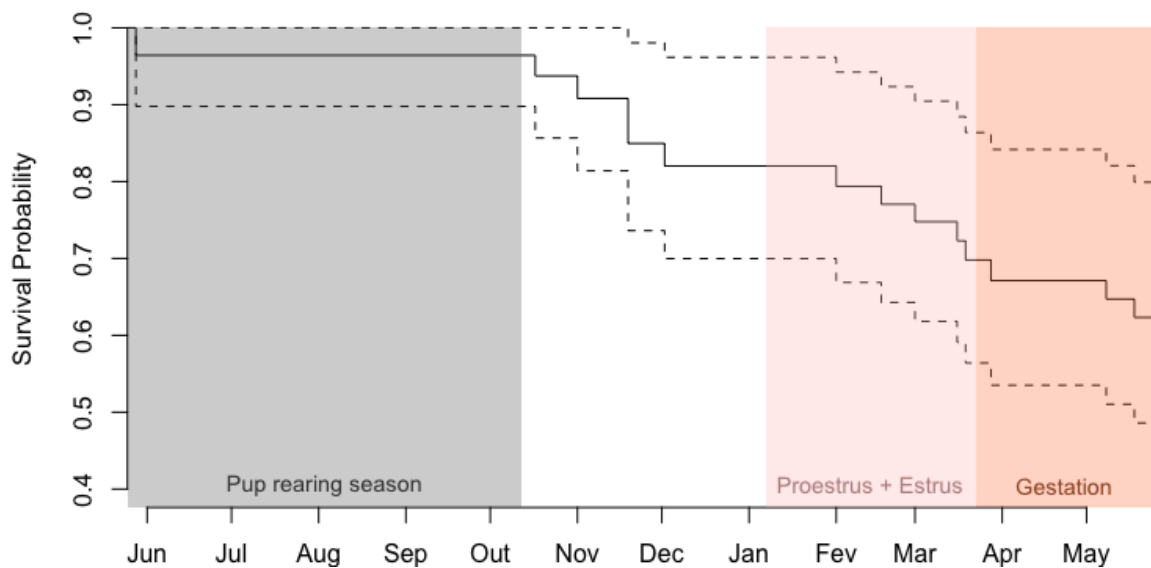


Figure 4 - Estimated Kaplan-Meier survival curve and 95% confidence interval for the 32 wolves radio-collared across Portugal between 1990 and 2017. Vertical drops correspond to mortality events.

1.3.3 Mortality risk

None of the tested models were superior ($\Delta AIC_c < 0$) to the null models (**Table 3**). In other words, the distinction between males (n = 17, 8 deaths) and females (n = 15, 6 deaths), residents (n = 26, 10 deaths) and nonresidents (n = 12, 3 deaths), adults (n = 26, 11 deaths) and the remainder age classes (yearling + pup) (n = 10, 3 deaths) or the effect of subpopulation failed to provide significant effects to explain the variation in the data any

more than the null model did. The result was the same in all environmental models (**Table 3**). The relationship between mortality risk and each of the selected environmental variables is illustrated in **Figure S1**.

Table 3 - Univariate models of mortality risk of all 32 radio collared wolves considered in the survival analysis (model 1), 29 radio collared wolves with available information on residency status (model 2) and 18 radio collared resident wolves (model 3), respectively, and corresponding null model. The hazard ratio is the multiplicative change in mortality risk and "95% CI" is its 95% confidence interval. Ph is the p-value of the proportional hazards assumption test, significant for $\alpha = 0.05$ indicates deviation from the critical assumption.

Model	AIC _c	Δ AIC _c	ph	p-value	Hazard ratio	95% CI	
						lower	upper
Null model 1	95.5	0	-	-	-	-	-
Age	97.4	1.99	0.988	0.789	1.19	0.332	4.28
Sex	97.5	2.03	0.576	0.863	0.92	0.355	2.38
Subpopulation							
Alto Minho	96.5	1.08	0.359	0.252	1.7	0.685	4.23
Montalegre	96.9	1.46	0.511	0.244	1.61	0.724	3.57
Bragança	95.9	0.422	0.086	0.276	0.323	0.0422	2.47
SDouro	96.3	0.893	0.186	0.33	0.378	0.0533	2.68
Null model 2	87.4	0	-	-	-	-	-
Residency	89.3	1.9	0.457	0.64	0.758	0.237	2.43
Null model 3	33.2	0	-	-	-	-	-
Land Cover							
Agriculture	35.1	1.97	0.832	0.62	0.312	0.00314	31.05
Forest	35.2	2.07	0.621	0.778	2.15	0.0103	452
Open	35.3	2.11	0.741	0.859	1.372	0.0419	44.96
Nightlight	34.65	1.48	0.273	0.401	1.075	0.908	1.272
Protected Areas	35.3	2.13	0.0108	0.954	0.917	0.0476	17.67
Roads							
All	35.03	1.86	0.13	0.625	1.262	0.497	3.2
Paved	35.1	1.93	0.351	0.658	1.4	0.316	6.21
Unpaved	35.02	1.85	0.046	-	-	-	-

Chapter 2 - Wolf Population Dynamics in Alto Minho

2.1 | Study area description at regional level

The study area for Chapter 2, comprising the Alto Minho region ($\cong 1727 \text{ km}^2$), is located in the northwest of Portugal and is confined by the Minho river (north), the Lima river (south), the Atlantic Ocean (west) and the Portuguese-Spanish border (east; **Figure 5**). The landscape is characterized by a mountainous profile with a dense hydrographic network, where the highest altitudes are located in *Serra d'Arga* (825m) *Serras do Soajo-Peneda* (1416m) and *Corno do Bico* (883m). The climate is typically Mediterranean, with dry-warm summers and rainy winters. The annual cumulative rainfall in this region is one of the highest in Portugal with an average of 2000 mm (IPMA 2018). Land uses in the region result from centuries of human presence and activity. Patches of natural vegetation are scarce, and the landscape is dominated by a matrix of agricultural fields, human settlements, artificial structures (e.g. wind farms) and a dense network of paved (2.61 km/km^2) and unpaved (2.16 km/km^2) roads. Human density is higher in the limits of the study area, particularly in the lowlands along the coastline and the two main rivers, Minho and Lima. In fact, parishes included in these areas with lower altitude have a human density of 182 hab/km^2 and comprise 61% of the Alto Minho human population despite covering only 32% of its area. The remaining parishes located in more mountainous areas average 54 hab/km^2 (INE 2011). From 2001 to 2011 the overall resident human population in Alto Minho suffered a small decline of 2%, with larger urban centers displaying population increments as high as 45% while more rural areas suffered a strong exodus with losses reaching 35% of the resident population (INE 2011). However, despite a decrease in the resident population in rural areas located at higher altitudes, these regions are still frequently used by humans, particularly for livestock grazing and human infrastructures, such as wind power development. In fact, Alto Minho region has one of the highest producing capacities for wind power in Portugal, with almost 200 wind turbines in operation (APREN and INEGI 2016), the majority located in mountain tops far from human settlements. The study area encompasses two regional protected landscapes (*Corno do Bico* and *Lagoas de Bertandos e São Pedro de Arcos*) and a portion of *Peneda-Gerês* National Park, adding up to 261 km^2 of protected area, which represents 15% of the entire study area (**Figure 5**).

Regarding potential food resources for wolves, there are 4 wild ungulate species, two of them widespread – wild boar and roe deer – and other two, recently reintroduced and still

with small and localized populations – red deer and Spanish wild goat – but with no available information on abundance and population size (Vingada et al. 2010, Álvares et al. 2015). The wild boar (*Sus scrofa*) recovered from a severe decline, at the national level, during the 20th century and is now the most widespread and abundant wild prey for wolves in the study area (Vingada et al. 2010). Populations of Roe Deer (*Capreolus capreolus*) have also recovered in the last decades, and are now widespread but apparently occurring at low densities (Vingada et al. 2010; Torres et al. 2015). The red deer (*Cervus elaphus*) and the Wild goat were reintroduced in early 2000s and are still confined to a small area inside Peneda-Gerês National Park (Vingada et al. 2010). Besides wild ungulates, there are also large numbers of several livestock species, such as goats, sheep, cattle and horses, under extensive grazing regimes in mountain pastures and which are the main food resource for wolves in the region (Álvares 2011). In particular, the Garrano horse, a semi-feral native pony breed occurring in a free-ranging regime all year round with minor herd management, is present in all mountain ranges of the study area and constitute up to 70% of wolf diet in some packs (Vingada et al. 2010, Álvares 2011, Álvares et al. 2015). The incidence of livestock depredation by wolves in Alto Minho is the highest at the national level, which generates a strong conflict with the local communities (Álvares 2011, Álvares et al. 2015, Pimenta et al. 2017).

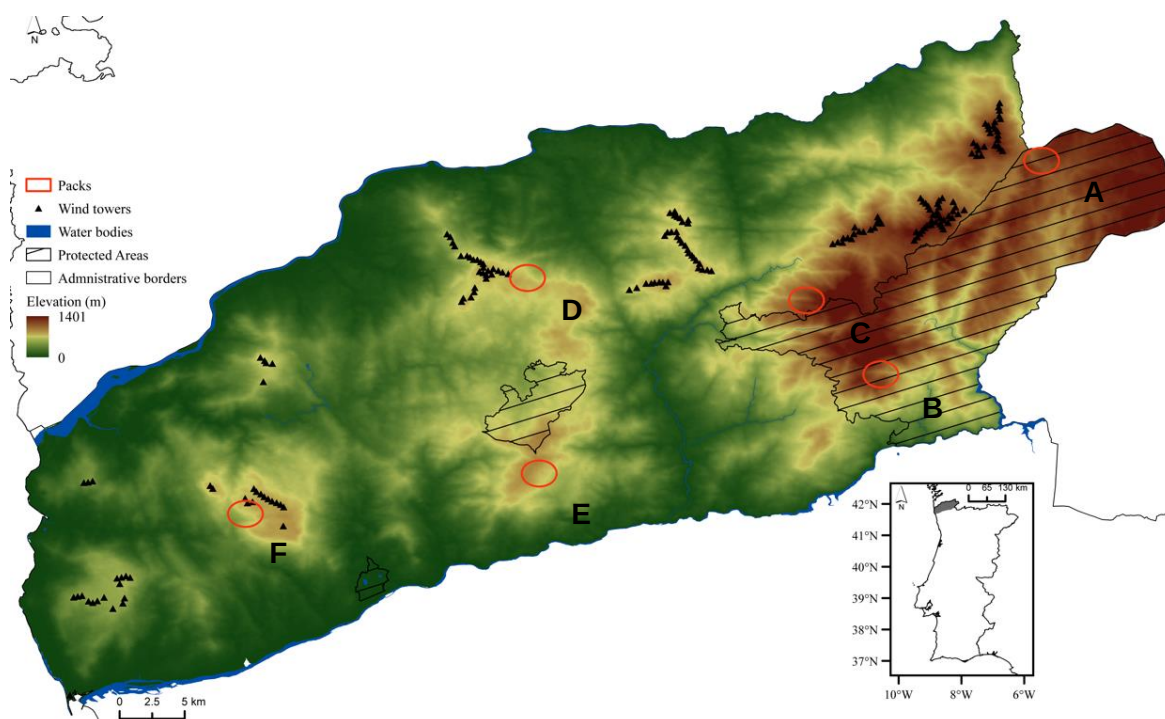


Figure 5 - Study area in Alto Minho and location of packs (red circles) and turbines from wind farms (black triangles). Pack location is based on breeding sites detected in 2011 and 2014 (Álvares, unpublished data). From the right to the left: A: Peneda pack, B: Soajo pack, C: Vez pack, D: Boulhosa pack, E: Cruz vermelha pack and F: Arga pack.

Despite centuries of human persecution, the wolf was never completely extirpated from the human-dominated landscape of Alto Minho (Álvares 2011). The maximum number of packs ever detected in the region was 6, which is considered to be the maximum number of territories that the region can hold (Pimenta et al. 2005, Álvares 2011, Álvares et al. 2015). The wolves in Alto Minho, although seemingly in continuity with the rest of the Iberian population to the west, have been shown to be an distinct genetic cluster with relatively high mean relatedness values compared to the remaining of the genetic cluster identified in wolves across Iberian Peninsula (Silva et al. 2018). This genetic differentiation suggests some level of demographic isolation which, considering the small population size, raises some conservation concerns regarding the possible impact of genetic inbreeding or even local extinction.

2.2 | Methods

2.2.1 Data collection

All data analyzed in this chapter was collected between May 2007 and October 2016 in Alto Minho under the scope of ongoing wolf monitoring projects led by CIBIO, related to environmental impact assessments for wind farm infrastructures (Rio-Maior et al. 2010, Nakamura et al. 2012, 2013, 2014, 2015, 2016b, 2017).

Pack presence was initially assessed based on sign abundance (e.g. scats, urine and scratches) detected during transects and other indirect indicators (e.g. livestock damages). Starting in mid-July, around the time pups are known to be able to vocalize, until early October, howling and observation surveys were performed in areas where high levels of territorial marking were suggestive of the occurrence of reproduction and proximity to pup-rearing sites, an approach that has been frequently used in the Iberian Peninsula (Llaneza et al. 2005, 2014). A positive response from a chorus or the visual detection of pups allows the confirmation of pack presence and occurrence of reproduction as well as estimation of pack and litter size by counting the minimum number of animals detected (during July-October). These surveys were performed by the same experienced technicians (M. Nakamura and H. Rio-Maior) throughout the entire studied period and never under unfavorable weather conditions, such as rain or heavy wind, which could influence the results. Survey effort was often unstandardized and built on continuous repetition of opportunistic howling and observation surveys until early October, the main focus being the assessment of reproduction at all known pack territories. Additionally, field effort was complemented with opportunistic camera trapping in areas where the probability of detecting wolves was higher, such as areas near breeding sites and predated livestock carcasses. The study design was preserved during the 10-year monitoring period, assuring the comparability of results between years. Previous knowledge on wolf presence and pack territories in the region was taken into account while designing the transect grids and choosing survey stations.

2.2.2 Field effort for pack and breeding detection

A total of 1,025 howling and 266 observation surveys were performed from mid-July to early October between 2007 and 2016. All surveys were spatially distributed between 6 pack territories that have remained relatively stable over the years, namely Argá, Boulhosa, Cruz Vermelha, Peneda, Soajo and Vez (**Figure 5**). Pack presence was continuous in the Vez and Soajo territories throughout the entire studied period (2007 - 2016). The same

happened in the Cruz Vermelha, Boulhosa and Arga territories, but only after first detection in 2010, 2011 and 2014, respectively (see **Table 5**). The Peneda territory was the only where pack presence was irregular after first detection in 2008 (see **Table 5**).

2.2.3 Pack and population size

I determined minimum pack size from the sum of the maximum number of adult and pups detected on each territory during pup rearing season from July to October, either through acoustic, visual or camera trapping detections. Pack size (P) was obtained based in the following equation:

$$P_{ti} = Adults_{ti} + Pups_{ti}$$

where t and i are indexes of time (year) and pack, respectively. Number of pups and number of adults were detected independently as the maximum number of individuals detected together belonging to each age class, which could be a product of two distinct observations. This is only possible because, during the reference period, pups and adults are easily distinguishable, both visually and acoustically.

I calculated population size (N_t), the sum of all detected pack sizes, in annual intervals (t), as illustrated by the following equation:

$$N_t = \sum_{i=1}^6 P_{ti}$$

These population counts are based exclusively on the results of the field methodologies reported above. Other types of data have been collected along the monitoring period, including non-invasive genetic samples and telemetry data, which can provide valuable information to improve and complement the population size estimates. However, the goal here was not to obtain precise estimates of population size but rather assess its trend and explore the dynamics that support it, which is possible if the observed sample is a relatively constant fraction of the whole population (Morris and Doak 2002). This applies even in the case of wolves counts obtained by field surveys that consider the pack as the basic monitoring unit and ignore a fraction of the natural structure of a wolf population, which are the dispersers and lone animals not affiliated with a pack (Blanco and Yolanda 2012). In this context I considered that the count data analyzed here results from the observation of a constant fraction of the wolf population in Alto Minho, the resident population, which is representative of its dynamics.

2.2.4 Population growth

I calculated annual exponential growth rates (λ_t), the amount by which population size (N) multiplied between subsequent years (e.g. from t to t+1), as illustrated by the following equation:

$$\lambda_t = \frac{N_{t+1}}{N_t}$$

and estimated average growth rate (λ) through linear regression of the observed annual rates, following a method proposed by Dennis et al. (1991) and described in detail in Morris and Doak (2002).

2.2.5 Count-based model

I assessed the growth of Alto Minho's resident wolf population between 2007 and 2016 through a stochastic count-based model. Given the context of this subpopulation and particularly the limited amount of available territories for packs to settle in, I took density dependent growth into account. Population growth will generally decline and eventually stabilize as the population reaches its carrying capacity, that can be imposed by any kind of limiting factor such as food, space or any other factor that correlates growth to abundance (Morris and Doak 2002).

Other two factors to have into consideration when modeling the growth of wild populations are Allee effects and inbreeding depression. The Allee effect is a negative response of population performance to low population densities that can occur, for example, due to the inability to find potential mates or difficulty in executing group defense or feeding strategies (Morris and Doak 2002). Allee effects have been observed in wolf populations and hypothesized to increase the probability of hybridization due to limited mate availability (Muñoz-Fuentes et al. 2010), conditioning recolonization rates due to the lowest probability of finding a mate in the low density zones of an expanding front (Hurford et al. 2006). On the other hand, inbreeding depression can reduce the performance of the population by decreasing the fitness of individuals and their reproductive success (Morris and Doak 2002), leading to an impact that has already been demonstrated on the insular population of wolves from Isle Royale, USA (Hedrick et al. 2014). Here, I do not account for Allee effects or inbreeding depression for the following reasons: first, because there's no data available on Iberian wolves from which to draw reasonable assumptions on how the population would behave in the presence of either factors; second, because, until the present day, there is no evidence that the Alto Minho wolf population is inbred to the point where individual fitness is impaired; and third, because the ten years of count data I have analyzed here are

considered the minimum requirements to build a count-based model (Morris and Doak 2002) and consequently not the most appropriate to fit more complex models than the one I describe below.

The theta logistic model is commonly used to describe the density regulation of growth in abundance time series and can produce a wide range density dependence forms: from the concave shape that approximates to the behavior of a density-independent growth into a ceiling, to the convex shape of the Gompertz form (Lande et al. 2003). However, Clark et al. (2010) suggested that the theta logistic model cannot be reliably used to fit real-world data because two of its parameters are not independent and therefore cannot be estimated simultaneously without prior knowledge on one or the other. Provided that, the authors suggest the use of a simpler model that describes a linear relationship between growth and population size, the Ricker model. Both models, the theta logistic and the Ricker's, are recommended by Morris and Doak (2002), as viable alternatives to fit census data and identify the best density dependence form of the observed data. Provided that, and taking into account the recommendations of Clark et al. (2010) I decided to use the Ricker model to test for density dependence on Alto Minho wolf abundance data.

The Ricker model was initially developed for quantitative fisheries management and can be illustrated by the following equation:

$$N_{t+1} = N_t e^{r(1-\frac{N_t}{K}) + \varepsilon(0, \sigma^2)}$$

where N_t is the abundance at time t , N_{t+1} is the abundance at time $t+1$, r is the maximum intrinsic growth rate, K is the carrying capacity and ε is the process error. The process error is estimated from the deviation between the observed values and the idealization of the model, being usually interpreted as environmental variation, or the stochasticity around growth, although it also contains variation due to observational errors (Morris and Doak 2002). Although the model includes stochasticity around the growth parameter (r), which is an approximation to reality, the density dependence term $(1-N_t/K)$ is deterministic. Furthermore, a constant carrying capacity (K), such as the way the Ricker model describes it, is not always an ideal simplification of biological processes where limiting factors can have seasonal patterns or other kind of fluctuations.

I also fitted the exponential growth model, a model that describes a density-independent growth, to evaluate which is the best approximation to the observed data, by using the following equation:

$$N_{t+1} = N_t \times \lambda$$

where N_t is the abundance at time t , N_{t+1} is the abundance at time $t+1$ and λ is the average annual growth rate.

I fitted each model to the observed data through nonlinear least-squares regression and compared the relative fit using Akaike's Information Criterion corrected for small samples (AICc; Burnham & Anderson 2002).

2.2.6 Breeding frequency and litter size

I obtained pack breeding frequency by calculating the ratio between detected reproductions and the number of times each pack were detected. I determined litter size from the maximum number of pups detected together on each pack during pup rearing season, from July to October, either through acoustic, visual or camera trapping detections, regardless of time of detection. Inevitably, estimates that were obtained in late-breeding season are less likely to be representative of the actual number of pups that were born in late May due to pup mortality along their first months of age.

2.2.7 Annual survival and opportunistic mortality events

To estimate survival in Alto Minho, I used data from 18 wolves collared between 2007 and 2017, comprising adults ($n = 15$) and yearlings ($n = 3$). One collared wolf (AM4) died due to capture related causes and was not included in the analysis, as reported in chapter 1. Capture, handling and tracking procedures are already described in Chapter 1 and are available in Rio-Maior et al. (2016). I used the staggered entry Kaplan-Meier approach (Kaplan and Meier 1958, Pollock et al. 1989) with an annual recurrent time scale (Fieberg and Delgiudice 2009) standardized to a biological year beginning on 25 May. The procedure is explained in detail in chapter 1.

Additionally, I was provided with 20 records of wolves found dead between 2002 and 2015, which contained information on date, cause of mortality, age and sex of the animal. These records were collected by the national authority for nature conservation, ICNF, that, since 1999, has a centralized system, the SMLM (Monitoring System for Dead Wolves), that collects, necropsies and stores biological samples of dead wolves opportunistically found in Portugal. Six of the dead wolves collected by the SMLM were animals radio-collared in the scope of the monitoring project and were consequently excluded from the dataset of opportunistic mortalities.

2.3 | Results

2.3.1. Pack detection, breeding frequency and litter size

Wolves were visually detected in 40% of the observation surveys (n=107) and acoustically detected in 16% of the howling surveys (n=160). Pups were detected in 44% (n=71) of the positive acoustic surveys and in 56% (n=60) of the positive observation surveys (“positive” meaning the detection of at least one individual). In 23 occasions, pups were detected in the absence of adults (adults could be present but were not detected), 17 of these events occurred during howling surveys and the remaining 6 during observation surveys.

A total of 33 breeding events were detected in Alto Minho subpopulation between 2007 and 2016, averaging 3.5 ± 1.8 pups per detected litter. The majority of pup detections occurred between mid-July and late-August. Reproduction was never detected on all 6 pack territories simultaneously in the same year, as the maximum number of breeding packs detected on each breeding season was 5 out of the 6 potentially existing in the study area. The number of pups detected in Alto Minho increased since the beginning of the study period in 2007 and reached its maximum in 2012 with 20 pups detected. In the following years, until the end of the study, an average of 15 pups were detected, with the exception of an extreme low value observed in 2015 (**Table 4**).

Table 4 - Number of pups and reproductions detected in Alto Minho between 2007 and 2016.

Year	Total number of pups	Reproductions detected	Mean litter size	Largest litter
2007	6	2	3	3
2008	8	2	4	5
2009	4	1	4	4
2010	10	3	3.3	5
2011	17	5	3.4	8
2012	20	4	5	7
2013	16	4	4	9
2014	14	4	3.5	4
2015	6	3	2	3
2016	15	5	3	4
Mean $\pm$ SD	11.6 $\pm$ 5.5	3.3 $\pm$ 1.3	3.5 $\pm$ 0.8	5.2 $\pm$ 2.1

The increase in the number of reproductions was probably the main driver of the positive trend observed in the number of pups between 2007 and 2011, provided that the mean litter

size remained fairly stable across that same period (**Table 4**). The smallest number of pups were detected in 2009 when only one pack bred (Vez). In 2015 the mean litter size was particularly small due an abnormal low number of pups detected in each of the packs that bred: 1 pup in Arga, 2 in Vez (the lowest recorded for this pack) and 3 in Soajo. Additionally, 2015 was the first year since 2010 when less than 4 packs produced a litter. The largest litter size (9 pups), was detected during 2013 in Vez pack. The largest litter was always detected in Soajo (n = 4 years) and Vez packs (n = 4 years). Pack specific mean litter size is presented in **Table 7**.

Packs in Alto Minho bred 83% of the times they were detected between 2007 and 2016. Breeding frequency was the highest in Arga and Peneda packs where reproduction occurred in the 3 and 4 years each pack was detected, respectively (100%), followed by 90% breeding frequency of the Vez and Soajo packs and 86% in Cruz Vermelha (**Table 5**). The Boulhosa pack was detected 6 times but reproduction was only detected in 2 of those years, corresponding to a 33% of breeding frequency (**Table 5**).

Overall, during the 10-year monitoring period analyzed here, a total of 116 pups were detected. Of these, 36% were detected in Vez, 30% in Soajo, 15% in Cruz Vermelha, 10% in Peneda, 6% in Arga and 3% in Boulhosa.

Table 5 - Detection of packs and reproduction between 2007 and 2016 in Alto Minho wolf subpopulation. Green cells represent years of pack presence and red cells represent year of pack absence.

Packs	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Arga								•	•	•
Boulhosa					•	⊙	⊙	⊙	⊙	•
Cruz Vermelha				•	•	•	•	•	⊙	•
Vez	•	⊙	•	•	•	•	•	•	•	•
Soajo	•	•	⊙	•	•	•	•	•	•	•
Peneda		•			•	•	•			
• - Reproduction confirmed					⊙ - Reproduction not detected					

2.3.2 Pack and population size

The number of packs detected increased in the first half of the studied period (2007-2012) and stabilized in the second (2013-2016) (**Table 6**). Mean pack size followed a similar trend to that of population size, increasing from 2007-2009 ($\bar{x} = 5.9 \pm 1.2$ SD) to 2010-2013 ($\bar{x} = 8.1 \pm 0.5$ SD) and then decreasing again in the last 3 years of the studied period ($\bar{x} =$

6.3 ± 0.8 SD) (**Table 6**). The largest pack size was observed during 2011 in Vez pack, with 16 individuals. The largest pack size was always observed in Vez (n = 4 years) or Soajo packs (n = 5 years). The year of 2009 is an outlier to the general trend, both regarding population size and mean pack size.

Table 6 - Demographic characteristics of the wolf subpopulation in Alto Minho during summer, between 2007 and 2016.

Year	Population size	Packs detected	Mean pack size	Maximum pack size
2007	13	2	6.5	7
2008	20	3	6.7	10
2009	9	2	4.5	5
2010	24	3	8	10
2011	38	5	7.6	16
2012	44	5	8.8	12
2013	40	5	8	15
2014	34	5	6.8	11
2015	27	5	5.4	7
2016	33	5	6.6	10
mean ± SD	28.2 ± 11.7	4 ± 1.3	6.9 ± 1.3	10.3 ± 3.5

The mean pack size was 7 ± 3.1 wolves. Mean pack size was higher in Soajo (8.9 wolves) and Vez packs (8.1 wolves) (**Table 7**). Boulhosa showed a mean pack size particularly small, with 3.5 wolves, relative to the remaining packs, mainly due to the small number of adults detected, $\bar{x}$ = 2.8 adults (**Table 7**). A large portion of the variation observed in mean pack sizes across different packs was due to the differences in the mean number of pups detected in each pack rather than the number of adults (**Table 7**). All summary statistics relative to wolf abundance were very similar across the Arga, Cruz Vermelha and Peneda packs (**Table 7**).

Table 7 - Mean number of adults, pups and pack size, with respective minimums and maximums observed per pack in Alto Minho between 2007 and 2016.

Pack	Adults		Pups		Pack	
	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max
Arga	4	3 - 5	2.3	1 - 3	6.3	6 - 7
Boulhosa	2.8	2 - 4	2.0	1 - 3	3.5	2 - 5
Cruz Vermelha	3.9	3 - 5	2.8	2 - 4	6.3	5 - 9
Peneda	3.8	3 - 4	2.8	2 - 4	6.5	5 - 8
Soajo	4.6	3 - 7	3.9	2 - 7	8.1	4 - 12
Vez	5.1	2 - 8	4.7	2 - 8	8.9	3 - 16

2.3.3 Population growth

The average population growth (λ) of the wolf subpopulation in Alto Minho between 2007 and 2016 was 1.109, 95% CI [0.752, 1.637]. This value suggests that the subpopulation grew in size by around 11% every year, although the obtained confidence interval ranges from negative ($\lambda < 1$) to positive values. The Alto Minho subpopulation grew by more than 3-fold between 2007 and 2012 (**Table 6**), representing an average annual growth rate of $\lambda = 1.28$, with a minimum of 9 wolves detected in 2009 and a maximum of 44 wolves in 2012. However, during the last 4 years (2013-2016) this subpopulation displayed a slight decrease in population size, with an average annual growth rate of $\lambda = 0.93$.

2.3.4 Count-based model

The model with the lowest AIC was the one describing a density independence growth, the Exponential model (**Table 8**). This model has a single parameter, r , that is the logarithmic form of λ . In this case, the fit of the exponential model to the observed data resulted in an $r = 0.104$, equivalent to the $\lambda = 1.109$ obtained through the Dennis method (see **2.3.3 Population growth**).

Regarding the fit of the Ricker model to the observed data, results suggests a carrying capacity of around 33 wolves. The estimated maximum intrinsic growth rate was particularly high and equivalent to $\lambda = 1.92$, suggesting that, as idealized by the model, the Alto Minho wolf subpopulation is capable of growths of around 90% of its size when density is very low (when N_t/K approximates 0).

Table 8 - Parameter estimates and AICc values for the density independent model (exponential) and the Ricker model applied to Alto Minho wolf subpopulation.

Model	r	K	σ^2	AICc	$\Delta AICc$
Exponential	0.104	-	0.256	18.23	0
Ricker	0.652	32.89	0.197	20.67	2.443

2.3.5 Annual survival and mortality events

Nine of the 18 collared wolves in Alto Minho died during the studied period, all of them due to human-related causes: five wolves (63%) were poached, by gunshot ($n = 2$), poison ($n = 1$) and undetermined human-related causes ($n = 2$) while three (37%) were killed accidentally in illegal snares set for wild boar. One collared wolf died due to capture related causes shortly after release, and was not included in the percentages above provided that it was caused by the sampling procedure. The annual survival of wolves in Alto Minho between 2007 and 2016 was 0.541 ± 0.119 SE, 95% CI [0.352, 0.831] (**Figure 6**).

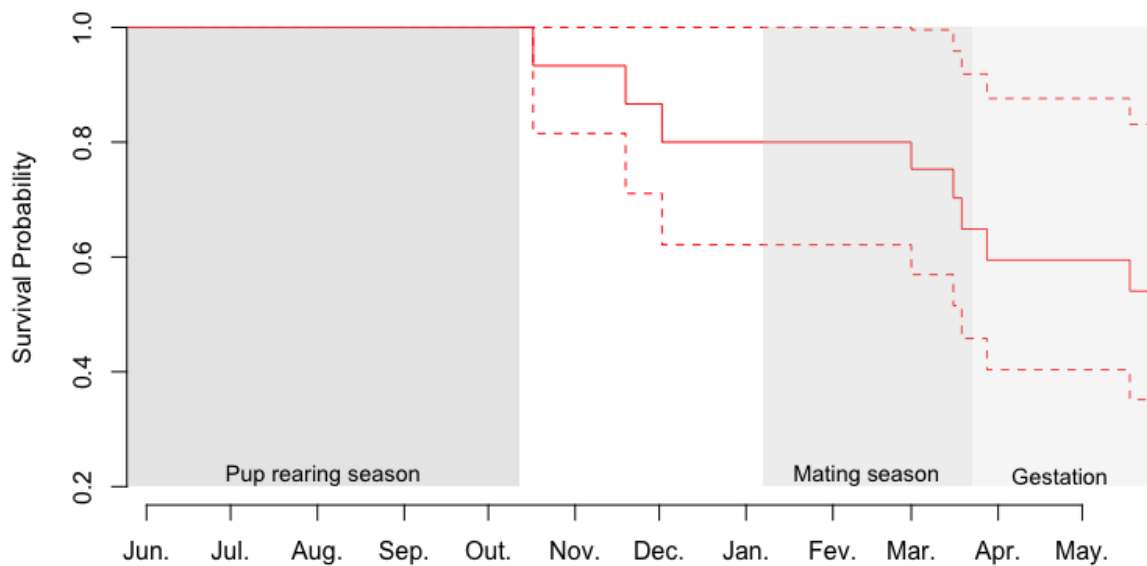


Figure 6 - Estimated Kaplan-Meier survival curve and 95% confidence interval for the 17 wolves radio-collared in Alto Minho between 2007 and 2016

A total of 14 dead wolves were collected opportunistically by the SMLM in Alto Minho subpopulation between 2002 and 2015, 9 of them (36%) during the second half of the sampled period (2009-2015). The maximum number of dead wolves per year was collected in 2009 ($n = 4$). The causes of mortality were also predominantly human-related: 5 wolves (36%) died by collision with vehicles, 3 (21%) were shot, 3 (21%) died in illegal snares, 2 (14%) by unknown causes and 1 (7%) was killed by canids, most probably other wolves.

Discussion

Survival and mortality risk of wolves in Portugal

This study revealed a high incidence of human-caused mortality in the Portuguese wolf population. Although legally protected, none of the 14 verified mortalities, from 32 collared wolves, observed between 1990 and 2017 were due to natural causes. Half of the animals that died were killed by poaching ($n = 7$), either through gunshot, poison or unknown means. The remainder were accidentally killed in illegal snares ($n = 5$), mostly thought to be aimed at wild boar, or in collisions with vehicles ($n = 2$). Accidental mortalities made up 38% of the deaths in Alto Minho subpopulation and 75% in Montalegre subpopulation. The prevalence of human-caused mortality and incidence of poaching reported here is consistent with results from Blanco & Cortés (2007) for wolves in north-central Spain, where only one natural mortality cause (i.e. killed by dogs) was observed in 9 verified mortalities between 1997 and 2007; 44% caused by poaching ($n = 14$ collared wolves). Conversely, in Picos de Europa, northern Spain, in 8 wolves collared between 2011 and 2015 only two died due to legal control (Fdez et al. 2014, Llana and Fdez 2016).

In the Scandinavian Peninsula, 19% poaching and 73% human-caused mortality were observed in 26 verified mortalities between 1998 and 2009 ($n = 104$ collared wolves; Liberg et al. 2011). In Finland out of 91 verified mortalities observed between 1998 and 2013, 97% were caused by humans and 57% by poaching ($n = 130$ collared wolves; Suutarinen & Kojola 2017). Despite human-caused mortality being prevalent throughout Europe, in all cases cited above some degree of legal control was allowed during the studied periods. In particular, legal control represented 34% of verified mortalities in the Scandinavian population (Liberg et al. 2011), 31% in the Karelian population (Suutarinen and Kojola 2017), 10% in Iberian population from north-central Spain (Blanco and Cortés 2007) and 100% also in the Iberian population in Picos de Europa (Fdez et al. 2014). Wolf protection in Portugal does not contemplate any kind of legal control but still anthropogenic mortality was prevalent and more frequent than in countries where the species is legally harvested, raising concern regarding the efficiency of the legal status, or at the very least its enforcement.

The estimated annual survival rate for wolves collared in Portugal, 0.623 ± 0.0791 SE, is similar to that observed by Webb et al. (2011) (0.628 ± 0.05 SE) in an harvested wolf population in west-central Alberta (Canada), that showed no signs of population decline. Fuller et al. (2003), reviewed the relationship between the mean rate of population increase and annual mortality of multiple exploited wolf populations in North America and suggested

that the growth of wolf populations should on average stabilize at an annual survival rate of 0.66 ± 0.08 SE. Adams et al. (2008), adopting a similar approach, suggested that the growth of wolf populations and mortality levels were strongly and inversely correlated below an annual survival rate of 0.71. Although demographic growth is a complex process modulated by multiple factors (Morris and Doak 2002), the annual survival estimated here for collared wolves in Portugal seems to be at, or slightly below, the threshold at which the growth in wolf populations is expected to remain stable. This is consistent with the fact that no critical signs of range contraction or population decline have been detected in Portugal over the last decades (Pimenta et al. 2005, Álvares et al. 2015). Nevertheless, it is important to note that the last national census was conducted 15 years ago (Pimenta et al. 2005) and recent efforts to quantify wolf abundance and distribution in Portugal were based on local and regional prospection carried out by independent teams, and did not result from a national wide program (Álvares et al. 2015). Therefore, the actual demographic condition of wolves in Portugal cannot be stated with certainty and the annual survival rate provided here cannot be paired with a properly quantified population trend. Furthermore, the confidence interval of my annual survival estimate, 95% CI [0.486, 0.799], is wide enough to encompass high annual survival rates, as those observed in fast growing populations (e.g. Gogan et al. 2004; Adams et al. 2008; Wydeven et al. 2009; Smith et al. 2010; range [0.75 - 0.791]), as well as very low annual survival rates like those observed in heavily exploited and declining wolf populations (Ballard et al. 1987; Ballard et al. 1997; Person & Russell 2008; range [0.54 - 0.55]), which further hampers the inference on the condition of the wolf in Portugal from the survival estimate alone.

Also in the Iberian Peninsula, Blanco & Cortés (2007) reported an annual survival rate of 0.82 for 14 collared wolves living in an area dominated by agricultural activities in north-central Spain, between 1997 and 2007, which is higher than the upper limit of the confidence interval obtained by me for the annual survival of collared wolves in Portugal. Even though these authors reported conditions that could potentially decrease natural mortality, such as low likelihood of starvation or intraspecific competition (Fuller et al. 2003), due to food-surplus in the form of livestock carrion, this still doesn't explain the disparity in survival provided that almost all mortality was caused by humans, with the exception of one wolf killed by dogs (Blanco & Cortés 2007). The fact that the survival reported for wolves in north-central Spain is higher than the upper limit of the confidence interval obtained for the annual survival of collared wolves in Portugal is yet again surprising, particularly considering the above-mentioned fact that wolves are fully protected in Portugal and not in Spain. In fact, one wolf was legally culled out of the 9 verified mortalities in Blanco & Cortés (2007).

The average number of years that wolves were tracked in Portugal, 0.9 wolf-years per collared wolf, was low compared to those reported in other studies, e.g. 1.4 wolf-years (Liberg et al. 2007) and 3.5 wolf-years (Blanco and Cortés 2007), a difference mostly due to methodological factors such as length of study and characteristic of collars (VHF or GPS). Inevitably, the length of individual tracking periods, as well as the small number of collared wolves, represented a significant limitation to the success of the mortality risk models. Therefore, the small sample size of my data-set had critical influence on the statistical power of the analysis, which might have caused the random structure of the null models to be the most parsimonious explanation to the variation of the observed data, instead of the complete lack of effect of the chosen factors. Additionally, for some of the tested variables, the upheld cohort was poorly represented compared to the reference group (e.g. Montalegre and Bragança; **Table 1**) or vice-versa (e.g. Sex and Residency status; **Table 1**) which further contributed to the lack of statistical power. Focusing on the models to assess the impact of the environmental context on the mortality of each individual, it would have been ideal to consider spatial covariates estimated at a finer temporal resolution (e.g. weekly or monthly). However, based on the available information for most wolves, I was limited to the “life-time” home range estimates, which may have created a temporal mismatch between the spatial covariates and the mortality risk of individuals at their time of death. In sum, the reliability of the mortality risk models was hampered by overall methodologic constrains, and the results I have obtained with the present data should not be used to infer on the mortality risk of wolves in Portugal.

Wolf population dynamics in Alto Minho

During the 10 breeding seasons monitored between 2007 and 2016 the Alto Minho wolf subpopulation displayed strong pattern of demographic growth and an East-to-West geographical expansion. The recovery of 3 packs that were absent from the study area in 2007 (Arga, Boulhosa and Cruz Vermelha), the stable reproductive output of two packs (Vez and Soajo) and a relatively high breeding frequency of most packs were the main contributors for the observed trend. During the first years of the monitored period (2007 – 2009) wolves in Alto Minho were restricted to 3 packs located inside the Peneda-Gerês national park (Vez, Soajo and Peneda) although the later was only detected in 2008. After this period the west-central packs made a comeback from the east, starting in Cruz Vermelha in 2010, then Boulhosa in 2011 and finally Arga in 2013. The recovery of these three packs was accompanied by an increase in the number of litters produced which in turn resulted in a higher wolf abundance between 2013 and 2016, averaging 8.1 wolves per

pack (~34 wolves in the study area per breeding season). An overall breeding frequency of 83%, higher than that reported by most studies in the Iberia peninsula (Barrientos and Fernández-Gil 2010), contributed to the maintenance of the high wolf abundance until the end of the monitored period, although a slight decline in population size due to a decrease in mean pack sizes was observed during the period of 2013 - 2016.

The relative importance of individual packs to the demographic vigor of this subpopulation was biased towards the role of Vez and Soajo packs, due to their stability and high reproductive success. These two packs were detected in all 10 monitored breeding seasons and each pack only failed to reproduce once, being responsible for the production of 66% ($n = 77$) of all pups detected in Alto Minho during the study period. This pattern is in agreement with the source-sink dynamic hypothesis that has been suggested by Álvares (2011) for the region and recently reinforced by Nakamura et al. (2016) where both Vez and Soajo are considered to be fundamental to the stability of Alto Minho's subpopulation and the recovery of peripheral packs through surplus of individuals. This demographic heterogeneity across neighboring packs suggest that habitat suitability, as well as limiting factors such as mortality and productivity, are not homogeneous across the study area, which was expected given the higher human pressure towards the coastline (west) and the river valleys that limit the study area to the north and south. On the other hand, the low stability of Peneda pack, detected only in 4 of the 10 monitored breeding seasons, contrasts with this general trend and seems quite unexpected given its location inside the National Park, where habitat suitability is higher, and its proximity to both Vez and Soajo packs, which are a likely source of dispersing wolves.

The demographic trend of Alto Minho's wolf subpopulation between 2007 and 2016 had a better fit to an exponential growth than to the density dependent realization of the Ricker model. This result was contrary to my expectation as I had initially hypothesized that the subpopulation was at carrying capacity, mainly due to the fact that never more than 6 packs were detected in this region, and the apparent negative relation between growth and population size (see **Figure S3**). Nevertheless, the documented trend seems to suggest that the subpopulation grew exponentially during the studied period, without hitting carrying capacity. The observed average exponential growth rate was positive ($\lambda = 1.109$), which is congruent with the overall pattern of geographical expansion, although the confidence interval associated to my estimate includes values that are indicative of population decline. This suggests that, although the subpopulation displayed a strong demographic growth between 2007 and 2016, it is still plausible that demographic decline will be observed in the following years. In fact, the average growth rate was negative during the last 4 years of the study period ($\lambda = 0.93$).

Alternatively, the population could have reached carrying capacity during the studied period, but I was unable to detect it. Density dependency is as much as a complex process as the wolf is a complex species, which leaves space to several reasons as to why density dependence could have gone unnoticed. First, with only 10 years of available data to work with, I was limited to construct a very simple count-based model with low statistical power, hampering a proper assessment of the growth of this subpopulation; Second, with the data available the Ricker model did not adequately characterize the population dynamics of wolves in Alto Minho, with one limitation being the model fitting growth under a constant carrying capacity (Clark et al. 2010). This assumption will fail if there is any kind of temporal variation on the factor that limits population growth. For example, although wolves in Alto Minho feed mostly on livestock (Álvares 2011), that is a relatively stable food source, an potential increase in food abundance between 2010 and 2013 could explain the increase in the average number of wolves per pack observed during that period (Mech and Boitani 2003), which would be indicative of a dynamic carrying capacity and therefore incompatible with model's idealization; Third, the monitored period was not long enough to fully capture the density dependent behavior. These alternatives arise from methodologic constrains that could be solved by the integration of data that allows for structured models as well as by a longer monitoring period.

An alternative hypothesis for not being able to detect density dependence is the potential effect of a cultural carrying capacity, related to the level of human tolerance to wolf presence. Both national and regional approaches, explored in chapter 1 and 2, show that despite the wolf being a fully protected species in Portugal, it is still affected by high levels of anthropogenic mortality. High wolf abundance in Alto Minho could motivate and facilitate persecution, leading this subpopulation to experience higher human-caused mortality rates as it reaches larger population sizes. Besides, persecution might not be equally distributed across the study area and can have particular incidence on certain pack territories, in particular those that during the studied period displayed lower stability or demographic vigor (e.g. Boulhosa, Peneda and Cruz Vermelha packs). This artificial regulation of density should have a distinct signature than the typical ecological carrying capacity and therefore not be so easily detected by the methods employed here.

Direct persecution was the main cause of mortality for collared wolves in Alto Minho, leading to a low estimate of average annual survival (0.541 ± 0.119). However, it's important to note its wide confidence interval, 95% CI [0.352,0.831] and the lack of significant difference relative to the national survival estimate provided in chapter 1. Consequently, there is no reason to interpret the survival estimate of Alto Minho alone. The incidence of poaching among collared wolf mortalities (56%) reflects the high level of conflicts in the

region, likely driven by livestock depredation as wolves feed almost exclusively on domestic ungulates (Álvares 2011; Pimenta et al., 2017). In fact, wolves in Peneda-Gerês population nucleus, an area including Alto Minho that comprises 25% of all packs detected in Portugal (Pimenta et al. 2005), were responsible for 50% of all reported attacks to livestock for compensation by the ICNF (Álvares et al. 2015).

I was unable to attain relevant information from the opportunistic collection of dead animals as the incidence of causes of mortality in the opportunistic data should be interpreted with caution given the bias towards causes that increase the chance of detection by man, such as road kills (Ciucci et al. 2007). For example, inference on the impact of poaching from the opportunistic collection of death wolves would suggest only an incidence of 21%, which is far below from that observed among the unbiased sample of collared wolves (56%). Additionally, the relatively small sample size of the opportunistic data set ($n = 14$ records collected across 13 years, excluding 6 collared animals) prevent me from detecting clear patterns from the temporal distribution of mortalities.

Possible effects on dispersal and breeder loss

The obtained low survival in Portuguese wolves in combination with a high abundance of livestock, can be one possible explanation for the reduced dispersal between subpopulations of Iberian wolves (e.g. Blanco and Cortés 2007; Silva et al. 2018). The wolf is a highly territorial and social species (Mech and Boitani 2003). Packs in saturated populations usually occur in a dense matrix of exclusive territories, while lone individuals lurk between resident packs (Packard & Mech 1980), and vacant territories are promptly occupied (Mech and Boitani 2003). Consequently, a high turnover of vacant territories caused by mortality of resident wolves might reduce the need for long-distance dispersal. Furthermore, packs may deviate from their naturally occurring kin-based structure (Mech and Boitani 2003) under high levels of harvest by increased adoption of unrelated pack members (Rutledge et al. 2010). This hypothesis is compatible with the dispersal patterns reported for the region of Alto Minho over this last decade. The 6 packs that form this subpopulation seem to be characterized by a source-sink dynamics in which few larger packs sustain the smaller and unstable peripheral packs through dispersal (Nakamura et al. 2016a). Therefore, with a local surplus of food in the form of free ranging livestock, the likelihood of Portuguese wolves dispersing longer distances and outside each subpopulation, might be reduced by their ability to successfully disperse and join packs within each subpopulation. This could explain why none of the collared wolves in Portugal

dispersed outside of the subpopulation where they were initially captured, and the high levels of genetic structure detected in the Iberian wolf population (Silva et al. 2018).

In Portugal, I found what seems to be some degree of temporal variation in human-caused mortality, as a large number of collared wolves were killed during breeding season, just before or shortly after the beginning of gestation. Packs are usually composed of two breeding wolves and their offspring, although in some cases this kin-based structure might be diluted by the presence of adoptees (Mech and Boitani 2003, Rutledge et al. 2010). The breeding pairs hold a key position in pack stability and they usually remain together unless one (or both) of the breeders dies (Milleret et al. 2017) which often leads to abandonment of a territory and reproductive failure in the subsequent breeding season (Brainerd et al. 2008, Borg et al. 2015). This effect is particularly severe when the loss occurs during breeding season, leaving little or no time for the remaining breeder to find a new mate. Borg et al. (2015) noted that packs were less likely to be maintained when breeders were killed by humans because most anthropogenic mortality was concentrated immediately before and during breeding season, as detected in the Portuguese wolf population. The data I have analyzed here does not allow for direct inference on the survival of breeders. Nevertheless, my results are suggestive of high incidence of mortality during critical stages of the reproductive cycle; a pattern that, if common to breeders, might have severe implications to pack stability and reproduction of wolves in Portugal.

Conservation Implications

The lack of reliable estimates of population trend and abundance is a constrain to the conservation of Iberian wolves in Portugal. In fact, it's surprising that a fully protected species that causes every year nearly €1 million of paid compensations for livestock damages (Álvares et al. 2015) has not been targeted by a national census in the last 15 years. This knowledge gap hampers any attempt to infer on the species current status, particularly across the two very different ecological contexts that exist on either bank of the Douro river (Álvares et al. 2015). The estimate of annual survival that I have provided here is not incompatible with a stable wolf population (Fuller et al. 2003; e.g. Webb et al. 2011). Wolf populations are known to be able to withstand high rates of mortality because of their high reproductive output (Fuller et al. 2003). In fact, among large carnivores, wolves have a particularly short generation time, as young animals mature relatively fast (Packard and Mech 1980), with records of animals with two years old reproducing in the wild, and females are able to breed every year, averaging 5 or 6 pups per litter (Fuller et al. 2003). Thus, it is possible that a high reproductive output of wolves in Portugal, particularly north of Douro

river, is compensating for the relatively high rate of estimated annual mortality. However, this pattern doesn't seem as likely for wolves South of the Douro river where a small number of breeding pairs display low reproductive rates (Álvares et al. 2015). It could be that survival of wolves south of the Douro River is higher, an hypothesis I was unable to test given the present sample sizes.

The interaction between the regional levels of tolerance towards wolves (Blanco and Cortés 2002, Gangaas et al. 2013) and the different environmental contexts where the species occur across its range likely results in small-scale spatial variation in survival and causes of mortality. Therefore, an adequate understanding of region-specific factors conditioning survival is very important to achieve effective management actions. For example, the main cause of accidental mortality in Alto Minho – snares - resulted from conflicts between farmers and wild boars, while most accidental mortalities in Montalegre were due to collisions with vehicles. Both mortality causes, although similar in nature (accidental), arise from distinct contexts and should have different solutions. Furthermore, the high levels of direct human-caused mortality related to poaching detected in Alto Minho possibly reflects the particular incidence of conflicts due to wolf predation on free-ranging cattle and horses in the region (Álvares et al. 2015, Pimenta et al. 2017), which is likely not felt so intensively on the remaining subpopulations.

Results from our regional approach (chapter 2) provided evidence that the estimated annual survival is not indicative of population decline. The Alto Minho wolf subpopulation displayed a positive growth between 2007 and 2016, growing from 13 wolves detected in 2 packs in 2007 to 33 wolves in 5 packs in 2016 ($\lambda = 1.09$). This increase in abundance happened despite strong evidence of anthropogenic pressure in the region, suggesting that the reproductive output of the subpopulation is compensating for low survival. One possible threat to high fecundity in wolf populations is the ill-timed death of breeders. I found only light evidences that the seasonal patterns of mortality might be particularly incident on crucial stages of the species breeding cycle. Nevertheless, given the implications of breeder loss on pack stability and reproductive success, I believe this hypothesis should be properly addressed in the near Future. Two packs contributed disproportionately to this population recover and were probably one of the main contributors to the recolonization of packs that had not been detected in the region for over 6 years (Nakamura et al. 2016a). Nevertheless, local random extinction is still a possibility. The complete disappearance of wolves from Alto Minho would be particularly problematic, conservation wise, given its semi-isolated state relative to the remainder of the population (Silva et al. 2018). In the future, understanding why wolves in the Iberia Peninsula do not disperse over long distances should be critical for the conservation of the species. Connectivity between subpopulations shall prove to be

a fundamental mechanism to allow recolonization of regions from where the wolf is locally extirpated.

The positive trend in wolf abundance observed in Alto Minho should not be interpreted as an increase of wolf abundance in Portugal. As discussed throughout this study, the several regions where the species is present offer different environmental and sociological conditions, which in turn have distinct implications for the population dynamics of local wolves.

In sum, there remains much to be learned regarding the status of wolves in Portugal. The survivorship analysis I have presented here results from a compilation of data that spans over 35 years. Although I believe the results are extremely valuable as a first attempt to understand wolf mortality in Portugal, it is critical to encourage new telemetry-based studies of wolves across the country, such as the one responsible for the large number of wolves collared in Alto Minho over the last decade. In this context, it would be advisable to promote further studies based on telemetry and review the annual limit imposed by ICNF on the number of wolves captured per project as it might constitute a considerable obstacle to meaningful sample sizes. A robust understanding of factors influencing mortality risk of wolves in Portugal, and ultimately their population dynamics and viability, will only be possible with studies focusing the multiple regions where the wolf is present in Portugal and with enough sample size to provide greater statistical power and inference.

References

- Adams, L. G., R. O. Stephenson, B. W. Dale, R. T. Ahgook, and D. J. Demma. 2008. Population dynamics and harvest characteristics of wolves in the Central Brooks Range, Alaska. *Wildlife Monographs* 170:1–25.
- Álvares, F., I. Barroso, C. Espírito-Santo, G. Ferrão da Costa, C. Fonseca, R. Godinho, M. Nakamura, F. Petrucci-Fonseca, V. Pimenta, S. Ribeiro, H. Rio-Maior, N. Santos, and R. Torres. 2015. Action plan for wolf conservation in Portugal - baseline knowledge.
- Álvares, F., J. Domingues, P. Sierra, and P. Primavera. 2011. Cultural dimension of wolves in the Iberian Peninsula: implications of ethnozoology in conservation biology. *Innovation -The European Journal of Social Science Research* 24:213+231.
- Álvares, F. J. 2011. Ecologia e conservação do lobo (*Canis lupus*, L.) no Noroeste de Portugal. PhD thesis. Universidade de Lisboa - Faculdade de Ciências.
- Andrén, H., J. D. C. Linnell, O. Liberg, R. Andersen, A. Danell, J. Karlsson, J. Odden, P. F. Moa, P. Ahlqvist, T. Kvam, R. Franzén, and P. Segerström. 2006. Survival rates and causes of mortality in Eurasian lynx (*Lynx lynx*) in multi-use landscapes. *Biological Conservation* 131:23–32.
- APREN, and INEGI. 2016. Parques Eólicos em Portugal - Wind Farms in Portugal.
- Ballard, W. B., L. A. Ayres, P. R. Krausman, D. J. Reed, and S. G. Fancy. 1997. Ecology of wolves in relation to a migratory caribou herd in northwestern Alaska. *Wildlife Monographs*:3–47.
- Ballard, W. B., J. S. Whitman, and C. L. Gardner. 1987. Ecology of an exploited wolf population in south-central Alaska. *Wildlife Monographs*:3–54.
- van Ballenberghe, V., and L. D. Mech. 1975. Weights, Growth, and Survival of Timber Wolf Pups in Minnesota. *Journal of Mammalogy* 56:44–63.
- Balme, G. A., H. S. Robinson, R. T. Pitman, and L. T. B. Hunter. 2017. Flexibility in the duration of parental care: Female leopards prioritise cub survival over reproductive output. *Journal of Animal Ecology*:1–11.
- Barrientos, L. M., and A. Fernández-Gil. 2010. Cómo Estimar Parámetros Reproductores en las Poblaciones Ibéricas de Lobos: Tamaño de Camada y Éxito Reproductor. Pages 55–68 in A. Fernández-Gil, F. Álvares, C. Vilá, and A. Ordiz, editors. *Los Lobos de la Península Ibérica: Propuestas para el diagnóstico de sus poblaciones*.
- Benson, J. F., S. J. Jorgensen, J. B. O'Sullivan, C. Winkler, C. F. White, E. Garcia-Rodriguez, O. Sosa-Nishizaki, and C. G. Lowe. 2018. Juvenile survival, competing risks, and spatial variation in mortality risk of a marine apex predator. *Journal of Applied Ecology*:1–10.
- Benson, J. F., P. J. Mahoney, J. A. Sikich, L. E. K. Serieys, J. P. Pollinger, H. B. Ernest, and S. P. D. Riley. 2016. Interactions between demography, genetics, and landscape connectivity increase extinction probability for a small population of large carnivores in a major metropolitan area. *Proceedings. Biological sciences* 283:51–60.
- Benson, J. F., B. R. Patterson, and P. J. Mahoney. 2014. A protected area influences genotype-specific survival and the structure of a *Canis* hybrid zone. *Ecology* 95:254–264.
- Berger, J., P. B. Stacey, L. Bellis, and M. P. Johnson. 2001. A mammalian predator-prey imbalance: Grizzly bear and wolf extinction affect avian neotropical migrants. *Ecological Applications* 11:947–960.

- Blanco, J. C., and Y. Cortés. 2002. Ecología, censos, percepción y evolución del lobo en España: Análisis de un conflicto. Málaga.
- Blanco, J. C., and Y. Cortés. 2007. Dispersal patterns, social structure and mortality of wolves living in agricultural habitats in Spain. *Journal of Zoology* 273:114–124.
- Blanco, J. C., S. Reig, and L. De La Cuesta. 1992. Distribution, status and conservation problems of the wolf *Canis lupus* in Spain. *Biological Conservation* 60:73–80.
- Blanco, J. C., and C. Yolanda. 2012. Surveying wolves without snow: a critical review of the methods used in Spain. *Hystrix, the Italian Journal of Mammalogy* 23:35–48.
- Boitani, L. 2000. Action Plan for the conservation of the wolves (*Canis lupus*) in Europe. *Council of Europe Publishing*.
- Boitani, L. 2003. Wolf Conservation and Recovery. Pages 317–340 *Wolves: Behavior, Ecology and Conservation*.
- Boitani, L., and P. Ciucci. 2009. Wolf management across Europe: Species conservation without boundaries. Page A *New Era for Wolves and People*.
- Borg, B. L., S. M. Brainerd, T. J. Meier, and L. R. Prugh. 2015. Impacts of breeder loss on social structure, reproduction and population growth in a social canid. *Journal of Animal Ecology* 84:177–187.
- Börger, L., N. Franconi, G. De Michele, A. Gantz, F. Meschi, A. Manica, S. Lovari, and T. Coulson. 2006. Effects of sampling regime on the mean and variance of home range size estimates. *Journal of Animal Ecology* 75:1393–1405.
- Brainerd, S. M., H. Andren, E. E. Bangs, E. H. Bradley, J. a Fontaine, W. Hall, Y. Iliopoulos, M. D. Jimenez, E. a Jozwiak, O. Liberg, C. M. Mack, T. J. Meier, C. C. Niemeyer, H. C. Pedersen, H. Sand, R. N. Schultz, D. W. Smith, P. Wabakken, and a P. Wydeven. 2008. The effects of breeder loss on wolves. *Journal of Wildlife Management* 72:89–98.
- Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Pratical Informarion-Theoretic Approach. Springer.
- Caniglia, R., E. Fabbri, S. Cubaynes, O. Gimenez, J. D. Lebreton, and E. Randi. 2012. An improved procedure to estimate wolf abundance using non-invasive genetic sampling and capture-recapture mixture models. *Conservation Genetics* 13:53–64.
- Chapron, G., P. Kaczensky, L. John, M. von Arx, D. Huber, H. Andrén, J. V. López-Bao, M. Adamec, F. Álvares, O. Anders, L. Balčiauskas, V. Balys, P. Bedő, F. Bego, J. Blanco, U. Breitenmoser, H. Brøseth, L. Bufka, R. Bunikyte, and L. Boitani. 2014a. Shapefiles of maps in Recovery of large carnivores in Europe's modern human-dominated landscapes (Science).
- Chapron, G., P. Kaczensky, J. D. C. Linnell, M. von Arx, D. Huber, H. Andrén, J. V. López-Bao, M. Adamec, F. Álvares, O. Anders, L. Balčiauskas, V. Balys, P. Bedő, F. Bego, J. C. Blanco, U. Breitenmoser, H. Brøseth, L. Bufka, R. Bunikyte, P. Ciucci, A. Dutsov, T. Engleder, C. Fuxjäger, C. Groff, K. Holmala, B. Hoxha, Y. Iliopoulos, O. Ionescu, J. Jeremić, K. Jerina, G. Kluth, F. Knauer, I. Kojola, I. Kos, M. Krofel, J. Kubala, S. Kunovac, J. Kusak, M. Kutal, O. Liberg, A. Majić, P. Männil, R. Manz, E. Marboutin, F. Marucco, D. Melovski, K. Mersini, Y. Mertzanis, R. W. Mysłajek, S. Nowak, J. Odden, J. Ozolins, G. Palomero, M. Paunović, J. Persson, H. Potočník, P.-Y. Quenette, G. Rauer, I. Reinhardt, R. Rigg, A. Ryser, V. Salvatori, T. Skrbinšek, A. Stojanov, J. E. Swenson, L. Szemethy, A. Trajçe, E. Tsingarska-Sedefcheva, M. Váňa, R. Veeroja, P. Wabakken, M. Wölfl, S. Wölfl, F. Zimmermann, D. Zlatanova, and L. Boitani. 2014b. Recovery of large carnivores in Europe's modern human-dominated landscapes. *Science* 346:1517–1519.

- Chapron, G., S. Legendre, R. Ferrière, J. Clobert, and R. G. Haight. 2003a. Conservation and control strategies for the wolf (*Canis lupus*) in western Europe based on demographic models. *Comptes Rendus - Biologies* 326:575–587.
- Chapron, G., P.-Y. Quenette, S. Legendre, and J. Clobert. 2003b. Which future for the French Pyrenean brown bear (*Ursus arctos*) population? An approach using stage-structured deterministic and stochastic models. *Comptes Rendus Biologies* 326:174–182.
- Ciucci, P., G. Chapron, V. Guberti, and L. Boitani. 2007. Estimation of mortality parameters from (biased) samples at death: Are we getting the basics right in wildlife field studies? A response to Lovari et al. (2007). *Journal of Zoology* 273:125–127.
- Clark, F., B. W. Brook, S. Delean, H. Reşit Akçakaya, and C. J. A. Bradshaw. 2010. The theta-logistic is unreliable for modelling most census data. *Methods in Ecology and Evolution*:253–262.
- Copernicus. 2018. CORINE Land Cover. land.copernicus.eu/pan-european/corine-land-cover/view.
- Creel, S., M. G. L. Mills, and J. W. McNutt. 2004. Demography and Population Dynamics of African Wild Dogs in Three Critical Populations. *Biology and Conservation of Wild Canids*.
- Cross, P. C., and S. R. Beissinger. 2001. Using Logistic Regression to Analyze the Sensitivity of PVA Models: a Comparison of Methods Based on African Wild Dog Models. *Conservation Biology* 15:1335–1346.
- Cuesta, L., F. Barcena, F. Palacios, and S. Reig. 1991. The trophic ecology of the iberian wolf (*canis lupus signatus cabrera*, 1907). A new analysis of stomach's data. *Mammalia* 55:239–254.
- Dennis, B., P. L. Munholland, and J. M. Scott. 1991. Estimation of Growth and Extinction Parameters for Endangered Species. *Ecological Society of America* 61:115–143.
- Duchamp, C., J. Boyer, P. E. Briaudet, Y. Leonard, P. Moris, A. Bataille, T. Dahier, G. Delacour, G. Millisher, C. Miquel, C. Poillot, and E. Marboutin. 2012. A dual frame survey to assess time- and space-related changes of the colonizing wolf population in France. *Hystrix* 23:14–28.
- Eggermann, J., G. F. da Costa, A. M. Guerra, W. H. Kirchner, and F. Petrucci-Fonseca. 2011. Presence of Iberian wolf (*Canis lupus signatus*) in relation to land cover, livestock and human influence in Portugal. *Mammalian Biology* 76:217–221.
- Fdez, E. J. G., L. Llana, V. Palacios, and V. J. López-Bao. 2014. Ecología espacial del lobo en el Parque Nacional Picos de Europa, 2013.
- Ferreras, P., J. J. Aldama, J. F. Beltrán, and M. Delibes. 1992. Rates and causes of mortality in a fragmented population of Iberian lynx (*Felis pardinus*). *Biological Conservation* 61:197–201.
- Fieberg, J., and G. D. Delgiudice. 2009. What time is it? Choice of time origin and scale in extended proportional hazards models. *Ecology* 90:1687–1697.
- Fuller, T. K. 1991. Effect of snow depth on wolf activity and prey selection in north central Minnesota. *Canadian Journal of Zoology* 69:283–287.
- Fuller, T. K., S. L. Berendzen, T. A. Decker, and J. E. Cardoza. 1995. Survival and cause-specific mortality rates of adult bobcats (*Lynx rufus*). *American Midland Naturalist* 134:404–408.
- Fuller, T. K., W. E. Berg, and D. W. Kuehn. 1985. Survival Rates and Mortality Factors of

- Adult Bobcats in North-Central Minnesota. *Journal of Wildlife Management* 49:292–296.
- Fuller, T. K., L. D. Mech, and J. F. Cochrane. 2003. Wolf Population Dynamics. Pages 161–191 *Wolves: Behavior, Ecology and Conservation*.
- Gangaas, K. E., B. P. Kaltenborn, and H. P. Andreassen. 2013. Geo-Spatial Aspects of Acceptance of Illegal Hunting of Large Carnivores in Scandinavia. *PLoS ONE* 8:1–9.
- Gaona, P., P. Ferreras, and M. Delibes. 1998. Dynamics and Viability of a Metapopulation of the Endangered Iberian Lynx (*Lynx pardinus*). *Ecological Society of America* 68:349–370.
- Gogan, P., W. Route, E. Olexa, N. Thomas, D. Kuehn, and K. Podruzny. 2004. Gray Wolves in and Adjacent to Voyageurs National Park, Minnesota Research and Synthesis 1987 – 1991. *Synthesis*:68.
- Goodrich, J. M., L. L. Kerley, E. N. Smirnov, D. G. Miquelle, L. McDonald, H. B. Quigley, M. G. Hornocker, and T. McDonald. 2008. Survival rates and causes of mortality of Amur tigers on and near the Sikhote-Alin Biosphere Zapovednik. *Journal of Zoology* 276:323–329.
- Grilo, C. 2002. Monitorização do lobo a sul do rio douro.
- Grilo, C., G. Moço, A. T. Cândido, A. S. Alexandre, and F. Petrucci-Fonseca. 2002. Challenges for the recovery of the Iberian wolf in the Douro river south region 20.
- van Haaften, J. L. 1982. Wolf Project - Bragança - Zamora.
- Harrell, F. E., K. L. Lee, and D. B. Mark. 1996. Tutorial in Biostatistics: Multivariable Prognostic Models: Issues in Developing Models, Evaluating Assumptions and Adequacy, and Measuring and Reducing Errors. *Statistics in Medicine* 15:361–387.
- Hedrick, P. W., R. O. Peterson, L. M. Vucetich, J. R. Adams, and J. A. Vucetich. 2014. Genetic rescue in Isle Royale wolves: genetic analysis and the collapse of the population. *Conservation Genetics* 15:1111–1121.
- Heppell, S. S., C. Hal, and L. B. Crowder. 2000. Life Histories and Elasticity Patterns: Perturbation Analysis for Species with Minimal Demographic Data. *Ecology* 81:654–665.
- Hindrikson, M., J. Remm, M. Pilot, R. Godinho, A. V. Stronen, L. Baltrūnaitė, S. D. Czarnomska, J. A. Leonard, E. Randi, C. Nowak, M. Åkesson, J. V. López-Bao, F. Álvares, L. Llaneza, J. Echegaray, C. Vilà, J. Ozolins, D. Rungis, J. Aspi, L. Paule, T. Skrbinšek, and U. Saarma. 2017. Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management. *Biological Reviews* 92:1601–1629.
- Hosmer, D. W., S. Lemeshow, and R. X. Sturdivant. 2013. Applied Logistic Regression. Wiley.
- Hurford, A., M. Hebblewhite, and M. A. Lewis. 2006. A spatially explicit model for an Allee effect: Why wolves recolonize so slowly in Greater Yellowstone. *Theoretical Population Biology* 70:244–254.
- ICNF. 2018. Áreas Protegidas, Rede Natura e Sítios Ramsar - Portugal continental. www2.icnf.pt/portal/pn/biodiversidade/cart.
- INE. 2011. Censos 2011 Resultados Definitivos - Região Norte. Lisbon.
- IPMA. 2018. Clima de Portugal Continental. <https://www.ipma.pt/pt/educativa/tempo.clima/index.jsp?page=clima.pt.xml>.

- Kaczensky, P., G. Chapron, M. Von Arx, D. Huber, H. Andrén, and J. Linnell. 2012. Status, management and distribution of large carnivores-bear, lynx, wolf & wolverine in Europe. Page European Commission.
- Kaplan, E. L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. *American statistical Association* 53:457–481.
- Knick, S. T. ., and W. Kasworm. 1989. Shooting Mortality in Small Populations of Grizzly Bears. *Wiley* 17:11–15.
- Knight, R. R., and L. L. Eberhardt. 1985. Population Dynamics of Yellowstone Grizzly Bears. *Ecological Society of America* 66:323–334.
- Kreeger, T. J. 2003. The Internal Wolf: Physiology, Pathology and Pharmacology. Pages 192–217 *Wolves: Behavior, Ecology and Conservation*.
- Lambert, C. M. S., R. B. Wielgus, H. S. Robinson, D. D. Katnik, H. S. Cruickshank, R. Clarke, and J. Almack. 2006. Cougar Population Dynamics and Viability in the Pacific Northwest. *The Journal of Wildlife Management* 70:246–254.
- Lande, R., S. Engen, and B.-E. Saether. 2003. Population viability analysis. Pages 1–29 *Stochastic Population Dynamics in Ecology and Conservation*.
- Liberg, O., G. Chapron, P. Wabakken, H. C. Pedersen, N. T. Hobbs, H. Sand, N. Thompson Hobbs, and H. Sand. 2011. Shoot, shovel and shut up: cryptic poaching slows restoration of a large carnivore in Europe. *Proceedings of the Royal Society B: Biological Sciences* 279:910–915.
- Liberg, O., H. Sand, P. Wabakken, and H. C. Pedersen. 2007. Dödlighet och illegal jakt i den skandinaviska vargstammen. *Fakta Skog* 9:4 [In Swedish].
- Lindzey, F. G., B. B. Ackerman, D. Barnhurst, and T. P. Hemker. 1988. Survival Rates of Mountain Lions in Southern Utah. *The Journal of Wildlife Management* 52:664–667.
- Linnell, J. D. C., and L. Boitani. 2012. Building biological realism into wolf management policy: the development of the population approach in Europe. *Hystrix, the Italian Journal of Mammalogy* 23:80–91.
- Linnell, J. D. C., J. E. Swenson, and R. Andersen. 2001. Predators and people : conservation of large carnivores is possible at high human densities if management policy is favourable. *Animal Conservation* 4:345–349.
- Llaneza, L., F. Álvarez, A. Ordiz, P. Sierra, and A. Uzal. 2004. Distribución y aspectos poblacionales del lobo ibérico (*Canis lupus signatus*) en la provincia de Lugo. *Ecología* 18:227–238 [In Spanish].
- Llaneza, L., and J. Blanco. 2005. Situación del lobo (*Canis lupus* L.) en Castilla y León en 2001. Evolución de sus poblaciones. *Galemys* 17:15–28 [In Spanish].
- Llaneza, L., and E. J. G. Fdez. 2016. Ecología espacial del lobo en el Parque Nacional Picos de Europa, 2014-16.
- Llaneza, L., E. J. García, and J. V. López-Bao. 2014. Intensity of territorial marking predicts wolf reproduction: Implications for wolf monitoring. *PLoS ONE* 9:e93015.
- Llaneza, L., J. V. López-Bao, and V. Sazatornil. 2012. Insights into wolf presence in human-dominated landscapes: The relative role of food availability, humans and landscape attributes. *Diversity and Distributions* 18:459–469.
- Llaneza, L., A. Ordiz, V. Palacios, and A. Uzal. 2005. Monitoring Wolf Populations Using Howling Points Combined With Sign Survey Transects. *Wildlife Biology in Practice* 1:108–117.

- Loveridge, A. J., M. Valeix, G. Chapron, Z. Davidson, G. Mtare, and D. W. Macdonald. 2016. Conservation of large predator populations: Demographic and spatial responses of African lions to the intensity of trophy hunting. *Biological Conservation* 204:247–254.
- Marucco, F., E. Avanzinelli, and L. Boitani. 2012. Non-invasive Integrated Sampling Design to Monitor the Wolf Population in Piemonte, Italian Alps. *Hystrix, the Italian Journal of Mammalogy* 23:5–13.
- Marucco, F., D. H. Pletscher, L. Boitani, M. K. Schwartz, K. L. Pilgrim, and J. D. Lebreton. 2009. Wolf survival and population trend using non-invasive capture-recapture techniques in the Western Alps. *Journal of Applied Ecology* 46:1003–1010.
- Mazerolle, M. J. 2017. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c).
- McLellan, B. N., F. W. Hovey, R. D. Mace, J. G. Woods, D. w. Carney, M. L. Gibeau, W. L. Wakkenin, and W. F. Kasworn. 1999. Rates and Causes of Grizzly Bear Mortality in the Interior Mountains of British Columbia, Alberta, Montana, Washington, and Idaho. *The Journal of Wildlife Management* 63:911–920.
- Mech, L. D. 1977. Productivity, Mortality, and Population Trends of Wolves in Northeastern Minnesota. *Journal of Mammalogy* 58:559–574.
- Mech, L. D. 1995. The Challenge and Opportunity of Wolf Populations Recovering. *Conservation Biology* 9:270–278.
- Mech, L. D., and L. Boitani. 2003. Wolf Social Ecology. Pages 1–34 *Wolves: Behavior, Ecology and Conservation*.
- Milleret, C., P. Wabakken, O. Liberg, M. Akesson, O. Flagstad, H. P. Andreassen, and H. Sand. 2017. Let's stay together? Intrinsic and extrinsic factors involved in pair bond dissolution in a recolonizing wolf population. *Journal of Animal Ecology* 86:43–54.
- Mills, K. J., B. R. Patterson, and D. L. Murray. 2008. Direct Estimation of Early Survival and Movements in Eastern Wolf Pups. *Journal of Wildlife Management* 72:949–954.
- Moreira, L. 1992. Contribuição para o estudo da ecologia do lobo (*Canis lupus signatus* Cabrera, 1907) no Parque Natural de Montesinho. Page Relatório de estágio profissionalizante para a
- Moreira, L., J. L. Rosa, J. Lourenço, I. Barroso, and V. Pimenta. 1997. Projecto Lobo - Relatório de Progressão 1996.
- Morris, W. F., and D. F. Doak. 2002. Quantitative Conservation Biology. SINAUER.
- Muñoz-Fuentes, V., C. T. Darimont, P. C. Paquet, and J. A. Leonard. 2010. The genetic legacy of extirpation and re-colonization in Vancouver Island wolves. *Conservation Genetics* 11:547–556.
- Murray, D. L. 2006. On Improving Telemetry-Based Survival Estimation. *Journal of Wildlife Management* 70:1530–1543.
- Murray, D. L., G. Bastille-Rousseau, J. R. Adams, and L. P. Waits. 2015. The Challenges of Red Wolf Conservation and the Fate of an Endangered Species Recovery Program. *Conservation Letters* 8:338–344.
- Nakamura, M., H. Rio-Maior, R. Godinho, and F. Alvares. 2016a. The role of core packs regulating peripheral packs: an approach based on long-term monitoring of a wolf population in NW Portugal. Page 58 IV Congresso Ibérico do Lobo.
- Nakamura, M., H. Rio-Maior, R. Godinho, and F. Álvares. 2012. Investigação aplicada à conservação do lobo no noroeste de Portugal.

- Nakamura, M., H. Rio-Maior, R. Godinho, and F. Álvares. 2013. Investigação aplicada à conservação do lobo no noroeste de Portugal.
- Nakamura, M., H. Rio-Maior, R. Godinho, and F. Álvares. 2014. Investigação aplicada à conservação do lobo no noroeste de Portugal.
- Nakamura, M., H. Rio-Maior, R. Godinho, and F. Álvares. 2015. Investigação aplicada à conservação do Lobo no Noroeste de POrtugal: Fase II - Ano I.
- Nakamura, M., H. Rio-Maior, R. Godinho, and F. Álvares. 2016b. Investigação Aplicada à Conservação do Lobo no Noroeste de Portugal: Fase III - Ano II.
- Nakamura, M., H. Rio-Maior, R. Godinho, and F. Álvares. 2017. 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.
- Nilsson, T. 2013. Population viability analyses of the Scandinavian populations of bear (*Ursus arctos*), lynx (*Lynx lynx*) and wolverine (*Gulo gulo*).
- NOAA. 2018. Version 4 DMSP-OLS Nighttime Lights Time Series. ngdc.noaa.gov/eog/dmsp/downloadV4composites.html.
- OpenStreetMap contributors. 2018. OpenStreetMap Data Extracts. download.geofabrik.de.
- Packard, J. M. 2003. Wolf Behavior: Reproductive, Social and Intelligent. Pages 35–65 *Wolves: Behavior, Ecology and Conservation*.
- Packard, J. M., and L. D. Mech. 1980. Population regulation in wolves. Pages 135–150 *in* M. N. Cohen, R. S. Malpass, and H. G. Klein, editors. *Biosocial mechanisms of population regulation*. Yale University Press, New Haven.
- Pereira, H. M., and L. M. Navarro. 2015. *Rewilding European Landscapes*. Springer.
- Pereira, M., F. Fonseca, and C. Magalhães. 1985. Wolf ecology in Portugal. Page Symposium *Predateurs*, Lisbonne 29/31.3.1985.
- Person, D., and A. Russell. 2008. Correlates of mortality in an exploited wolf population. *Journal of Wildlife Management* 72:1540–1549.
- Persson, J., G. Ericsson, and P. Segerström. 2009. Human caused mortality in the endangered Scandinavian wolverine population. *Biological Conservation* 142:325–331.
- Petrucchi-Fonseca, F. 1990. O Lobo (*CANIS LUPUS SIGNATUS* Cabrera, 1907) em Portugal. *Problemática da sua Conservação*.
- Pianka, E. R. 1970. On r- and K-Selection. *The American Naturalist* 104:592–597.
- Pimenta, V. 1998. Estudo comparativo de duas alcateias no nordeste do distrito de Bragança. Utilização do espaço e do tempo e hábitos alimentares. Universidade de Lisboa - Faculdade de Ciências.
- Pimenta, V., I. Barroso, F. Álvares, J. Correia, F. da C. Costa, L. Moreira, J. Nascimento, F. Petrucci-Fonseca, S. Roque, and E. Santos. 2005. Censo Nacional de Lobo 2002/2003. Lisboa.
- Pimenta, V., I. Barroso, L. Boitani, and P. Beja. 2017. Wolf predation on cattle in Portugal: Assessing the effects of husbandry systems. *Biological Conservation* 207:17–26.
- Pollock, K. H., S. Winterstein, C. Bunck, and P. Curtis. 1989. Survival Analysis in telemetry studies: The staggered entry design. *Journal of Wildlife Management* 53:7–15.
- Pullin, A. S., and T. M. Knight. 2001. Effectiveness in Conservation Practice: Pointers from Medicine and Public Health. *Conservation Biology* 15:50–54.

- QGIS Development Team. 2018. QGIS Geographic Information System. Open Source Geospatial Foundation Project.
- R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Reinhardt, I., G. Kluth, and S. Nowak. 2015. Standards for the monitoring of the Central European wolf population in Germany and Poland.
- Riley, S. P. D., R. M. Sauvajot, T. K. Fuller, E. C. York, D. a Kamradt, C. Bromley, and R. Wayne. 2003. Effects of Urbanisation and Habitat Fragmentation on Bobcats and Coyotes in Southern California. *Conservation Biology* 17:566–576.
- Rio-Maior, H., P. Beja, M. Nakamura, and F. Álvares. 2018. Use of space and homesite attendance by Iberian wolves during the breeding season. *Mammalian Biology* 92:1–10.
- Rio-Maior, H., P. Beja, M. Nakamura, N. Santos, R. Brandão, R. Sargo, I. Dias, F. Silva, and F. Álvares. 2016. Rehabilitation and post-release monitoring of two wolves with severe injuries. *Journal of Wildlife Management* 80:729–735.
- Rio-Maior, H., M. Nakamura, and F. Alvares. 2010. Programa de Monitorização do Lobo no Parque Eólico do Alto Minho I – Relatório Final.
- Ripple, W. J., J. A. Estes, R. L. Beschta, C. C. Wilmers, E. G. Ritchie, M. Hebblewhite, J. Berger, B. Elmhagen, M. Letnic, M. P. Nelson, O. J. Schmitz, D. W. Smith, A. D. Wallach, and A. J. Wirsing. 2014. Status and Ecological Effects of the World ' s Largest Carnivores 343.
- Robinson, H. S., J. M. Goodrich, D. G. Miquelle, C. S. Miller, and I. V. Seryodkin. 2015. Mortality of Amur tigers: The more things change, the more they stay the same. *Integrative Zoology* 10:344–353.
- Roque, S., R. Godinho, D. Cadete, S. Pinto, A. S. Pedro, J. Bernardo, F. Petrucci-Fonseca, and F. Álvares. 2011. Plano de Monitorização do Lobo na Área dos Projectos Eólicos das Serras de Montemuro, Freita, Arada e Lomil.
- Rutledge, L. Y., B. R. Patterson, K. J. Mills, K. M. Loveless, D. L. Murray, and B. N. White. 2010. Protection from harvesting restores the natural social structure of eastern wolf packs. *Biological Conservation* 143:332–339.
- Silva, P., J. V. López-Bao, L. Llaneza, F. Álvares, S. Lopes, J. C. Blanco, Y. Cortés, E. Garcia, P. Palacios, H. Rio-Maior, N. Ferrand, and R. Godinho. 2018. Cryptic population structure reveals low dispersal in Iberian wolves. *Scientific Reports*:1–14.
- Smith, D. W., E. E. Bangs, J. K. Oakleaf, C. Mack, J. Fontaine, D. Boyd, M. Jimenez, D. H. Pletscher, C. C. Niemeyer, T. J. Meier, D. R. Stahler, J. Holyan, V. J. Asher, and D. L. Murray. 2010. Survival of Colonizing Wolves in the Northern Rocky Mountains of the United States, 1982–2004. *Journal of Wildlife Management* 74:620–634.
- Southwood, T. R. E., R. M. May, M. P. Hassel, and G. R. Conway. 1974. Ecological strategies and population parameters. *The American Naturalist* 108:791–804.
- Suutarinen, J., and I. Kojola. 2017. Poaching regulates the legally hunted wolf population in Finland. *Biological Conservation* 215:11–18.
- Suutarinen, J., and I. Kojola. 2018. One way or another: predictors of wolf poaching in a legally harvested wolf population. *Animal Conservation*:1–9.
- Terborgh, J., L. Lopez, P. Nun, M. Rao, G. Shahabuddin, G. Orihuela, T. D. Lambert, and L. Balbas. 2001. Ecological Meltdown in Predator-Free Forest Fragments. *Science* 294:1923–1926.

- Therneau, T. M., and P. M. Grambsch. 2000. Modeling Survival Data: Extending the Cox Model.
- Therneau, T. M., and P. M. Grambsch. 2015. A Package for Survival Analysis in S.
- Torres, R. T., J. Miranda, J. Carvalho, and C. Fonseca. 2015a. Expansion and Current Status of Roe Deer (*Capreolus capreolus*) at the Edge of Its Distribution in Portugal. *Annales Zoologici Fennici* 52:339–352.
- Torres, R. T., N. Silva, G. Brotas, and C. Fonseca. 2015b. 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* 10:1–12.
- Treves, A. 2009. Hunting for large carnivore conservation. *Journal of Applied Ecology* 46:1350–1356.
- Treves, A., and J. Bruskotter. 2014. Tolerance for predatory wildlife. *Science* 344:476–477.
- Treves, A., and K. U. Karanth. 2003. Human-Carnivore Conflict and Perspectives on Carnivore Management Worldwide. *Conservation Biology* 17:1491–1499.
- Vickers, T. W., J. N. Sanchez, C. K. Johnson, S. A. Morrison, R. Botta, T. Smith, B. S. Cohen, P. R. Huber, H. B. Ernest, and W. M. Boyce. 2015. Survival and Mortality of Pumas (*Puma concolor*) in a Fragmented, Urbanizing Landscape. *PLoS ONE* 10:1–18.
- Vingada, J., C. Fonseca, J. Cancela, J. Ferreira, and C. Eura. 2010. Ungulates and their management in Portugal. Pages 392–418 *European ungulates and their management in the 21st century*.
- Vos, J. 2000. Food habits and livestock depredation of two Iberian wolf packs (*Canis lupus signatus*) in the north of Portugal. *Journal of Zoology* 251:457–462.
- Wabakken, P., H. Sand, O. Liberg, and A. Bjärvall. 2001. The recovery , distribution , and population dynamics of wolves on the Scandinavian peninsula , 1978 – 1998 725:710–725.
- Webb, N. F., J. R. Allen, and E. H. Merrill. 2011. Demography of a harvested population of wolves (*Canis lupus*) in west-central Alberta, Canada. *Canadian Journal of Zoology* 89:744–752.
- Wielgus, R. B., and F. L. Bunnell. 2000. Possible negative effects of adult male mortality on female grizzly bear reproduction. *Biological Conservation* 93:145–154.
- Wielgus, R. B., D. E. Morrison, H. S. Cooley, and B. Maletzke. 2013. Effects of male trophy hunting on female carnivore population growth and persistence. *Biological Conservation* 167:69–75.
- Woodroffe, R., H. Davies-Mostert, J. Ginsberg, J. Graf, K. Leigh, K. McCreery, G. Mills, A. Pole, G. Rasmussen, R. Robbins, M. Somers, and M. Szykman. 2007. Rates and causes of mortality in Endangered African wild dogs *Lycaon pictus*: Lessons for management and monitoring. *Oryx* 41:215–223.
- Wydeven, A. P., J. E. Wiedenhoeft, R. N. Schultz, R. P. Thiel, R. L. Jurewicz, B. E. Kohn, and T. R. Van Deelen. 2009. History, Population Growth, and Management of Wolves in Wisconsin.

Annex

A.1 | Description of the social status inferred for the compiled collared wolves

I obtained the social status of collared wolves in each subpopulation by compiling the information available in the respective reports and publications or, whenever possible, by direct contact to the authors, as described below.

For Alto Minho subpopulation (n=18), I obtained the residency status for all collared wolves directly from the authors (Francisco Álvares and Helena Rio-Maior, pers. communication), and the criteria used for the classification can be found in Rio-Maior et al. (2016, 2018).

For Montalegre subpopulation (n=4), the dispersal patterns of the collared wolves MAE1, MAE2 and MAE3, were validated through direct observations, howling surveys and interpretation of signs (tracks) that allowed to determine if the collared animals were integrated into the local pack (Álvares 2011). MAE1 was resident and a pack member when collared, dispersed and later joined another pack in an adjacent territory (Álvares 2011). MAE2 was also a resident pack member when collared, dispersed but, unlike MAE1, became resident in an area where there were no signs of a resident pack (Álvares 2011). The same was observed for MAE3 who was captured while dispersing and established residency in that same area. Although these two animals stabilized their dispersal behavior in the same area during partially overlapping periods, they were never detected together or in the presence of another wolf (Álvares 2011). I've followed the interpretation of the author and classified 3 different periods for MAE 1 and MAE 2, (Resident -> Disperser -> Resident), and 2 for MAE3 (Disperser -> Resident). For one additional individual from Montalegre (MAE4) social status was obtained directly from the authors (Francisco Álvares and Helena Rio-Maior, pers. communication), and the criteria used for the classification can be found in Rio-Maior et al. (2016).

Regarding Bragança subpopulation (n=7), the wolves BG4, BG5 and BG6 never left the territories of their pack (Pimenta 1998) and therefore I have classified them as resident for the entire tracking period. The wolf BG7 was collared while a member of BG4's and BG5's pack and the three animals were frequently detected together (Pimenta 1998). The author reports dispersal behavior for BG7 from mid-August 1997 on (Pimenta 1998), without providing a more accurate date for the change in behavior. Due to the need of a specific day for the change in social status for the Cox proportional hazard model, I assigned the transition from resident to disperser of the wolf BG7 on the 15/08/1997, as I believe it is the

date that best fits the author's notes. The wolf BG8 wasn't tracked continuously because the majority of its home range was located outside the study area covered by the project (Moreira 1992). Consequently, I was unable to obtain enough information to classify the residency status of this animal. The other two wolves collared during this same project, BG9 and BG10, are discussed as residents by the author and their home ranges were described as being "*stable*" (Moreira 1992). Accordingly, I classified them as residents.

Regarding South Douro subpopulation (n=4), the collar deployed on resident wolf SD1 failed in August 1998 (Grilo 2002). The animal was later recaptured in the territory of another pack in September of that same year (Grilo 2002). The author reports that, prior to battery malfunction, the animal was changing its behavior, spending most of its time on the periphery of one pack's territory. For 7 months following capture, the animal floated between the territories of two packs and showed no signs of affiliation to any of the two groups (Grilo 2002). This was confirmed through the locations obtained for two other collared wolves during the same time period, SD2 and SD3, each resident in one of the two packs, and with a single recorded encounter with SD1 (Grilo 2002). The author states that, although no data were obtained on SD1's position between August and September 1998 and that the animal was confirmed as a disperser between September 1998 to March 1999, the dispersal of SD1 began in August 1998. I followed the author's notes and classified SD1 as a resident from the date of its capture until August 1998 (when the signal was lost), a disperser between Sep 1998 and March 1999 and then finally as resident from March 1999, when it settled in another pack, until the end of the tracking period. Here, I also had to establish a specific date for the change in residency status: from resident to disperser (August 1998) and disperser to resident (March 1999). The author reports that the animal became a resident around mid-March, so I proceeded as above and assigned the change of status on the 15th of March. Regarding the change that occurred in August, the author did not specify the timing of the change in status within the monthly scale. Given that the animal was captured on 15 September, and in the absence of any other information, I chose to assign the start of the dispersal behavior on 15th August. The other collared wolves, SD2 and SD3, were residents and never dispersed (Grilo 2002). Wolf SD4 was resident in a pack during its entire monitoring period (Roque et al. 2011).

A.2 | Supplementary Tables

Table S1 - Compiled information for all collared wolves in Portugal, including gender, age (A for Adults, Y for yearlings and P for pups), tracking period (Capture and End date), available home range (MCP100), fate and cause of mortality. The Subpopulation of collared wolves is implicit in each ID, AM for Alto Minho, MA to Montalegre, BG to Bragança and SD to SDR.

ID	Sex	Age	Capture	End	MCP100	Fate	Cause
AM1	F	A	16/12/2007	29/02/2008	Yes	Died	Human-related
AM2	M	Y	20/08/2008	02/12/2008	Yes	Died	Poison
AM3	F	A	24/09/2008	04/03/2010	Yes	Battery failure	-
AM4	F	A	12/11/2008	09/12/2008	No	Died – capture	Capture
AM5	F	A	09/02/2010	12/02/2011	Yes	Battery failure	-
AM6	M	A	24/09/2010	07/07/2011	Yes	Battery failure	-
AM7	F	A	19/10/2010	01/11/2010	No	Collar failure	-
AM8	F	A	11/04/2012	19/03/2013	Yes	Died	Gunshot
AM9	M	Y	22/08/2012	20/04/2014	Yes	Drop-off	-
AM10	F	A	26/09/2012	17/10/2013	Yes	Died	Gunshot
AM11	F	A	22/09/2012	19/11/2012	Yes	Died	Snare
AM12	F	A	06/02/2013	18/05/2014	Yes	Died	Snare
AM13	F	Y	09/02/2013	04/06/2013	Yes	Collar failure	-
AM14	F	A	10/02/2013	18/03/2014	Yes	Drop-off	-
AM15	F	A	13/02/2013	13/12/2013	Yes	Battery failure	-
AM16	M	A	04/08/2015	16/09/2015	Yes	Collar failure	-
AM17	M	A	26/02/2016	15/03/2016	No	Died	Snare
AM18	M	A	31/08/2016	28/03/2017	Yes	Died	Human-related
MA1	M	A	27/04/1998	01/11/1999	Yes	Died	Snare
MA2	M	P	21/12/1999	17/02/2002	Yes	Died	Road-kill
MA3	M	A	17/11/2000	01/02/2003	Yes	Died	Poison
MA4	M	Y	04/07/2012	19/11/2012	Yes	Died	Road-kill
BG1	M	A	13/05/1982	15/12/1982	No	End of study	-
BG2	F	A	20/08/1982	03/12/1982	No	Died – shot	Gunshot
BG3	M	A	12/11/1982	15/12/1982	No	End of study	-
BG4	M	A	24/04/1996	18/08/1997	Yes	Loss of signal	-
BG5	F	A	24/05/1996	23/06/1997	Yes	Loss of signal	-
BG6	M	A	11/09/1996	31/10/1997	Yes	End of study	-
BG7	M	P	25/05/1997	31/10/1997	Yes	End of study	-
BG8	M	A	10/12/1990	18/03/1992	No	Left study area	-
BG9	F	A	14/04/1991	28/05/1991	Yes	Died	Poison
BG10	F	Y	11/10/1991	25/03/1992	Yes	End of study	-
SD1	M	Y	15/09/1997	31/10/1999	Yes	End of study	-
SD2	F	A	18/08/1998	31/10/1999	Yes	End of study	-
SD3	M	Y	18/09/1998	31/10/1999	Yes	End of study	-
SD4	M	P	29/01/2010	04/03/2011	Yes	Battery failure	-

Table S2 - Reclassification criteria used to obtain the land cover variables and respective Corine Land Cover (CLC) grid code.

Variable	CLC Label 3	CLC grid code
Agriculture		
	Non-irrigated arable land	12
	Permanently irrigated arable land	13
	Vineyards	15
	Fruit trees and berry plantations	16
	Annual crops associated with permanent crops	19
	Complex cultivation patterns	20
	Land principally occupied by agriculture, with significant areas of natural vegetation	21
	Agro-forestry areas	22
Forest		
	Broad-leaved forest	23
	Coniferous forest	24
	Mixed forest	25
	Transitional woodland-shrub	29
Open areas		
	Pastures	18
	Natural grasslands	26
	Moors and heathland	27
	Bare rock	31
	Sparsely vegetated areas	32

Table S3 - Association criteria used for each collared wolf in Portugal between its tracking period and variable year for Corine Land cover (CLC) and Nightlight. "End year" corresponds to the year when contact with the collared animal was lost, either due to death or other causes mentioned in the methods. The CLC was only available for years 1990, 2000, 2006, and 2012. Nightlight was available for the period 1992 – 2013.

Wolf ID	Year of capture	End year	CLC	Nightlight
AM1	2007	2008	2006	2008
AM2	2008	2008	2006	2008
AM3	2008	2010	2012	2009
AM5	2008	2011	2012	2010
AM6	2010	2011	2012	2011
AM8	2010	2013	2012	2012
AM9	2012	2014	2012	2013
AM10	2012	2013	2012	2013
AM11	2012	2012	2012	2012
AM12	2013	2014	2012	2013
AM13	2013	2013	2012	2013
AM14	2013	2014	2012	2013
AM15	2013	2013	2012	2013
AM16	2015	2015	2000	2013
AM18	2016	2017	2000	2013
MA1	1998	1999	2012	1999
MA2	1999	2002	1990	2001
MA3	2000	2003	1990	2002
MA4	2012	2012	1990	2012
BG4	1996	1997	1990	1996
BG5	1996	1997	1990	1996
BG6	1996	1997	2000	1997
BG7	1997	1997	2000	1997
BG9	1991	1991	1990	1991
BG10	1991	1992	1990	1992
SD1	1997	1999	1990	1998
SD2	1998	1999	2000	1999
SD3	1999	1999	2000	1999
SD4	2010	2011	2012	2010

A.3 | Supplementary Figures

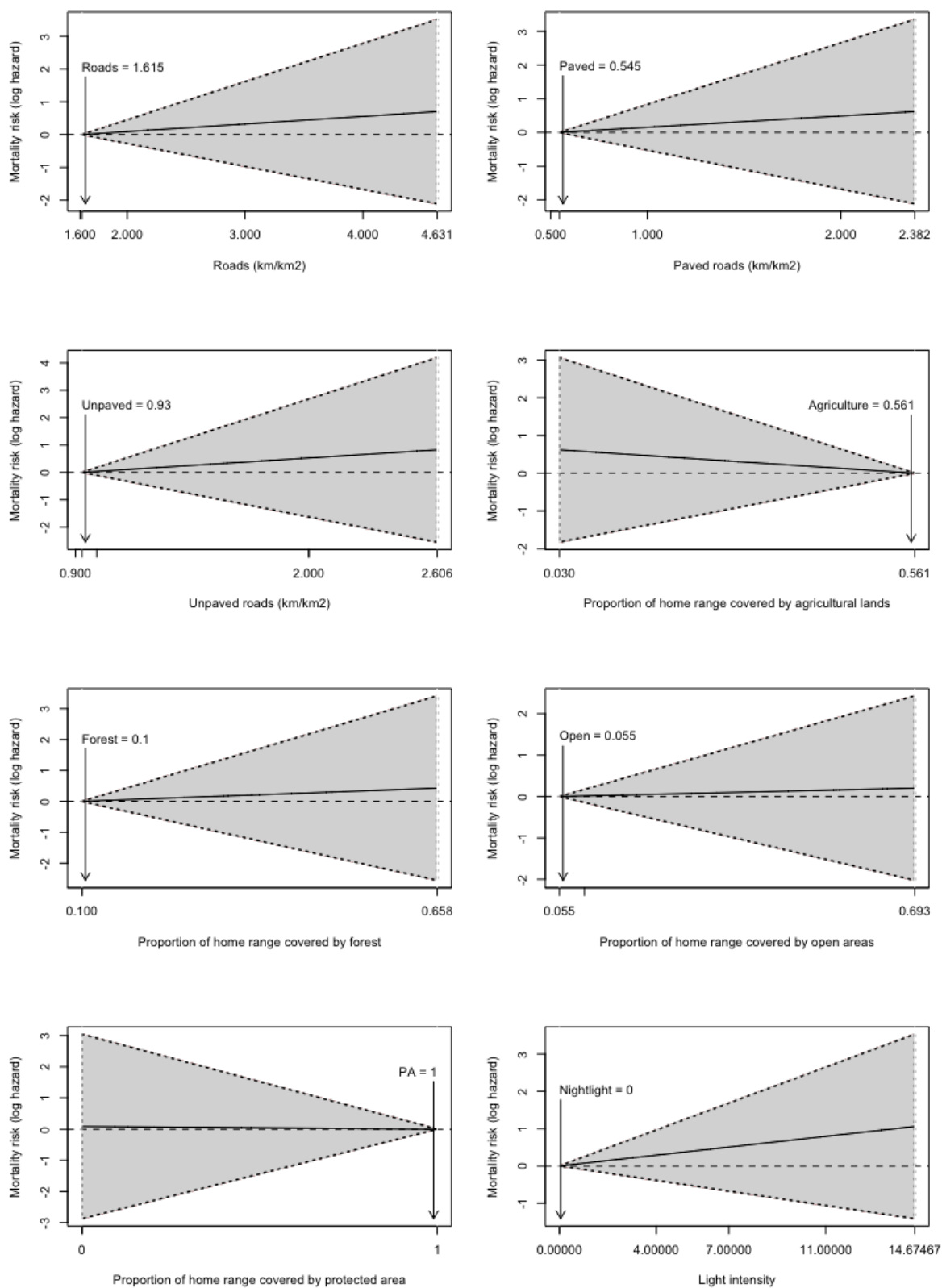


Figure S1 - Relationship between mortality risk and each of the environmental predictors for 29 collared wolves in Portugal.

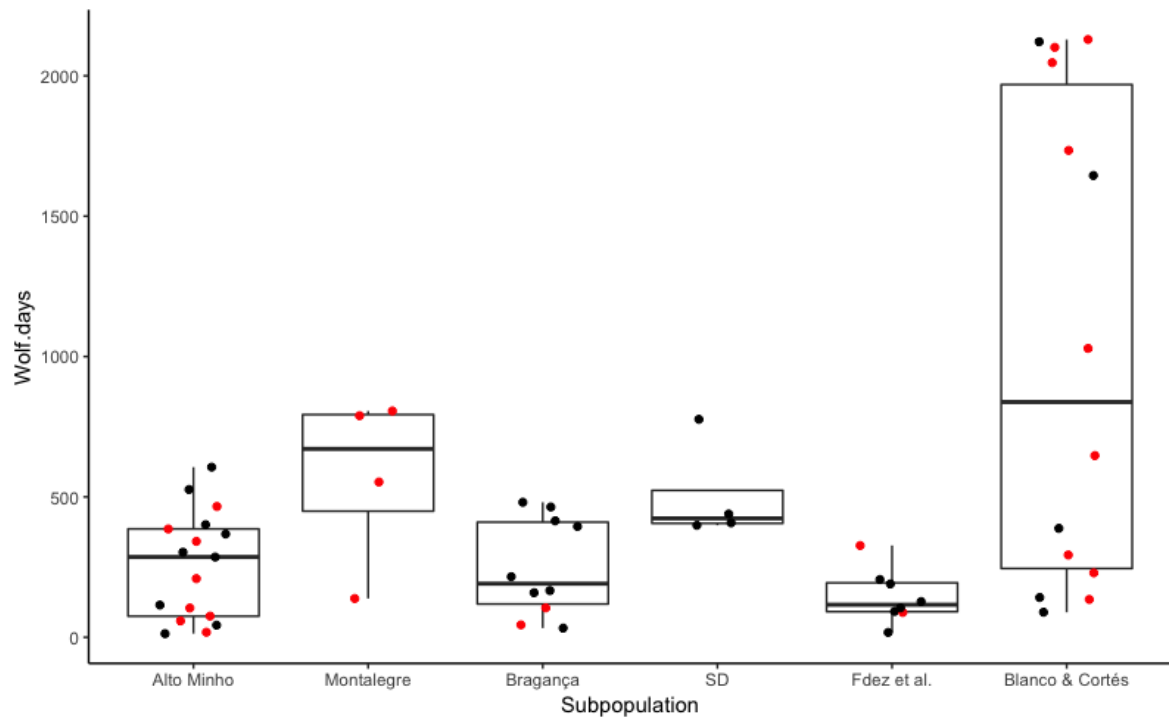


Figure S2 - Tracking periods for all radio-collared wolves in Portugal by subpopulation (this study) and for wolves radio-collared in two different areas of Spain (Picos de Europa according to Fdez et al. 2014; Llana & Fdez 2016; and central Spain according to Blanco & Cortés 2007). Red dots represent tracking period that ended with the death of the collared individual.

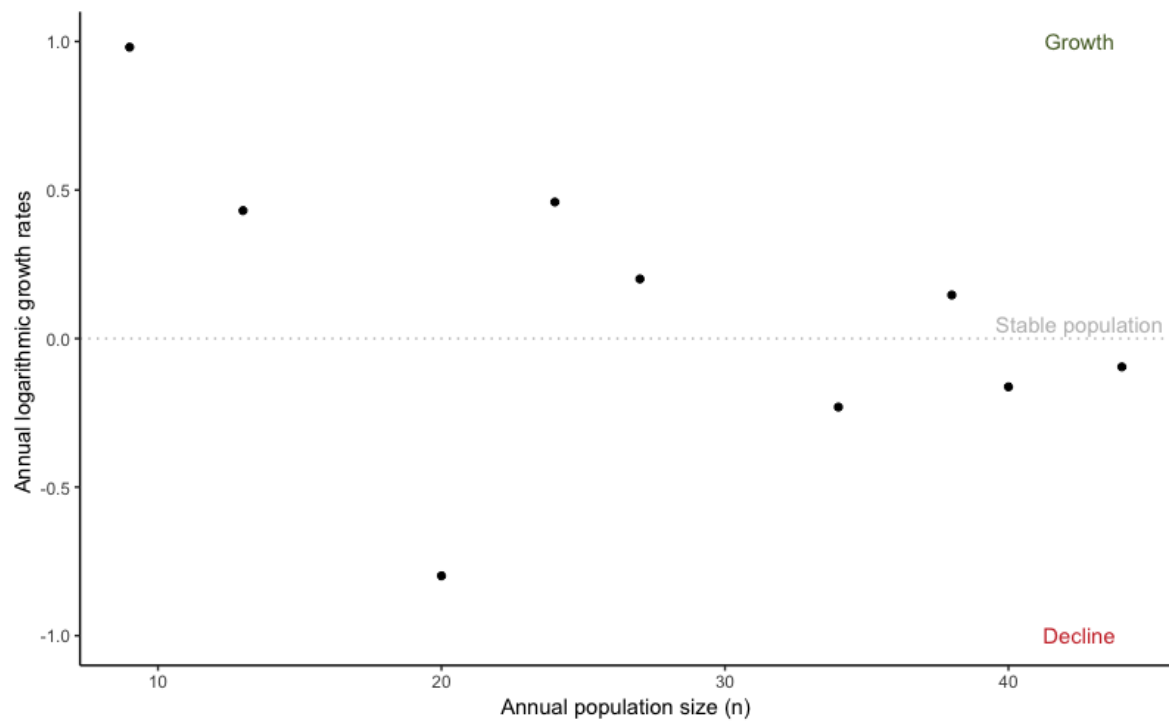


Figure S3 - Relationship between the annual logarithmic growth rates and annual population size of Alto Minho wolf subpopulation between 2007 and 2016.