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ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA DE MONTES,
FORESTAL Y DEL MEDIO NATURAL

**EFFECTS OF ENVIRONMENTAL VARIATION ON
THE REPRODUCTION OF TWO WIDESPREAD
CERVID SPECIES**

DOCTORAL DISSERTATION

MARTA PELÁEZ BEATO

Ingeniera Técnica Forestal

Máster en Investigación Forestal Avanzada

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FORESTAL AVANZADA

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Abstract

Populations living at the edge of a species' range of distribution often experience sub-optimal conditions and different selective pressures than those located at more central locations. Thus, in the context of climate change, edge populations may provide key information to understand the evolution of species under harsher climatic conditions, helping to predict future demographic changes and distribution shifts. Our main goal was to assess the effect of environmental variation on the reproduction of red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) populations living at the edge of their range (i.e. Mediterranean and Alpine ecosystems). This thesis is structured in three chapters that contain 6 research studies.

Chapter I analyzed how environmental conditions affected female reproductive timing of both species. **Study 1** revealed that red deer displayed high phenotypic plasticity in conception dates in Mediterranean environments, being population traits and the effect of climatic conditions on plant productivity the most influential factors. Thus, dry springs with low plant productivity and high densities produced delayed conception dates. **Studies 2 and 3** assessed the ability of roe deer parturition timing to track environmental changes along two different axes: space and time. While roe deer parturitions were delayed with increasing latitudes and elevations, they displayed little plasticity to rapidly adjust them to the increasingly earlier plant phenology of Alpine environments. **Chapter II** studied the variation of demographic parameters along geographical gradients. Thus, **study 4** showed an increase in roe deer litter size with latitude but a decrease with elevation. This opposite relationship, known as the fecundity gradient paradox, has never been reported for a large mammal. Finally, **Chapter III** analyzed how environmental conditions and individual factors influenced the development of male secondary sexual traits in Mediterranean environments. **Study 5** indicated

that population traits and food availability were the main factors influencing antler size in red deer, in line with Study 1. Finally, roe deer early-life investment on antlers was a reliable indicator of future adult performance (i.e. antler and body size) in Mediterranean environments (**Study 6**).

Overall, the increasingly longer and more severe drought in Mediterranean areas and the advanced spring phenology and higher temperatures in Alpine locations induced by climate change may produce a misalignment between the timing of key reproductive events (i.e. parturition or antler formation) and the peak of plant productivity. Therefore, this could negatively impact the performance of red- and roe deer living in these environments.

We highlight the importance of lowering red deer population density in Mediterranean environments to decrease the impact of severe drought events. Finally, the existence of evolutionary constraints for roe deer parturition dates along the elevational gradient advocates for adaptive management practices under the rapid climate change in Alpine environments.

Keywords: Alpine environments, antler size, birth timing, climate change, conception date, red deer, roe deer, litter size, Mediterranean environments, parturition time.

Resumen

Las poblaciones, que se encuentran al límite del área de distribución de su especie, a menudo experimentan condiciones extremas y presiones selectivas diferentes a las poblaciones ubicadas en hábitats más centrales. Por ello, estas poblaciones marginales pueden aportar una información muy valiosa en el contexto actual de cambio climático ya que han evolucionado y se han adaptado a condiciones climáticas subóptimas, pudiendo ayudar a predecir futuros cambios demográficos y de distribución de las especies. El objetivo principal de esta tesis ha sido evaluar el efecto de la variación ambiental sobre la reproducción de poblaciones de ciervo (*Cervus elaphus*) y corzo (*Capreolus capreolus*), situadas en el límite del área de distribución de ambas especies, como son los ambientes mediterráneos y alpinos. Esta tesis está estructurada en tres capítulos que incluyen seis estudios científicos.

En el **capítulo I** se analiza el efecto de diferentes variables ambientales en la fenología reproductiva de ambas especies. En ambientes mediterráneos (**estudio 1**), las hembras de ciervo ibérico mostraron una gran variabilidad interanual en su época de concepción, siendo los rasgos poblacionales y la disponibilidad de alimento los factores más importantes. De este modo, las hembras concibieron más tarde los años con primaveras secas o con altas densidades poblacionales. Los **estudios 2 y 3** evalúan la capacidad del corzo de modificar su época de partos, tanto en el espacio como en el tiempo. Si bien se observó que, a nivel poblacional, los partos de las corzas se atrasaron al incrementar la latitud o la altitud, estas mostraron poca plasticidad para adelantar rápidamente los partos y así sincronizarse con el —cada vez más temprano— comienzo de la primavera en los hábitats alpinos.

En el **capítulo II** se estudia la variación de un parámetro demográfico del corzo, como es el tamaño de la camada, a lo largo de ambos gradientes

geográficos, observándose un aumento con la latitud, pero una disminución con la elevación (**estudio 4**). Esta relación opuesta, conocida como la paradoja del gradiente de fecundidad, no se había observado anteriormente en ningún otro gran mamífero.

Finalmente, el **Capítulo III** analiza cómo las condiciones ambientales y los factores individuales afectan al desarrollo de los caracteres sexuales secundarios de los machos en ambientes mediterráneos. El **estudio 5** indica cómo los factores que más influyen en el tamaño de las cuernas son los rasgos poblacionales y la disponibilidad de alimento, acorde con los resultados del estudio 1. Finalmente, se observa que una alta inversión en cuernas en los corzos jóvenes es un buen indicador de su rendimiento futuro (i.e. cuernas y tamaño corporal) en ambientes mediterráneos (**estudio 6**).

En conclusión, el incremento en la duración e intensidad de las sequías en ambientes mediterráneos y el adelantamiento del comienzo de la primavera en ambientes alpinos como consecuencia del cambio climático pueden provocar un desfase entre eventos clave del ciclo reproductivo de ambas especies (como los partos o la formación de las cuernas) y el máximo de producción primaria. Todo esto podría afectar negativamente al rendimiento de los ciervos y corzos que viven en estos ambientes.

Es necesario destacar la importancia de reducir la densidad de las poblaciones de ciervos en ambientes mediterráneos, para así disminuir el impacto de eventos de sequía severa. Por último, la poca plasticidad mostrada por los corzos para adelantar rápidamente los partos a lo largo del gradiente altitudinal pone en evidencia la necesidad de implementar medidas de gestión adaptativa para contrarrestar los efectos negativos del cambio climático en ambientes alpinos.

Palabras clave: ambientes alpinos, ambientes mediterráneos, cambio climático, ciervo, corzo, tamaño de la cuerna, época de cría, fecha de concepción, partos, tamaño de la camada.

Table of contents

Abstract xiii

Resumen xv

1 GENERAL INTRODUCTION	5
1.1 Context of the study. Past, present and future.....	7
1.2 Reproduction in seasonal environments	11
Life-history theory and reproductive trade-offs.....	12
Phenotypic plasticity vs. evolutionary adaptation.....	14
The role of each sex on reproduction	15
1.3 Female Reproduction.....	17
The importance of breeding timing.....	17
Breeding timing and local adaptation across environmental gradients...	22
Reproductive investment strategy and phenotypic plasticity in breeding timing.....	24
Litter size variation across environmental gradients	26
Response of female reproductive traits to climate change.....	28
1.4 Male Reproduction.....	31
Secondary sexual characters	32
Male deer reproductive cycle and antler cycle	33
Trade-offs in antler formation.....	36
2 SPECIES AND STUDY SITES.....	39
2.1 The red deer and the roe deer. Comparison of our model species.....	41
2.2 Study sites and climatic conditions	45
Mediterranean environments	45
Alpine environments.....	47
2.3 Data collection	51
From inter-specific level analysis to intra-individual analysis.	51

3 OBJECTIVES AND HYPOTHESES	55
4 RESULTS.....	61
 CHAPTER I: Environmental variation and female reproductive timing in the context of climate change	 63
Study 1: Climate, female traits and population features as drivers of breeding timing in Mediterranean red deer populations..	65
Study 2: Large scale variation in birth timing and synchrony along latitudinal and altitudinal gradients of a large herbivore.....	87
Study 3: Advancing plant phenology causes an increasing trophic mismatch in an income breeder across a wide elevational range.....	121
 CHAPTER II. Effects of environmental variation on female litter size .	143
Study 4: Roe deer litter size increases with latitude but decreases with elevation. First example of the fecundity gradient paradox for a large mammal.....	145
 CHAPTER III. Environmental and individual factors affecting male secondary sexual traits	 173
Study 5: Use of cast antlers to assess antler size variation in red deer populations: effects of mast seeding, climate and population features in Mediterranean environments.	175
Study 6: Early life antlers and body size as reliable indicators of adult performance in roe-deer Mediterranean populations.....	197

5 GENERAL DISCUSSION	223
I Environmental variation and female reproductive timing in the context of climate change	225
II Effects of environmental variation on female litter size.....	229
III Environmental and individual factors affecting male secondary sexual traits	231
Implications for management and conservation	235
6 CONCLUSIONS.....	237
7 REFERENCES.....	239
8 OTHER SCIENTIFIC OUTCOMES DURING THE PRE- DOCTORAL TRAINING.....	291

1 GENERAL INTRODUCTION

1.1 Context of the study. Past, present and future

Deer (family Cervidae) have fascinated humans since the beginning of times. They are easily identifiable in the landscape thanks to their exaggerated and conspicuous male traits (i.e. antlers). Throughout history, deer have constituted an important source of food and played a central role in the development of ancient art and culture as antlers are a symbol of male strength and competition but also of fertility and growth rhythms as they are periodically renewed. More recently, deer have inspired naturalists as they display a variety of social structures, mating systems and patterns of parental care (Clutton-Brock, Guinness, & Albon, 1982). Furthermore, the growth of such conspicuous male traits challenged the foundation of natural selection, promoting the earlier grounds of sexual selection theory (Darwin, 1859).

From an ecological perspective, deer are considered ecosystem engineers (*sensu* Jones, Lawton, & Schackak, 1994) as they modulate the availability of resources for other species. They contribute to landscape heterogeneity by creating and maintaining a mosaic of woody vegetation and open patches (Rooney & Waller, 2003). They are also important seed dispersers (Malo & Suárez, 1995; Gill & Beardall, 2001; Perea et al., 2013), an essential component in the soil nutrient cycle and a control agent for abiotic disturbance regimes, such as fires (Hobbs, 1996). Therefore, by modifying plant species abundance and distribution (Perea et al., 2014), deer in turn modify animal diversity (Côté et al., 2004). At moderate densities, they can increase the abundance of ground-dwelling invertebrates that benefit from open habitats such as worms, beetles (Coleoptera), moths (Lepidoptera), orthoptera and flies (Diptera) and hence, the

abundance of many species that feed on them; reptiles, amphibians, birds and mammals (see Côté et al., 2004 for a review). Deer are also a key component in the trophic web as they constitute potential prey for large carnivores, a major food source for scavengers and the main source of dung for coprophagous insects. Therefore, deer are essential, for example, in the conservation of necrophagous birds protected by EU Birds Directive (79/409/ECC). For these reasons, they are considered keystone species (*sensu* Paine, 1995) as they strongly modify the structure and composition of ecological communities (McShea & Rappole, 1992).

Deer are among the most widely distributed, and most numerous, large mammals in the northern hemisphere. Two species, roe deer (*Capreolus capreolus* Linnaeus, 1758) and red deer (*Cervus elaphus* Linnaeus, 1758), are particularly common and widespread throughout most of continental Europe, occupying nearly the same habitats. Therefore, these sympatric and native species occur from the Mediterranean area to the Scandinavian region and from sea level up to more than 2400 m a.s.l. (Fig. 1.1). Hence, due to their adaptability to a wide range of different environments, their European populations are estimated to be in the order of millions of individuals (15 million for roe deer: Lovari et al., 2016; 2.4 million for red deer: Lovari et al., 2018).

Figure 1.1 Spatial distribution of: a) red deer and b) roe deer in Europe. Source: IUCN (<https://www.iucnredlist.org/>)

Deer also provide high economic benefits as game species since they are appreciated by both, their trophy and meat (venison). Hunting trends have increased during the last decades in Europe where yearly hunting bags are estimated to be 429 thousand individuals of red deer and at least 2.7 million roe deer (Burbaité & Csányi, 2009; Burbaité & Csányi, 2010). Similarly, venison price has raised during the last decades as deer meat is highly appreciated by consumers for its tenderness, fatty acid composition and high levels of minerals but also for the sustainability of its production (Hoffman & Wiklund, 2006).

At the beginning of the 20th century, overexploitation, habitat loss and habitat fragmentation led to big declines in red and roe deer populations (Apollonio, Andersen, & Putman, 2010). However, during the last 50 years, they have experienced an unprecedented population increase (Gortázar et al., 2000; Milner et al., 2006; Burbaité & Csányi, 2009; Burbaité & Csányi, 2010). Their geographical expansion and demographic success are the result of both, anthropogenic causes and their high reproductive and dispersal potential (Milner et al., 2006). Land use changes (i.e. rural abandonment, access to crop fields, extensive livestock decrease), low densities of natural predators and the interest of hunters to increase their populations (i.e. supplementary feeding, reintroductions) are the main causes of this increase (Cederlung et al., 1998; Côté et al., 2004; San Miguel et al., 2010). Unfortunately, alongside this increase in range and number, serious ecological and socio-economic problems have arisen (Côté et al., 2004; Apollonio et al., 2010). Even though deer are a key component of the ecosystems in moderate numbers, at high densities, they can produce cascading effects over lower trophic levels and negatively affect different ecosystems components and processes (Garrott et al., 1993; Hobbs, 1996). In addition, socio-economic conflicts have raised exponentially during the last decades, such as crop and forest plantation damage (browsing, trampling and rubbing; Gill, 1992; Côté et al., 2004), road accidents (Putman, 1997) and transmission of diseases to livestock and human (Gortázar et al., 2006). All these problems translate into high economic and environmental costs (Cederlung et al., 1998). Therefore, the

understanding of the factors that influence population dynamics, especially in terms of reproduction and survival, have become a main focus for ecologists and wildlife managers.

Over the last decades, red and roe deer populations have been extensively monitored and studied in many parts of Europe (see Vanpé, 2007, p. 65). In fact, the greatest contribution to our understanding of these species population dynamics was based on long-term deer population monitoring from central and northern Europe (i.e. Isle of Rum in Scotland: Clutton-Brock, Guinness, & Albon, 1982; Trois Fontaines and Chizé in France: Gaillard et al., 1993a; Bogesund in Sweden: Kjellander et al., 2004). However, more long-term studies describing how population dynamics vary at the edge of the species distribution range (i.e. Mediterranean ecosystems or Alpine environments) are still needed (but see Rodríguez-Hidalgo et al., 2010; Torres-Porras et al., 2014; Torres et al., 2015).

Populations living at the edge of a species' range often experience extreme conditions and different selective pressures than their analogous living in more central locations of their geographic distribution (Kawecki, 2008). Therefore, long-term studies performed on these regions are crucial to understand the evolution of life-history traits under extreme climatic conditions and the mechanisms that drive adaptation to these environments. Furthermore, climate changes due to global warming are predicted to be especially marked in these regions, with more frequent and severe drought in Mediterranean areas (Peñuelas et al., 2002; IPCC, 2007; Hoerling et al., 2012) and the advance of spring phenology and increasing temperatures in alpine locations (Menzel, 2000; IPCC, 2013; Klein et al., 2016). Hence, in the context of climate change, these types of studies are even more relevant as they may provide a clue about how populations may respond to future climatic scenarios, helping to predict demographic changes and distribution shifts (Plard et al., 2014a; Büntgen et al., 2017; Chevin & Hoffmann, 2017).

1.2 Reproduction in seasonal environments

Seasonal environments are characterized by the occurrence of certain abiotic and biotic events during limited periods of the year (Lieth, 1974; Tonkin et al., 2017). This annual variation in abiotic events (e.g. temperatures, day-length, snow or precipitation) and associated biotic interactions (e.g. food availability, predation risk, parasitism) divides the annual cycle into productive periods (with favorable conditions) and unproductive periods (with limiting conditions; Varpe, 2017). Thus, this environmental variation greatly affects the phenology of species, which are forced to restrict certain vital functions only to favorable periods of the year (Boyce, 1979).

A major adaptation of specie to seasonality is the timing of reproduction, an energy-demanding function that should be aligned with optimal time window of favorable conditions (Boyce, 1979). Other activities timed with the favorable periods are growth, development of sexual secondary traits and energy storage. In addition, species also need to develop strategies to minimize energy expenditure during the unproductive season such as hibernation, lower metabolism, diapause or fasting, but also migratory movements to areas with more favorable conditions (Varpe, 2017).

Seasonality has been used to explain life-history adaptation among species (Boyce, 1979) but also within species, to explain life-history trait variation along geographical gradients (Ferguson & McLoughlin, 2000).

Life-history theory and reproductive trade-offs

Populations of widespread species, such as red deer and roe deer, have to cope with multiple limiting environmental factors that can be highly variable across their distribution area. Life-history theory (Stearns, 1976) predicts that organisms should maximize their fitness (i.e. their success passing their genes to the next generation) by allocating resources to different vital functions according to environmental conditions. Thus, we expect life-history traits (such as age and size at maturity, number and size of offspring, number of reproductive attempts during life-time or life-span) to vary across environmental gradients. A conceivable way to maximize an organism fitness could be achieved by maximizing all life-history traits simultaneously. If existed, this hypothetical organism, the Darwinian Demon (Law, 1979), would start to reproduce just after birth, reproduce continuously, produce many offspring at a time and live forever. However, as individuals live in resource-limited environments, the energy obtained has to be distributed between the requirements derived from its growth, reproduction and survival (Williams, 1966a). Thus, based on the principle of allocation, trade-offs explain that any extra energy allocated to one biological process can only occur at the expense of another (Cody, 1966; Williams, 1966a; Gadgil & Bossert, 1970). For this reason, trade-offs have played a central role in the development of life-history theory (Stearns, 1976).

Lifetime reproductive success (LRS), the number of surviving offspring produced by an individual during its lifetime, has often been used as an estimate or proxy of individual fitness (Clutton-Brock, 1988; Brommer et al., 2004), although note that some authors have criticized it as it assumed constant generation time (McGraw & Caswell, 1996) and the same value for all recruits (Benton & Grant, 2000). Deer are long-lived **iteroparous** mammals, which means that they reproduce more than once in their life-time (opposite to semelparous animals; Cole, 1954), and thus, they partition reproductive effort among different attempts at different time intervals. Consequently, to maximize LRS, and

therefore their fitness, deer face multiple trade-offs between reproduction at one time and subsequent growth, reproduction or survival. For example, if males invest too much energy on fighting one year, they may be injured and their survival might be therefore compromised (Clutton-Brock et al., 1979). If females loose much body condition during lactation, they may not be able to ovulate the following season or even die during winter (Clutton-Brock, Guinness, & Albon, 1983) or if males invest too much on fighting for access to females during early life they could show earlier reproductive senescence (Lemaître et al., 2014). According to Stearns (1989), the main reproductive trade-offs are: 1) current reproduction vs. survival; 2) current reproduction vs. future reproduction; 3) current reproduction vs. parental growth; 4) current reproduction vs. parental condition and 5) number vs. size of offspring.

In addition, one key trade-off, current reproduction vs. survival, has been described to constrain the co-variation of life-history traits along an axis known as the slow-fast life-history continuum (Gaillard et al., 1989; Ricklefs, 2000), similar to the concept of r- and K-selection (MacArthur & Wilson, 1967). On the “slow” end of this axis, we find individuals characterized by higher investment on survival over reproduction and thus, individuals that start to reproduce relatively late in life, have low fecundity but a high lifespan (Stearns, 1983). On the “fast” end of this continuum, we find individuals with the opposite suites of traits (see Réale et al., 2010 for some recent progress on this topic). Furthermore, this co-variation of life-history traits is independent of the scale studied (i.e. among different species or between populations of the same species; Stearns, 2000). Therefore, we can expect key life-history traits of red deer and roe deer to vary, not only between both species, but also for each species across its latitudinal and elevational range (Hopkins, 1938; Ashmole, 1963; Rutberg, 1987; Réale et al., 2010; Hille & Cooper, 2015). Finally, as the variation of life-history traits can greatly affect population growth (McAdam et al., 2007), understanding the factors that influence such variation for both species is crucial to predict future demographic changes.

Phenotypic plasticity vs. evolutionary adaptation

The mechanisms that explain population responses to environmental conditions can be explained by evolutionary adaptation, adaptive phenotypic plasticity, or a combination of both (Pigliucci, 2001). Evolution is the mechanism which allows species to “*genetically adapt to new conditions through mutations or selection of existing genotypes*” (Bellard et al., 2012). On the other hand, phenotypic plasticity (Bradshaw, 1965) is “*the change in the expressed phenotype of a genotype as a function of the environment*” (Scheiner, 1993). Accordingly, the latter can be graphically represented by the “reaction norm” of a genotype, which represents the different phenotypes that can be expressed by one genotype across an environmental gradient (Woltereck, 1909; Johannsen, 1911). However, note that phenotypic plasticity can be adaptive, if it improves the fitness of the organism (Pigliucci et al., 2006; Ghalambor et al., 2007), or non-adaptive as a consequence of unavoidable constraints imposed by the organism biology (i.e. *inevitable plasticity*; Sultan, 1995).

In the context of climate change, one of the central questions is whether species would be able to adapt fast enough to the rapid environmental changes and what mechanisms would be involved in this response (Visser, 2008). As micro-evolutionary responses occur across generations, large mammals, with long generation times, need long time periods to adapt (Gienapp et al., 2008). Conversely, adaptive phenotypic plasticity is a mechanism that allows an organism to adjust to the environment within its lifetime. Thus, recent empirical evidence signaled towards a higher importance of plastic responses over micro-evolutionary responses in the rapid adaptation of long-lived species to current climate change (Bradshaw & Holzapfel, 2006; Williams et al., 2008; Hoffmann & Sgrò, 2011). However, in the long term and due to the strength of the environmental changes, phenotypic plasticity alone may not be sufficient and some degree of microevolution might be needed (Visser, 2008).

The role of each sex on reproduction

Large herbivores, and deer in particular, are usually **polygynous**, as one male mates with several females while each female mates with only one male (Emlen & Oring, 1977). A key determinant in the mating system adopted by a species is the degree of parental care provided by each sex (Emlen & Oring, 1977). For large herbivores, it is the female which allocate more reproductive effort to rear the offspring because of the cost of the long gestation and lactation periods (Oftedal, 1985). On the contrary, for males, the cost of copulation and fertilization is minimal (i.e. producing sperm), and hence, their contribution to directly rear the progeny is negligible compared to females (Fisher, 1930; Clutton-Brock, Guinness, & Albon, 1982). For this reason, males can father more offspring during a reproductive season than females can produce and hence, a polygynous mating system was adopted.

While the reproductive success of females depends on their ability to provide quality food resources and a safe environment for their young, male reproductive success depends on their ability to gain access to females (Trivers, 1972). Therefore, males compete to monopolize the scarce number of females on estrous, a limited resource (*sensu* Bateman, 1948), by engaging in intense or aggressive competitions that are likely to give the advantage to animals with developed traits to either fight or attract females (Clutton Brock et al., 1982).

1.3 Female Reproduction

Female deer in northern temperate environments usually reproduce every year once reached maturity (Asher, 2011), having highly seasonal and synchronous birth seasons (Gaillard et al., 1993b; Adams & Dale, 1998; Bowyer, Van Ballenberghe, & Kie, 1998). Like for many other ungulates, the start of the breeding season is cued by photoperiodic changes, being the majority of species **short-day breeders** (i.e., they initiate mating activity when days start to shorten, during late summer and autumn; Lincoln & Short, 1980). Females are usually **seasonally polyestrous** as they have multiple ovulations during the mating season if they fail to conceive (Sadleir, 1987). Calving occurs in spring or early summer (Clutton-Brock, Guinness, & Albon, 1982) and the lactation period extends over the summer (see red deer reproductive cycle as an example; Fig. 1.2). Hence, the annual reproductive cycle of female deer is highly energy-demanding as they usually start a new breeding cycle just while they are still lactating their previous fawn (Clutton-Brock, Guinness, & Albon, 1982; Gaillard et al., 1993b).

The importance of breeding timing

Production of offspring is costly for females, with the late gestation and early lactation being the periods of highest energetic demands (Oftedal, 1985). At this time, females require a substantial amount of resources, not only for the survival and growth of their offspring but also for the recovery of body condition in order to successfully mate and breed the following season (Clutton-Brock, Guinness, & Albon, 1983). Therefore, as they have long gestation periods, they should time in advance their mating season to match parturition dates with the time of highest resource availability (Oftedal, 1985).

According to the forage maturation and green wave hypotheses (Fryxell & Sinclair, 1988; Rivrud et al., 2016), the leading edge of the growing season is the optimal parturition time for large herbivores in temperate environments (Ofstedal, 1985; Rutberg, 1987; Merkle et al., 2016). The early vegetative stage of herbaceous plants, although low in biomass production, has higher protein content and digestibility than later stages of plant development (Albon & Langvatn, 1992; Hebblewhite, Merrill, & McDermid, 2008). This is because a significant drop in quality occurs during flowering and seed production as plants produce fewer leaves and more stems, increasing the fiber content (Albon & Langvatn, 1992). Thus, the energy intake capacity of herbivores becomes limited by the increasing fiber content as herbaceous biomass production increases, making early development stage with low vegetative biomass nutritionally superior than mature, high-biomass vegetation (Fryxell, 1991). Indeed, the availability of nutritious forage during early lactation promotes the production of quality milk with high protein concentration (Landete-Castillejos et al., 2000) as most of the milk secreted by hinds is the result of their daily food intake (Sadleir, 1987).

Furthermore, if parturitions occur at the start of the growing season, fawns can benefit from the whole length of plant productivity, having more time to grow before the onset of winter (Clutton-Brock et al., 1987). Therefore, for many deer species, fawns born early in the season have shown increased early-life survival (Clutton-Brock, Guinness, & Albon, 1982; Plard et al., 2015) and higher body mass before the onset of winter, the limiting season in temperate environments (Gaillard et al., 2000b).

Figure 1.2 Reproductive cycle of red deer. Reference dates are adapted to Mediterranean environments.

Furthermore, the importance of correctly time reproductive cycle is such, that roe deer have developed an evolutionary adaptation that is unique among ungulates. As we can see in Fig. 1.3, roe deer gestation length is 10 months approximately, nearly twice as much as expected for a large herbivore of its size (Huggett & Widdas, 1951). In fact, red deer, which are between 5-6 times larger at birth than roe deer, have a shorter gestation length (7.5 months; Fig. 1.2). The unusually long gestation period of roe deer can only be explained by the evolutionary phenomenon of delayed implantation or obligate embryonic diapause (Short & Hay, 1966; Aitken, 1974). This mechanism allows the uncoupling of mating and fertilization from parturition by pausing the embryonic development at blastocyst stage for 5 months before it attaches to the uterine epithelium (Fig. 1.3; Aitken, 1974). This strategy, which evolved to increase gestation length, helps synchronizing both, calving date and mating season with the time of favorable environmental conditions (Sandell, 1990). Roe deer “real” gestation lasts approximately 5 months (Short & Hay, 1966) and therefore, for calving season to be synchronized with spring peak in forage availability, mating should occur during winter. However, as winter is an unfavorable period with low resource availability, males should accumulate enough fat during summer and fall to cover simultaneously the cost of mating and overwintering (Williams et al., 2017). As previously explained, male-male competition is key to maximize reproductive success and therefore it should only occur when environmental conditions are optimal (Sandell, 1990). Therefore, this strategy that has evolved independently across many mammal species helps roe deer to adapt their complete breeding cycle to the optimal moments in terms of resource availability. However, this has caused some singularities in roe deer reproduction compared to most northern ungulates. For example, roe deer are **long-day breeders** (Sempéré & Boissin, 1981) with a summer mating season (July and August) and females are **monoestrous**, with only one ovulation per reproductive season lasting just 24–36 h (Hoffmann et al., 1978).

Figure 1.3 Reproductive cycle of roe deer. Adapted from Aitken, (1974).

Breeding timing and local adaptation across environmental gradients

Across large regions and distinct topographies, variation in the timing and duration of resource availability is known to shape the timing of large herbivores reproductive cycle. Therefore, as the growing season starts later at high latitudes, females of large herbivores delay their parturition to match the later onset of vegetation growth (Weber, 1966; Bunnell, 1982; Linnell, & Andersen, 1998a; Loe et al., 2005; Plard et al., 2013). Furthermore, at these latitudes, the growing season also becomes shorter, which limits the time available for offspring growth (Ofstedal, 1985; Clutton-Brock et al., 1987; Rutberg, 1987) and female body condition recovery before the onset of the winter, the limiting season (Clutton-Brock, Guinness, & Albon, 1983). Thus, we expect a strong selection pressure against late births, which leads to an increased birth synchrony (Sadleir, 1969; Rutberg, 1987; Gaillard et al., 1993b).

On the contrary, in European Mediterranean habitats, earlier and less synchronized parturitions are expected as the growing season starts sooner and lasts longer than in northern environments (Mateos-Quesada & Carranza, 2000). Furthermore, in Mediterranean environments, distinct environmental conditions impose different constraints to deer reproductive cycle: 1) the limiting period in terms of resource availability is the summer while winters are milder and shorter and 2) there is a high inter-annual variation in the start of the spring flush, the length of the summer drought and autumnal seed production (Bugalho & Milne, 2003). Therefore, due to the high inter-annual variability of these environments, it is not unusual to find years on which either i) the birth season takes place after the spring peak of quality forage, ii) the lactation period extends over long summer droughts or iii) the rutting season starts before the arrival of autumn rains and the mast season. Thus, in Mediterranean environments, the depletion of female reserves during the nursing period leaves them with little time to recover body condition before the start of the next rutting season (San Miguel et al., 1999;

Bugalho & Milne, 2003; Landete-Castillejos et al., 2003; Martínez-Jauregui et al., 2009; Rodríguez-Hidalgo et al., 2010). Hence, reproductive trade-offs, such as current reproduction vs. future reproduction, may be more easily observable in these extreme environments and thus, the importance of an early parturition date (Landete-Castillejos et al., 2001). However, there is little information available about the factors that influence variation in conception and parturition timing in these environments (but see Mateos-Quesada & Carranza, 2000; Rodríguez-Hidalgo et al., 2010).

Finally, while most attention has been focused on the variation of birth timing and synchrony along latitudinal gradients, studies specifically addressing changes along the elevational gradient for large herbivores (Weber, 1966 on white tailed deer; Bunnell, 1982; Thompson & Turner, 1982; Hass, 1997 on wild sheep, *Ovis* sp.), or even other mammals (Dunmire, 1960 on deer mouse, *Peromyscus maniculatus*; Zammuto & Millar, 1985 on ground squirrels, *Spermophilus columbianus*; Zhao, 1994 on Tibetan macaques, *Macaca thibetana*) are very scarce. Both latitudinal and elevational gradients show increasingly harsh climatic conditions towards their higher ends, leading to delayed plant phenology, shorter growing season and, thus, stronger seasonality in food availability (Hopkins, 1938). However, despite these similarities, latitudinal and elevational gradients differ mainly in the spatial scale at which environmental changes occur (i.e. kilometers vs. meters). Hence, in areas of complex topography, there is large variation of environmental conditions over short geographical distances. This may have different implications for the large herbivores: 1) the scale at which changes occur may be smaller than the scale at which individuals move (through migration or dispersion) and thus, gene flow may limit evolutionary adaptation of birth timing across this gradient (Lenormand, 2002; Loe et al., 2005) and 2) the topographic heterogeneity provides them with a wide range of plant phenology phases in a short spatial scale, which may help large herbivores to track resource availability in space by adopting a multi-range tactic (Couriot et al., 2018).

Therefore, the study of these two distinct environmental gradients (i.e. latitudinal and elevational) has important management implications particularly in the current context of climate change as animals may adapt and migrate more quickly in local mountain ranges (elevational gradient) compared to large-scale latitudinal gradients.

Reproductive investment strategy and phenotypic plasticity in breeding timing

A mismatch between resource availability and parturition timing is expected to have important demographic consequences for deer as it can reduce female annual reproductive success (Clutton-Brock et al., 1987; Plard et al., 2014a) and future reproductive performance of both females and newborn fawns (Clutton-Brock et al., 1983). However, the severity of the consequences of a trophic mismatch can vary depending on the reproductive investment strategy along the capital-income breeding continuum (C-I continuum; Drent & Daan, 1980; Jönsson, 1997; Williams et al., 2017).

Both species, roe and red deer, have different positions within this continuum. On the one hand, roe deer reproductive investment strategy is located towards the income end of the C-I continuum as they strongly rely on current resource intake to offset increased energy requirements due to reproduction (Andersen et al., 1998). On the other hand, red deer are considered to be in the capital end of the spectrum as they store reserves in advance that allow a partial temporal decoupling of energy acquisition and reproduction (Festa-Bianchet, Gaillard, & Jorgenson, 1998; Moyes et al., 2006). Income breeding has the advantage of avoiding the costs of accumulating and carrying capital (i.e. loss in mobility, increased predation risk, metabolic costs, etc.; Houston et al., 2006), but instead, they must follow a ‘bet-hedging’ strategy in which parturition timing coincides with a long-term average of favorable conditions (Post &

Forchhammer, 2008) and thus, they rely strongly on photoperiodic cues to time their breeding season (Jönsson, 1997).

Recent studies have demonstrated that capital could be used, not only to lessen the need for matching optimal timing in parturition (Kerby & Post, 2013), but also to facilitate temporal synchrony between resource need and supply (Williams et al., 2017). Indeed, many studies have demonstrated the ability of red deer to adjust gestation length during the last third of the gestation if there are abundant resources, as an increase in female body condition during this period increases fetal growth and thus advances parturition time (Albon, Clutton-Brock, & Guinness, 1987; García et al., 2006; Moyes et al., 2011). This allows them to precisely match year-to-year birth timing with peak resource availability (Moyes et al., 2011). However, this is not the only mechanism to modify parturition timing, variation in ovulation dates also showed phenotypic plasticity (Langvatn et al., 2004). For red deer, delayed ovulation has been discussed to be the result of decrease female condition as it has been frequently reported in high density populations and also for females carrying a calf from previous year (Langvatn et al., 2004). In addition, environmental conditions at early life can affect body size and phenotypic quality during adulthood influencing reproductive performance later in life (Albon et al., 1987). Therefore, all variables affecting female phenotypic quality or inter-annual variation in maternal body condition (i.e. early life and annual environmental conditions such as habitat quality, weather conditions and density) may in turn affect parturition timing for this capital breeder. Finally, other population factors such as sex ratio or male age structure, although not having a direct influence in the physical condition of the females, can influence ovulation or conception dates when not balanced (Noyes et al., 1996; Bonenfant et al., 2004).

In contrast, roe deer have shown limited phenotypic plasticity on parturition dates and restricted potential to track temporal variation in the onset of vegetation growth (Plard et al., 2014a). In fact, contrary to red deer, inter-

annual changes in female roe deer body condition had no influence on its parturition date (Plard et al., 2014b). Thus, parturition dates are highly repeatable for a single female throughout its lifetime (Plard et al., 2013).

Therefore, capital breeding seems to be a better strategy under changing environmental conditions, whereas income breeding seem to work superior under predictable environmental conditions (Jönsson, 1997). Furthermore, it is expected that the negative consequences of a trophic mismatch will be more pronounced for income breeders compared to capital breeders as the first group lack both, phenotypic plasticity on parturition timing and mechanisms to decouple resource acquisition and energetic needs. Therefore, in the context of climate change, the income breeding strategy may turn out to be maladaptive (Kerby & Post, 2013).

Finally, it is important to highlight that, for roe deer, high quality females have been shown to give birth consistently earlier in the season than low quality females (Plard et al., 2013). Note that female quality in this context is defined as a set of time-invariant female attributes that are positively linked to individual fitness (i.e. year of birth, median adult mass and longevity; Plard et al., 2013). Furthermore, female age also influenced parturition dates, with older females giving birth earlier (Plard et al., 2013). Therefore, although within a female lifetime, we expect no phenotypic plasticity on parturition date, we can expect this trait to vary at population level according to population structure and environmental conditions.

Litter size variation across environmental gradients

Litter size (i.e. annual fecundity *sensu* Pincheira-Donoso & Hunt, 2017) is an important component of recruitment for large herbivores. Annual recruitment is the result of the proportion of females that give birth, the number of offspring produced by those females each season (litter size), and offspring survival (Gaillard et al., 2013). The red deer is a **monocotous** species, which means that it gives birth to only one fawn at a time. Therefore, recruitment for this species

depends on both the proportion of females that give birth and offspring survival. In contrast, the roe deer is a **polytocous** species that can have one, two or three fawns per litter (quadruplets and quintuplets parturitions are very rare although possible; Stubbe et al., 1982; Flajšman et al., 2017). As female fertility is high among adults, litter size and fawn survival generate most of the variation in annual recruitment (Plard, 2014). Hence, the importance of litter size to determine demographic tendencies. However, little is known about geographical variation on litter size for large herbivores and its impact on population dynamics.

Two main trade-offs are involved in determining the optimal litter size: 1) the number of offspring vs. their quality (Lack, 1954) and 2) current parental reproductive investment vs. future fecundity (Williams, 1966b). These trade-offs depend on two main ecological pressures: 1) climate (i.e. seasonality, length of the growing season, day length, temperature) and 2) predation. As both selection pressures display a geographical gradient (i.e. increased seasonality and reduced predation at higher latitudes), Lack (1954) proposed a macroecological fecundity gradient based on the observation that birds clutch size increased with increasing latitude. However, attempts to demonstrate a positive link between litter size and latitude in larger mammals had led to contradictory results: positive relationship (wild boar *Sus scrofa*; Bywater et al., 2010; Artiodactyla; Tökölyi et al., 2014), no relationship (canids and felids; Lord, 1960; Gaillard et al., 2014) or opposite results (polar bears *Ursus maritimus*; Derocher, 1999).

A problem encountered when studying geographical clines on litter size is that this trait displays a high phenotypic plasticity (Hewison & Gaillard, 2001; Nilsen, Linnell, & Andersen, 2004; Macdonald & Johnson, 2008; Flajšman et al., 2013). Litter size for large herbivores has been described to be mainly driven by female body mass/condition and age (Andersen et al., 2000; Gaillard et al., 2000b; Macdonald & Johnson, 2008). Therefore, all variables affecting maternal body mass (such as environmental conditions, habitat quality, density) may in turn affect litter size (Gaillard et al., 2000a; Flajšman et al., 2017). This is likely to

introduce noise in inter-population analysis, reducing the chance of detecting a clear geographical trend on this trait, as close populations, genetically similar, show large inter-population variation in litter size.

Finally, despite the relevance of this topic, there is virtually no information on the variation of litter size along an elevational gradient for wild large mammals. As both sources of selection (i.e. climate and predation) also co-vary in similar direction with increasing elevation, it was hypothesized that litter size would also display an increase along the elevational gradient. Surprisingly, however, most studies performed on birds reported a decrease of clutch size with increasing elevation, creating the so-called fecundity gradient paradox (Pincheira-Donoso & Hunt, 2017).

Response of female reproductive traits to climate change.

Rising temperatures due to climate change have resulted in an earlier start of plant growth across the Northern Hemisphere (Menzel, 2003; Parmesan & Yohe, 2003; Roberts et al., 2015). As a consequence, phenological mismatches may occur if a differential rate of response disrupts previously synchronous trophic interactions (Visser et al., 1998; Koh et al., 2004) leading to a decoupling between the date of key life-history events and optimal environmental conditions (Bronson, 2009; Usui, Butchart, & Phillimore, 2017). As explained above, an increasing mismatch between parturition date and peak resource availability during the breeding season may have negative demographic consequences (Plard et al., 2014a on roe deer). Therefore, in order to cope with the rapid changes in plant phenology due to climate change, long-lived mammals have three main options (Bellard et al., 2012; Gaillard et al., 2013): track resources in time, space or adapt *in situ*.

Firstly, to keep up with plant phenology, individuals can **reschedule the timing** of key life-history events, such as breeding timing. Among deer, observed advancements in breeding timing as a response to recent climate change have

been reported to be mainly consequence of phenotypic plasticity (red deer: Moyes et al., 2011; reindeer *Rangifer tarandus* Linnaeus, 1758; Mysterud et al., 2009; semi-domesticated reindeer Paoli et al., 2018). However, as indicated earlier, there are some species that lack phenotypic plasticity on this trait (i.e. roe deer: Plard et al., 2014a). Furthermore, even for those species that display plastic phenotypic responses, if the rate of environmental change is too fast and/or large in magnitude, individuals might reach a physiological limit that make them unable to adapt beyond a certain threshold point (Chevin, Lande, & Mace, 2010). For both cases, lack of phenotypic plasticity or great environmental changes, the species may need micro-evolutionary adaptations which may take longer periods as large mammals have long generation times (Gaillard et al., 2005).

Secondly, instead of shifting reproduction timing in situ to track increasingly earlier plant phenology over the years, deer can also **move in space** to minimize the increasing mismatch between birth timing and vegetation flush (Fryxell & Sinclair, 1988). This type of behavioural plasticity can be achieved through dispersion movement (latitudinal and elevational range shifts; Büntgen et al., 2017) or through seasonal migratory movements (temporarily changing their ranges). Migration in the deer family is common at high latitudes where, for example, reindeer or caribou travel across long distances to their breeding grounds (Festa-Bianchet et al., 2011). However, not only large migratory movements are important but also short movements to close habitats at the local scale. Indeed, partial migration is a frequently reported phenomenon in populations inhabiting heterogeneous topographical habitats, which allows females to decrease the temporal mismatch by changing their home range to higher elevations during the breeding season (Albon & Langvatn, 1992; Mysterud et al., 2001; Ramanzin, Sturaro, & Zanon, 2007; Hebblewhite, Merrill, & McDermid, 2008; Cagnacci et al., 2011). Furthermore, species also follow a multi-range tactic at the local scale within seasons to track spatio-temporal variation of resource availability in heterogeneous and unpredictable environments (Gaudry et al., 2015; Couriot et al., 2018). For roe deer, this tactic allows them to move to

secondary habitats such as agricultural landscapes during the critical mismatch period where they seem to perform better than in their typical forest habitat (Gaillard et al., 2013). However, although deer obtain benefits by tracking resources in space, each movement outside their home range has an associated cost or risk (i.e. increase predation; energy expenditure or road accidents, etc.; Putman, 1997; Hein, Hou, & Gillooly, 2012). Furthermore, in the new sites they may face environmental constraints different to their origin areas (different type and level of predation; competition with residents; Hebblewhite & Merrill, 2009). Finally, this option may not be available in highly anthropized landscapes where habitat fragmentation (i.e. fencing, infrastructures, etc.) prevents wild animals from tracking the most suitable environment.

Finally, species can keep in balance with the environment by **altering** some of their **physiological functions *in situ*** (i.e. body size, metabolic rate or reproductive output; Bellard et al., 2012). This option involves that they remain in the same habitat and maintain demographic performance invariant without modifying the timing of key life-history events (Gaillard et al., 2013). As we have discussed above, this is more likely to be achieved by capital breeders, which have the ability to decouple energetic need and resource acquisition, and it has been suggested that it is not likely to occur for an income breeder such as roe deer (Gaillard et al., 2013). Indeed, for red deer, the advanced growing season due to climate change showed no quantifiable consequences on either birth weight or juvenile winter survival (Moyes et al., 2011) even though there was an observable mismatch between life-history events and environment. On the contrary, a lower early survival has been already reported for roe deer as a consequence of the increasingly earlier springs (Plard et al., 2014a).

Further research is required to improve our understanding of how large herbivores track resource dynamics across different environments as it is particularly relevant in the context of climate change (Vitasse, Signarbieux, & Fu, 2018).

1.4 Male Reproduction

As explained before, reproductive performance of males in polygynous species depend on their ability to compete for access to females (Trivers, 1972). Thus, in order to successfully mate, polygynous males have to distribute in such a manner so they have access to as many females as possible (Emlen & Oring, 1977). Among deer species, monopolization of mates can be achieved following different strategies, which vary according to the distribution of females in space (i.e. size of female range and core areas, size/stability of groups and density; Clutton-Brock, 1989). These strategies can be classified into two groups depending on how males gain access to females (Emlen & Oring, 1977): 1) direct competition for access to females or 2) defense of high quality territories by anticipating female distribution during the breeding season.

Red deer usually **defend harems**, as females, during the rut, distribute in large groups relatively stable. Therefore, one male monopolizes a big group of females and defend them against other males in direct competition (Clutton-Brock, Guinness, & Albon, 1982). In contrast, roe deer are **territorial**, as female distribution during the breeding season is disperse (one mother with their calves) but their home range is predictable and small. Therefore, males defend a territory that can include several female home ranges concentrated in patches of high resource availability (Linnell & Andersen, 1998b). However, note that intraspecific variation in mating strategies has been described for some species, as males might exhibit some degree of behavioral plasticity according to environmental conditions (see red deer resource defense polygyny; Carranza, Álvarez, & Redondo, 1990; Carranza, 1995; Carranza, 2000). Conversely, other

species such as roe deer show little plasticity in mating strategies (Hewison et al., 1998; Liberg et al., 1998).

Secondary sexual characters

Independently of the mating strategy, male deer reproductive success depends on their ability to outcompete members of their same sex through fighting or dominance rank (Clutton-Brock, Guinness, & Albon, 1982). Therefore, traits such as body size, strength and antler development are key for their reproductive success (Clutton-Brock, Guinness, & Albon, 1982).

Deer antlers have fascinated ecologists since the beginning of times. Antlers are conspicuous traits that male deer grow and shed yearly. Their formation entails a great cost, an extravagance in terms of energy expenditure that seemed to contradict the “*struggle for existence*” of ordinary natural selection theory (Darwin, 1859; Andersson, 1994). However, the decrease in fitness due to the formation of these costly traits is compensated for the fitness gained due to mating success (Darwin, 1859). Darwin termed the process in which members of the same sex “*struggle*” for the possession of opposite sex mates “**sexual selection**” and named the traits used for intra-sexual competition “**secondary sexual characters**” to distinguish them from the primary sexual characters (i.e. reproductive organs). Secondary sexual traits are those traits that increase the chances to gain access to females during intra-sexual competition. Therefore, male-biased **sexual size dimorphism** and antlers are thought to have evolved principally as the result of sexual selection (Clutton-Brock et al., 1982).

The higher the level of polygyny, the larger the opportunity for sexual selection as the variance in reproductive success between males would be higher (Darwin, 1871; Huxley, 1938). Therefore, it is expected that the degree of polygyny would increase the divergence between phenotypic traits of females and males (sexual dimorphism; Clutton-Brock et al., 1982). For example, red deer are **highly polygynous**, as one male can monopolize harems of more than 20

females (Clutton-Brock, Guinness, & Albon, 1982). This strategy to monopolize females by defending large harems produces a high variance in male reproductive success (i.e. while just few males breed with all females, many do not have the chance to mate; Clutton-Brock, et al., 1979). Therefore, as there is a large opportunity for sexual selection, red deer present **high sexual size dimorphism**, with males being almost 35% larger than females. In addition, antler investment relative to body weight is high (antler weight around 2.53-4.91% of their body mass; Huxley, 1931). Alternatively, roe deer are **oligogynous** (i.e. weakly polygynous; Vanpé, 2007). As they display resource-defense territorial systems, each territorial male mates normally with the few females within its territory, presenting a lower variance in male reproductive success (but see Liberg et al., 1998; Vanpé et al., 2007 on female excursions). As they have reduced potential for sexual selection through competition between males, they present low sexual dimorphism, being males around 10% bigger than females (Andersen et al., 1998; Andersson, 1994; Vanpé, 2007) and their relative investment on antlers in relation to body weight is small compared to other Cervid species (antler weight is 0.92-1.52 % of body weight; Huxley, 1931).

Interestingly, at interspecific level, sexual selection for body mass also affects litter size, with increasing levels of sexual size dimorphism selecting for smaller litter sizes (Carranza, 1996). This relationship can be observed between red deer (high sexual dimorphism and monogamous) and roe deer (low sexual size dimorphism and polygamous).

Male deer reproductive cycle and antler cycle

Reproductive cycle of male deer is timed so that physical and reproductive conditions are at their maximum for the breeding season (Goss, 1969). Photoperiod has been described to be the main environmental cue that promotes deer antler cycle and governs behavior during the breeding season (Goss, 1969). Day length changes produce seasonal increase in gonadotrophin secretion (LH),

which controls testosterone levels (Lincoln & Kay, 1979; but see Sempéré, Mauget, & Bubenik, 1982 on roe deer). Low levels of this hormone promote antler cast and subsequent regrowth and high levels cause velvet shed, antler hardening and rutting behavior (Lincoln, Guinness, & Short, 1972 on red deer, Sempéré & Boissin, 1981 on roe deer).

Red deer male reproductive cycle starts at the beginning of autumn, when testosterone levels are at their maximum and antlers are fully formed and hardened (Fig. 1.2). In this period, males display typical rutting behavior such as roars, chasing off rivals, fights and scent marking (Lincoln et al., 1972). Males retain their antlers and their fertility throughout the autumn and winter as females are seasonally polyestrous and may show estrous over this period (Lincoln, 1992). After the peak of the rutting season, there is a steady decline in testosterone that reaches its lower levels at early spring, causing antler cast (Goss, 1968; Gaspar-López et al., 2010). Immediately afterwards, antlers start to grow very fast, and become fully hardened and clean (i.e. without velvet) around 1 month before the rut (Gaspar-López et al., 2010). Because antlers are costly to produce, they should grow during the period of maximum availability of food (spring-summer in northern temperate environments; Schmidt et al., 2001). Indeed, red deer antler formation requires partial demineralization of the skeleton (19–24% of the bone mass; Gómez et al., 2012) as the amount of minerals required for antler growth (13.7 g/day on average at peak deposition; Muir, Sykes, & Barrell, 1987) cannot be supplied exclusively via nutrition in such a short period of time (95% of final antler length is formed in a mean of 112 days; Muir et al., 1987). Due to the relationship between bone mass and antlers, any environmental factor affecting body growth, such as early life conditions, may in turn affect antler development during adulthood (red deer; Schmidt et al., 2001; Mysterud et al., 2005; Cappelli et al., 2017). In addition, red deer also allocate part of the daily intake into antler formation and therefore, any factor that affect per capita resource availability (i.e. climatological conditions, plant productivity or density) may affect antler growth (Mysterud et al., 2005; Landete-Castillejos et al., 2010).

Contrary to red deer, roe deer antlers are cast in late autumn and regrown during winter, being the only ungulate in the northern hemisphere presenting long-day active testicular function (Sempéré & Boissin, 1981; Fig. 1.3). Antlers are formed in 105 days approximately (60 days of growth and 45 days of mineralization; Sempéré & Boissin, 1981), being clean usually by early spring. As long day breeders, their antlers should be formed for the summer rut. However, antlers are fully formed several months earlier (Sempéré & Boissin, 1981) for the start of their territorial period (i.e. early spring). At this time, males have high levels of testosterone (Sempéré, Mauget, & Bubenik, 1982), which increases their aggressive behavior towards other males, defending their territories for a period of around 5-6 months (Johansson, 1996). During the territorial period, males use their antlers for rubbing (fraying) them against stems of trees or shrubs as visual marking behavior, but also as scent mark as they have skin glands in the head (Johansson, Liberg, & Wahlström, 1995). Whenever there is an interaction between males, antlers are first used as indicators of rank or fighting ability before engaging in any aggression (Hoem et al., 2007). Most encounters resolve with no actual fight and only if the males are very similar in body or antler size they are used as weapons during fights (Hoem et al., 2007). In contrast to other cervids, roe deer antler size has been described to vary very little during the adult stage (i.e. without accounting for yearling and senescent stages; Vanpé et al., 2007). Environmental conditions such as high density or poor climatic conditions had, in general, little effect on intra-individual antler size variation during adulthood (Vanpé et al., 2007). In fact, although some studies observed an influence of environmental conditions on roe deer antlers (Pélabon & van Breukelen, 1998), these effects are likely reflecting early life environmental effects on body size rather than the impact of yearly environmental conditions on energy allocation to antler growth (Vanpé et al., 2007). Therefore, roe deer antler size has been related to male phenotypic quality rather than condition (note that phenotypic quality in this context is a time-invariant attribute linked to average adult body mass; Toïgo et al., 2006; Vanpé et al., 2007).

Finally, while red and roe deer antlers have been widely studied in northern regions, more information is needed to understand the individual and environmental factors that influence antler growth in Mediterranean environments (but see Gaspar-López et al., 2010; Gómez et al., 2012; Cappelli et al., 2017) where the environmental conditions are characterized by long dry summers (the limiting season) and mild winter with greater food availability as compared to temperate regions.

Trade-offs in antler formation

As antlers are costly to produce, main trade-offs described between allocation to antler growth and other vital functions are: 1) early life antler growth vs. body growth and 2) antler growth vs. survival. Indeed, it has been hypothesized that if males invest much on antlers early in life (i.e. while they are still growing), this investment would influence body growth and hence, body size during adulthood (Metcalf & Monaghan, 2003; Lee, Monaghan, & Metcalfe, 2013). In addition, it is also expected that males carrying larger antlers with more points during early life would show increased senescence in reproductive traits later in life (disposable soma theory of ageing; Kirkwood & Holliday, 1979).

However, the hypothesis of an existing cost of growing large antlers during early in life has been challenged for both species (Lemaître et al., 2014 on red deer; Lemaître et al., 2018 on roe deer). Red deer between 4 and 9 years old showed no detrimental effects of antler allocation on later antler size (Lemaître et al., 2014) while, roe deer showed a positive relationship between absolute and relative antler investment during early life and adult body size (note that “relative antler size” refers to the antler length relative to individual body mass; Lemaître et al., 2018). As roe deer body mass has been described as a proxy of individual fitness, early life antler investment acts as a sign of male phenotypic quality (Lemaître et al., 2018). However, while cervid antlers have been widely studied in northern regions, more information is needed to understand the individual and

environmental factors that influence antler growth in Mediterranean environments (but see Gaspar-López et al., 2010; Gómez et al., 2012; Cappelli et al., 2020) as trade-offs may be more easily detected at the edge of a species range of distribution (Kawecki, 2008).

2 SPECIES AND STUDY SITE

2.1 The red deer and the roe deer. Comparison of our model species.

Throughout the introduction, we have described the main characteristics of our model species, the red deer and the roe deer, and highlighted their main differences between their reproductive cycle, reproductive investment strategies, mating system and energy allocation to secondary sexual characters. This section aims to summarize and compare the main characteristics of each species (Table 2.1) and to briefly explain how all these characteristics are intrinsically interrelated.

If we compare both species in terms of body size, the red deer is a larger species than the roe deer but also displays higher sexual size dimorphism (SSD). This interspecific difference in SSD can be explained by their relative position along the **continuum of opportunity for sexual selection** (Gaillard, 1988). The red deer, which inhabits open areas where females aggregate in larger groups, is a strongly polygynous species that has a high opportunity for sexual selection (Carranza, 2000). Thus, red deer display high SSD, comparatively higher investment on sexual secondary characters and a female defense strategy (Table 2.1). On the other hand, roe deer that live in forest environments where females are dispersed, have developed a territory defense strategy and an oligogynous mating system (i.e. nearest to monogamy). Thus, as they face lower opportunity for sexual selection, they display lower SSD (i.e. closer to monomorphism) and lower investment on antlers (Table 2.1).

Similarly, if we place both species along the **fast-slow continuum of life-history strategies** (Gaillard et al., 1989; Ricklefs, 2000), red deer, the largest of both species, would be situated towards the slower side of the continuum while roe deer would occupy the faster side. Red deer investment in survival over

Comparison of species

reproduction is high, with relatively late mean age at primiparity (3 years), long generation times, low reproductive rates (monotocous species) and long lifespans (lives up to 15-18 years; Table 2.1). In contrast, the vast majority of roe deer females are fertilized as yearlings (Gaillard et al., 1992), are polytocous (give birth usually to one, two or even three fawns) and live on average 10 years. For both species, the inverse relationship between the level of SSD and litter size (Carranza, 1996) can be observed (i.e. red deer with high SSD and monotocous and roe deer with a low SSD and polytocous).

Finally, red deer reproductive cycle seems to be, in general, in accordance with most Northern-temperate deer and ungulate species while roe deer seem to be somewhat against the norm. For example, red deer are polyestrous short-day breeders with an autumnal rut while roe deer are monoestrous, long-day breeders with a summer rut and a unique strategy to delay parturition (embryonic diapause).

Throughout this dissertation, we will analyze the effects of environmental conditions on reproduction for each species separately but, in the discussion section, we will have the opportunity to analyze and compare how the main biological and reproductive differences described above may condition the response of each species to environmental changes.

Table 2.1 Summary of the main differences among the studied species.

	RED DEER	ROE DEER	REFERENCES
<u>HABITAT AND FOOD PREFERENCES</u>			
Habitat	Open /forest edge	Forested areas	Hofmann (1989)
Feeding strategy	Mixed feeder	Browser	Putman (1988)
<u>BODY MEASUREMENTS</u>			
Mean size female	100-150 kg*	20- 29 kg	Asher (2007); Gaillard et al. (2000a)
Mean size male	145-230 kg	20-30 kg	Whitehead (1950); Vanpé, (2007)
Sexual size Dimorphism (SSD)	High <i>Males 35% bigger</i>	Low <i>Males 10% bigger</i>	Andersen, Duncan and Linnell (1998); Vanpé (2007)
<u>LIVE-SPAN</u>	15-18 years	10 years. Max: 15 (males) – 18 (fem.)	Nussey et al. (2006); Plard (2014); Loison et al. (1999)
<u>REPRODUCTION</u>			
Investment strategy	Capital breeder	Income breeder	Festa-Bianchet et al. (1998); Andersen et al. (1998)
Breeding cycle	Short-day breeder	Long-day breeder	Sempéré and Boissin (1981); Lincoln and Short (1980)
Ovulatory cycle	Polyestrous	Monoestrus	Sadleir (1987); Hoffmann et al. (1978)
Gestation length	7.5 months	10 (5 months diapause)	Asher (2007); Short and Hay (1966)
Average age at primiparity	3 years	20 months (pregnant); 2 years (give birth)	Clutton-Brock et al. (1983); Moyes et al. (2009); Gaillard et al. (1992); Plard (2014)
N° offsprings per litter	1 fawn/litter (Monocotous)	1,2,3 fawns/litter (Polytocous)	Stubbe et al. (1982)
Fawn weight at birth	6.5 kg	1.6 kg	Clutton-Brock et al. (1982); Gaillard et al. (1993b)
Calves behaviour after birth	Hider (2 weeks)	Hider (1-2 months)	Espmark (1969); Linnell (1994); Mitchell (1971)
Mating system	Strongly polygynous	Oligogynous	Vanpé (2007)
Monopolization of mates (strategy)	Female defense (Harem-defense)*	Resource defense (Territorial)	Vanpé (2007)
Antler investment**	High (2.5-4.9 %)	Low (0.9-1.5%)	Huxley (1931)

* Note that the information provided here may change according to variations in latitude, elevation or other environmental parameters. We have included in the reference from which each value or piece of information was obtained. ** *Percentage of antler weight per kg of total body weight.*

2.2 Study sites and climatic conditions

This thesis was based on the analysis of different deer populations located in areas with highly contrasting environmental conditions, the **Mediterranean and the Alpine region**. Both regions have in common that they constitute the edge of both species range of distribution and hence, impose a severe environmental constraint on the life histories of these cervid species (Stearns, 1992). On the one hand, the Mediterranean environment of Spain constitutes the **southernmost latitudinal limit** of the species distribution (Figs. 1.1 and 2.1). On the other hand, alpine environments of Switzerland represent the **upper altitudinal limit** of the species. We will briefly describe the main climatic features of each environment and how they affect resource availability for deer.

Mediterranean environments

The main difference between Mediterranean environments and other temperate environments located at a more central range of the species is the distribution of resources throughout the year. Mediterranean climate is characterized by its hot and dry summer period and the high inter-annual variation in precipitation (low predictability). Thus, instead of presenting one major growing season (i.e. from spring to autumn) and a winter period of nutritional constraint, Mediterranean environments have two annual peaks in vegetative growth (a major one in spring and the other in autumn) and two periods of nutritional constraints (the summer drought and the end of winter; San Miguel, Pérez-Carral, & Roig, 1999; Table 2.2).

During spring and autumn, pastures provide a good source of high-quality food. However, the high inter-annual variation in rainfall quantity and spread (e.g. the date of the first autumnal rains or rainfall during winter-spring) produces a low predictability of plant biomass and quality production (San Miguel, 2001; Bugalho & Milne, 2003). In addition, during autumn, mast-seeding trees, mostly oaks (*Quercus* spp.), provide a high-energy source of food although highly irregular (San Miguel, Roig, & González, 2000). The Mediterranean summer impose a huge constraint in the reproductive cycle of deer as it is the period of highest nutritional constraint with most pastures withered and yet a time of high energy requirements for lactating females (San Miguel et al., 1999). Winters are also low in resources although, depending on the year and the degree of continentality, there could be some herbaceous pasture production during mild winters (San Miguel et al., 1999). Finally, contrary to other northern environments, evergreen shrubs also constitute an important and permanent source of low–medium food quality for deer, especially during the periods of nutritional constraint when high-quality food (fresh fodder or acorns) is scarce (San Miguel et al., 1999). As a consequence, the number of deer that some Mediterranean environments can support might be higher compared to other more temperate or Eurosiberian regions and thus population densities reported in Mediterranean areas are typically higher (Acevedo et al., 2008). However, the combined effect of high densities and increase browse intake during the periods of nutritional constraints (Bugalho & Milne, 2003) can cause serious problems of regeneration for some shrub and tree species (Perea, Girardello, & San Miguel, 2014).

Two of our study sites where located in Mediterranean environments: 1) Los Quintos de Mora (QM) situated in south-central Spain with continental Mediterranean climate and 2) Valsemana roe deer research station (VLSM) located in north-western Spain with sub-Mediterranean climate; Fig. 2.1; Table 2.2).

Figure 2.1 Study sites location and study species. Adapted from: European Environment Agency (www.eea.europa.eu). QM = Los Quintos de Mora; VLSM = Valsemana.

Alpine environments

Deer species living in alpine or sub-alpine environments have to cope with the multiple limiting factors imposed by the severity of winter, the limiting season (Schmidt, 1993). Compared with other temperate environments and similar to arctic environments, the period of plant growth in alpine environments is characterized by a delayed spring plant phenology, a short growing season length and a strong and fairly predictable seasonality in resource availability concentrated during spring and summer, when high-quality food is abundant (Suttie & Webster, 1995)

This short period of plant growth forces deer species to complete the breeding cycle rapidly and thus, calving seasons are expected to be brief, highly synchronized and precisely timed so that parturitions occur at the leading edge of the growing season to guarantee that fawns attain a minimum size before the onset of winter (Ofstedal, 1985). Throughout the winter, snow cover creates a shortage of quality food as low-lying plants are buried (Schmidt, 1993), snow depth increases the cost of locomotion (Parker, Robbins, & Hanley, 1984) and

low temperatures increase the cost of thermoregulation. Furthermore, deciduous plants are leafless and the nutritional quality of the remaining vegetation during winter is poor as plants have developed strategies to cope with the impact of climatic stress such as frost damage, wind desiccation and solar radiation (Bertrand, & Castonguay, 2003). Thus the available perennial plants such as conifers and cushion-like evergreen plants have reduced leaf/stem ratios, higher fiber content, lower protein content, and higher concentrations of plant secondary metabolites (Mooney, Winner, & Pell, 1991), which drastically reduces palatability (Sauvé & Côté, 2007). For deer, the strategy during winter is to conserve energy and minimize expenditures (Schmidt, 1993) although, under these extreme conditions, many individuals opt to migrate in elevation to avoid these severe effects (i.e. partial migration; Gaudry et al., 2015).

Finally, compared to Mediterranean environments, the high intra-annual variation in food availability of alpine habitats produces a bottleneck effect during winter with high mortality rates, which in turn translates into an overall lower carrying capacity of these areas (Bonardi et al., 2017).

Our alpine study site was situated in a very large area of Switzerland that included three regions known as the Swiss Plateau, Norther Swiss Alps and Eastern Central Alps (Table 2.2 for more details).

Table 2.2 Summary of the climatic features and vegetation characteristics of the study areas.

	Los Quintos de Mora	Valsemana Research Station	Switzerland
Study Area	6864 ha fenced	856 hectares fenced	~ 4 Million hectares unfenced
Elevation	740-1235 (m a.s.l.)	1040-1160 (m a.s.l.)	288-2366 (m a.s.l.)
Climate	Continental Mediterranean	Sub-Mediterranean	Humid Oceanic (SP); Oceanic (NA) and Continental (ECA)
Limiting season	Summer: 3-5 months of drought (May- September)	Summer: 2-3 months of drought (mid-June to mid-September)	Winter: 1-4 months snow cover ≥ 30 cm (December-March)
Mean Rainfall [Min; Max]	581 mm/year [236-945]	886 mm/year [637; 1281]	SP: 1117 [859; 1549] NA: 1556 [1213; 2011] ECA: 987 [708; 1607]
Annual T° [January; July]	14.7 °C [5.7; 25]	11.1 °C [3.3; 19.8]	SP: 9.6 [0.8; 18.6] NA: 2.4 [-1.7; 15.2] ECA: 6.6 [-6.7; 11.6]
Soils	Poor and acidic (pH $\approx$ 5.2) with a lithological substrate of quartzites and slates	Siliceous	
Vegetation • Dominant tree species • Shrub genus • Plantations	Mediterranean veg Sclerophyll/Evergreen • <i>Quercus ilex</i> • <i>Cistus</i> , <i>Rosmarinus</i> , <i>Rubus</i> , <i>Erica</i> , <i>Phillyrea</i> and <i>Cytisus</i> sp. • <i>Pinus pinea</i> and <i>Pinus pinaster</i>	Mediterranean vegetation • <i>Quercus pyrenaica</i> • <i>Adenocarpus</i> , <i>Cytisus</i> or <i>Genista</i> • <i>Pinus sylvestris</i> and <i>Pinus pinaster</i>	Temperate forest • Deciduous forests (oak, beech, maple, and chestnut) and coniferous forest (spruce, pine, and fir).
Annual variation in resource availability*

* Colors on each graph represent, by order of color: 1) late gestation period, 2) parturition timing and 3) lactation period for red deer (Quintos de Mora) and roe deer (Valsemana and Switzerland).

2.3 Data collection

From inter-specific level analysis to intra-individual analysis.

This thesis was based on the analysis of different databases derived from the long-term monitoring of three different deer populations: 1) a Mediterranean red deer population living in a public hunting state called “Los Quintos de Mora”, 2) a sub-Mediterranean roe deer population from the Valsemana research station and 3) a meta-population of roe deer living along a wide elevational gradient in Switzerland (See Fig. 2.2; Table 2.3).

Each population was studied or analyzed at a different scale in relation to the level of detail of information available (i.e. inter-specific level, inter-population level, population level, or intra-individual level) and thus, each database helped answer different types of research questions. Indeed, even though natural selection operates at individual level, life-history variation can be studied at very different scales, such as those described above (Stearns, 2000). Therefore, each scale gives information about different aspects of the species adaptation to the environment.

Collecting data on populations at a given level of detail is a decision that managers or scientific teams have to make based on a cost-benefit analysis where they balance the quality of the data obtained against its quantity, as they are usually inversely related (Cai & Zhu, 2015). Because deer are elusive animals, the access to individual-level data requires the capture of the animal, either alive or death, which can be costly in terms of resources and time. In this regard, any information that does not require handling animals may be more accessible. For example, the

collection of cast antlers is a non-invasive and cost-effective method to gather larger amount of information. However, when using this type of data there are also drawbacks because important information such as the age of the individual, its weight or size is missing. Thus, as certain life-history traits and reproductive traits (such as parturition date, litter size and antler investment) are modified by developmental stage or body condition, the inferences that can be made with this type of data are limited (Hewison, & Gaillard, 2001; Kruuk et al., 2002; Plard et al., 2014b). Throughout this thesis, we used different databases with different levels of detail to perform our analysis. For example, we used the results obtained on both deer species to perform an **interspecific** comparison to understand the causes of the different responses of each species to environmental changes (Discussion section). We also performed **inter-population** analyses to compare the variation of key life-history events such as parturition dates or litter size along latitudinal or elevational gradients (geographical variation; Study 2 and 4). Furthermore, we analyzed the **population-level** response to changes in the environment through time (temporal variation; Study 1, 3 and 5). Finally, we used **intra-individual level** data (i.e. repeated measurements on “marked” individuals throughout their lives) to, among others, understand main trade-offs between early life antler investment and adult quality (Study 6).

Figure 2.2 A) Red deer male with harem during the rut at Los Quintos de Mora (Author: Alfonso San Miguel), B) Oak stands *Quercus pyrenaica* in Valsemana (Author: Ramón Perea) and C) Roe deer female during winter in the Alps.

Table 2.3 Summary of the characteristics of the databases used.

	Los Quintos de Mora (Spain)		Valsemana (Spain)	«Rehkitzmarkierung Schweiz» project (Switzerland)		
Deer species	Iberian red deer (<i>Cervus elaphus hispanicus</i>)		Roe deer (<i>Capreolus Capreolus</i>)	Roe deer (<i>Capreolus Capreolus</i>)		
Study	Study 1	Study 5	Study 6	Study 2	Study 3	Study 4
Type of data	Population level	Population level	Intra-individual level	Population and inter-population	Population level	Population and inter-population
Sex studied	Females	Males	Males	Female and male	Female and male	Female and male
Method data collection (Dead/alive)	Culled females (dead)	Cast antler. Selective culled males (dead)	Net drive captures (alive)	Marked fawns (alive)	Marked fawns (alive)	Marked fawns (alive) Recaptured adults (dead/ hunting).
Years of study	12 years (2000-2011)	10 years (2002-2011)	14 years (2003-2017)	26 years (1989-2015)	45 years (1971-2015)	45 years (1971-2015)
Type of data and sample size	829 pregnant females	4756 cast antlers 2835 selective males*	488 males 1162 measured times 146 captured >2 times	7444 parturition dates	8986 parturition dates	4555 litters 563 adult recaptured
Measurement taken on the species	Body mass, age, conception date (fetal weight)	Antler measurements (see Table S5.5.1)	Body mass, body size (snout-tail length, cross height), age, antler measurements	Date marking and fawn age	Date marking and fawn age Vegetation height	Date marking and fawn age, adult body mass
Period of data collection	November-February	February-April	October-November	April-July	April-July	April-July
Environmental variables	Population density and sex ratio, Spring plant productivity (RBI) End of summer drought	Population density Autumn and Spring productivity (RBI) Acorn yield Male age structure		Spring plant phenology (SPP) Elevation	Growing season start (GSS) Flowering start (FS) Elevation	Elevation Land use (CLC)

* used to reconstruct population male age structure (data collected between years 2002-2016). RBI= Real bioclimatic index (Montero & González-Rebollar, 1974); SPP and FS = the date of first hay cut; GSS= calculated as the date after six consecutive days with mean daily temperatures above 5°C; CLC=Corine Land Cover.

3 OBJECTIVES AND HYPOTHESES

The main objective of this thesis is to provide new scientific knowledge about how environmental conditions affect red and roe deer reproductive traits at the edge of their distribution range (i.e. Mediterranean and Alpine ecosystems). Both ecosystems are among some of the most vulnerable habitats to the effects of rapid climate change. Yet, there is a lack of understanding of how deer populations may cope with the different limiting factors and selecting pressures imposed by these distinct habitats. Such knowledge is, however, necessary to elucidate how these species have evolved under sub-optimal climatic conditions and to predict population demographic changes and distribution shifts under future climate scenarios.

This thesis has been divided into three main chapters, including six research articles. Each chapter includes different studies that will help address the research questions. Finally, based on the current knowledge on both species, we have formulated the specific hypotheses for each study.

CHAPTER I aims to analyze how **environmental variation affects female reproductive timing of both species**, with a special focus on the implications for the species **in the context of climate change**. Therefore, we addressed the following research questions: *Which are the key environmental factors driving the time of breeding in Mediterranean and Alpine environments? Are these cervid species able to modify breeding timing in response to rapid changes in plant phenology or productivity? Will they be able to adapt to the new environmental conditions imposed by climate change?*

In our **Study 1**, we focused on a red deer population inhabiting a Mediterranean environment to study the environmental factors that influenced the inter-annual variation of conception dates in this region. As explained in the introduction, red deer parturition timing in northern temperate environments was influenced by many factors. Environmental conditions at birth (i.e. date of birth and density at birth) and yearly environmental variation (i.e. annual weather

conditions and density) are considered the most important. Other factors described were individual and population features such as age or male age structure. In Mediterranean environments, early life conditions influence hind pregnancy rates, creating cohort effects for those born at high density or after a severe drought year (Rodríguez-Hidalgo et al., 2010). Based on these findings, **we hypothesize** that, red deer conception dates in Mediterranean environments would be influenced by a mixture of climatic conditions (spring and end of summer conditions before ovulation) and population features (density and sex ratio). Individual features such as body mass and hind age would also play an important role. Finally, due to the high phenotypic plasticity of red deer breeding timing reported for northern environments, we expect to find a high inter-annual variation in conception dates for this capital breeder.

In **Studies 2 and 3**, we assessed the variation of roe deer parturition timing and synchrony. Firstly, we studied this variation along two different geographical gradients (latitude and elevation). As mentioned in the introduction, the timing of birth has been widely studied for other northern hemisphere ungulates, being delayed and more synchronized with increasing latitude. On the contrary, much less attention has been paid to the changes along the elevational gradient. As both gradients display similar environmental conditions (i.e. delayed plant phenology, shorter growing seasons and stronger seasonality in food availability), we hypothesized that roe deer births would be delayed and more synchronous with both, increasing latitude and elevation. Secondly, we studied the temporal variation of roe deer parturition timing over a period of 45 years to elucidate if they were able to adapt their breeding timing to the rapid changes in plant phenology/productivity occurring in Alpine environments. In a flat, lowland area of France, this income breeder showed a lack of phenotypic plasticity on this trait (Plard et al., 2014), with parturition dates highly repeatable for a given female over its lifetime (Plard et al., 2013). However, little is known about the response of species across large regions with highly heterogeneous topographies, which offer the possibility of tracking optimal environmental conditions by moving in

space along the elevational gradient (i.e. behavioral plasticity). Therefore, due to the lack of phenotypic plasticity of roe deer parturition dates, we hypothesize that the mismatch between roe deer parturitions and plant phenology will increase over the study period but also that this mismatch will be larger in low areas compared to higher elevations.

CHAPTER II aims to elucidate the **effect of environmental conditions** (latitude and elevation) **on female fecundity**. We assessed the following questions based on roe deer litter size data: *How do demographic parameters such as fecundity vary along the latitudinal and elevational gradient? Does fecundity vary along both gradients in the same direction?*

In **Study 4**, we hypothesized that litter size would increase with latitude, similarly to what has been observed for other northern-hemisphere ungulates. However, based on the results obtained from birds (the most widely studied group of species), we predicted a decrease in litter size with increasing elevation. We proposed this hypothesis based on the fact that both gradients differ substantially in the spatial scale at which environmental changes occur. Similar to birds, roe deer are highly mobile animals with high dispersal capacities and therefore, we hypothesized that gene flow at such a small scale could constrain selection for optimal litter size along the elevational gradient, or even, indirectly, constrain other life-history traits that influence litter size such as body size (Williams, 1966b).

CHAPTER III analyzes how **environmental conditions and individual factors affect the development of male secondary sexual traits** in Mediterranean environments. Two main research questions were posed: *Which are the key environmental factors that influence antler size in Mediterranean environments? Are there any trade-offs between early-life investment on antlers and future adult performance?*

The first research question was addressed using population-level data from red deer cast antlers and the second question was addressed using an intra-

individual level database of live-captured roe deer. Therefore, note that the results of each question would not apply to the other species. In our **study 5**, similar to red deer females, we hypothesized that the effects of early life conditions on body growth and yearly environmental conditions (food availability, climate and population density) would influence antler growth. However, as we based our inference on cast antlers for which we lacked important information of the male that carried them, such as age, body size or condition, we could not test the effects of early life conditions.

Finally, in our **study 6**, based on an intra-individual database of roe deer in Mediterranean environments, we tested whether there was a trade-off between early life investment on antlers and adult phenotypic quality in Mediterranean environments. Based on the results of previous studies performed in more northern roe deer populations, we predicted that there would be no negative consequence of a strong allocation to antlers as fawns on antler size during adulthood. Thus, fawn antler size would act as an honest signal of future phenotypic quality (Lemaître et al., 2018) even in highly variable Mediterranean environments.

4 RESULTS

CHAPTER I: Environmental variation and female reproductive timing in the context of climate change

Study 1: Peláez, M., San Miguel, A., Rodríguez-Vigal, C., & Perea, R. (2017)

Climate, female traits and population features as drivers of breeding timing in Mediterranean red deer populations

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Abstract

Understanding the factors that lead to variation in the timing of breeding in widespread species such as red deer (*Cervus elaphus*) is crucial to predict possible responses of wild populations to different climate scenarios. Here, we sought to further understand the causes of inter-annual variation in the reproduction timing of female deer in Mediterranean environments. An integrative approach was used to identify the relative importance of individual, population and climate traits in the date of conception of free-ranging deer, based on a dataset of 829 hinds culled during 12 years. We found that a population trait, density, was the most important factor explaining the variation in conception dates, with greater densities causing later conception dates. Body mass was the second in importance, with heavier females conceiving earlier than lighter ones. Almost equally important was the spring real bioclimatic index, a measure of plant productivity, causing later conception dates in the least productive springs (drier and hotter). Another climatic component, the end of summer drought, showed that the sooner the autumn arrives (greater rainfalls and cooler temperatures) the earlier the conception dates. Interestingly, age class was found to be a minor factor in determining conception date. Only older females (≥ 10 years old) conceived significantly later, suggesting reproductive senescence. This study highlights not only the importance of population and individual traits but also the influence of climatic parameters on the deer reproductive cycle in Mediterranean environments, giving valuable insight into how reproductive phenology may respond to seasonality and global climate changes.

INTRODUCTION

Seasonal reproduction is an evolutionary adaptation to variable but predictable environments observed in long-lived mammals at all latitudes (Lincoln & Short, 1980). However, among large herbivores, such as deer, the factors that lead to variation in breeding and reproduction dates are still far from clear (Nussey et al., 2006; English et al., 2012; Stopher et al., 2014). In fact, our understanding of the environmental factors (e.g. climate) that trigger or cue breeding phenology remains elusive, especially at extreme latitudes or in dry environments (Aung et al., 2001; Stokkan et al., 2007; Bronson, 2009; Dye et al., 2012). Similarly, individual or population features related, for instance, to body mass, age, density or sex ratio have also been reported to be important in driving reproduction dates (Taber, 1953; Mitchell & Lincoln, 1973; Guinness et al., 1978; Clutton-Brock et al., 1983; Noyes et al., 2002; Cook et al., 2004; Holand et al., 2004; Langvatn et al., 2004; Dye et al., 2012; Plard et al., 2014a). However, we still lack a more integrative approach that accounts for all climatic, population and individual traits which could help disentangle the relative importance of each in driving reproduction phenology.

As a short-day breeder, many deer species usually conceive in autumn and calve in early summer (Clutton-Brock, Guinness, & Albon, 1982). In cool temperate areas, red deer (*Cervus elaphus* L.) fawns are born at the most favorable time of the year when the constant availability of palatable food contributes to a high-protein diet (Lincoln & Guinness, 1973). Thus, the time of breeding (conception date) aligns parturition dates and lactation with periods of increased resource availability (Lincoln & Guinness, 1973) to maximize calves survival and to compensate for the high cost of lactation (Oftedal, 1985). In contrast, in European Mediterranean habitats, red deer births occur after the spring peak in pasture production (the green flush), and lactation extends over the 3–5 months of dry summer, the limiting season (Asher et al., 1996; San Miguel et al., 1999; Perea & Gil, 2014). In addition, the next rutting season starts while many calves

are still nursing, leaving the female deer (hinds) with little time to recover from the physical depletion of the nursing period (Bugalho & Milne, 2003; Landete-Castillejos et al., 2003).

In the temperate zone, red deer mating is rigidly controlled by photoperiod, which has been considered the principal factor influencing the timing of the rutting season (Jaczewski, 1954; Lincoln, 1998; Asher et al., 2011). In Mediterranean areas, it has been manifested in the popular literature that the onset of the deer breeding season is triggered, apart from the photoperiod, by environmental cues, such as the moon phase (although the theory has been repeatedly rejected; Moe et al., 2007; Dye et al., 2012) or the beginning of autumn rains, which to the extent of our knowledge, has not yet been scientifically tested on red deer (but see Fisher & Johnstone, 2002).

In addition, many authors of studies performed in temperate areas agree that other secondary factors related to individual female traits (e.g. age, body mass, body condition and breeding history) or population features (e.g. density and sex ratio) influence the sexual cycle of deer (Mitchell & Lincoln, 1973; Clutton-Brock et al., 1983). In Mediterranean unsupplemented populations, hind body mass (once controlled by age) has been linked mainly to early life-history traits (e.g. density and rainfall at the time of birth [Rodríguez-Hidalgo et al., 2010], birth date [Landete-Castillejos et al., 2001] and maternal quality [sensu Landete-Castillejos et al., 2005a]) rather than to current year conditions (Rodríguez-Hidalgo et al., 2010). Therefore, this long-lasting influence of birth conditions on the attainment of a certain body mass in Mediterranean environments may produce delayed quality effect on some reproductive traits (Rodríguez-Hidalgo et al., 2010). In contrast, in both Mediterranean and temperate areas, yearly variations in herd nutritional status (population level) have been linked to climatic variables (e.g. rainfall or temperatures at certain times of the year) and also to population features (mainly density) as both influence intraspecific competition for resources. Rainfall and temperatures shape the vegetative growth and determine the quality

and quantity of forage available, whereas density limits the share of resources per individual (Bonenfant et al., 2002; Martínez-Jauregui et al., 2009; Rodríguez-Hidalgo et al., 2010; Veeroja et al., 2013). Along with these variables, there are other population factors such as sex ratio or male age structure that might not have direct influence in the physical condition of the females but can have a long-term negative influence in deer population dynamics when not balanced (Noyes et al., 1996; Langvatn & Loison, 1999; Bonenfant et al., 2004).

Therefore, in this study, we aimed to integrate the influence of individual level factors (body mass and age class), population level factors (density and sex ratio) and climatic variables on the date of conception of a Mediterranean red deer population for a period of 12 years. We hypothesize that: (1) climate would play an important role in determining the inter-annual variations in conception dates in Mediterranean environments because of the high intra-annual and inter-annual variability in rainfall and greater irregularity in the availability of food resources, and (2) conception dates will also be influenced by individual and population features. We posit that the understanding of the different factors regulating the inter-annual variations in the timing of conception is crucial to detect possible shifts in population dynamics. In addition, as timing of breeding tends to respond rapidly to natural selection under variable climatic conditions (Coulson et al., 2003; Moyes et al., 2011; Stopher et al., 2014), a better understanding of the impact of environmental changes on reproduction phenology and individual fitness will allow us to better predict population dynamics under different climate and management scenarios.

MATERIALS AND METHODS

Study area and estate management

The study area is a public hunting estate called Los Quintos de Mora, located in Toledo Province, south-central Spain (39°26'N, 4°2'W). This fenced game estate comprises 6864 ha and elevation ranges from 740 to 1235 m a.s.l. The

climate is continental Mediterranean, characterized by 3–5 months of dry, hot summer (May–September) and high inter-annual variability of rainfall (from 200 mm to more than 1500 mm per year). Mean annual temperature is 14.7 °C. Temperatures are very high in summer (mean temperature of July 25 °C) and low in winter (mean temperature of January 5.7 °C), with frequent frosts from December to March. Soils are poor in nutrients and acidic ($\text{pH} \approx 5.2$) with a lithological substrate of quartzites and slates. The state is a hilly area dominated by Mediterranean vegetation. The most common tree species in this area is Holm oak (*Quercus ilex*), while other evergreen and deciduous oaks (*Quercus suber*, *Quercus faginea* and *Quercus pyrenaica*) are less predominant. These 4 species usually coexist with a wide variety of evergreen shrubs such as those belonging to the genera *Cistus*, *Rosmarinus*, *Rubus*, *Erica*, *Phillyrea* and *Cytisus*. There are also plantation areas with pine trees (*Pinus pinea* and *Pinus pinaster*). In the lower areas, there are scattered woody grasslands and croplands. Iberian red deer (*Cervus elaphus hispanicus*) is the main wild ungulate in the study area. Every year, approximately 210 ha (approximately 3% of the total estate area) of non-irrigated crops are sown to provide some food supply for wildlife in the 2 main periods of food shortage in continental Mediterranean environments (winter and summer; San Miguel et al., 1996). No food supplements are provided and animals move freely throughout the estate. Evergreen shrubs also constitute an important and permanent source of low–medium food quality for deer, especially in summer and winter when high-quality food (green grass or acorns) is scarce.

From 2000 to 2011 (study period) the 2 main management objectives were: (i) to reduce deer density to an average of 23–24 deer/100 ha and (ii) to approach sex ratio structure to 1:1. This was achieved by placing more emphasis on culling females than males (approximately 25% more females than males).

Data collection and study variables

In this study, we analyzed 829 pregnant hinds collected from November to February during a 12-year period. These females were a random subsample of

the population as no hunting selection by age, body size or condition was performed.

The response variable (conception date) was calculated using the fetal aging criteria (Huggett & Widdas, 1951). First, the uterine cavity from all hinds culled was explored to determine the presence of a fetus. For fetuses older than 34 days, weight was measured with a precision balance (younger fetuses usually have insignificant body mass; Mitchell & Lincoln, 1973). Second, fetus age (t) was calculated based on the formula described by Huggett and Widdas (1951):

$$t = t_0 + \frac{(W)^{\frac{1}{3}}}{a}$$

where W is the fetus weight, t_0 is the embryonic period when weight is considered insignificant, being calculated as 14.6% of the total gestation length and (a) the fetal growth rate. This formula has been adapted to Iberian red deer according to its mean gestation length (234.1 ± 0.7 days for Iberian red deer; García et al., 2006) and mean weight at birth, based on 139 Iberian red deer calves (8.12 kg; Carrión et al., 2008). In addition, as fetal growth rate in red deer may vary among females with different body condition in the last third of gestation, only fetuses younger than 150 days were included in the analysis (Scott et al., 2014). In this study, the formula used for the calculation of the fetus age was as follows:

$$t = 34.18 + \frac{(W)^{\frac{1}{3}}}{0.100}$$

Finally, by subtracting the fetus age from the harvesting date of the hind, the date of conception (*Julian date*) was obtained.

Subsequently, for each pregnant hind culled, 7 predictors were compiled: 2 individual-level factors (body mass and age class), 2 population level factors

(density and sex ratio) and 2 climatic variables (spring real bioclimatic index and end of summer drought).

Individual level factors

(1) Body mass (*Weight*): To be able to compare hinds at different stages of pregnancy, which might affect the data on body mass (Carrión et al., 2008), we subtracted 1.3 times the fetus weight from the hind body mass (Robbins & Robbins, 1979) to compensate for the enlargement of uterus, fluid and membrane weight. In addition, as in the study area female body mass increases from October to February (Martínez-Jauregui et al., 2009), we have also modeled weight fluctuation in relation to the number of days past 1 October up to the date of capture (controlling by age). Then, we used 1 January (approximately the midpoint of the culling period) as a reference date to control for variation in individual body mass.

(2) Age-class: Histological examinations of incisors were performed by either Matson's Lab (USA) or by staff at the Los Quintos de Mora's laboratory using cementum annuli counts. Hinds used in this study were all females found pregnant with ages ranging from 16 months (yearlings) to 15 years. For the following statistical analysis females were divided into 4 groups (yearlings = 1 year old, subadults = 2 years old, adults = 3–9 years old and senescent ≥ 10 years old). Grouping criteria followed that of Landete-Castillejos et al. (2004) to distinguish between yearlings, subadults and adults. However, we used a different threshold for the senescent group based on our own data, as we only detected a delay in the conception date from 10 years onwards.

Population level factors

Census data were collected every year in 2 consecutive days during the peak of the rut (end of September/beginning of October). The survey design comprised 9 systematically spaced transects (total transects length = 54.53 km and

mean transect length $\pm$ SE = 6.06 ± 0.36 km). Nine trained observers simultaneously started each transect 3 h before dusk when deer become more active and, thus, more visible (Carranza et al., 1991). The following data were recorded for each deer or deer group encountered: (i) perpendicular distances to the transect line; (ii) age class and sex grouped in 4 categories (fawns [with no sex information], young males [between 1 and 2 years], male adults [over 2 years] and female adults [over 1 year]); and (iii) vegetation type and (4) location. The total area surveyed was 21.47 km² and the sampling effort was 7.94 km/10 km².

(3) Density: Absolute density estimates and confidence intervals were computed according to distance sampling methodology using R software package “Distance” (Buckland et al., 2004). Data collected during the survey were post-stratified by habitat stratum due to the difficulty of designing a pre-stratified survey in Mediterranean mosaic landscape (Acevedo et al., 2008). Six different habitat strata were defined by combining: (i) vegetation structure (open habitats [dehesas], pine woodlands and Mediterranean scrublands); and (ii) topography (plains and mountain chains). Then, we fitted 3 detection functions (half-normal cosine, uniform cosine and hazard-rate with simple polynomial adjustment) to each habitat stratum and selected the best model based on the lowest Akaike information criterion (AIC). We also tested correlations between cluster size and distance from the transect line searching for cluster-size bias. When detected, regression techniques were used to determine an unbiased cluster size estimate for density calculations (Buckland et al., 2001). Year 2005 with no census data was calculated by averaging the previous and following year as the hind culling rate was very similar in the previous 4 years (274.25 ± 17.85 hinds/year [approximately 20% of the estimated hind population]).

(4) Sex ratio: The pre-hunt adult sex ratio was calculated based on census counts as the proportion of males per hind (all but fawns). We included yearling males (spikes and button males) because although they are unable to defend

harems during the peak rut (Clutton-Brock, Guinness, & Albon, 1982), they are fertile and, hence, they can mate (Lincoln, 1971).

Climatic variables

(5) Spring real bioclimatic index (*spring RBI*): Developed by Montero de Burgos and González-Rebollar (1974), it is an adaptation of the Ombrothermic diagrams described by Gaussen (1955) that measures monthly soil water availability using monthly precipitation and temperatures, soil water retention capacity and water runoff. Hence, it is a plant productivity index that determines the vegetative growth and, thus, is a proxy of resource availability for deer. We averaged RBI values of March and April (late gestation) and May and June (early lactation), which is most energetically demanding period for hinds. We excluded July and August as plant productivity during these months is negligible in Mediterranean environments. A positive value indicates good conditions for vegetative growth (precipitation and warm mean temperatures), whereas negative values indicate vegetative rest or little productivity, which are usually associated with low winter temperatures or limited water availability (drought). Meteorological data was obtained from a weather station located at Los Cortijos, 2 km south of the estate (Spanish National Meteorological Agency).

(6) End of summer drought (*PC1*): To test the popular belief that the rut starts with the first autumnal rains or with the reduction of summer temperatures we have selected 3 climatic variables for September. We chose September because it usually marks the transition between the long summer drought and the arrival of autumn in Mediterranean environments. Thus, we calculated all variables from 1 to 30 September. We used the following 3 climatic variables: (i) total amount of rainfall during September (ASR); (ii) mean September temperature (MST); and (iii) number of days elapsed from 1 September until the first rainfall greater than 5 mm (DESR). Hence, as it is common practice when multiple moderately correlated candidate variables are considered ($-0.67 < r < 0.62$), we calculated a synthetic variable combining the effects of our 3 variables by performing a

principal component analysis (PCA) and used the first principal component axis as it captured 72% of the total inertia. Coefficients of the dominant eigenvector (*PC1*) were 0.56 for ASR, -0.55 for MST and -0.61 for DESR. A high positive value of *PC1* indicates that precipitation started in early September, cumulative precipitations were higher and mean temperature was lower.

Statistical analysis

The response variable for the model was conception date (*Julian date*) and the predictors were: *age class*, *body mass* (*Weight*), *density*, spring real bioclimatic index (*spring RBI*) and end of summer drought (*PC1*). We also included in the maximal model the interactions between: (i) *age class* and *body mass* to assess whether a weight variation on hinds of different age classes affected conception dates differently; and (ii) the interaction between *density* and *spring RBI* to search for a density threshold under which the *spring RBI* effect on conception date could be minimized. Sex ratio was excluded from the analysis due to moderate correlation with density ($|r| = -0.51$; Dormann et al., 2013). Thus, only *density* was included in the final model (maximal model) because models with *density* as the predictor were better-fitting models (lower AIC and higher deviance) than those containing *sex ratio*. Pearson's χ^2 -test among the rest of independent variables resulted in weak correlations ($P < 0.001$; $R^2 < 0.09$) and the variation inflation factors (VIF) of the variables included in the model showed no signs of multicollinearity (maximum VIF = 1.15).

To quantify the effect size of covariates and obtain estimates relative to conception dates, we used generalized linear models (GLM, gamma errors and reciprocal link function, $n = 829$ hinds). We could not use linear models as the response variable was not normally distributed (Kolmogorov–Smirnov test, $D = 0.501$, $P < 0.001$).

With the intention of having easily interpretable main effects and model estimates, even when variables are involved in interactions, we centered all

independent variables to their mean value during the study period (Schielzeth, 2010).

All the analyses were performed using R 3.2.1 statistical software (www.r-project.org). We used the function “glm,” within the package “MASS,” to fit the model. We used a model averaging approach to compare and rank multiple candidate models containing predictor variables of interest based on the weighting provided by the Akaike information criterion (AIC; Burnham & Anderson, 2002). Therefore, a full array of models was performed using the function “dredge” in the package “MuMIn.” Based on the idea that there is no single best model but multiple competing models (Grueber et al., 2011), instead of selecting a single best model, we selected the top models, for which the accumulated sum was 95% of AIC weight. Afterwards, estimates of each predictor were averaged (using AIC weights) across the selected models that contain each predictor (Burnham & Anderson, 2002). Subsequently, residuals of the top model were plotted against the explanatory variables to detect nonlinear relationships.

In addition, a relative importance was assigned to each predictor based on the sum of model weights across all models containing that predictor. However, as weightings apply to each model as a whole rather than to individual variables, many variables contained in the top ranked models received a relative importance of near one. Therefore, to elucidate the proportion of independent variance explained by each predictor, we used a hierarchical partitioning approach (Chevan & Sutherland, 1991) for all predictors with a relative importance greater than 0.9, using the function “hier.part” of the R package “hier.part.”

Finally, to observe the unique effects of a single covariate on the response variable (controlling for all other covariates) we used the function “predict” within the package “MuMIn” and we made predictions based on the averaged model.

RESULTS

Throughout the 12 years of study, the average mean conception date was the 28 September (*Julian date* $\pm$ SD: 270.60 ± 12.11 ; all years pooled) and 80% of conception dates were concentrated over a period of 26 days. Hence, most conceptions occurred from mid-September to mid-October (Fig. 4.1.1). Inter-year variation in the mean conception date ranged from the 20 September in year 2010 (*Julian day* 263) to 9 October in year 2005 (day 282). On the other hand, within-year synchrony also varied; year 2010 was the most synchronous (80% of hind conceiving within 15 days) and year 2005 the least synchronous (34.6 days).

Figure 4.1.1 Inter-annual variation in conception dates (*Julian date* [JD]) in a Mediterranean red deer population over 12 years.

Within the subpopulation of females that successfully conceived, adult hinds were on average 16%, 8% and 3% heavier than yearlings, subadults and senescents, respectively (Table 4.1.1). Population density throughout the study years varied from 41.94 to 29.55 (number of deer/100 ha), with an overall decreasing tendency through time, as also occurred with *sex ratio* (Table 4.1.1 and Fig. 4.1.2a).

Table 4.1.1 Characteristics of pregnant red deer hinds, population features and the main weather conditions during the study period

Descriptor	
Mean pregnant hind body mass (kg $\pm$ SE)	74.30 $\pm$ 0.33
	Senescents = 74.13 $\pm$ 1.25; 95% CI = [54, 97]
	Adults = 76.11 $\pm$ 0.36; 95% CI = [60, 93]
Mean pregnant hind body mass by age class (kg $\pm$ SE) and the 95% confidence intervals	Subadults = 69.80 $\pm$ 0.81; 95% CI = [54, 90]
	Yearlings = 64.30 $\pm$ 1.02; 95% CI = [50, 75]
Mean red deer absolute density (No. of deer/100 ha $\pm$ SD)	35.17 $\pm$ 3.98; Range [29.55 (2011), 41.94 (2001)]
Mean sex ratio [(No. of males/female) $\pm$ SD]	0.81 $\pm$ 0.11; Range [0.71 (2004), 1.05 (2010)]
Mean spring real bioclimatic index $\pm$ SD	0.96 $\pm$ 0.45; Range [-0.20 (2005), 1.39 (2004)]
Mean end of summer drought (<i>PCl</i> $\pm$ SD)	-0.84 $\pm$ 1.61; Range [-2.70 (2004), 2.77 (2010)]
Data for the study period: 2000–2011. <i>N</i> = 829 hinds	

The real bioclimatic index showed no trend through the years although it presented great variability among years (Table 4.1.1 and Fig. 4.1.2b).

Figure 4.1.2 Observed (•) mean conception dates by year (*Julian date* [JD] $\pm$ SE) in relation to (a) yearly deer population estimates (density ($\square$) $\pm$ CV) and (b) spring climatic conditions (*spring RBI* ($\square$) $\pm$ SD). RBI, real bioclimatic index.

A set of 128 different models was obtained and ranked based on AIC weights (Table 4.1.2).

Table 4.1.2 Model comparison based on Akaike information criterion weights to analyze the variation on conception date

MODEL	k	LL	ΔAIC	w	Δw
<i>AGE_CLASS</i> + <i>WEIGHT</i> + <i>DENSITY</i> × <i>SPRING_RBI</i> + <i>PCI</i>	10	-3120.409	0.00	0.535	0.535
<i>AGE_CLASS</i> + <i>WEIGHT</i> + <i>DENSITY</i> + <i>SPRING_RBI</i> + <i>PCI</i>	9	-3122.559	2.30	0.169	0.704
<i>AGE_CLASS</i> × <i>WEIGHT</i> + <i>DENSITY</i> × <i>SPRING_RBI</i> + <i>PCI</i>	13	-3118.628	2.44	0.158	0.862
<i>AGE_CLASS</i> × <i>WEIGHT</i> + <i>DENSITY</i> + <i>SPRING_RBI</i> + <i>PCI</i>	12	-3120.750	4.68	0.051	0.913
<i>AGE_CLASS</i> + <i>WEIGHT</i> + <i>DENSITY</i> × <i>SPRING_RBI</i>	9	-3123.961	5.10	0.042	0.955
<i>WEIGHT</i> + <i>DENSITY</i> × <i>SPRING_RBI</i> + <i>PCI</i>	7	-3126.686	6.56	0.020	0.975
<i>AGE_CLASS</i> × <i>WEIGHT</i> + <i>DENSITY</i> × <i>SPRING_RBI</i>	12	-3122.263	7.71	0.011	0.986
<i>WEIGHT</i> + <i>DENSITY</i> + <i>SPRING_RBI</i> + <i>PCI</i>	6	-3128.859	8.90	0.006	0.992
<i>WEIGHT</i> + <i>DENSITY</i> × <i>SPRING_RBI</i>	6	-3129.293	9.77	0.004	0.996
<i>AGE_CLASS</i> + <i>WEIGHT</i> + <i>DENSITY</i> + <i>SPRING_RBI</i>	8	-3128.144	11.47	0.002	0.998
<i>AGE_CLASS</i> × <i>WEIGHT</i> + <i>DENSITY</i> + <i>SPRING_RBI</i>	11	-3126.495	14.17	<0.001	0.999

The maximal model included as explanatory variables: Interaction between *body mass* and *age class*, interaction between *density* and the real bioclimate index during spring (*Spring_RBI*) and a synthetic variable combining 3 measurement of rainfall and temperature prior the rut (*PCI*). $N = 128$ models. When the model contains an interaction, the variables involved are in bold. K indicates the number of model parameters. LL denote maximum log likelihood, ΔAIC is the difference in Akaike information criterion scores between 2 competing models, w is AIC weights and Δw is the cumulative AIC weight. Top model deviance (ranked 1) = 0.225.

The model that best described the variation in conception dates and had the strongest statistical support ($w = 0.535$) included the additive effects of *age class*, *weight*, end of summer drought (*PCI*) and the interaction between *density* and *spring RBI*. The subsequent model averaging assigned to all predictors (without interactions) a relative importance of 1 (based on the sum of model weights across

all models containing each predictor; Table 4.1.3). Subsequently, the outcomes of the hierarchical partitioning analysis showed that *density*, *hind body mass* and *spring RBI* explained 36.52%, 23.12% and 21.95% of the total variation, respectively (Table 4.1.3), whereas end of summer drought (*PC1*) and *age class* explained 9.87% and 8.51% each.

Table 4.1.3 Summary of the generalized linear model averaging to analyze the factors affecting conception date in red deer

Explanatory variable	Estimates	Standard error	P-value	Relative importance	Indep. effect
<i>Density</i>	-1.136×10^{-5}	1.565×10^{-6}	<0.001	1.00	36.52%
Body mass (<i>Weight</i>)	3.195×10^{-6}	6.434×10^{-7}	<0.001	1.00	23.12%
<i>Spring RBI</i>	8.605×10^{-5}	1.350×10^{-5}	<0.001	1.00	21.95%
End of summer drought (<i>PC1</i>)	1.083×10^{-5}	4.019×10^{-6}	0.007	1.00	9.87%
<i>Age class</i>				1.00	8.51%
Yearlings	-4.143×10^{-5}	3.033×10^{-5}	0.172		
Subadults	3.692×10^{-6}	1.544×10^{-5}	0.811		
Senescent	-4.915×10^{-5}	1.775×10^{-5}	0.006		
<i>Density</i> × <i>Spring RBI</i>	1.190×10^{-5}	5.878×10^{-6}	0.043	0.76	
(<i>Age class</i>) × (<i>Weight</i>)				0.23	
Yearlings × (<i>Weight</i>)	3.255×10^{-6}	2.923×10^{-6}	0.266		
Subadults × (<i>Weight</i>)	5.768×10^{-7}	1.659×10^{-6}	0.728		
Senescent × (<i>Weight</i>)	-2.109×10^{-6}	1.668×10^{-6}	0.206		

Relative importance was calculated by summing the Akaike information criterion weights across all models (>0.90 means a strong effect, 0.60–0.90 a moderate effect and <0.60 a weak effect). The independent effect percentage is a result of hierarchical partitioning of variables showing relative importance >0.9 and indicates the proportion of independent variance explained by each variable relative to the others (sums to 100%). Bold type indicates a significant effect ($P < 0.05$)

Therefore, density was the strongest variable explaining the variation in conception date. Estimates from the averaged model (when controlling for all the other variables) predict that the mean conception date for the year with lowest density (29.55 deer/100 ha) would be 10 days earlier than the mean conception date for the year with the highest (41.94 deer/100 ha; Table 4.1.3). The second most important variable explaining the variation in conception date was *body mass*, although it was relatively close to *spring RBI* (Table 4.1.3). Conception dates

strongly varied with body mass, showing a shift toward earlier dates as hind increased in weight (for each additional 5 kg body mass, hinds advanced their conception date approximately 1.1 days; Fig. 4.1.3a). Mean conception date also decreased with increasing spring real bioclimatic index, with a difference of 9 days between the year with the lowest RBI (2005) and the year with the highest (2004; Table 4.1.1). A high value in the predictor end of summer drought (*PC1*) (i.e. earlier and higher amount of rainfall during September and lower mean temperatures) produced earlier conception dates (approximately 4 days between the highest *PC1* and the lowest) and *age class* was the least important variable (8.51% of independent variance explained) as only the senescent group showed significantly later conception dates than the adult group and the subadult group (approximately 4 days later [Table 4.1.3 and Fig. 4.1.3b]).

Figure 4.1.3 Influence of individual traits on conception date (Julian date [JD] $\pm$ SE): (a) predicted relationship between body mass (10-kg intervals) and conception dates ($\pm$ SE) and (b) observed ($\bullet$) and predicted ($\square$) conception dates by age class.

Finally, *spring RBI* and *density* had a significant interactive effect (Table 4.1.3); RBI had a stronger effect on conception dates at higher densities in comparison to lower densities (Fig. 4.1.4).

Figure 4.1.4 Interactive effects of *spring* RBI and *density* on the date of conception in a red deer population of south-central Spain. Predicted conception dates are plotted (with 95% confidence intervals) against the variable *spring* RBI at high density (40 individuals/km²; black lines) and low density (30 individuals/km²; dashed lines). RBI, real bioclimatic index. JD, *Julian date*.

DISCUSSION

Our results indicate that all individual, population level and climatic predictors affected breeding phenology in a Mediterranean deer population. We found that the most important variable was a population level variable (*density*) followed by *body mass* and *spring* RBI (ranked second and third, respectively, and each explaining a similar amount of independent variance; Table 4.1.3). Finally, end of summer drought (*PCT*) and *age class* were less important variables, although they were statistically significant.

The high relative importance of *spring* RBI and *density* in determining conception date can be explained by the fact that both combined represent a good proxy of per capita resource availability (Langvatn et al., 1996). In Mediterranean environments, a high spring forage production (from March to June) is crucial for deer reproductive success because of the increase of energetic demand on hinds during late pregnancy and early lactation (Landete-Castillejos et al., 2003). In addition, in Mediterranean environments, characterized by long and dry summers, the importance of a high forage productivity during spring is greater than in other

temperate areas because it shortens the effects of the aestival drought and guarantees the hinds a minimum body condition during the lactation period (Martínez-Jauregui et al., 2009; Torres-Porras, Carranza, & Pérez-González, 2009). In contrast, population density is considered as an important parameter to estimate intra-specific competition for resources (Langvatn et al., 1996) and has been described as highly influencing breeding success (Albon et al., 1983; Forchhammer et al., 1998). Sex ratio could be another important population trait that was not fully evaluated (because of its correlation with density) but it should show a similar pattern to that found for deer density.

Importantly, we found an interactive effect of both *density* and *spring RBI* on conception date (Table 4.1.3). At high deer densities the model predicts 17-days delay in the mean conception date between the least and the most productive spring (the lowest and highest RBI, respectively). However, in the same weather scenario, with lower densities the influence of spring productivity would only be 5 days (Fig. 4.1.4). Therefore, in the current context of global change, we can expect an increasing delay of conception dates based on the additive effects of both climate change, predicting longer and more severe summer droughts (IPCC, 2007), and the land use change, favoring a clear increase in wild ungulate populations across the Northern Hemisphere (Côté et al., 2004; Acevedo et al., 2008; Perea et al., 2014). In Mediterranean environments, this delay in conception dates will potentially misalign, even more, parturition dates with the vegetation flush, leaving newly calved hinds with a short period of green quality food available before the onset of the summer drought. This could have consequences on calf survival and hind reproductive success the following season. This is in line with the results of Plard et al. (2014b) for roe deer (*Capreolus capreolus*), predicting an impact on fitness and population dynamics as spring phenology progressively advanced in France over the past 30 years. However, contrary to roe deer, red deer parturition date is not only driven by conception date but also by gestation length (García et al., 2006; Moyes et al., 2011). Hence, further investigations on Mediterranean populations are necessary to elucidate whether gestation length

can compensate for later conception dates and to what extent this compensation can impact fitness or population dynamics.

As expected, body mass was an important driver in determining conception dates, as has been previously recognized in other regions (Mitchel & Lincoln, 1973; Hamilton & Blaxter, 1980; Clutton-Brock et al., 1983). In Mediterranean environments, body mass is, indeed, highly related to the history of the individual and, therefore, it is a delayed density-dependence factor (Landete-Castillejos et al., 2001; Rodríguez-Hidalgo et al., 2010). Hence, our results support the assertion that early life-history traits in Mediterranean environments are very important in the life reproductive performance of hinds (Rodríguez-Hidalgo et al., 2010). However, when controlling for body mass, we only found significant differences between the senescent class and adult and subadult classes, but not between the other age classes (Fig. 4.1.3b). The explanation underlying this might be that differences in conception dates between yearlings, adults and subadults must be driven by body mass, independently from age class. In contrast, the delayed conception date of older hinds (independent from weight) suggests that reproductive senescence in this population occurred after 10 years old. In Scotland, Nussey et al. (2006) described similar senescence for maternal traits such as offspring birth weight and parturition dates in red deer hinds older than 9 years. We attribute this senescence in conception dates to the higher sensitivity of older hinds to environmental variations (Gaillard et al., 2000b), which may produce later ovulation dates and even lower ovulation rates within this group.

In addition, our results reveal that another climate component, end of summer drought (*PC1*), also affected the conception dates of red deer in Mediterranean environments, although it only explained 9.87% of independent variance (Table 4.1.3). Thus, years with earlier and higher levels of precipitations and lower temperatures before the rut slightly advanced conception dates (total difference of 4 days) compared with years with no rain at all or high mean

temperatures. However, this small influence contrasts with the popular belief, which seems to attribute a larger importance to this variable. We suspect the popular belief is based on male deer activity observations because their roaring seems to be more noticeable as temperatures are lower (e.g. after rainfall; Clutton-Brock, Guinness, & Albon, 1982; Douchard et al., 2013). Therefore, we can only speculate that: (i) the conception advance observed might be associated with higher male activity as vocalization, smell or visualization of preferred males can advance hind ovulation (McComb, 1987; Komers et al., 1999); (ii) rainfall at the end of summer (September) has been described as a crucial factor for acorn maturation (García-Mozo et al., 2012), allowing hinds to faster recover body condition and, hence, ovulation is advanced (Asher & Pollard, 2003); and (iii) rainfall or temperature act as a predictive cue to induce estrus along with photoperiod (Fisher & Johnstone, 2002; Wolcott et al., 2015; Kourkgy et al., 2016). Nevertheless, these hypotheses should be further explored in an experimental study. Therefore, as it was shown with *spring RBI*, we would expect that changes on the length of summer drought would delay the onset of autumn rainfalls (IPCC, 2007) and, thus, would also delay conception dates in Mediterranean red deer populations.

In conclusion, this study reveals that multiple factors interplay to drive the reproduction timing of red deer in Mediterranean environments. We posit that population level factors (mainly *density*), along with a combination of climatic factors (*spring RBI* and the end of summer drought) and individual level factors (e.g. *body mass* and *age class*) are essential in determining the time of conception. We want to highlight the importance of climatic parameters (*spring RBI* and end of summer drought) in Mediterranean environments as they may delay conception dates, producing misalignment of parturition with resources availability if the predicted future drought intensification materializes.

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**Large scale variation in birth timing and
synchrony along latitudinal and altitudinal
gradients of a large herbivore**

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Abstract

Hopkins' Bioclimatic Law predicts geographic patterns in phenological timing by establishing a correspondence between latitudinal and altitudinal gradients. First proposed for key phenological events of plants, such as leaf sprouting or flowering dates, this law has rarely been used to assess the geographical equivalence of key life-history traits of mammals. We hypothesize that (H1) parturition dates of European roe deer (*Capreolus capreolus*) are delayed and more synchronized at higher latitudes and altitudes, (H2) parturition timing varies along latitudinal and altitudinal gradients in a way that matches the Hopkins' Bioclimatic Law, and (H3) females adjust parturition timing to match the period of high energy demand with peak resource availability.

We used parturition dates of 7,444 European roe deer from Switzerland to assess altitudinal variation in birth timing and synchrony from 288 to 2,366 m a.s.l. We then performed a literature survey to compare altitudinal results with those from different populations along the species' latitudinal range of distribution. Finally, we performed spatial analysis combining our highly resolved altitudinal data on parturition dates with plant phenology data. As expected, parturition dates were delayed with increasing latitude and altitude. This delay matched the Bioclimatic Law, as the effect of 1° increase in latitude was similar to 120 m increase in altitude. However, while parturitions were more synchronized with increasing altitude, we did not detect any trend along the latitudinal gradient. Finally, plant phenology explained altitudinal variation in parturition timing better than a linear effect of altitude. Our findings clearly demonstrate the ability of a large herbivore to match parturition timing with phenological conditions across the altitudinal gradient, even at the smallest spatial scales.

INTRODUCTION

Climate induced variation in the seasonal availability of resources is known to influence individual life-history trajectories (Larter & Nagy, 2001; Ferguson, 2002). Populations of species distributed across large geographical ranges have to cope with multiple limiting factors of different magnitudes. Thus, key life-history traits can be expected to vary with climatic norms, readily approximated by latitude and altitude (Hopkins, 1938; Rutberg, 1987). Latitudinal and altitudinal patterns are comparable in the sense that harsher climatic conditions at one end of the gradients lead to stronger seasonality and fewer limiting factors (e.g. less predation and less disease) in these regions (Hopkins, 1938). Thus, Hopkin's Bioclimatic Law (Hopkins, 1938) links spatial patterns in phenological timing with changes in latitude and altitude (i.e. 1-degree increase in latitude corresponds to approximately 120 m increase in altitude). First established from observations of key phenological events for plants such as leaf sprouting or flowering dates, this law is assumed to influence traits such as the timing of reproduction in mammals, due to the high energetic needs of females during lactation (Oftedal, 1985). However, Hopkin's Law has rarely been used to explain the breeding patterns of mammals (but see Bunnell, 1982).

Large herbivores are suitable model species to study how climate drives and shapes life-history evolution because they are distributed over large areas covering a wide range of latitudes and altitudes, and consequently, growing conditions (Pagel, May, & Collie, 1991). They are also plant consumers, and thus vegetation phenology is likely to influence key life-history traits such as the timing and synchrony of reproduction (Loe et al., 2005). Large herbivores often match late gestation and early lactation, which are the most energy demanding stages of reproduction (Oftedal, 1985), with the onset of the growing season (Albon & Langvatn, 1992; Post, Boving, Pedersen, & MacArthur, 2003). Indeed, protein content and digestibility of graminoids and herbs are highest at early herbaceous development stage, and then display an exponential quality drop from the time of

snowmelt till the start of flowering, when fiber content dramatically increases (Albon & Langvatn, 1992). Therefore, a good match between birth timing and the peak availability of high-quality forage provides large herbivores with fitness advantages (Boyce, 1979). On the contrary, a mismatch could jeopardize offspring survival, thereby reducing annual reproductive success (Festa-Bianchet, 1988 on bighorn sheep, *Ovis canadensis*; Coulson, et al., 2003 on red deer, *Cervus elaphus*; Plard et al., 2014a on roe deer, *Capreolus capreolus*) or have legacy effects on the future reproductive performance of offspring (Clutton-Brock, Guinness, & Albon, 1983; Festa-Bianchet, Jorgenson, & Réale, 2000).

As the growing season of plants is delayed with increasing latitude or altitude, females of large herbivores delay their parturition to match the peak in vegetation quality and biomass (Weber, 1966; Bunnell, 1982; Linnell & Andersen, 1998a; Loe et al., 2005). Furthermore, the growing season also becomes shorter, which limits the time available for offspring to grow during the period of abundant forage (Ofstedal, 1985; Rutberg, 1987; Clutton-Brock, Albon, & Guinness, 1987). In addition, the time available for females to recover body condition after lactation and prior to the onset of winter is limited. Thus, a strong selection pressure against late births can be expected, which may lead to an increased birth synchrony (Sadleir, 1969; Rutberg, 1987; Gaillard et al., 1993b). Alternatively, the delayed and more synchronized births could be interpreted as a plastic response of females to the constraint imposed by the short growing season (Loe et al., 2005; Moyes et al., 2011).

However, although latitude and altitude can reliably predict plant phenology and thereby parturition dates at large spatial scales (Hopkins, 1938), the effect of latitude/altitude can be altered by local climatic conditions at finer scales. For example, the onset of the growing season occurs later in Arizona (34-35 °N) than in northern Utah (41-42° N; Stoner et al., 2016), which is opposite to the expected trend (Hopkins, 1938). This unusual pattern is caused by the different local climatic drivers that determine the onset of the growing season in

each region; first, the spring snowmelt at the northern latitudes and second, the summer thunderstorms (Monsoon rains) in the south. Furthermore, under this situation, mule deer (*Odocoileus hemionus*) parturition dates closely tracked the growing season at local scale rather than the expected latitudinal trend (Stoner et al., 2016). Similarly, in areas of complex topography, thermal inversion is a common phenomenon that disrupts the expected linear decrease in air temperature with increasing altitudes (Hopkins, 1938), producing delayed plant phenology at lower altitudes compared to higher altitudes (Beniston & Rebetez, 1996; Bendix, 2002). However, little is known about how local conditions across the altitudinal gradient would influence timing of parturition for large mammals. Furthermore, in this context, the altitudinal gradients differ from the latitudinal gradient mainly regarding the spatial scale at which environmental changes occur (i.e. meters vs. kilometers, respectively). Hence, across the altitudinal gradient, there is large variation in environmental conditions over short geographical distances. Therefore, if environmental changes occur at a smaller spatial scale than the scale at which individuals mix (i.e. through dispersal or migration), gene flow may impose a limit on local adaptation (Lenormand, 2002; Loe et al., 2005). Finally, most studies have focused on variation in parturition timing and synchrony along latitudinal gradients, whereas studies specifically addressing altitudinal variation in large herbivores (Weber, 1966 on white tailed deer; Bunnell, 1982; Thompson & Turner, 1982; Hass, 1997 on wild sheep, *Ovis* sp.) or other mammals are very scarce (Dunmire, 1960 on deer mouse, *Peromyscus maniculatus*; Zammuto & Millar, 1985 on ground squirrels, *Spermophilus columbianus*; Zhao, 1994 on Tibetan macaques, *Macaca thibetana*). On the other hand, altitudinal variation in reproduction has been widely studied in birds where results showed delayed and more synchronized laying dates with increasing altitude (i.e. Beldal et al., 1998; Fargallo, 2004; Both & te Marvelde, 2007; Bears, Martin, & White, 2009; López-López, García-Ripollés, & Urios, 2007).

Here, we used the European roe deer (*Capreolus capreolus*) as a model species to assess the link between birth timing and altitudinal and latitudinal

gradients. This species is widely distributed in western Eurasia (from Spain to northern Scandinavia) and has a large altitudinal range (from sea level up to 2,400 m a.s.l.; Lovari, et al., 2016). Roe deer are close to the income end of the Capital-Income breeder continuum (Andersen et al., 2000) because they use daily food intake instead of relying on stored energy reserves for reproduction (*sensu* Jönsson 1997). Furthermore, they are also concentrate selectors that need “high quality” diets because they are unable to digest plants with high fiber content (Hofmann, 1989). Therefore, due to their physiological constraints related to reproduction and feeding tactics, the need for matching parturition timing with the optimal time of resource availability is high for roe deer (Kerby & Post, 2013). Finally, females are monoestrous with a summer rut followed by an obligatory embryonic diapause until late December or early January, when blastocyst implantation occurs (Aitken, 1974). After a gestation period of about 5 months, they usually give birth to one or two (rarely three) fawns in spring (Aitken, 1974).

We used a highly resolved data set of female parturition dates along an altitudinal continuum and searched for published data on roe deer birth timing and synchrony of different populations across the latitudinal gradient in order to test the following hypotheses:

H1: Roe deer females give birth later and have more synchronized parturitions at higher latitudes and altitudes.

H2: According to the Bioclimatic Law (Hopkins, 1938), the rate of change of parturition timing is similar between the latitudinal and altitudinal gradients, being 1-degree increase in latitude equivalent to approximately 120 m increase in altitude.

H3: Plant phenology is a better predictor of parturition date than altitude because females should maximize resource availability to meet the high energy demand of late gestation and early lactation.

MATERIALS AND METHODS

Latitudinal data

To explore potential latitudinal trends in both roe deer birth timing and synchrony, we carried out a literature survey of reproductive traits across the whole range of the roe deer distribution (Fig. 4.2.1). For this purpose, we searched for publications reporting information on mean population birth dates and synchrony. Therefore, we entered the following keywords and operators into the Web of Science search engine: TS = (“*Capreolus capreolus*” SAME “roe deer”) AND TS = (“timing” OR “birth date” OR “parturition” OR “synchrony” OR “pregnancy”). A total of 128 peer-reviewed publications were obtained through this search (Supplementary material Fig. S4.2.1). However, after reading the title and abstract of all publications, we only found 15 research articles reporting roe deer mean birth date and/or birth synchrony.

Figure 4.2.1 Location of study sites (stars) retrieved from the literature review and used to assess latitudinal variation in birth timing and synchrony across Europe.

By scanning the citations in these articles, we were able to add another ten publications. When parturition date was assessed more than once for the same

study site, we recorded values based on the largest sample size or the longest period of data collection. Also, we only included articles that measured birth synchrony as the number of consecutive days over which 80% of the year's fawns were born because it is the most commonly used metric in studies of large herbivores (Rutberg, 1987). Additionally, we excluded publications that obtained data on birth phenology from captive females. Finally, we constructed a database including information about the country, latitude (from 40 – 64°N), birth date, synchrony, number of study years and study site altitude (classified into four altitudinal intervals of 500 meters: < 500, 500–1,000, 1,000–1,500 and > 1,500 m a.s.l.). We performed this classification because mean study site altitude was not reported in many studies (mainly those located in lowland areas), and therefore, an altitudinal interval was assigned based on topographical maps (see Supplementary material Table S4.2.2).

Altitudinal data

Study area

Roe deer altitudinal data was collected across three biogeographic regions of Switzerland, which encompassed the whole altitudinal and climatic range of the species in the country (Christen, Janko, & Rehnus, 2018). These adjacent and connected regions are, from north to south and from low to high altitude: the Swiss Plateau, the northern flank of the Alps (the Pre-Alps and the Northern Alps) and the Eastern Central Swiss Alps (Gonseth, et al., 2001; Fig. 4.2.2).

The Swiss Plateau (SP) is the region with the lowest altitudinal range. It forms a large basin that is partly flat but mostly hilly (mean altitude 546 m a.s.l.; ranging from 240 to 1,280 m a.s.l.; Fig. 4.2.2). The climate is humid oceanic (see Supplementary material Table S4.2.1 for more details). This region is separated from the Northern Alps by a sub-alpine mountain range called the Pre-Alps (PA; mean altitude 1,022 m; ranging from 381 to 2,361 m a.s.l.). From autumn to spring, thermal inversion commonly occurs in the Swiss Plateau and the Pre-Alps

because a lack of wind promotes the formation of a layer of fog or stratus clouds between 500 and 1,000 m a.s.l (Beniston & Rebetez, 1996). This causes a cold air pool under the fog or stratus that makes the Swiss Plateau and foothills of the Pre-alps to have often colder ambient temperatures at lower altitudes than at higher altitudes (Beniston & Rebetez, 1996; Bendix, 2002). To capture this phenomenon, we analyzed the Swiss Plateau and the Pre-Alps together (hereafter called SP/PA; Fig. 4.2.2).

Figure 4.2.2 Distribution of roe deer parturition events (grey dots) across the three biogeographical areas of Switzerland (SP/PA = Swiss Plateau and the Pre-Alps, NA = Northern Alps, ECA = Eastern Central Alps). Grey triangles represent location of phenological stations.

The Northern Alps (NA) is a high alpine mountain range located between the SP/PA and the Central Alps. It is cut by numerous deep valleys that feed into the Swiss Plateau, most of which have a north–south orientation. The mean altitude is 1,389 m a.s.l. and the climate is oceanic (Supplementary material Table S4.2.1).

The Eastern Central Swiss Alps (ECA) region has the largest altitudinal range of our study area (from 560 to 3,981 m a.s.l.), with a mean altitude of 2,111

Study 2

m a.s.l. It is characterized by a continental climate (Table S4.2.1). The steep mountain chains of the ECA are cut by deep glacial valleys that mostly run west to east.

Roe deer data

Roe deer data originated from the project «Rehkitzmarkierung Schweiz» (Roe deer marking Switzerland), which has been running since 1971 (Rehnus & Reimoser, 2014). Every year, roe deer fawns are systematically searched and marked across Switzerland. For each fawn marked, the following data were recorded; the date of capture, the estimated fawn age, its sex, and the geographic coordinates of its location. Fawn age was assessed using characteristics of the umbilical cord and fawn behavior at marking (Jullien, Delorme, & Gaillard, 1992; Rehnus, Arnold, Elliger, & Reimoser, 2018). Birth date was calculated by subtracting the estimated age of the fawn from the date of capture. To avoid pseudo-replication problems (*sensu* Hurlbert, 1984), individual fawns from the same litter were pooled within a single birth event, thereafter referred to as parturition date (expressed as day of year; DOY). Altitude was obtained from the geographic coordinates by superposing a digital terrain model (DTM) using only data that could be assigned to a spatial resolution of one hectare or less. We used data from the period between 1989 and 2015, when the dataset best represented the whole altitudinal range. Finally, we only included fawns aged ten days or less in the analyses because the accuracy of age assessment markedly decreases for older fawns (Jullien et al., 1992).

Plant phenology data

Phenological data was obtained from MeteoSwiss, which has a broad phenological network with 160 stations distributed throughout Switzerland. In particular, we used the date of first hay cut as a proxy of spring plant phenology (SPP). Hay is cut when the ratio of forage quantity/quality is at its maximum (just before flowering) to obtain high quality fodder. This phenological measure is a

good proxy to test our hypothesis for several reasons: 1) it covers the full altitudinal range of our study data, 2) it represents the phenological state of the plant community, not just of a single species, 3) herbaceous plants are very sensitive to temperature (Hemming & Trevaskis, 2011), 4) meadows constitute an important source of food for roe deer during the breeding season and are actively selected by females (Tixier & Duncan, 1996; Dupke et al., 2017) and 5) female productivity is known to increase in these type of open landscapes (Hewison et al., 2009; Gaillard et al., 2013). Similarly, previous studies on roe deer used date of flowering as a proxy of spring plant phenology (Plard et al., 2014a).

In the study region, 105 phenological stations reported hay cut dates. However, we only selected stations with at least 10 years of phenological recordings. Furthermore, we imposed the restriction of having a minimum of five years of phenological information during the first half of the study period (1989-2002) and five years during the second half (2003-2015). The reason behind this restriction was to avoid a bias as plant phenology has advanced during the study period in response to climate change (Güsewell, et al., 2018). Finally, for each station, we calculated the mean date of hay cut between these two periods. This process resulted in the selection of 86 phenological stations distributed across the study area from 330 to 1,800 m a.s.l. (see Fig. 4.2.2).

Latitudinal analysis

Linear regressions were fitted to explore the effect of latitude (predictor) in both birth timing and birth synchrony (response variables; H1). In addition, study site altitude (classified into 500 m intervals) was included as an explanatory variable to control for altitudinal variation among the different study sites. When the mean birth date was not available for a study site ($N=2$), we used the median birth date. As female roe deer are monoestrous, their parturition dates are normally distributed and thus, the mean and the median are very close (Gaillard et al., 1993b; Supplementary material Table S4.2.2).

Altitudinal analysis

To assess variation in birth timing and synchrony in relation to changes in altitude (H1), we analyzed data from 7,444 parturition events (1,745 in SP/PA, 1,418 in NA and 4,281 in ECA).

Variation in parturition date in relation to altitude

We used spatial linear mixed models (SLMMs) to assess relationships between parturition timing (response variable) and altitude while accounting for spatial autocorrelation. We also included the interaction between altitude and the factor region to control for differences between areas. We used the function *corrHLfit* within the *spaMM* package (Rousset & Ferdy, 2014) using R (Version 3.4.3; R Core Team, 2016). X-Y coordinates of each parturition event were included as a spatial random effect in a Matérn correlation model, and fawn cohort nested within region was also included as a random effect. We assumed that spatial autocorrelation among locations declined exponentially with distance by fixing *nu* at 0.5 and used Gaussian family errors.

In addition to the SLMM analysis, we also applied different statistical methods to evaluate the robustness of the results. Therefore, we used linear mixed models (*lme* function from the package “nlme”) and ordinary linear regressions (function *lm* from the “stats” R package) on our complete dataset (N=7,444). Furthermore, we also performed LM and LMM using a reduced dataset (N=2,018) on which a spatial filtering approach was applied to account for spatial autocorrelation and to homogenize sampling intensity by using the function *ecospat.mindist* within the R package “ecospat” (Broennimann et al., 2018). We set this function to select randomly parturitions separated by a minimum of 667 meters, equivalent to the mean annual home range of roe deer females calculated from twelve GPS-collared roe deer in the Swiss Plateau (44.5 ha; Ineichen, 2015). Finally, for both LMM (i.e. with and without spatial filtering), we included fawn cohort nested within region as a random effect.

Finally, we compared each region's results (SLMM, LM, LMM and spatial filtering approach) and assessed whether there were differences in the slope among regions. If there were differences, we studied the area separately to check for non-linearity between parturition date and altitude by fitting GAMMs models, using the function *gam* from the “mgcv” R package. The flexibility of these models, associated with a smoothing spline, allowed us to explore the data to determine the functional form of the relationship. We used the effective degrees of freedom (edf) resulting from the GAMMs to obtain the best fitting function.

Variation in birth synchrony in relation to altitude

To assess birth synchrony, we first divided the whole dataset (N=7,444) into four altitudinal quartiles (~1,860 parturitions each; (288,786]; (786,1232]; (1232,1564; (1564,2366]). Similarly, we generated three separate datasets, one per region (SP/PA, NA and ECA), and distributed parturition dates into 250 m altitude intervals (Supplementary material Fig. S4.2.2). Then, we calculated, for each dataset and altitudinal quartile or interval (250 m), the period when 80% of births occurred to compare our results with those obtained from other roe deer populations across Europe. However, we only calculated birth synchrony and mean parturition date when the number of parturition events was ≥ 180 per altitudinal interval and region, which corresponded to an accuracy of ± 1.5 days for estimation of the mean (See Supplementary material Fig. S4.2.3). We estimated this threshold using a sequential sampling approach by using the R “Resampling” package (Schönbrodt & Perugini, 2013; Hehman et al., 2018). Two main concepts were used for this calculation: 1) “corridor of stability” (COS), defined as the acceptable variation of the mean that could be assumed for the scale being used; and 2) “point of stability” (POS), defined as the sample size at which 95% of averages do not exceed the boundaries of the COS (Supplementary material Fig. S4.2.3). We resampled, for each region, 2,000 times the sample size (N) and calculated the POS at different COS levels (± 1 , ± 1.5 and ± 2 days).

In addition, for each of the four datasets described above, we tested whether the variance of parturition dates changed with increasing altitude. Therefore, at each altitudinal quartile or interval, we calculated the log-likelihood of the distribution of parturition dates under a normal distribution with a specified mean and variance. The log-likelihood of all the data can then be calculated as the sum of these log-likelihoods. We then used the subplex algorithm to find the maximum likelihood intercept and altitudinal slope for the mean and the variance. To assess whether the altitudinal gradient in the variance was statistically significant, we conducted a likelihood ratio test by comparing the maximum log-likelihood of the full model to a model with the altitudinal slope of the variance forced to 0.

Phenological analysis across the altitudinal gradient

To assess whether parturition date was better correlated to altitude or plant phenology (H3), we firstly assigned to each parturition location the station with highest probability of presenting similar phenological conditions. Therefore, we selected from all the phenological stations located within a 10 km distance of each litter, the one that was at most similar altitude (within an altitudinal interval of ± 100 m; Supplementary material Fig. S4.2.4). Unfortunately, for some litters, no station met these characteristics. This resulted in a subset of 1691 parturitions events and 60 phenological stations (Supplementary material Fig. S4.2.4).

Then, we performed a linear mixed model (LMM) with parturition date as response variable, altitude and spring plant phenology (the date of first cut of hay) as predictors and fawn cohort as random factor. Firstly, however, we performed Pearson correlation tests between fawn altitude and plant phenology to assess collinearity problems as both predictors are expected to be correlated. Pearson correlations for the whole study area and for two of the regions separately were indeed high between altitude and plant phenology (whole study site: $r=0.95$; $P<0.001$; within NA region: $r=0.79$, $P<0.001$; within ECA region: $r=0.91$; $P<0.001$). However, in the SP/PA both predictors were not correlated ($r=0.06$,

$P=0.192$), which offered a unique opportunity to test the hypothesis 3. Therefore, as altitude and plant phenology were strongly associated in NA and ECA regions, we only performed LMM for the SP/PA region by using a subset of 410 parturitions events and 27 phenological stations (Supplementary material Fig. S4.2.4) to avoid multicollinearity (Dormann et al., 2013).

RESULTS

Latitudinal results

From the literature review, we retrieved 19 articles encompassing 20 study sites for which mean birth dates were reported and 11 articles from 11 study sites for which birth synchrony was reported. These studies covered most of the range of roe deer distribution, from Spain (40°N) to Norway (64°N; Fig. 4.2.1; Supplementary material Table S4.2.2).

The selected model revealed a delay in the date of parturition with increasing latitude (0.72 ± 0.30 days per degree of increase in latitude, $P=0.027$; $N=20$; Fig. 4.2.3a), which resulted in a difference of 18 days in mean parturition date between the sites at the lowest and highest latitudes (40°N – 64°N). Along the latitudinal gradient, parturition date also seemed to be delayed with increasing altitude. However, due to the small number of observations, we only used this factor as a control variable (Supplementary material Table S4.2.3) and did not interpret the estimate. Birth synchrony did not show any detectable trend across the geographical distribution of roe deer ($P=0.505$, Fig. 4.2.3b; Table S4.2.3).

Figure 4.2.3 a) Roe deer mean (or median) birth date along the latitudinal gradient controlling for altitude and b) differences in roe deer birth synchrony with latitude. Dots represent observations from each study site, solid lines indicate results from linear regressions and dashed lines represent 95% confidence intervals. Colors indicate the altitudinal range of each study site: black (<500 m a.s.l.), grey (500-1000 m a.s.l.) and light grey (>1500 m a.s.l.).

However, birth synchrony markedly varied among populations, with 80% of females giving birth within only 17 days in the most synchronous population (Apennines, Italy) vs. 30 days in the least synchronous one (Kalo in Denmark and Aurignac in France; Supplementary material Table S4.2.2). Unfortunately, owing to the small number of studies reporting roe deer birth synchrony (N=11), we could not control for the possible confounding effect of altitudinal variation.

Altitudinal results

Variation in parturition date in relation to altitude across Switzerland

The SLMM analysis encompassing all regions of Switzerland revealed an overall delay in mean parturition date with increasing altitude (Fig. 4.2.4a). Across the three regions, SLMM predicted a delay of mean birth date from day 143 (23 May) at 450 m a.s.l. in the SP/PA region to day 160 (9 June) at 2,100 m a.s.l. in

the Eastern Central Alps, which corresponds to a 17-day delay between the lowest and highest altitudes (99% of fawn locations were between 431 m and 2,080 m a.s.l.).

Figure 4.2.4 a) Date of parturition of roe deer along the altitudinal gradient by region (dots; Swiss Plateau and the Pre-Alps (SP/PA), N=1,745; Northern Alps (NA), N=1,418; and Eastern Central Alps (ECA), N=4,281). Solid lines represent predicted mean birth dates from the SLM model for each region. b) Density plot displaying birth synchrony for each altitudinal range and region.

Furthermore, SLMMs showed similar altitudinal patterns in parturition timing within NA and ECA regions, with a delay of 0.65 ± 0.07 days per each 100 m increase in altitude ($t=8.407$) in ECA and of 0.75 ± 0.12 days per 100 m ($t=6.443$) in NA (Fig. 4.2.4a). Among these regions, there were no detectable differences in the slope or the intercept (Supplementary material Table S4.2.4). The other models fitted (LM, LMM and spatial filtering method) all gave very similar estimates for the relationship between altitude and birth phenology (see Table S4.2.4).

A non-statistically significant trend occurred between parturition date and altitude in SP/PA region (0.40 ± 0.23 days per 100 m increase in altitude; $t=1.752$; Fig 4a; Table S4.2.4). Furthermore, the estimates of the altitudinal variation in the

SP/NA were not as stable as in NA and ECA when fitting different models (Table S4.2.4). Parturition dates did not detectably change with altitude or even tended, surprisingly, to be earlier with increasing altitude (for LM and LMM; Table S4.2.4). We investigated the causes of these inconsistent results by fitting GAM models only to the SP/PA region. Results revealed a more complex (cubic) relationship between parturition date and altitude (edf=3.29; $P=0.02$; Supplementary material Fig. S4.2.5). This result indicates that parturition timing did not vary with altitude between ~500–800 meters or even tended to advance with increasing altitude.

Variation in birth synchrony in relation to altitude across Switzerland

Parturition dates were not only delayed with increasing altitude but also more synchronized (Table S4.2.5). For each altitudinal quartile, a total of ~1,860 parturition events were available. In the lowest altitudinal quartile [288–786 m a.s.l.] 80% of parturitions took place within 26 days, whereas in the highest altitudinal quartile (1,564–2,366 m a.s.l.) 80% of parturitions occurred within only 20 days, which corresponds to a 30% increase in birth synchrony (Supplementary material Table S4.2.5; Fig. S4.2.2). Likelihood ratio tests indicated that there was an altitudinal gradient in the variance of parturition date as statistically significant differences were found when comparing the maximum log-likelihood of the full model with a model where the altitudinal slope in variance was forced to 0 (Likelihood Ratio Test (LRT) =77.73; $P<0.001$).

However, region-specific analyses showed mixed results (Fig. 4.2.4b; S4.2.2). With increasing altitude, variance in parturition date (i.e. birth synchrony) showed no detectable differences in SP/PA and ECA regions ($P=0.219$ and $P=0.889$, respectively), but statistically significant differences occurred in NA ($P=0.001$).

Comparison among altitudinal and latitudinal gradients

Hopkin's Bioclimatic Law (Hopkins, 1938) linked spatial patterns in phenological timing with changes in latitude and altitude with a 1-degree increase in latitude analogous to approximately 120 m increase in altitude (H2). Latitudinal results indicated a delay of parturition date of 0.72 days with each degree increase north (while holding altitude constant). If we adapt our altitudinal results to the scale described by the Bioclimatic law, parturition date was delayed by 0.78 and 0.90 days per each 120 m increase in altitude for the NA and ECA regions, respectively. Finally, in the latitudinal gradient, there was a 19-day difference between the lowest and highest latitude (from 40°N – 64°N) while in the altitudinal gradient this difference was 17 days (431 m and 2,080 m a.s.l.).

Phenological analysis across the altitudinal gradient

Results of the LMM performed in the SP/PA region supported our hypothesis (H3) that parturition date was better correlated to spring plant phenology than to altitude (Fig. 4.2.5). The full model indicated a marked delay of parturition date with delayed spring plant phenology (0.29 ± 0.08 days/day in SPP; $t=3.527$; $P<0.001$) but found no detectable effect of altitude in the SP/PA region ($t=-1.605$; $P=0.109$; see Supplementary material Table S4.2.6). The variation inflation value (VIFs) for this model was 1.004, which is below the suggested threshold of 4 that indicates autocorrelation problems (Neter et al., 1996).

Figure 4.2.5 Relationship between: a) Spring plant phenology (SPP) and parturition date and b) altitude and parturition date in the Swiss Plateau and the Pre-Alps (SP/PA).

DISCUSSION

Our study provides clear evidence from a large herbivore that parturition timing is progressively adjusted along the altitudinal and latitudinal gradients, in support of H1. Further, we identified a clear trend towards more synchronized parturitions along the altitudinal continuum, but failed to detect an increased birth synchrony with latitude. In addition, we demonstrated that Hopkins' Bioclimatic Law can also be applied to the breeding phenology of a large herbivore (H2), as the observed rate of change in parturition timing was similar with both, 120 meters increase in altitude and one-degree increase in latitude.

The lack of correlation between spring plant phenology and altitude in one region of Switzerland allowed us to test our hypothesis (H3) that plant phenology would be a better predictor of parturition date than altitude itself. As explained above, in this region the frequent fog during spring and autumn disrupts the expected linear decrease in temperature with increasing altitude, producing delayed plant phenology at lower altitudes compared to higher altitudes (Beniston

& Rebetez, 1996). Roe deer inhabiting this region matched the specific phenological trend caused by these local climatic conditions, which supports our hypothesis (H3) that females give birth at the time of maximum forage availability and quality, thereby optimizing fawn growth and survival (Clutton-Brock et al., 1987; Festa-Bianchet, 1988; Keech et al., 2000; Coulson et al., 2003; Plard et al., 2014a). Therefore, we could demonstrate that plant phenology, rather than altitude per se, drives the timing of births in roe deer. Plant phenology has also been reported as playing a major role in birth timing of other large herbivore species (Bunnell, 1982 on wild sheep; Stoner et al., 2016 on mule deer). However, being an income breeder and concentrate selector (Andersen et al., 2000; Hofmann, 1989), roe deer are expected to be especially sensitive to the optimal timing for resource availability (Plard et al., 2014a). Therefore, further research should investigate how large herbivores with different reproductive or feeding tactics, such as capital breeders or grazers, respond to small scale changes in plant phenology along the altitudinal gradient.

Surprisingly, we could only support hypothesis H1, that populations experiencing the most pronounced seasonality would display the highest birth synchrony, for the altitudinal gradient, but not so for the latitudinal gradient. The small number of studies reporting population birth synchrony across Europe ($n=11$) may be one reason for it. However, other studies on large herbivores similarly failed to detect a latitudinal trend in birth synchrony (Sigouin, Ouellet, & Courtois, 1995 on moose; Linnell & Andersen, 1998a on roe deer; Loe et al., 2005 on red deer). Loe et al., (2005) proposed that confounding effects of variation in population sex and age structures and in environmental factors, such as altitudinal variation, might explain this lack of a latitudinal pattern. Indeed, our findings clearly demonstrate that altitude is a key factor determining birth synchrony, and hence variation in altitude should always be accounted for when studying latitudinal variation of life-history traits. Furthermore, in the highest altitude region (Eastern Central Alps), birth remained highly synchronized throughout the whole region without showing an altitudinal trend even though parturition timing

matched the delay in plant phenology. This finding may suggest that the mean regional altitude should be controlled for, in addition to absolute altitude, to determine reliably the pattern of birth synchrony (see also Loe et al., 2005). Finally, collecting a large number of parturitions is of paramount importance for accurately assessing birth synchrony. Some of the studies included in the latitudinal analysis were based on quite small sample sizes compared to our proposed minimum of approx. 180 parturition events. A large sample size is indeed crucial to obtain high precision in the estimation of birth synchrony, as the total variation in birth synchrony along the whole altitudinal and latitudinal range of the species is quite small (a maximum of 7 and 13 days, respectively). Furthermore, big sample sizes collected over large geographical regions can be expected to dampen the potential confounding effect of changes in sex and age structures of different subpopulations, which have been reported to influence the intensity of birth synchrony (Mysterud, Coulson, & Stenseth, 2002; Loe et al., 2005). Therefore, further research is needed to disentangle the relationship between latitude and birth synchrony.

Unfortunately, we were unable to disentangle the potential evolutionary mechanisms (i.e. phenotypic plasticity vs. local adaptation) that could account for the response of parturition date along the altitudinal gradient. On one hand, the adjustment of birth timing and synchrony likely represents a response to the strong selection pressure for matching the optimal parturition time, which is increasingly delayed and reduced at higher altitudes (Gaillard et al., 1993b). This adjustment has been suggested to be caused by: (1) the short time period between fawning (May to June) and rut (July to August) in roe deer, which restricts the time window when females can recover a sufficient body condition to mate again (Gaillard et al., 1993b), and (2) the short period when fawns can grow rapidly before winter (Clutton-Brock, Guinness, & Albon, 1982), when their growth slows down (Hewison et al., 2002). However, on the other hand, evolutionary adaptations require long time periods (Plard et al., 2014a) and may only operate among distant populations (i.e. between large geographical regions). Therefore,

for some of the observed changes occurring at small spatial scales, such as those observed within regions, we consider gene flow a probable process constraining evolution (Lenormand, 2002). Hence, an alternative to the ultimate (evolutionary) explanation is needed, for example, that phenotypic plasticity or migratory movements are involved in the response. As overall phenotypic plasticity of roe deer, in terms of parturition timing, has been demonstrated to be limited in a flat study area (Plard et al., 2013), the better match of roe deer birth timing in areas of topographically more complex landscapes, may be explained by migratory movements toward higher altitudes (behavioural plasticity; Cagnacci et al., 2011). Indeed, partial migration is a frequently reported phenomenon in populations inhabiting mountainous and northern environments, which allows females to decrease a temporal mismatch by changing their home range to higher altitudes or latitudes during the breeding season (Mysterud, 1999; Ramanzin, Sturaro, & Zanon, 2007; Cagnacci et al., 2011; Gaudry et al., 2015; Couriot et al., 2018). Therefore, further research is needed to understand the relative effect of evolutionary adaptation and phenotypic plasticity on the response of populations to environmental variation along the altitudinal gradient.

A better understanding of the effect of seasonal movements in roe deer adaptation to the environment could be achieved by studying the temporal variation of birth timing in response to climate change. In Trois Fontaines (a flat, low-altitude forest in France), roe deer females did not change their birth timing over the last 35 years to track earlier plant phenology derived from recent climate warming. The resulting mismatch between birth timing and optimal plant phenology led to increased fawn mortality and decreased female reproductive output (Plard et al., 2014a). Likewise, the effect of climate change is expected to be particularly pronounced in the seasonally distinct environments such as mountains (IPCC, 2013). However, mountain environments may provide a possibility for escaping from the adverse effects of climate change because large herbivores have the possibility to track resources in space as distances are short in comparison to long migratory movements along the latitudinal axis. Therefore,

instead of having the only option of shifting reproduction timing *in situ* to track increasingly earlier plant phenology over the years, roe deer in mountain environments have the opportunity to flexibly select higher altitude areas to give birth and thereby minimize the mismatch between birth timing and vegetation flush that is increasing over time (Fryxell & Sinclair, 1988). Hence, we expect that the proportion of migratory females will increase in the future, especially in lower areas of the mountain regions, as the fitness benefits of giving birth at higher altitude will most likely increase over time. In this context, the maintenance of habitat connectivity is of high priority to allow migratory animals moving easily between summer and winter ranges. Thus, effective measures should be implemented, such as maintaining corridors to mitigate the effects of habitat fragmentation (Coulon et al., 2004) or combining fencing along with wildlife crossings structures to reduce road-kills (Olsson et al., 2008). Finally, further research is required to improve our understanding of how large herbivores track resource availability in mountain regions (Vitasse, Signarbieux, & Fu, 2018), which will allow us to develop adaptive management plans for the species in times of rapid climate change.

Acknowledgments

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Authors' contributions

M.R and K.B compiled and organized the long-term data. K.B. and M.R conceived the ideas for the work with input from J.M.G, M.H and MP. M.P. analyzed the data and led the writing of the manuscript. All authors contributed critically to the manuscript and gave final approval for publication.

Data availability statement

The species data were provided by courtesy of the Federal Office for the Environment. Data associated with this manuscript are deposited in the Dryad Digital Repository. The same applies for the R-Script used for the analyses.

Supplementary material

Supplementary tables

Table S4.2.1 Mean monthly temperatures and annual precipitation for the three Swiss regions (Swiss Plateau, Northern Alps and Eastern Central Alps) during the study period (1989-2015). Data were obtained from the homogeneous data series of the Swiss Federal Office of Meteorology and Climatology.

Region	Monthly temperature ± SD (°C)		Annual precipitation ± SD (mm)	Meteorological Station	
	<i>January</i>	<i>July</i>		<i>Location</i>	<i>Altitude (m a.s.l.)</i>
Swiss Plateau (SP)	0.8 ± 1.7	18.7 ± 1.7	1,118 ± 166	Zurich / Fluntern	556
Northern Alps (NA)	-1.7 ± 1.7	15.2 ± 1.5	1,556 ± 169	Engelberg	1,036
Eastern Central Alps (ECA)	-6.7 ± 1.6	11.6 ± 1.3	987 ± 220	Segl – Maria	1,804

<https://www.meteoswiss.admin.ch/home/climate/swiss-climate-in-detail/homogeneous-data-series-since-1864.html>

Table S4.2.2 Roe deer reproductive timing across Europe.

Country	Latitude	Mean birth date	Mean(Median) birth date	N	Synchrony (days)	N	Years of data recording	Reported / assigned altitudinal interval (m)			References
Norway (Jøa)	64 °N	22 May	142	45	28	24	1992-1993	----	/	<500	In Linnell & Andersen (1998a): Aanes & Andersen (1996)
Norway(Storfosna)	63 °N	22 May	142 (143)	296	26	296	1991-1994	----	/	<500	Linnell & Andersen (1998a)
Norway (Hedmark and Østfold Akershus)	60 °N	8 June	160 (161)	153	24	153	1995-1998, 2001-2004	----	/	500-1,000	Panzacchi et al. (2008)
Sweden (Grimsö)	59 °N	29 May	149 (150)	26			2000-2009	----	/	<500	Plard et al. (2013)
Sweden (Bogesund)	59 °N	4 June	155 (156)	43			2001-2006	----	/	<500	Plard et al. (2013)
Sweden (Ekenäs)	59 °N	2 June	153	231	25 ¹			----	/	<500	Jarnemo et al. (2004)
Sweden (Öster-Malma)	59 °N	11 June	163	15			1957, 64-67	----	/	<500	Espmark (1969)
Denmark (Kalo)	56 °N	29 May	149		30 ²			----	/	<500	In Linnell & Andersen (1998a): Strandgaard (1972)
Denmark (Borris)	56 °N	14 June	165	1,692	19	1,692	1956-1980	----	/	<500	Strandgaard (1997)
England (Chedington)	52 °N	22 May	142		18		1961-1967, 1973-1989	75-150	/	<500	In Linnell & Andersen (1998a): Gill (1994)
Poland	52 °N	2 June	153 ³	129			1976-1980	----	/	<500	Kaluzinski (1982)
Germany	51 °N	1 June	152	12,340			1938-1945	----	/	<500 ⁴	Rieck & Munden (1955)
Germany (Bavaria)	49 °N	23 May	143				1972-1976	~ 500	/	<500	In Linnell & Andersen (1998a): Ellenberg (1979)
France (Trois Fontaines)	48 °N	15 May	136 (136)	784	28	141	1985-2010	lowland	/	<500	Gaillard et al. (1993a); Plard et al. (2014)
Switzerland (Bern)	46 °N	2 June	(153)	793			1965	450-1000	/	500-1,000	Sägesser & Kurt (1965)
Switzerland	46 °N	27 May	(147)	3,972			1971-1995	----	/	500-1,000 ⁵	Muri (1999)
Italy (Tyrol)	46 °N	13 June	164	113	21	10	1983-1992	1400-2050	/	>1,500	In Linnell & Andersen (1998a): Wotschikowsky & Schwab (1994)
France (Aurignac)	43 °N	14 May	134 (132)	87	~ 30 ¹	87	2007-2012	----	/	<500	Plard et al. (2013)
Italy (Apenines)	44 °N	30 May	150	117	17	117	1997-2003	350-980	/	500-1,000	Raganella-Pelliccioni et al. (2007)
Spain (Madrid)	40 °N	19 May	139 ³					----	/	500-1,000	In Plard (2014); FIDA (2009)

¹ Calculated based on 79% of birth dates. ² Data based on marking dates. ³ Mean birth date calculated using absolute length of the birth season. ⁴ Mean altitude of all country. ⁵ Mean fawn altitude calculated with our own data. ---- not reported. Mean and median birth dates calculated as day of year (DOY) and birth synchrony (80% of parturitions in days) are shown.

Table S4.2.3 Results of the linear models accounting for latitudinal variation on birth timing and synchrony across Europe.

Response variable	Fixed factors	estimates	error	t-value	P-value
Mean (median) birth date	Latitude	0.72	0.30	2.432	0.027
	Study site altitude (m a.s.l.)				
	500-1000	6.97	4.68	1.489	0.156
	>1500	22.03	8.52	2.586	0.020
Birth synchrony (days)	Latitude	0.14	0.20	0.695	0.505

*Dummy study site level: <500 m a.s.l.

Table S4.2.4 Model results using different methodologies to account for altitudinal variation on birth phenology (SLMM, LM, LMM and spatial filtering). All models included *parturition date* as response variable and the interaction between the predictor *altitude* and the factor *region* to control for differences between areas (NA = Northern Alps, ECA = Eastern Central Alps, SP/PA = Swiss Plateau/Pre-Alps). For all Mixed Models, we included *fawn cohort* nested within *region* as a random effect. For the filtering approach we used a home range size of 44.5 ha (Ineichen, 2015).

Type of model	Fixed factors	estimates	error	t-value	P-value
SLMM N=7444	Altitude	0.0065	0.0008	8.408	
	Region (NA)	-3.2847	1.7595	-1.867	
	Region (SP)	-6.8146	2.0201	-3.373	
	Alt*Region (NA)	0.0010	0.0014	0.715	
	Alt*Region (SP)	-0.0025	0.0024	-1.028	
LM N=7444	Altitude	0.0069	0.0004	16.524	<0.001
	Region (NA)	-1.5189	1.0550	-1.440	0.150
	Region (SP)	-1.4994	1.2468	-1.203	0.229
	Alt *Region (NA)	-0.0000	0.0009	-0.044	0.965
	Alt*Region (SP)	-0.0086	0.0016	-5.382	<0.001
LMM N=7444	Altitude	0.0069	0.0004	16.667	<0.001
	Region (NA)	-1.5387	1.1017	-1.397	0.167
	Region (SP)	-1.7122	1.2850	-1.332	0.187
	Alt *Region (NA)	-0.0000	0.0009	-0.039	0.970
	Alt*Region (SP)	-0.0084	0.0016	-5.251	<0.001
Spatial filtering	Altitude	0.0069	0.0008	8.178	<0.001
LM N=2018	Region (NA)	-3.4309	1.9123	-1.794	0.073
	Region (SP)	-6.5837	2.1469	-3.067	0.002
	Alt *Region (NA)	0.0010	0.0016	0.631	0.528
	Alt*Region (SP)	-0.0029	0.0026	-1.118	0.264
Spatial filtering	Altitude	0.0069	0.0008	8.136	<0.001
LMM N=2018	Region (NA)	-3.5482	1.9366	-1.832	0.073
	Region (SP)	-6.7847	2.1659	-3.133	0.003
	Alt*Region (NA)	0.0011	0.0016	0.666	0.505
	Alt*Region (SP)	-0.0028	0.0026	-1.089	0.277

Statistically significant variables highlighted in bold. SLMM parameters calculated: Rho = 0.0008358684

Table S4.2.5 Altitudinal variation in mean parturition date and birth synchrony (measured as the period when 80% of parturitions occurred). We analyzed all regions together and also each region separately (SP/PA = Swiss Plateau/Pre-Alps, NA = Northern Alps, ECA = Eastern Central Alps).

Region	Altitudinal interval (m a.s.l.)	Mean parturition date (DOY)	Dates 80% of births occurred (DOY)	Birth synchrony (days)	Sample size (N)
All (altitudinal quartiles)	[288–786]	144	132–158	26	1,867
	(786–1,232]	150	138–163	25	1,859
	(1,232–1,564]	155	145–165	20	1,861
	(1,564–2,366]	158	148–168	20	1,857
SP/PA	[500–750)	143	131–156	25	995
	[750–1,000)	142	129–155	26	574
NA	[500–750)	149	137–161	24	246
	[750–1,000)	150	138–161	23	459
	[1,000–1,250)	152	142–164	22	406
	[1,250–1,500)	153	143–163	20	205
ECA	[750–1,000)	152	142–161	19	214
	[1,000–1,250)	154	143–164	21	611
	[1,250–1,500)	155	146–165	19	1,167
	[1,500–1,750)	157	147–167	20	1,193
	[1,750–2,000)	158	148–168	20	859

Supplementary figures

Figure S4.2.1 PRISMA diagram of our systematic literature review.

Figure S4.2.2 Density plot displaying birth synchrony for each altitudinal interval and region. A) whole dataset divided into four altitudinal quartiles and B) data from each region divided into 250 m altitude intervals.

Figure S4.2.3 Dashed horizontal lines represent ± 2.0 , ± 1.5 and ± 1.0 corridor of stability (COS), defined as the acceptable variation of the mean that could be assumed for the scale being used. Solid curved lines contain 95% of all average parturition dates at each sample size N. Solid vertical lines indicate the Points of stability (POS), defined as the sample size at which 95% of parturition dates do not exceed the boundaries of each COS level.

Figure S4.2.4 A) Location of the phenological stations included in the analysis and their 10 km buffer area (black triangles and black circumferences, respectively). Dashed rectangle indicates the extent of Figure S4.2.4B. B) Light grey dots represent locations of litters excluded from the phenological analysis and dark-grey crosses represent locations selected for analysis. All parturitions included in the analysis were closer than 10 km from their phenological station and within an altitudinal interval of ± 100 m.

Figure S4.2.5 Gam model results to account for altitudinal variation in birth phenology in the Swiss Plateau and the Pre-Alps regions. The GAM included *parturition date* as response variable and *altitude* as predictor.

Study 3: Rehnus, M.*, Peláez, M.*, & Bollmann, K., (2020) * *equal first authors*

Advancing plant phenology causes an increasing trophic mismatch in an income breeder across a wide elevational range

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Abstract

To avoid trophic mismatches, large herbivores should reschedule the breeding timing to keep in synchrony with the recent advance of plant phenology caused by climate change. To do so, individuals must track resources along two main non-exclusive axes: time (rescheduling the breeding timing) and space (i.e. shifting their range). While the vast majority of studies have focused on the study of specific population inhabiting small and homogeneous environments, little is known about the response of species across large regions with highly heterogeneous topographies.

Here, we studied roe deer (*Capreolus capreolus*), an income breeder that has demonstrated a lack of phenotypic plasticity on birth timing in a densely forested lowland area, and evaluated its ability to track resources along a wide elevational gradient (288–2366 m a.s.l.). We used data from 8986 parturition events collected in Switzerland during the period 1971–2015. We hypothesize that the mismatch between roe deer parturition dates and peak resource availability will increase over the study period. In addition, we expect this mismatch to be larger in low areas compared to higher elevation areas.

Contrary to previous studies, we did find a consistent, but small, trend towards more advance parturition dates through time across all elevations. However, this rate of advance was less than that of the plant phenology indicators, resulting in an increasing mismatch at all elevations. Parturition dates changed on average at a rate between 7.5 times slower than the Growing Season Start and 5 times slower than the Flowering Start. Thus, at the lowest elevations, parturition timing has already fallen outside the optimal time window for high-quality forage while at high elevations, parturitions are still synchronized with the peak availability of quality forage.

Our study emphasizes the need for large-scale study designs along climatic gradients to help understand the complex relationships that shape adaptation across environments. This would allow for better understanding on how

populations will respond to the new environmental conditions imposed by climate change.

INTRODUCTION

Rising temperatures as a consequence of climate change have led to an overall spring phenology advancement for many organisms across the Northern Hemisphere (Menzel, 2003; Cleland et al., 2007; Roberts et al., 2015). However, the magnitude of this response might differ among interacting species (Parmesan & Yohe, 2003; Root et al., 2003). Therefore, phenological mismatches may occur if a different magnitude of response disrupts previously synchronous trophic interactions (Visser et al., 1998; Koh et al., 2004). A decoupling between the date of key life-history events and resource availability may lead to strong negative consequences on species demography and population viability (Brook et al., 2009; Bronson, 2009; Miller-Rushing et al., 2010; Usui et al., 2017).

For plant consumers, such as large herbivores, breeding timing is the period of peak energy demand for females which should match the optimal time in terms of resource availability (Post et al., 2003; Hämäläinen et al., 2017). Otherwise, a mismatch can reduce annual reproductive success (Clutton-Brock et al., 1987; Plard et al., 2014; Paoli et al., 2018) and future reproductive performance of both females and newborns (Clutton-Brock et al., 1983; Festa-Bianchet et al., 2000).

For most large herbivores, the leading edge of the growing season has been defined as the optimal timing to give birth (Merkle et al., 2016). The Forage Maturation hypothesis states that the early vegetative stages are nutritionally superior to later stages of plant development such as flowering, despite being lower in biomass production (Albon & Langvatn, 1992; Hebblewhite, Merrill, & McDermid, 2008). During flowering and seed production, plants produce fewer leaves and more stems, increasing the fiber content and reducing the protein content, and thus reducing their quality (Albon & Langvatn, 1992; Duru, 1997;

Bumb et al., 2016). Therefore, forage at early development stage with low vegetative biomass is of higher nutritional quality than mature, high-biomass vegetation (Fryxell, 1991).

In order to track the rapid changes in plant phenology due to climate change, long-lived mammals must have adaptive mechanisms such as phenotypic or behavioral plasticity (within individual lifetimes; Przybylo et al., 2000; Lane et al., 2012; Hoy et al., 2018), as micro-evolutionary responses in animals with long generation times is suggested to occur at much slower rates than current climate change. Recent studies investigating temporal shifts in breeding timing of large herbivores as response to climate have found two reaction types: 1) one not being able to keep track of the changing environment because its reproductive cycle mainly depends on photoperiodicity, and 2) another that shows temporal flexibility because it relies in other cues such as temperature and food availability to keep synchrony with the environment. Most capital breeders belong to the second type as they have shown some degree of phenotypic plasticity, for example, by advancing conception dates (e.g., Pyrenean chamois *Rupicapra pyrenaica pyrenaica*: Kourkgy et al., 2016; red deer *Cervus elaphus*: Peláez et al., 2017) or shortening gestation length (e.g., reindeer *Rangifer tarandus tarandus*: Mysterud et al., 2009; red deer: Moyes et al., 2011; semi-domesticated reindeer: Paoli et al., 2018). In fact, recent studies have demonstrated that capital can be used, not only to lessen the need for matching optimal timing in parturition, but also to facilitate temporal synchrony between resource need and supply (Williams et al., 2017). On the contrary, income breeders exhibited limited potential to track early-spring plant growth because their reproduction relies strongly on photoperiodic cycles (e.g., West Greenland caribou *Rangifer tarandus groenlandicus*: Post & Forchhammer, 2008; Kerby & Post, 2013; roe deer *Capreolus capreolus*: Plard et al., 2014). Income breeding under stable conditions allows animals to synchronize their energy demands with supply (Jönsson, 1997) following a ‘bet-hedging’ strategy in which parturition timing coincides with a long-term average of favorable conditions (Post & Forchhammer, 2008). Thus, capital breeding seems to be beneficial under

changing environmental conditions, whereas income breeding is superior under predictable environment conditions. However, in the context of climate change, the income breeding strategy may turn out to be maladaptive.

Roe deer stand out as a species particularly unable to modify plastically parturition dates, as it has been demonstrated in a densely forested lowland area of France (Plard et al., 2014). However, little is known about the response of roe deer in other habitats, such as topographically heterogeneous landscapes composed by a mosaic of forests, croplands and pastures (Gaillard et al., 2013). Topographically heterogeneous landscapes integrate a larger range of climatic conditions per area (Büntgen et al., 2017; Rehnus et al., 2018) than flat landscapes (Bellard et al., 2012) and thus, females have access in a short spatial scale to a wide range of plant phenology phases. Hence, this type of landscape may help individuals to minimize the mismatch between parturition timing and vegetation flush (Fryxell & Sinclair, 1988) by following a multi-range tactic as a response to resource heterogeneity and unpredictability (Couriot et al., 2018). Finally, while populations in topographically homogeneous areas might be at risk of falling outside the optimal climate niche, populations living in heterogeneous areas might still find suitable environmental conditions on nearby higher elevations.

Here, we investigated the response of roe deer parturition timing to track changes in plant phenology during 45 years (1971–2015) along a distinct elevational gradient (288–2366 m a.s.l.) in Switzerland. We hypothesize that:

H1: The mismatch between roe deer parturition dates and peak resource availability will increase over the study period because of the lack of phenotypic plasticity of roe deer to track temporal changes in plant phenology.

H2: The mismatch between roe deer parturition dates and peak resource availability will be larger in low areas compared to higher elevations.

MATERIALS AND METHODS

Study area

The study area comprises three biogeographic regions that cover the whole elevational and climatic range of roe deer in Switzerland (Christen et al., 2018). These adjacent and connected regions are, from north to south and from low elevation to high: the Swiss Plateau and the Pre-Alps (SP/PA; mean elevation 639 m a.s.l.; ranging from 240 to 2361 m a.s.l.), the Northern Alps (NA; mean elevation 1389 m; ranging from 370 to 4045 m a.s.l.), and the Eastern Central Swiss Alps (ECA; mean elevation of 2111 m a.s.l.; ranging from 560 to 3981 m a.s.l.; Fig. 4.3.1; Gonseth et al., 2001). Details of the study area have been published elsewhere (Christen et al., 2018).

Roe deer fawn data collection

Data originated from the project «Rehkitzmarkierung Schweiz» (i.e., roe deer marking in Switzerland), which has been running since 1971. As of 2015, 15380 roe deer fawns had been marked by hunters, gamekeepers, and researchers in the study area with varying marking efforts in number of marked fawns over 45 years. Although the application of an ear mark is simple, only people experienced in handling wildlife were allowed to participate in this project. Fawns were found by repeatedly observing the mother during late pregnancy until the day of parturition. For each fawn marked, the following information was recorded: the date of marking, the estimated age in days, the geographic coordinates and the herbaceous layer height at the marking site (< 20 cm, 20–50 cm and > 50 cm). Fawn age was assessed by examining the umbilical cord and observing fawn behavior during marking (Jullien et al., 1992). Parturition date was then calculated by subtracting the estimated age of the fawn (in days) from the date of capture. Marking site elevation, in meters, was derived from a digital elevation model (DEM) with a 25-m resolution (Zimmermann and Kienast, 1999) using ArcGIS 10.6 (ESRI, 2018). Finally, elevation was classified into 250 meters intervals.

Figure 4.3.1 Distribution of roe deer marking sites (grey dots, $N = 8986$) along an elevational gradient in Switzerland from 1971 to 2015. Triangles indicate MeteoSwiss stations ($N = 98$) and squares phenological stations ($N = 59$). Grey circles mark the six small areas of 7.5 km of radius used for the local scale analysis. SP = Swiss Plateau, PA = the Pre-Alps, NA = Northern Alps, ECA = Eastern Central Alps (Gonseth et al., 2001).

Environmental variables

Although roe deer has been described as a forest-dwelling and browser species, open herbaceous patches such as meadows constitute an important diet source for roe deer during the breeding season and are actively selected by females (Tixier & Duncan, 1996). Indeed, their recent colonization of modern agricultural landscapes throughout Europe has demonstrated a better performance in these habitats where they have access to high quality food such as crops and wild forbs (Tixier & Duncan, 1996; Hewison et al., 2009; Gaillard et al., 2013). Therefore, we characterized the optimal timing for roe deer births, as the period between the start of the growing season (GSS) and the start of the herbaceous flowering (FS). We selected two different variables to define this period: 1) the date after six consecutive days with mean daily temperatures above 5°C as a proxy for GSS (Zimmermann & Kienast, 1999) and 2) the date of the first cutting of hay as a proxy for FS. Reasons to select the date of first cut of hay as proxy for FS were:

1) hay is cut when the ratio of forage quantity/quality is at its maximum, just before the start of flowering, 2) hay cut information matched the full temporal and elevational range of our roe deer data and 3) it represents the phenological state of the plant community, not just of a single species. Similarly, previous studies on roe deer used the date of flowering as a proxy of spring plant phenology (Plard et al., 2014).

GSS was calculated from the temperature measurement network of MeteoSwiss (Federal Office of Meteorology and Climatology, 2018). Dates of the first cutting of hay were obtained from the national phenology network of MeteoSwiss (www.meteoswiss.admin.ch/home/measurement-and-forecasting-systems/land-based-stations/swiss-phenology-network.html). We selected the most representative stations for our study area (those inside the minimum convex polygon including 99% of roe fawns locations). In addition, we only used stations with at least 3 years of data collected per decade throughout the study period. In total, we used data from 59 phenological stations and 98 meteorological stations located at elevations ranging from 330 to 1900 m a.s.l. Station elevations were also we classified into elevational intervals of 250 m. The minimum number of years collected by any station included in our analysis was 24 years.

Statistical analysis

Spatial scale of analysis

We performed analysis at three different scales: 1) inter-regional scale, 2) regional scale and 3) local scale. For the inter-regional scale analysis, we pulled all data from the study area. For the regional analysis, we divided the three datasets (parturition date, GSS and FS) by biogeographic region (SP/PA, NA and ECA). Finally, at the local scale, we performed a spatial analysis to find small areas (maximum of 7.5 km radius) with the highest concentration of parturition recorded (at least 200 parturitions) and a maximum of 250 m a.s.l. difference between the highest and the lowest parturition recorded (see Data S1 for more

details). We assumed that, within these small areas, there would be little genetic differentiation among individuals, as the 85 percentile of adult individuals in Switzerland dispersed below this distance (Euclidean distance, own unpublished data). Finally, we selected the two closest meteorological or phenological stations for each small area. Parturition date and phenological information (GSS, FS) was expressed in Julian days (JD).

Temporal changes in plant phenology

To assess temporal changes in plant phenology at inter-regional, region and local scale, we performed linear models with GSS and FS as response variables. Predictors were year, elevational interval and the interaction between the two. In addition, to provide an overall estimate of the rate of advance of GSS and FS during the study period, we calculated the average rate of advance across elevational intervals for each spatial scale and then, calculated the average rate among the three spatial scales.

Temporal changes in parturition date

We used parturition dates instead of date of birth of individual fawns to avoid pseudo-replication problems (*sensu* Hurlbert, 1984) caused by the presence of twins or triplets (i.e. two or three fawns from the same mother in the same year). To ensure that parturition dates were calculated accurately, we only used information from fawns aged ten days or younger because ageing precision decreases with increasing fawn age (Jullien et al., 1992; mean fawn age 4.69 ± 2.79 days). We only included marking sites with a data resolution ≤ 1 ha. Ultimately, we used information from 8986 parturition events recorded during the period 1971–2015. Finally, for the three scales of analysis, we calculated the median parturition date by year and elevational interval.

To assess temporal changes in parturition dates, we fitted linear regressions with a Gaussian error distribution using median parturition date as

the response variable. As predictors, we included year, elevational interval and their interaction. In addition, we included the number of parturitions used to calculate the median as a weighting variable to adjust the relative influence of each year based on the number of parturition dates recorded. Finally, we calculated the average rate of advance in parturition date across elevational intervals for each spatial scale and then, calculated the average rate among the three spatial scales.

Mismatch quantification

Results of the models were used to compare differences in the temporal trend between peak parturition and peak resource availability (GSS and FS) as an indicator of mismatch.

As additional indicator, we analyzed the temporal variation of the herbaceous layer height at the moment of fawn marking. For this analysis, we only used information on individual fawns found in meadows, as the development of the ground vegetation layer is related to tree canopy closure and light conditions in forests (N = 6219 fawns; 71% of the total fawns found with known vegetation height). Therefore, we created a binomial response variable by giving value one to the marking sites with low herbaceous layer height (< 20 cm) and value zero to the marking sites with medium (20–50 cm) or high height (> 50 cm). We then fitted a generalized linear model with a binomial link function including elevational interval, year, and their interaction as predictors. Because this corresponded to a smaller subset of our data, we only analyzed it at the inter-regional and regional scale.

All analyses were performed using R 3.2.1 statistical software (R Development Core Team, 2018). We used the function “lm” to fit the models and the “plot” function to check model assumptions through diagnostic plots (Pinheiro and Bates 2000). Both functions were part of the “stats” package.

RESULTS

Temporal changes in plant phenology

Results showed that GSS and FS advanced significantly through time independently from the scale studied (Fig. 4.3.2; Supplementary material Fig. S4.3.1). Furthermore, the rates of advance of GSS and FS were very similar across different elevation intervals and scales, advancing on average at a rate of 0.45 and 0.32 days per year, respectively (Table 4.3.1). Thus, the date of the GSS and FS advanced on average 20 and 14 days, respectively, over the 45 years of study.

Temporal changes in parturition date

A slight trend towards more advanced parturition dates through time was found at different scales and elevational intervals (Table 4.3.1; Fig. 4.3.2; Supplementary material Fig. S4.3.1 and S4.3.2). On average, the advance in parturition date was 0.06 days per year (ranging between 0.05–0.06 depending on the scale studied; Table 4.3.1). However, note that significance levels varied among elevation intervals and scales but the direction of this trend signaled almost in all cases towards an advance in parturition date (Table 4.3.1; Supplementary material Fig. S4.3.2). Across the 3 different scales studied, we calculated a total of 25 independent slopes for the relationship between median parturition date and time, for 23 of them this relationship was negative (Table 4.3.1). Results obtained by using mean parturition dates instead of the median were very similar (Supplementary material Table S4.3.1). Therefore, parturition date is now about 3 days earlier than 45 years ago.

Table 4.3.1 Results of the model performed to assess temporal variation of parturition date, the start of the growing season (GSS) and the start of the herbaceous flowering (FS) for the selected elevational intervals at the inter-regional and local scale for the period 1971–2015. Significance levels: ‘***’ = $p < 0.001$; ‘**’ = $p < 0.01$; ‘*’ = $p < 0.05$; ‘.’ = $p < 0.1$; ‘ns’ = $p > 0.1$.

Scale	Region and elevational interval (m a.s.l.)	Median parturition date (days per year $\pm$ SE)	GSS (days per year $\pm$ SE)	FS (days per year $\pm$ SE)
Inter-regional	(250–500]	-0.04 $\pm$ 0.06 ns	-0.49 $\pm$ 0.04 ***	-0.21 $\pm$ 0.04 ***
	(500–750]	-0.02 $\pm$ 0.02 ns	-0.45 $\pm$ 0.04 ***	-0.25 $\pm$ 0.04 ***
	(750–1000]	-0.09 $\pm$ 0.03 **	-0.39 $\pm$ 0.05 ***	-0.36 $\pm$ 0.04 ***
	(1000–1250]	-0.10 $\pm$ 0.04 **	-0.41 $\pm$ 0.08 ***	-0.30 $\pm$ 0.05 ***
	(1250–1500]	-0.02 $\pm$ 0.04 ns	-0.48 $\pm$ 0.10 ***	-0.45 $\pm$ 0.10 ***
	(1500–1750]	-0.07 $\pm$ 0.04 .	-0.57 $\pm$ 0.15 ***	-0.34 $\pm$ 0.10 ***
	(1750–2000]	-0.06 $\pm$ 0.05 ns	-0.32 $\pm$ 0.13 *	-0.31 $\pm$ 0.10 **
	Average	-0.06	-0.45	-0.32
Region	SP/PA (250–500]	-0.02 $\pm$ 0.08 ns	-0.51 $\pm$ 0.05 ***	-0.08 $\pm$ 0.05 .
	SP/PA (500–750]	-0.07 $\pm$ 0.03 *	-0.49 $\pm$ 0.04 ***	-0.20 $\pm$ 0.05 ***
	SP/PA (750–1000]	-0.14 $\pm$ 0.04 ***	-0.44 $\pm$ 0.07 ***	-0.58 $\pm$ 0.09 ***
	NA (500–750]	0.08 $\pm$ 0.04 .	-0.35 $\pm$ 0.08 ***	-0.32 $\pm$ 0.06 ***
	NA (750–1000]	-0.07 $\pm$ 0.04 .	-0.35 $\pm$ 0.07 ***	-0.31 $\pm$ 0.05 ***
	NA (1000–1250]	-0.07 $\pm$ 0.05 ns	-0.34 $\pm$ 0.11 **	-0.44 $\pm$ 0.08 ***
	NA (1250–1500]	-0.01 $\pm$ 0.08 ns	-0.48 $\pm$ 0.15 **	---
	ECA (750–1000]	-0.10 $\pm$ 0.07 ns	-0.35 $\pm$ 0.19 .	-0.31 $\pm$ 0.11 **
	ECA (1000–1250]	-0.10 $\pm$ 0.04 *	-0.49 $\pm$ 0.09 ***	-0.19 $\pm$ 0.06 ***
	ECA (1250–1500]	-0.02 $\pm$ 0.03 ns	-0.47 $\pm$ 0.11 ***	-0.45 $\pm$ 0.08 ***
	ECA (1500–1750]	-0.07 $\pm$ 0.03 *	-0.57 $\pm$ 0.13 ***	-0.34 $\pm$ 0.07 ***
	ECA (1750–2000]	-0.06 $\pm$ 0.04 ns	-0.32 $\pm$ 0.11 **	-0.31 $\pm$ 0.08 ***
	Average	-0.05	-0.43	-0.32
Local	SP [460–651]	-0.13 $\pm$ 0.07 .	-0.49 $\pm$ 0.19 **	-0.38 $\pm$ 0.09 ***
	SP/PA1 [620–870]	-0.02 $\pm$ 0.06 ns	-0.52 $\pm$ 0.19 **	-0.35 $\pm$ 0.09***
	SP/PA2 [600–850]	-0.16 $\pm$ 0.06 **	-0.39 $\pm$ 0.19 *	-0.22 $\pm$ 0.09 *
	NA/ECA [1177–1425]	0.06 $\pm$ 0.09 ns	-0.50 $\pm$ 0.19 **	-0.36 $\pm$ 0.09 ***
	ECA1 [1443–1693]	-0.04 $\pm$ 0.08 ns	-0.54 $\pm$ 0.19 **	-0.24 $\pm$ 0.09 *
	ECA2 [1754–2004]	-0.02 $\pm$ 0.09 ns	-0.35 $\pm$ 0.19 .	-0.29 $\pm$ 0.09 **
	Average	-0.05	-0.47	-0.31

--- not enough data to perform lm

Figure 4.3.2 Temporal trends at the beginning of the growing season (GSS; black solid line) and the herbaceous flowering (FS; black dashed line) and at the median parturition date (grey solid line) for different elevational intervals at A) inter-regional and B) local scale. GSS was calculated based on climatological data (the date after six consecutive days with temperatures above 5°C). FS was calculated as the mean date of the first cutting of hay. Dots represent parturition dates for each elevational interval and year. Dot size indicates the number of parturitions used to calculate the median. SP/PA = Swiss Plateau and the Pre-Alps, NA = Northern Alps, ECA = Eastern Central Alps.

Mismatch quantification

The magnitude of advance in parturition dates through time was much smaller than that of plant phenology (GSS and FS) at all elevations and scales. Parturition dates changed at a rate 7.5 times slower than GSS and 5 times slower than FS. Thus, due to the slower rate of advance of parturition date in relation to plant phenology, we observed that, below 1000 m a.s.l., mean parturition timing has already fallen outside the optimal time window for high-quality forage (Fig. 4.3.2; Supplementary material Fig. S4.3.1). In contrast, parturitions occurring at elevations above 1000 m a.s.l. still occurred within the optimal period for high-quality forage (Fig. 4.3.2; Supplementary material Fig. S4.3.1).

The faster rate of advance of plant phenology compared to parturition dates was also supported by the measurements of vegetation height at marking sites. Throughout the study period, the percentage of fawns found in low height meadows (< 20 cm) decreased from 61% to 20% at 1750 m a.s.l., from 33% to 10% at 1250 m a.s.l. and from 13% to 5% at 750 m a.s.l (inter-regional scale: Fig. 4.3.3).

Figure 4.3.3 Temporal trend in the number of fawns found at meadows with low herbaceous layer height (< 20 cm) during the period 1971–2015 at the inter-regional scale. Size of shapes is proportional to the sample size.

Shapes indicate different elevation intervals (m a.s.l.):

- ▲ = (1500 - 2000]
- = (1000 - 1500]
- = (500 - 1000]
- ◆ = (250 - 500]

We found no significant changes at the lowest elevation interval because the percentage of fawns born at sites with low vegetation was already less than 7% at the beginning of the study. Overall, the same trend was obtained at the regional scale (Supplementary material Fig. S4.3.3).

DISCUSSION

Here, we found a consistent advance toward earlier parturition dates across all scales and most elevation ranges that could be associated with a response to climate change. However, this advance was much slower than that of plant phenology, creating a trophic mismatch between parturition dates and peak resource availability over the 45 years of study (1971–2015). Furthermore, at low elevations, the phenological decoupling has already caused mean parturition dates to fall outside the temporal window of optimal forage quality while, at high elevations, parturitions are still synchronized with peak resource availability.

Clear evidence was found for a strong climate change signature in plant phenology, resulting in an earlier spring plant growth in the study area (-0.45 and -0.32 days per year for start of the growing season and the start of the herbaceous flowering, respectively). This result is in line with previous studies performed across Switzerland (Klein et al., 2016) and Europe (e.g. Menzel et al., 2006). In addition, vegetation height measurements at the marking sites revealed that the percentage of fawns found in low height meadows progressively decreased throughout the study period. This indicates that roe deer fawns were born each year at a later stage of herbaceous development and therefore, further away from the leading edge of the growing season and closer to the flowering start.

Despite the increasing mismatch observed between plant phenology and parturition date, we did find a consistent advance toward earlier parturition dates across all scales and most elevation ranges (although with varying levels of significance; see Yoccoz, 1991). Nevertheless, plant phenology advance was between five and seven times larger than the rate observed for roe deer parturition

dates, which was on average -0.06 days per year. Similarly, in Trois Fontaines, a densely forested lowland area of France, the different rate of advance between plant phenology (-0.60 days per year) and roe deer parturition dates (mean birth date $= -0.05 \pm 0.06$ days per year; $p=0.421$ and median $= -0.087 \pm 0.070$, $p=0.232$) also lead to a phenological mismatch (Plard et al., 2014). Interestingly, the minor advance in roe deer parturition timing observed in our study area was of the same magnitude and direction of that obtained by Plard et al. (2014), although for this study the trend was always not significant. Our results suggest that, although very slowly and at population level, roe deer parturition timing has changed in the direction of advancing peak resource availability. This is an important biologically meaningful finding for the species which, at least in highly heterogeneous environments, is showing some degree of response to climate change. This highlight the importance of performing large-scale studies across a wide gradient of environments to detect subtle phenological trends on key life-history events that may otherwise remain undetected.

Unfortunately, in comparison to other northern hemisphere large herbivores studied, the roe deer is the species that showed the smallest response to climate change (-0.06 days per year). West Greenland caribou, another income breeder, advanced $-0.11 (\pm 0.03 \text{ SE})$ days per year over 33 years (Kerby & Post, 2013). Capital breeders like red deer have advanced parturition by $-0.42 (\pm 0.08 \text{ SE})$ days per year over 26 years in Scotland (Moyes et al., 2011). In Finland, semi-domesticated reindeer, which are also described as capital breeders, advanced parturitions $-0.15 (\pm 0.04 \text{ SE})$ days per year over 45 years (Paoli et al., 2018). For Pyrenean chamois in France, a 10-day advance in the onset of autumn or spring plant phenology led to an advance of four and one days in birth dates, respectively (Kourkgy et al., 2016). For all the studies above, the observed changes in parturition date have been attributed mainly to phenotypic plasticity (Moyes et al., 2011; Kerby and Post, 2013; Kourkgy et al., 2016; Paoli et al., 2018). Only recently, a study demonstrated that adaptive evolution likely explained some of the advance in red deer parturition dates during the last four decades on the Isle of Rum,

Scotland (Bonnet et al., 2019), suggesting that up to a total of -0.05 days' delay per year (95% CI -0.10 to 0.02) could be explained by evolution (Bonnet et al., 2019).

Unfortunately, we lack information to infer the mechanism responsible for the small advance in parturition date observed for roe deer. An overall advanced parturition dates has been reported for older females (Plard et al., 2014) or high quality females (note that quality here is defined as a set of time-invariant attributes positively linked to individual fitness; Plard et al., 2013). However, we have no information on population demography or on female age, body weight and longevity in our study area. Therefore, we could not assess whether 1) female quality at population level varied through time as a response of changes in population density or land use (i.e. increased resource availability) or 2) an increase in the mean age of female at population level was responsible for the observed trend. However, the large sample size used in this study should render the effect of changes in age structures or population demography. Thus, due to the consistency of the results across scales and elevations, we do not expect that our results are biased by these variables. Alternatively, (micro)evolutionary processes may be the cause of the slight advance observed in parturition date. However, a previous study found no statistical support for heritability of birth date for female roe deer based on 28 pair of daughter–mother pairs, even though birth date was highly variable within a population and a trait under strong directional selection (Plard et al., 2014). Therefore, the study predicted that a micro-evolutionary response of parturition date to climate change would be limited for roe deer. Nevertheless, this question is still under discussion as a much larger sample size is required to confirm these results (Plard et al., 2014). Finally, the last possible explanation would be that the observed advance corresponded to migration or dispersion movements towards higher elevations. This would mean that, instead of rescheduling breeding timing *in situ* to counteract the phenological mismatch via phenotypic plasticity or microevolution, roe deer tracked appropriate conditions in space by moving towards higher elevations (i.e. behavioral

plasticity). Such an adaptive behavior is likely to occur in topographically heterogeneous ecosystems due to their small-scale spatial heterogeneity (Büntgen et al., 2017). Indeed, in mountainous areas, roe deer population exhibited partial migration, being a percentage of the population migratory and the rest, stationary (Myserud, 1999; Cagnacci et al., 2011). For this species, migration and dispersion movements may constitute a good strategy to match parturition dates and peak resource availability during the breeding season. However, we were unable to discriminate between both options, microevolution or behavioral plasticity. Therefore, more research is needed to understand the mechanisms that led to the small response observed along the elevational gradient. Only then, better populations-specific predictions on the rate of response of roe deer birth timing to climate change would be possible.

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Supplementary material

Supplementary tables

Table S4.3.1 Results of models performed at the inter-regional, regional and local scales to assess temporal variation of mean parturition date (instead of median parturition date) at different elevational intervals and their significance levels ('***' = $p < 0.001$; '**' = $p < 0.01$; '*' = $p < 0.05$; '.' = $p < 0.1$; 'ns' = $p > 0.1$). SP/PA = Swiss Plateau and the Pre-Alps, NA = Northern Alps, ECA = Eastern Central Alps.

Scale		Elevational interval	Mean parturition date (days per year $\pm$ SE)
Inter-region		(250-500]	0.03 $\pm$ 0.04 ns
		(500-750]	-0.02 $\pm$ 0.02 ns
		(750-1000]	-0.09 $\pm$ 0.02 ***
		(1000-1250]	-0.07 $\pm$ 0.03 **
		(1250-1500]	-0.00 $\pm$ 0.03 ns
		(1500-1750]	-0.09 $\pm$ 0.03 ***
		(1750-2000]	-0.06 $\pm$ 0.03 .
		Average	-0.04
Region	SP/PA	(250-500]	0.06 $\pm$ 0.05 ns
		(500-750]	-0.05 $\pm$ 0.02 *
		(750-1000]	-0.14 $\pm$ 0.03 ***
	NA	(500-750]	0.06 $\pm$ 0.04 ns
		(750-1000]	-0.04 $\pm$ 0.04 ns
		(1000-1250]	-0.06 $\pm$ 0.04 ns
		(1250-1500]	0.01 $\pm$ 0.07 ns
	ECA	(750-1000]	-0.15 $\pm$ 0.05 **
		(1000-1250]	-0.06 $\pm$ 0.03 *
		(1250-1500]	-0.01 $\pm$ 0.02 ns
		(1500-1750]	-0.08 $\pm$ 0.02 ***
		(1750-2000]	-0.07 $\pm$ 0.03 *
		Average	-0.04
Local	SP	[460-651]	-0.14 $\pm$ 0.05 **
	SP/PA1	[620-870]	-0.10 $\pm$ 0.04 *
	SP/PA2	[600-850]	-0.15 $\pm$ 0.04 ***
	NA/ECA	[1177-1425]	0.07 $\pm$ 0.06 ns
	ECA1	[1443-1693]	-0.01 $\pm$ 0.05 ns
	ECA2	[1754-2004]	-0.07 $\pm$ 0.06 ns
		Average	-0.07

Supplementary figures

Figure S4.3.1 Temporal trend in the start of the growing season (GSS; black solid line), the date of the beginning of the herbaceous flowering (FS; black dashed line) and median parturition date (grey solid line) for different elevational intervals at the regional scale: A) Swiss Plateau and the Pre-Alps, B) Northern Alps and C) Eastern Central Alps. Grey dots represent median parturition dates for each elevational interval and year. Dot size indicates the number of parturitions used to calculate the median.

Figure S4.3.2 Temporal variation in the date of median parturition for different elevational intervals at the regional scale: A) Swiss Plateau and the Pre-Alps, B) Northern Alps and C) Eastern Central Alps. Dot color and shape indicates the median parturition date for each year and elevational interval (m a.s.l.): ♦ (250-500]; ■ (500-750]; ■ (750-1000]; ● (1000-1250]; ● (1250-1500]; ▲ (1500-1750]; ▲ (1750-2000]. Dot size indicates the number of parturitions used to calculate the median.

Figure S4.3.3 Temporal trend in the number of fawns found in low vegetation meadows (< 20 cm) during the study period (1971 to 2015) for the three biogeographic regions: A) Swiss Plateau and the Pre-Alps, B) Northern Alps and C) Eastern Central Alps. Each shape indicates a different elevational interval (m a.s.l.): ■ = (500-1000], ● = (1000-1500] and ▲ = (1500-1000]. Shape size is proportionate to sample size.

CHAPTER II: Effects of environmental variation on female litter size

Study 4: Peláez, M., Perea, R., Bollmann, K., Rehnus, M. (in preparation)

Roe deer litter size increases with latitude but decreases with elevation. First example of the fecundity gradient paradox for a large mammal

Abstract

Lack's rule and Bergmann's rule describe the positive latitudinal gradient in clutch size and body size, respectively. As there is a positive allometry between body size and clutch size at the intraspecific level, both ecogeographical rules have often been used together to explain the macroecological fecundity gradient. Seasonality and predation have been posited as main drivers of fecundity selection and, as both display the same latitudinal/elevational trend, it was hypothesized that an elevational fecundity gradient should also exist. However, most studies suggest that bird clutch size decreases with elevation creating a fecundity gradient paradox. This paradox has seldom been tested for mammals.

Here, we used data on European roe deer (*Capreolus capreolus*) to assess litter size and body size variation along both gradients. We hypothesize that: (H1) both traits increase with latitude similar to other northern-hemisphere ungulates, (H2) body size does not increase with elevation, constraining litter size increase and (H3) as a result of phenotypic plasticity, body mass and litter size increase with habitat quality.

We performed a literature survey to assess the latitudinal trend in body size and litter size along the species' range of distribution. We then used data on roe deer fawns marked at birth (N= 4556) and recaptured as adults (N=563) across Switzerland (from 200 to 2,400 m a.s.l.) to assess the elevational trends of both traits. Finally, we performed a spatial analysis combining our elevational data with land use data to assess how habitat quality influenced phenotypic plasticity on both traits.

We observed an increase in roe deer litter size with latitude but a decreasing trend with elevation. Furthermore, although body mass displayed a latitudinal trend across its European range of distribution, this relationship did not occur along the elevational gradient.

This is the first time to observe this opposite relationship for a large mammal, suggesting that the fecundity gradient paradox not only applies to bird species but also to some mammal species. We believe, that the most likely explanation of this paradox may involve both, different selection pressures along the latitudinal and elevational gradient and the existence of evolutionary constraints on body mass along the elevational gradient.

INTRODUCTION

Obtaining accurate estimates of demographic parameters across geographical and climatic clines is important to predict population trends of widespread species. This is particularly critical under the current context of climate change due to the potential impacts of global warming on terrestrial organisms (Brook et al., 2009). Litter size, defined as the number of offspring produced by a female per single reproductive event (transient fecundity *sensu* Pincheira-Donoso & Hunt, 2017), is an important component of recruitment that modulates demographic tendencies. However, there is still a great research gap on how current environmental conditions influence litter size of large mammals at macroecological scales.

According to life-history theory (Stearns, 1992), litter size should be modified in space and time according to environmental variation to maximize female lifetime reproductive success and therefore, individual fitness. Two main trade-offs are involved in determining the optimal litter size: 1) the number of offspring vs. their quality (Lack, 1954) and 2) current parental reproductive investment vs. future fecundity (Williams, 1966b). These trade-offs depend on both, life history of species and environmental conditions (Gaillard et al., 2013). In addition, two main ecological selective pressures have been described to affect litter size: 1) climate (i.e. seasonality, length of the growing season, day length or temperature) and 2) predation (MacArthur, 1972). As both selection pressures display a geographical gradient (i.e. increased seasonality and reduced predation

probability at higher latitudes), Lack (1954) proposed a macroecological fecundity gradient based on the observation that litter size increased with increasing latitude at the intraspecific level (hereafter Lack's rule). This prediction, firstly postulated to explain clutch size variation on birds, has been also applied to other groups of vertebrates. But note that the vast majority of research has focused on birds (see Pincheira-Donoso & Hunt, 2017 for a review).

Interestingly, both sources of selection (i.e. climate and predation) also co-vary in similar direction with increasing elevation. Thus, latitudinal and elevational gradients show similar patterns, with an increase in seasonality and a decrease in predation as the mean temperature declines. Therefore, if the abovementioned environmental factors are the main drivers of litter size variation along the latitudinal gradient, they should also operate in the same direction along the elevational gradient (Pincheira-Donoso & Hunt, 2017). Surprisingly, however, the vast majority of studies assessing the macrogeographic variation of bird clutch size has reported opposite trends between both gradients (Hille & Cooper, 2015 for a review), revealing larger clutch sizes with increasing latitude but smaller clutches with increasing elevation. This finding is still under discussion as the selective pressures driving this contradictory trend have not been identified and thus, this paradox remains an open question (Hille & Cooper, 2015; Pincheira-Donoso & Hunt, 2017).

Although similar, elevational and latitudinal gradients differ substantially in the spatial scale at which environmental changes occur. While along the latitudinal gradient, environmental variation occurs over long geographical distances (i.e. hundreds of kilometers), this variation occurs at much smaller spatial scales along the elevational gradient (i.e. meters to few kilometers). Thus, for mobile animals with high dispersal capacities, such as large mammals, gene flow could constrain selection for optimal litter size along the elevational gradient, or even, indirectly, constrain other life-history traits that influence litter size. Indeed, female body size is such a trait that has often been linked to fecundity

selection (see Pincheira-Donoso & Hunt, 2017 for a review). It has been posited that, at intraspecific level, larger females are able to accommodate more offspring (Williams, 1966b) but see Calder's rule (1984), for interspecific level. Therefore, as body size displays a latitudinal trend (Bergmann's rule; Bergmann, 1847), the two ecogeographical rules (Lack's rule and Bergmann's rule) have usually been used together to explain variation in litter size (Boyer, Cartron, & Brown, 2010). However, while the compliance of Bergmann's rule at intraspecific level (Rensch's modification of the rule; Rensch, 1938; Blackburn, Gaston, & Loder, 1999) has been demonstrated for several species of large mammals along the latitudinal gradient (Langvatn & Albon, 1986 on red deer *Cervus elaphus*; Flajšman et al., 2018 on roe deer *Capreolus capreolus*; see Ashton, Tracy, & Queiroz, 2000 for a review), body size variation along the elevational gradient has seldom been studied within this group.

Furthermore, attempts to demonstrate a latitudinal trend on litter size for large mammals are scarce and had led to contradictory results: those that correspond with Lack's rule (Bywater et al., 2010 on wild boar *Sus scrofa*; Tökölyi, Schmidt, & Barta, 2014 on the order Artiodactyla), those that found no trend (canids and felids; Lord, 1960; Gaillard et al., 2014) and one that found the opposite relationship (Derocher, 1999 on polar bears *Ursus maritimus*). Furthermore, along the elevational gradient, to date, Lack's rule has only been tested on four genera of small-medium sized mammals leading to inconclusive results (see Supplementary material Table S4.4.1). Surprisingly, despite the relevance of this topic, there is virtually no information on the variation of litter size along an elevational gradient for wild large mammals.

A common problem encountered when studying geographical clines on litter size or body size of large mammals is that both traits display a high phenotypic plasticity (Boutin & Lane, 2014). This is likely to introduce noise in inter-population analysis, making it harder to obtain a clear geographical trend on this trait. In particular, as close, genetically similar populations, show large

inter-population variation on litter size depending on habitat quality and environmental conditions (Nilsen, Linnell, & Andersen, 2004). Thus, it is very important to control for these variables.

Ungulates constitute good model species to study life-history variations at the macroecological scale due to their abundance and widespread distribution, which includes regions of very different climates. Contrary to most small mammals, with multiple litters per breeding season, most large herbivores only have one breeding attempt per reproductive season. Hence, there is no trade-off between number of litters and litter size, which simplifies the analysis (Whorley & Kenagy, 2007). Polytocous large ungulates (those with variable litter size) are perfectly suited for this type of studies and, within this group, some species are among the most abundant and widespread mammalian species in the northern hemisphere (e.g. white tailed deer, roe deer and wild boar).

Here, we studied, how roe deer, a polytocous large herbivore, varies litter size and body size along the latitudinal and elevational gradient. Roe deer females are income breeders (Andersen et al., 2000) which only accumulate little body fat. Therefore, body mass is used as a proxy of phenotypic quality, highly correlated to body size (Hewison, 1996). Interestingly, a recent meta-analysis has demonstrated a positive latitudinal trend on roe deer body mass (Flajšman et al., 2018). Based on these findings, we performed a literature search to gather litter size data from different populations of roe deer across the latitudinal range of the species' distribution. Secondly, to assess the variation of body mass and litter size along the elevational gradient, we used an extensive database on roe deer fawns marked at birth (N= 4556 roe deer litters) and recaptured as adults (N=563) across Switzerland from 200 to 2,400 m a.s.l. Finally, we compared results of both gradients. More specifically, the following expectations were formulated and tested.

H1: We expect litter size to increase along the latitudinal gradient, similarly to what it has been described for other ungulates (Bywater et al., 2010; Tökölyi et al., 2014).

H2a: Given the dispersal ability of a large herbivore, we do not expect body size to vary greatly along the elevational gradient as gene flow would constrain genetic adaptation at such a small scale. H2b: Consequently, if body size variation is one of the main drivers of litter size selection for roe deer (Flajšman et al., 2018), we do not expect litter size to increase with elevation.

H3: However, due to the reported high phenotypic plasticity of body mass and litter size for roe deer (Hewison & Gaillard, 2001; Nilsen, Linnell & Andersen, 2004; Macdonald & Johnson, 2008; Flajšman et al., 2013; Flajšman et al., 2018), we do expect habitat quality to influence both traits between individuals living at the same elevation.

MATERIALS AND METHODS

Data collection

Latitudinal gradient

We performed a literature survey to explore the potential latitudinal trends in roe deer litter size across its European range of distribution. Previous research studying macrogeographic variation in litter size on large mammals have preferably used either *corpora lutea/embryo* counts per ovulating/pregnant females (Bywater et al., 2010 on wild boar) or number of offspring per parturition (i.e. litter size; Gaillard et al., 2014 on Eurasian lynx; Derocher, 1999 on polar bear). Each technique used to calculate female fecundity points to a different step in female reproduction (Hewison & Gaillard, 2001). On the one hand, *corpora lutea* count gives information about potential or maximal litter size. Therefore, *corpora lutea* count is usually higher than embryo counts in utero due to fertilization/

implantation failure (Hewison & Gaillard, 2001). Furthermore, fetal reabsorption, stillbirths and early life mortality further decrease the observed litter size after birth, which highlights the importance of using only data of newborns collected on the first days of life. Therefore, we expect for the same population, *corpora lutea* count per ovulating female to be greater than *embryo* count per pregnant female and both higher than litter size after birth.

Therefore, we searched for research publications reporting any of the three fecundity parameters described above (i.e. *corpora lutea*, *embryo* counts and fawns per litter). A previous study assessing roe deer body mass variation across the latitudinal gradient also assessed the relationship between body mass and litter size for different populations (Flajšman et al., 2018). However, this study treated data on *corpora lutea*, *embryo* counts and fawns per litter (i.e. Norway) indistinctively (Flajšman et al., 2018). We are convinced that the differentiation among these parameters is important for any analysis. Furthermore, mean *corpora lutea* and *embryo* calculations should be calculated only among ovulating or pregnant females, respectively, and not among all female adults studied. This is particularly important to distinguish between two different processes; fertility and transient fecundity (*sensu* Pincheira-Donoso & Hunt, 2017).

In order to perform a literature review, we entered the following topic search (TS) and operators into the Web of Science database: TS = (“*Capreolus capreolus*” SAME “roe deer”) AND TS = (“litter size” OR “*corpora lutea*” OR “*embryo*” OR “pregnant” OR “pregnancy” OR “fawns” OR “reprod*”). A total of 642 peer-reviewed publications were obtained. After reading the title and abstract of each publication, we selected 29 research articles reporting roe deer litter size, *corpora lutea* or number of embryos per ovulating/pregnant female across Europe. By scanning the citations in these articles, we were able to add 12 publications (see Supplementary material Fig. S4.4.1).

We applied the following criteria to include information from an article in the analysis: 1) to have collected information on a minimum sample of 80 ovaries,

uteri or litters, as sample size is very important to infer small changes in litter size across latitudes, 2) when litter size was assessed more than once for the same population, we recorded values from the study reporting the largest sample size and among similar values the study that specify the number of litters with singles, twins and triplets, 3) we discarded all articles where it was not clear if calculations were made only among pregnant females or among all female adults (including non-pregnant females) and 4) we only used data coming from wild populations and thus discarded studies in captivity or fed *ad libitum* (Supplementary material Fig. S4.4.1).

Therefore, for the analysis, we finally used information on 24 different study sites (from 27 different research articles) that covered most of the geographical distribution of roe deer, from Norway (64°N) to the south of Spain (39°N). Then, we constructed a database including information on: country, latitude, number (N) of fawns per litter (mean age at capture), N embryos per pregnant female, N *corpora lutea* per ovulating female, percentage of singletons, twins, triplets, quadruplets and quintuplets, N litter, N *corpora lutea*/embryo/fawns, year of study and references (Supplementary material Table S4.4.2).

Elevational gradient

Data was obtained from the project “Rehkitzmarkierung Schweiz” (roe deer fawn marking project) of Switzerland. Since 1989, yearly systematic searches have been performed to find and mark newborn fawns across Switzerland, from 289 to 2366 m a.s.l (Peláez et al., 2020). For each fawn found, the following information was recorded: the date of capture, the estimated age in days, the geographic coordinates and the number of fawns per litter (Rehnus, Peláez, & Bollman, 2020 for more information). Fawn age was determined based on the characteristics of the umbilical cord and behavior while handling (Jullien, Delorme, & Gaillard, 1992). Finally, each fawn was released with an ear mark to be able to identify them if recaptured.

The area of study encompassed four characteristic and adjacent regions: Swiss Plateau (SP), Pre-Alps (PA), Northern Alps (NA) and Eastern Central Alps (ECA; Peláez et al., 2020). These regions constitute a continuous elevational gradient, with the Swiss Plateau at the lowest elevation, the Pre-Alps and Northern Alps as middle/transitional regions and the Eastern Central Alps at the highest elevation (Fig. 4.4.1).

Figure 4.4.1 Map of Switzerland showing all locations included in the litter size elevational analysis. Yellow colors represent agricultural areas and green areas forests and shrublands. Different signs and colors represent litter size: ● one fawn, ▲ two fawns; ■ three fawns.

Litter size variation along the elevational gradient

Litter size was calculated based on the observations of field workers who recorded the number of fawns per parturition event. As siblings generally hide within 50 meters of each other (Linnell, 1994), once a fawn was found repeated searches around it allowed the capture or observation of siblings determining litter size (stillbirth fawns were accounted for but not marked). However, not in all cases litter size could be assessed with certainty and in those cases no information

on litter size was provided. Additionally, we used fawns ≤ 10 days old to minimize the risk of accounting for the effect of early life mortality (Aanes & Andersen, 1996; Panzacchi et al., 2008). Furthermore, fawns younger than 10 days are in the hiding phase so that they do not try to escape or move when approached, which makes it easier to find the siblings, as they will stay in the same place (Jullien et al., 1992). Therefore, for the analysis, we used litter size information from 4556 parturition events collected between 1989 and 2011. Mean age at marking for the subset used in the analysis was 4.6 ± 2.8 days (mean $\pm$ SD) and was very similar for the three regions (SP/PA= 4.9 ± 2.8 ; NA= 4.4 ± 2.6 ; ECA= 4.6 ± 2.8).

Body size variation along the elevational gradient

Also from the roe deer fawn marking project, we obtained data on 563 recaptured adults (aged 2 years or older) hunted between August and December across Switzerland. As these adults were marked as fawns (179 females and 384 males), the following information was available: date of birth, birth location, age, capture location and body mass calculated from the clean carcass with head, with an accuracy of ± 500 grams.

Land use or habitat quality information along the elevational gradient

For the elevational analysis, we assessed the type of land use in the surroundings of each litter or recaptured adult to control for habitat quality which is known to influence both, female litter size (Hewison, 1996; Gaillard et al., 1992; Gaillard et al., 2000b; Mateos-Quesada & Carranza, 2000; Macdonald & Johnson, 2008) and fawn body mass during adulthood (Pettorelli et al., 2002). In fact, for an income breeder such as roe deer, body mass varies little during adult stage and therefore early life conditions have been described to be more determinant of body mass than adult home range quality (Andersen et al., 2000). Also, litter size for large herbivores has been described to be mainly driven by female body mass but also by female age (Gaillard et al., 2000b; Hewison & Gaillard, 2001). However, our data on litter size came from marked fawns whose mothers were

not captured. Therefore, we could not control for maternal body mass or age but instead, we controlled by habitat quality around the location of birth.

Firstly, we drew a circular buffer area around each litter or recaptured adult, equivalent to the area of a mean annual home range calculated from twelve GPS-collared roe deer in Switzerland (radius of 376 m; 44.5 ha; Ineichen, 2015). From this area, we extracted the percentage of each habitat type by using first and second level classification of Corine land cover (CLC) maps (<https://land.copernicus.eu/pan-european/corine-land-cover>). Furthermore, because our data came from the period 1989-2011, we used three different maps to control for possible temporal changes in land use; CLC2000 (from 1989-2002), CLC2006 (from 2003-2008) and CLC2012 (from 2009-2011). We used CLC2000 instead of CLC1990 for the period 1989-2000 because Switzerland does not have fully compatible maps for CLC1990 and using the map available was likely to introduce more bias than simply using CLC2000. Finally, because coordinate precision is very important to assess elevation and land use type around the place of capture, we only used coordinates with a data resolution ≤ 1 ha.

Overall, 51.2% of the area surrounding the litters were *forest and seminatural areas* (43.8% forest and 7.3% *scrub and/or herbaceous vegetation associations*) while the rest 48.7% were occupied by agricultural areas [*pastures* (36.6%), *arable land* (9.9%) *heterogeneous agricultural areas* (2.2%)]. Other land uses occupied less than 1%. *Water bodies, wetlands, and artificial surfaces* were excluded from the calculation of these percentages.

Finally, we used the percentage of agricultural areas surrounding the birth place as proxy variable for habitat quality. Other studies have shown that open agricultural landscapes are important drivers of female body size and litter size for roe deer (Gaillard et al., 2000b; Hewison et al., 2009).

Statistical analysis

Latitudinal analysis

Linear regressions were fitted to detect latitudinal trends in litter size from the 24 studies considered. Therefore, we used fecundity as the response variable. Latitude and the method used to calculate fertility (mean number of *corpora lutea* per ovulating female or mean number of embryos per pregnant female or litter size after parturition) were included in the model as co-variables. Finally, diagnostic tests were performed to check for normality of residuals and heteroscedasticity.

Elevational analysis

We performed Generalized Linear Mixed Models (GLMMs) with Poisson error distribution to assess elevational trends in female litter size. Litter size was the response variable and elevation, fawn age and fawn age² and the percentage of agricultural areas surrounding each litter as predictors. Year was included in the model as a random factor. Fawn age² was included in the model to capture the non-linear decreasing tendency in the observed litter size with increasing fawns age. Fawn age at marking was included in the model as control variable to avoid problems associated with early life mortality and the percentage of agricultural areas was included to account for variations in habitat quality.

To assess elevational variation in adult body size, we performed Linear Mixed Models (LMMs) with body mass of adults as the response variable (carcass weight with head). We included elevation, sex and their interaction as the predictors. Similarly, habitat quality at birth and during adulthood were included as control variables. Cohort (year of birth) was included as a random effect. We studied both, male and female variation in body size along the elevational gradient because: 1) there is high genetic correlation in body size between the sexes

(Fairbairn, 1997) and 2) data on one sex may validate the results obtained from the other and 3) a larger sample size leads to less error and higher statistical power.

All analyses were performed using R 3.2.1 statistical software (R Development Core Team, 2018). We used the function “lm” from the “stats” package to fit linear models and the function “lme” from the “nlme” package to fit generalized linear mixed model with Poisson error distribution.

RESULTS

Latitudinal results

Linear model results revealed a positive relationship between latitude and the three measures of female fecundity (N of *corpora lutea*, N of embryos per pregnant female and litter size at birth; Fig. 4.4.2A; Supplementary material Table S4.4.1). Although sample size was small ($n=24$), female fecundity significantly increased with each degree increase in latitude (estimate= 0.0264 ± 0.0067 ; $t=3.884$; $p<0.001$ for all three fecundity parameters measured; Fig. 4.4.2A).

Figure 4.4.2 Roe deer litter size variation along the latitudinal and elevational gradients. A) Variation of the three fecundity parameters along the latitudinal gradient: *corpora lutea* (mean number of *corpora lutea* per ovulating female), embryo count (mean number of embryos per pregnant female) and fawns per litter (the number of fawns per litter after birth). B) Litter size variation (number of fawns per litter after birth) with increasing elevation for different ages of fawns at capture. Points represent raw data and lines full model results. N indicates sample size.

As expected, number of *corpora lutea* per ovulating female was higher than number of embryos per pregnant female and both higher than the number of fawns per litter after birth. However, the difference was only significant between embryo count and the number of fawns per litter ($t=-2.759$; $p=0.012$). Finally, our data suggested a higher percentage of litters with triplets in the Scandinavian region compared to sites further south (Fig. S4.4.2A).

Elevational results

Litter size results

Predictions from the generalized linear mixed model indicated an overall decrease in the number of fawns per litter with increasing elevation ($t=-5.58$, $p<0.001$; Table 4.4.1; Fig. 4.4.2B), even when controlling by age and habitat quality. In fact, age at marking had an important influence on the observed litter size; the later the age at marking, the lower the estimated litter size (Table 4.4.1; Supplementary material Fig. S4.4.3A). In addition, we found a non-significant increase of litter size with adult habitat quality (Table 4.4.1; Supplementary material Fig. S4.4.3B). Finally, with increasing elevation, we observed a decrease in the percentage of females with triplets and an increase in the percentage of singletons (Supplementary material Fig. S4.4.2B).

Table 4.4.1 Results of the models describing female fecundity variation at different latitudes and elevations.

	Fixed effect	Estimate	Std.Error	T value	p-value
Litter size (Latitude)	Latitude (°N)	0.0264	0.0068	3.88	<0.001
	Technique [Embryo/ pregnant]	-0.1868	0.1250	-1.49	0.151
	Technique [Fawns/litter]	-0.3722	0.1349	-2.76	0.012
Litter size (Elevation)	Elevation	-0.0682	0.0122	-5.58	<0.001
	Age	-0.0390	0.0133	-2.93	0.003
	Age²	0.0390	0.0122	3.17	0.002
	Percentage of agricultural land surrounding the birth place	0.0125	0.0122	1.02	0.306
	Elevation	0.0003	0.0003	1.10	0.273
Body mass (Elevation)	Sex (males)	1.4340	0.4664	3.08	0.002
	Elevation*sex	-0.0002	0.0004	-0.64	0.517
	Percentage of agricultural land surrounding the birth place	0.7290	0.2531	2.88	0.004
	Percentage of agricultural land in the adult territory	0.0444	0.2691	0.17	0.869

Latitudinal model: LM: Fecundity ~ Latitude + Technique. Dummy factor was corpora lutea per pregnant female. Elevational models: GLMM: Litter size ~ Elevation + age + age2 + Percentage of agricultural land surrounding the birth place + (1 | year of capture). Poisson error family. Variables were scaled. LMM: Body mass ~ Elevation * sex + Percentage of agricultural land surrounding the birth place + Percentage of agricultural land in the adult territory + (1 | year of birth). Gaussian error family. Variables were scaled.

Body size results

Results from the generalized linear mixed model did not report a significant increase in body weight with elevation either for males (estimate=0.01 ± 0.02 kg per 100 m increase, t=0.44, p=0.663) nor for females (estimate=0.03 ± 0.03 kg per 100 m increase, t=1.10, p=0.273; Table 4.4.1; Fig. 4.4.3A). However, there were significant differences between female and male body mass, being males on average 1.43 ± 0.021 kg heavier than females (t=-3.075, p= 0.002). In addition, we found a significant relationship between adult body weight and

habitat quality at birth ($t=2.88$, $p=0.004$; Fig. 4.4.3B), while we did not find any significant correlation with habitat quality during adulthood ($t=0.17$, $p=0.869$; Fig. 4.4.3C). Finally, the interaction between elevation and sex was non-significant ($t=0.65$, $p=0.517$).

Figure 4.4.3 Linear mixed model results describing the variation of adult body weight in relation to: A) elevation, B) the percentage of agricultural land surrounding the birth place and C) the percentage of agricultural land in the adult territory. ● = mean weight for females (f) and ● = mean weight for males (m; based on raw data). N indicates sample size.

Comparison among both gradients

We predicted an increase in litter size with latitude from 1.34 fawns per litter at 39°N to 1.98 at 64°N. On the contrary, the elevational model predicted a decrease of litter size with increasing elevation, varying from 1.95 fawns per litter at 400 m a.s.l. to 1.53 at 2000 m a.s.l (Table 4.4.2). These estimates were calculated for fawns found at the age of one day on an area surrounded by 50% of agricultural lands.

Contrary to previous studies (Flajšman et al., 2018), the elevational model showed a non-significant increase of body mass from ~16.9 kg at 400 m a.s.l. to 17.5 kg at 2000 m a.s.l (Table 4.4.2).

Table 4.4.2 Comparison of the models performed to assess variation on litter size and body size along the latitudinal and elevational gradient.

	Gradient	Lowest range	Highest range	Trend	References
Litter size (fawns/ litter)	Latitude	1.34 (39°N)	1.98 (64 °N)	Increase	This study
	Elevation	1.95 (400 m a.s.l.)	1.53 (2000 m a.s.l.)	Decrease	This study
Body size (kg)	Latitude	16.0 (46 °N)	21.0 (63 °N)	Increase	(Flajšman et al., 2018)
	Elevation	16.9 (400 m a.s.l.)	17.5 (2000 m a.s.l.)	No trend	This study

DISCUSSION

In this study, we observed an increasing trend in roe deer litter size with latitude but a decreasing trend with increasing elevation. To the best of our knowledge, this is the first time to observe this opposite relationship for a large mammal, although the same contradictory results have been obtained in most bird species (Hille & Cooper, 2015). Furthermore, although roe deer body mass shows a latitudinal trend across its European range of distribution (Flajšman et al., 2018), we demonstrated that this relationship does not occur along the elevational gradient (from 289 to 2366 m a.s.l.) of this study.

Roe deer latitudinal results on litter size are in line with the results of the only study on another large ungulate (wild boar; Bywater et al., 2010) and with a study across species of the order Artiodactyla (Tökölyi et al., 2014). Hence, our results further support the theory that litter size of large ungulates increases with higher latitudes (Lack, 1954). However, Lack's latitudinal fecundity gradient among different orders of medium-size or large mammals (see Lord, 1960; Gaillard et al., 2014 on canids and felids; Derocher, 1999 on polar bears) appears to be far less consistent than across bird species (Cardillo, 2002). Therefore, as suggested previously by some authors (Lord, 1960; Bywater et al., 2010), different groups of mammals should be studied separately by, for example, investigating species with different food habits (carnivores vs. herbivores or omnivores;

Bywater et al., 2010) or between hibernating vs. non hibernating behaviour (Derocher, 1999; Lord, 1960).

On the contrary, our study revealed that roe deer litter size at population level decreased with elevation across Switzerland, even after controlling by fawn age at marking and habitat quality at birth places. There are only few species of mammals for which elevational and latitudinal clines on litter size are available to compare with our results and, most of the species studied are small mammals which usually have multiple litters per year. These species showed increasing litter sizes with elevation and a decreasing trend or no variation on the number of litters (Dunmire, 1960 on *Peromyscus maniculatus*; Smith & McGinnis, 1968 on *Peromyscus* sp.; Dolbeer & Clark, 1975 on snowshoe hare: *Lepus americanus*; Schai-Braun et al., 2017 on Alpine mountain hare: *Lepus timidus*; Supplementary material Table S4.4.1). These patterns correspond with the trend along the latitudinal gradient (Rowan & Keith, 1956 on snowshoe hare; Lord, 1960 on deer mice; Schai-Braun et al., 2017 on Alpine mountain hare). For other medium-sized mammals such as the ground squirrel (*Spermophilus* spp.), with only one reproductive attempt per year, no trend or a decrease in litter size with elevation was observed (Murie et al., 1980; Zammuto & Millar, 1985 on *S. columbianus*; and Bronson, 1979 on *S. lateralis*). However, there was no relationship between litter size and latitude when pooling all species from *Spermophilus* and *Ammospermophilus* genera (Lord, 1960) although later, studies showed a positive and a negative trend for *A. leucurus* and *Spermophilus beecheyi*, respectively (Chapman & Lind, 1973; Whorley & Kenagy, 2007). Thus, elevational and latitudinal trends on litter size for mammals are far from clear. Future comparative, interspecific studies should consider the number of litters per year, as this factor may influence the observed trend.

In this study, we demonstrated that the fecundity gradient paradox not only applies to bird species (Pincheira-Donoso & Hunt, 2017) but also to the roe deer. We hypothesized that selection on female body mass would be required for an increase in litter size as suggested by Williams, (1966). Indeed, female body

mass (carcass weight) increased strongly in Scandinavian populations compared to populations in central and southern Europe (Flajšman et al., 2018) but did not significantly increase along the elevational gradient in Switzerland (H2a). Thus, as body mass did not increase with elevation (also see Nägeli & Rehnus, 2018), we predicted that it would constrain a possible increase in litter size (H2b).

We hypothesize that the particular evolutionary scenarios provided by the highly heterogeneous topography along the elevational gradient, which significantly differs from those provided by the latitudinal gradient (Pincheira-Donoso & Hunt, 2017), prevents a trend on body mass with elevation. Further, body mass selection may be constrained by gene flow, as dispersion distances of roe deer might be larger than the scale at which environmental changes occur. Therefore, selection on body mass variation in this environment is low (Lenormand, 2002; Loe et al., 2005). On the other hand, an increase in body mass might not be beneficial as seasonal migration is common in the populations inhabiting the harsher regions of the Alps (Ramanzin, Sturaro, & Zanon, 2007; Gaudry et al., 2015). This may indicate that instead of increasing body size to compensate for the cost of enduring the harsh winter conditions, as it happens along the latitudinal gradient, they simply move towards lower, more suitable elevations (i.e. valley areas). This behavioural plasticity would allow them to optimize the energy metabolism efficiently without an increase in body size, which would in turn constrain litter size selection. This could explain why large mammals with long-distance dispersal or seasonal movements show different trends along the elevational gradient compared to small mammals.

Finally, it is not clear why we observed a decreasing trend in litter size with elevation despite the lack of variation in female body size. To answer this question, firstly, we would have to elucidate which adaptive mechanisms (phenotypic plasticity, local adaptation or both) are predominantly involved in the adaptation of large herbivores to each environmental gradient. While along the latitudinal gradient, we could argue that body size and litter size are the

consequence of genetic differentiation (Lenormand, 2002), the underlying mechanisms driving litter size variation along the elevational gradient remain highly elusive. One possible explanation is that different selection pressures are interacting on fecundity at higher elevations (other than those described for the latitudinal gradient; growing season length and predation pressure, Skutch, 1949). Some climatic particularities that occur at high elevations but not at high latitudes are: increased solar radiation, lower atmospheric pressure, lower oxygen concentration and the fact that day length does not change with increasing elevation, with the same number of foraging hours at low and high elevations (day length hypothesis; Hille & Cooper, 2015). Another alternative would be that, without a favorable scenario for body mass selection along the elevational gradient, the decrease of litter size at higher elevations would be a plastic response of females to the decrease in the growing season length and delayed parturition dates (Peláez et al., 2020). Finally, the observed decrease in litter size could be the product of some factors that could not be accounted for in our model (i.e. mother body size or age, population density, etc.). Indeed, roe deer females have been described to have higher litter sizes with increasing age (Hewison, 1996; Flajšman et al., 2017) and therefore, the observed decrease of litter size with increasing elevation could be the outcome of a younger female age structure at higher elevations. Further, we demonstrated that adult body mass was more influenced by habitat quality at birth than during adulthood (see Pettorelli et al., 2002) but, in our litter size model, we only controlled by habitat quality at the mother adult home range and not by the maternal habitat quality at birth. Nevertheless, we do not expect maternal body mass to be the major causal factor of the observed decrease in litter size, as overall body mass of females remained almost constant along the elevational gradient. Therefore, further studies should confirm our results based on embryo counts of females with known age and body mass or alternatively, elucidate whether there is a trend towards younger female age structure at higher elevations.

Finally, we think, that the most likely explanation of this paradox may involve both, different selection pressures along the latitudinal and elevational gradient and the existence of evolutionary constraints along the elevational gradient that cause a different plastic response on litter size (Pincheira-Donoso & Hunt, 2017). Thus, a given evolutionary outcome can include genotypes that both show some plasticity, yet also differ adaptively across a spatial gradient—a combination of plasticity and local adaptation (Scheiner & Holt, 2012).

Recommendations for further research

In our latitudinal analysis, we demonstrated that the time at which female fecundity is assessed is determinant as each technique (corpora lutea, embryo per female, litter size per female or per ovulating/pregnant female) produces indicators of different female reproductive stages. Therefore, they should not be treated as equal in any analysis. Ideally, the best way to determine fecundity for females would be through embryo counts as closest as possible to birth date. However, for obvious ethical reasons hunting is forbidden at this stage and capturing and handling live animals for ultrasonography could produce an unwanted outcome on females (i.e. increased abortions). Therefore, most of the data available is usually either at the beginning of pregnancy or after birth. Therefore, we must assume that when assessing litter size at the beginning of pregnancy, we may account for fetus that would be aborted or reabsorbed before birth and when assessing litter size after birth, we risk not accounting for stillbirths, early life mortality or predation. Therefore, when using the latter parameter, it is very important to control by fawn age (in days) as the number of fawns strongly decreased with increasing fawn age at marking.

Acknowledgements

The authors thank all gamekeepers, hunters, and researchers who participated in the marking of fawns over the study period. We also acknowledge the Federal Office for the Environment for the permission to use data from the project «Rehkitzmarkierung Schweiz». Marta Peláez acknowledges financial support from the Spanish Ministry of Education (FPU13/00567 and EST16/00095).

Supplementary material

Supplementary tables

Table S4.4.1 Litter size variation for different mammal species along the latitudinal and elevational gradients.

Order	Species	Litters per season	Latitudinal trend (low to high)		Elevational trend (low to high)		References
			Litter size	N litter per season	Litter size	N litter per season	
Lagomorpha	<i>Lepus timidus varronis</i>	>1	+	-	+	=	Schai-Braun et al. (2017)
Lagomorpha	<i>Lepus americanus</i>	>1	+	-	+	-	Rowan & Keith (1956); Dolbeer & Clark (1975)
Lagomorpha	<i>Sylvilagus floridanus</i>	>1	+	NA	-	NA	Conaway, Sadler, & Hazelwood (1974)
Rodentia	<i>Peromyscus maniculatus</i>	>1	+		+	-	Lord (1960); Dunmire (1960)*
Rodentia	<i>Peromyscus</i> spp.	>1			+	NA	Smith & McGinnis (1968)
Rodentia	<i>Spermophilus</i> spp.	one	= - (<i>S. beecheyi</i>)		= / - (<i>S. columbianus</i>) - (<i>S. lateralis</i>)		Chapman & Lind (1973); Bronson (1979); Murie, Boag & Kivett (1980); Zammuto & Millar (1985)
Artiodactyla	<i>Capreolus capreolus</i>	one	+		-		This study
Artiodactyla	<i>Sus scrofa</i>	one	+				Bywater et al. (2010)
Artiodactyla	<i>Many species</i>	one	+				Tökölyi, Schmidt & Barta (2014)
Carnivora	<i>Ursus</i>		=				Derocher (1999)

*small sample size, n=40

Table S4.4.2 Summary of the information extracted from the 41 articles pre-selected during the literature review. On black, information used for the latitudinal analysis, on grey articles excluded.

Country	Latitude °N	Fawns / litter	Mean age at capture	Embryo / pregnant female	Corpora lutea / pregnant fem.	Singles %	Twins %	Triplets %	Quad. %	Quint %	N litters	N fawns	Years	References
Norway	64	1.88	7 ± 0.6			37.5	37.5	25			24*	45	1992-1993	Aanes & Andersen, (1996)
Norway	63	2.1												Linnell (1994) In: Aanes & Andersen, (1996)
Norway (Stofosna)	63	2.17	≤ 5			12.3	59.2	28	0.8		130	282	1991-1994	Andersen & Linnell (1997)
Norway (Stofosna)	63	2.02									146	296	1991-1994	Linnell & Andersen (1998a*) **
Norway (Hedmark)	61	1.74									19*	35	1995-1997	Linnell & Andersen (1998a*)
Norway (Hedmark, Østfold and Akershus)	61	1.9	5.9±5.5								81	151	1995-1998; 2001-2004	Panzacchi et al. (2009); Panzacchi et al. (2008) (birth date)
Norway (Bogesund)	59			1.8							61*			Walhström & Kjellander (1995) ****
Sweden	59		2.2			15.7	50	33	1.1		362			Borg, (1971): In Stubbe, Stubbe, & Stubbe (1982)
Sweden	59			2.29										Borg (1971): In Andersen & Linnell (2000)
Sweden (Grimsö)	59		2.2								23			Cederlund & Lindström (1983)****
Denmark (Kalo)	56		1.91			13	82.6	4.3			46*	88		Andersen (1953): In Stubbe et al. (1982)

Table S4.4.2 (continued)

Lithuania	55				2.16	12.6	61.8	22	3.5					Prusaite, Blazys, & Baleisis (1976); In Stubbe et al. (1982)
Poland (Zielonka)	52			1.82							61	111	1975-1980	Fruziński & Łabudzki (1982)
Poland (Czempiń)	52			2.02		13.2	72.9	13	0.8		129		1973-1978	Kałuźiński (1982)
Germany (Hakel)	52			1.85		21.5	71.8	6.7	0		149	276		Stubbe et al. (1982)
Bielowesha	52			1.4		58	41.4	0.6			169	241		Sablina (1955); In Stubbe et al. (1982)
England	53			1.69							> 1000			Hewison (1996) **
England	53				1.81						> 1000			Hewison (1996)
England (Wales and England)	52			1.73		26.6	72	1.4			1536	2684	1994-2005	Macdonald & Johnson (2008); Hewison (1996); Hewison & Gaillard (2001)
England (Kielder Forest)	55			1.7							34*			McIntosh, Burlton & McReddie (1995)
England (Dorset, Wiltshire)	51			1.904		16.7	77.8	3.7	1.9		54	103	1963-1966	Chapman & Chapman (1971); Chaplin, Chapman & Prior (1966)
England (Dorset, Wiltshire)	51				2						42*	84	1964-1965; 1965-1966	Chapman & Chapman (1971); Chaplin & Chapman & Prior (1966)
Belgium	51			1.92							74*	142	1987-1993	Wauters et al. (1995)
Poland (Krakov)	50				1.85									Bobek et al. (1974); In Bobek (1977)
France (Trois Fontaines)	48	1.35	~ 7			65.1	34.6	0.4			784	1062	1985-2010	Plard thesis (2014) pp 69; Gaillard et al. (1998)

Table S4.4.2 (continued)

Hungary	47			1.66		36.2	61.4	2.4			246	409		Szederjei & Szederjei (1971); In Stubbe et al. (1982)
France (Chizé)	46			1.49^a		53	47	0			73 ^b		1987-1988	Sempéré, Renaud & Bariteau (1989)
France (Chizé)	46			1.95							20*			Portier et al. (2000) ***
France (Chizé)	46			1.72							358	614	1988-2001	Gaillard et al. (2003)
Switzerland	46	1.67	≤ 3 wks			39.1	53.4	7.2			573	959		Kurt (1968); In Stubbe et al. (1982) * it is embryo count but it is fawns per litter
Switzerland (Bern)	46	1.66	≤ 3 wks			38.9	56.1	5			384	638	1965	Sägesser & Kurt, (1965)
Slovenia	46			1.85		19.8	75.8	4	0.3	0.1	1477		2013-2016	Flajšman et al. (2017) (Table S1)
Ukraine (Crimea)	45			1.4		59.8	40.2				92	129		Heptner & Naumov (1966); In Stubbe et al. (1982)
Italy (Apenines)	44	1.16									117	136	1997-2003	Raganella-Pelliccioni, Scremin & Toso (2007)
France (Petit Luberon)	43	1.59									29*	46*		Maillard et al., (2002)
Italy (Pisa)	43			1.95		10.9	83	6.2			292	569	2009-2014	Lombardini, Varuzza, & Meriggi (2017)
Italy (Arezzo)	43			1.89		19.3	72.8	7.7	0.2	0.1	2617			Flajšman et al. (2017) (Table S1)
Italy (Arezzo)	43				2.03	7.8	80.9	11	0.2		2689			Flajšman et al. (2017) (Table S1)
Spain (Villuercas)	39	1.46				55.8	42.5	1.8			113	165	1994-1995	Mateos-Quesada & Carranza (2000); Mateos-Quesada (2002)

a Mean calculated from 1.47 and 1.51; b Seven litters inconclusive between 1 and 2. Asterisks represent the cause of exclusion: ‘*’: Litter size below 80, ‘**’: duplicated information (same database as other study), ‘***’: Study performed on captive females and ‘****’: not clear if calculation was made only among pregnant females or among all females captured.

Supplementary figures

Figure S4.4.1 PRISMA diagram of our systematic literature review.

Figure S4.4.2 A) Percentage of female roe deer with 1, 2, 3 or 4 corpora lutea/embryos or fawns at different latitudes. **B)** Percentage of litters with 1, 2, or 3 fawns at different elevations.

Figure S4.4.3 Litter size variation with: **A)** increasing marking age of the fawns at different elevational ranges and **B)** for habitat quality with different percentage of agricultural lands surrounding birth places at different elevations: low (<25%), medium-low (25-50%), medium-high (50-75%) and high (>75%). Signs of different size indicate sample sizes.

CHAPTER III. Environmental and individual factors affecting male secondary sexual traits

Study 5: Peláez, M., Perea, R., Díaz, M., San Miguel, A., Rodríguez- Vigal, C., & Côté, S. D. (2018)

Use of cast antlers to assess antler size variation in red deer populations: effects of mast seeding, climate and population features in Mediterranean environments

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Abstract

Fundamental understanding of the factors influencing cervid antler size, development and investment has been traditionally drawn from harvest data. However, depending on the hunting tactic, harvest data may not represent a random sample of the population leading to possible inferential biases. Cast antlers may represent an alternative, cost-effective and non-invasive method. We used 4756 red deer (*Cervus elaphus* L.) cast antlers collected during a 10-year period to evaluate the relationship between annual antler gross score and three key environmental components that determine habitat quality and resource availability in Mediterranean systems: (1) population traits (density and male age structure), (2) acorn yield and (3) a proxy of plant productivity [Real Bioclimatic Index (RBI)]. Population traits and acorn yield were measured before antler formation (autumn/winter) whereas RBI was calculated before (autumn/winter) and during (spring) antler formation. Population traits explained the highest amount of variance in antler score, followed by acorn yield and spring RBI, while no effect was found for autumn/winter RBI. Antler gross score was negatively related to population density but positively associated with acorn yield, spring RBI and male age structure. Interestingly, a significant interaction between population traits and acorn yield suggests a disproportional effect of population traits on antler size during non-mast years (poor acorn crops), whereas no significant population effect was observed during mast years. Similarly, we found a positive effect of spring RBI on antlers when density was medium or low and/or age structure was balanced or older. These findings have important ecological implications in environments with high inter-annual resources variability where high population densities lead to strong intraspecific competition during years of low food availability (e.g. during non-mast years or drier springs), producing large antler size variation. Finally, although cast antlers reflect changes in environmental conditions we do not recommend their use unless reliable data on age structure is available.

INTRODUCTION

Multiple factors have been identified as determinant in red deer (*Cervus elaphus* L.) antler development. Beyond genetic control and individual age, the most relevant factors described are early life conditions and yearly environmental factors (e.g. density and weather parameters; Schmidt et al., 2001; Kruuk et al., 2002; Mysterud et al., 2005; Landete-Castillejos et al., 2012). Antler formation requires partial demineralization of the skeleton (19-24 % of the bone mass; Gómez et al., 2012) as the amount of minerals required for antler growth (13.7 g/day on average at peak deposition; Muir, Sykes, & Barrell, 1987) cannot be supplied exclusively via nutrition in such a short period of time (95% of final antler length is formed in a mean of 112 days; Muir et al., 1987). Hence, poor early life nutrition exerts negative delayed effects on adult body and skeleton size limiting potential allocation to sexual secondary traits (Moore, Littlejohn, & Cowie 1988; Schmidt et al., 2001; Michel et al., 2016). In addition, antler size relative to body size was found to be greater during years with favorable environmental conditions (Mysterud et al., 2005). In particular, weight recovery after the rut (~25% of weight is lost during the rut; McMahon, 1994; Strickland et al., 2017) and body size/condition at antler casting have been suggested to strongly correlate with allocation to antler growth (Hyvärinen, Kay, & Hamilton 1977; Muir et al., 1987; Gaspar-López et al., 2010; Gómez et al., 2012), although weight gain during antler development is also important (Gaspar-López et al., 2010). An example of high-quality food after the rut is that provided by mast-seeding trees (e.g. acorns, beechnuts, and chestnuts) which offer an irregular but highly nutritious food source in many temperate and Mediterranean environments (red deer: Picard, Oleffe, & Boisaubert, 1991; San Miguel, Roig, & González, 2000; Azorit et al., 2012, white tailed deer *Odocoileus virginianus*: Wentworth et al., 1992). Most studies on red deer, however, have overlooked the potential influence of mast-seeding trees on antler development (but see Wentworth et al., 1992).

Importantly, most research on cervid antler growth has been traditionally drawn from harvest data. However, depending on the hunting tactic, harvest data may not represent a random sample of the population and may incorporate a major source of inferential bias (Strickland et al., 2001; Martínez-Jauregui et al., 2005; Andersen et al., 2007). In deer populations, cast antlers may provide a non-invasive and less biased method for gathering large amount of data as cast antlers' sample size and representativeness are not limited by hunting quotas or methods (Schoenebeck & Peterson, 2014). However, very few studies have focused on this accessible source of information (red deer: Fierro et al., 2002; Kruuk et al., 2002; Landete-Castillejos et al., 2010, white-tailed deer: Schoenebeck & Peterson, 2014; Ditchkoff, Welch, & Lochmiller et al., 2000). The main limitation of using cast antlers is that the age of deer is unknown. Because deer antler size varies dramatically with age (Kruuk et al., 2002; Mysterud et al., 2005), the absence of control for changes in male age structure could lead to inferential error. Because accurate data on population age structure is difficult to obtain for free ranging wild populations, most studies have dismissed the use of antlers. Here, we aimed to evaluate the association between antler size variation at the population level and different environmental components (population density, age structure, mast seeding and weather conditions) based on a large cast antler database. We hypothesized that 1) inter-annual variation of acorn crop (masting) would influence variations in antler size due to its role in the re-establishment of body condition after the rut and previous to antler casting, 2) weather variation before and during antler formation would also affect antler size due to its influence on plant productivity and hence nutritional status, 3) inter-annual variation in antler size would be influenced by population traits (i.e., population density and/or male age structure). Hence, our study represents the first long term investigation (10 years) based on more than 4700 red deer antlers aimed at identifying the environmental factors influencing antler size at the population level.

MATERIALS AND METHODS

Study area

Cast antlers from Iberian red deer were collected from Quintos de Mora hunting estate (68.6 km²; south-central Spain) with a Continental Mediterranean climate [more details on the study area in Peláez et al. (2017)]. Peak of rutting activity usually occurs at the end of September with 80% of conceptions concentrated from mid-September to mid-October (Peláez et al., 2017). During spring and autumn, pastures provide high-quality food along with the autumnal highly nutritious acorns, mostly from the abundant Holm oak (*Quercus ilex* L.), but also from the less prevalent *Q. faginea* Lam. Throughout periods of nutritional constraints (usually summer and some winters), deer increase their browse intake (Bugalho & Milne, 2003) but also have access to 2.1 km² of rainfed crops (oats, barley, rye, common vetch and clover) which are protected by an electric fence the rest of the year. During the study period (2002-2011) deer management goals were: (1) to reduce deer density to an average of 23-24 deer/km² and (2) to approach a sex ratio structure of 1:1.

Data collection

A total of 4756 fresh and unbroken cast antlers were collected during the period 2002-2011, with an annual average of 476 ± 86 ($\pm$ SD). These antlers belonged to males ≥ 2 years old because of the difficulty to find yearling antlers due to their later cast date and the high percentage of button-stage yearlings in the population.

The following study variables were collected:

1) Gross score of antlers was calculated using the C.I.C. method (International Council for Game and Wildlife Conservation; see Supplementary material Table S4.5.1), similar to the North American Boone and Crockett scoring method (Nesbitt & Wright, 1981). Both metrics have been widely used in many studies of

antlers (Ditchkoff et al., 2000; Azorit et al., 2002; Fierro et al., 2002; Gaspar-López et al., 2010; Strickland et al., 2013).

2) Deer density: Line transect surveys were performed every year at dusk during the rutting period (end of September/ beginning of October). Absolute deer density and confidence intervals were estimated by distance sampling (Buckland et al., 2001) using the “Distance” package in R software. More details can be found in Peláez et al. (2017). For analysis purposes we used population density calculated during the rut before antler formation.

3) Real Bioclimatic Index (RBI) is a Gaussen derived index developed by Montero de Burgos and González-Rebollar (1974) and used as a proxy of plant productivity in studies on Iberian Red deer (see Appendix S3 Martínez-Jauregui et al., 2009; Peláez et al., 2017). We used: 1) *average RBI during autumn and winter* (prior to antler formation; from September to February) and 2) *RBI during spring* (while antlers developed; from March to June). We used March as a threshold because cast antlers start to be found in our study area in late February. Weather parameters were obtained from a weather station located inside the study area.

4) Acorn yield index was obtained each year during October (before antler formation). Data was collected from Cabañeros National Park, located 10 km from our study area, assuming geographical synchrony of “masting” (Liebhold, Koenig, & Bjørnstad, 2004). We used a semi-quantitative method (Díaz et al., 2011) ranking acorn production of 150 Holm oak visually, using an acorn yield index that represents the percentage of the crown covered by viable acorns. This index varies from 0 to 4 (0: no acorns observed; 1: <10% of the crown covered by acorns; 2: 10–50%; 3: 50–90% and 4: >90%) and was validated with estimates of acorn production obtained from seed traps (Koenig et al., 2013; Morán-López et al., 2016). Although we only have acorn production data on the abundant *Q. ilex*, we expect *Q. faginea* to have synchronous acorn production based on species similarities (see Koenig et al., 1994): 1) acorns mature in one year, 2) similar flower and seed crop phenology, and 3) same taxonomic Section (white oaks, Section

Leucobalanus). Finally, the presence of cork trees (*Q. suber* L.) was anecdotal and *Quercus pyrenaica* Willd. Coppices presented negligible acorn production.

5) Age structure index. Based on the data of known-age males harvested during the period 2002 to 2016 (n=2835), we reconstructed the age structure of the male population retrospectively (i.e. knowing the age of each male at death we calculated its age during the previous years). Hence, we obtained a yearly male age structure based on a mean of 790 ± 62 known-age males which corresponded to more than 80% of the estimated male population. Age was determined by histological examinations of incisors using cementum annuli counts (Low & Cowan, 1963). Finally, we obtained the age structure for the subset of the male population ≥ 2 years old (Supplementary material Fig. S4.5.1). Then, we calculated a set of different indices to account for inter-annual variation in male age structure: 1) mean age, 2) median age, 3) percentage of males ≥ 4 years, and 4) percentage of males ≥ 6 years (Supplementary material Table S4.5.2). Because correlations among all indices were high ($0.73 < r < 0.97$; p 's < 0.05), we selected the percentage of males ≥ 6 years old as the most biologically meaningful variable because antler size increase is small for stags ≥ 6 years old compared with younger ages (Supplementary material Fig. S4.5.2).

Statistical Analyses

Correlations between predictors were tested to avoid co-linearity problems. Density and age structure (percentage of males ≥ 6 years old) showed high correlation ($r = -0.82$, $p < 0.005$; Supplementary material Table S4.5.2). Therefore, we calculated the Variance Inflation Factors (VIFs) for all predictors and only predictors with $VIFs < 4$ remained in the model (Neter et al., 1996). The variable percentage of males ≥ 6 years old was therefore removed ($VIF = 4.7$). Because age structure could not be included in the model, the variable density partly included changes in age structure. To avoid interpretation problems, this variable was called "Population traits" (POP_t). Therefore, we built a linear model with left antler gross score (n=2350) as the response variable and the following

predictors: Population traits (POP_t), Acorn yield index (ACORN), *RBI during autumn and winter* (RBI_aut_win) and *RBI during spring* (RBI_spr). We centered all predictors (Schielzeth, 2010) and included two-way interactions of population traits and the other variables to account for food availability per individual.

Because we considered left and right antlers as independent samples, we kept the right-side subset (n=2406) to validate the results from the left side model. Hence, we performed Pearson's χ^2 tests to assess correlations between left-side model predictions and mean right antler gross scores by year. All analyses were performed using the R statistical software v3.2.1 (<http://www.r-project.org/>). To fit the model, we used the function "lm" of the package "stats". We used the function dredge to rank all possible models based on the lowest weighting provided by the Akaike information criterion (AIC) and selected the simplest model (least number of parameters) among all candidate models within $\Delta AIC < 2$ following the principle of parsimony (Burnham & Anderson, 2002). To estimate the amount of variance explained by each predictor we performed variance partitioning with the function "varpart" included in the R package "vegan". Finally, as density and age structure were correlated, the effect size of the variable Population traits accounted for both variables. To assess their relative importance, we calculated to what extent the observed changes in age structure during our study period could have modified mean population antler size. Firstly, we calculated mean antler gross score based on data of males harvested during a management regime called "Monterías" before and after the study period (1997; density of 36 ind/km², 2012 and 2013; density of 26 ind/km², n=247; Supplementary material Fig. S4.5.2). Martínez-Jauregui et al., (2005) concluded that size or age selection by hunters was less pronounced in Monterías than for other hunting methods such as trophy-stalking and management hunting. Using the annual percentage of males of each age and the mean antler size by age, we calculated the yearly mean population gross score and the potential increase in antler size caused by changes in age structure.

RESULTS

Over the 10 years of the study, mean left antler gross score was 126 ± 19 (score $\pm$ SD; $n=2350$). Inter-annual variation in mean gross score varied from 111 ± 15 in 2005 to 138 ± 18 in 2011 (Fig. 4.5.1).

Figure 4.5.1 Inter-annual variation in cast antler gross score for Iberian red deer from south-central Spain during 2002-2011 (observed mean gross score (●) 95% CI) and predicted (■) values from the top model.

Mean deer population density decreased throughout the years (40.3 deer/km² in 2003 and 29.6 deer/km² in 2011) while all male age structure variables followed an increasing trend (Figs. 4.5.2b, c). Acorn production showed high inter-annual variability, with lower acorn crops during 2005 and 2006 and highest in 2009 (Fig. 4.5.2d).

RBI during spring and *RBI during autumn and winter* showed high inter-annual variability (mean 0.99 ± 0.40 and 0.22 ± 0.21 , respectively; Figs. 4.5.2e, f). Finally, it is worth noting the low values of 2005 for all variables reflecting plant productivity (Acorn yield, *RBI during spring* and *RBI during autumn and winter*). Following the principle of parsimony we selected the best fitting model that contained the following predictors: Acorn yield, *RBI during spring* and their interactions with Population traits (Table 4.5.1).

Figure 4.5.2 (a) Observed (●) mean left antler gross score of Iberian red deer from south-central Spain (2002-2011), (b) annual deer population estimates by year (density (■) ± CV), (c) age structure variation through time (% of males ≥ 6 years old (d) mean acorn yield (acorn (■) ± SD); (e) mean RBI during spring (RBI (■) ± CV); (f) mean RBI during autumn and winter (RBI (■) ± CV).

Table 4.5.1 Model selection describing the factors affecting Iberian red deer antler size (gross score) in south-central Spain, 2002-2011

Models	df	LogLik	Δ AIC	w
Left antler gross score				
1 ACORN + POP_t + RBI_spr + POP_t * ACORN + POP_t * RBI_spr	7	-10123.4	0.0	0.38
2 ACORN + POP_t + RBI_spr + RBI_aut_win + POP_t * ACORN + POP_t * RBI_spr	8	-10123.0	1.2	0.21
3 ACORN + POP_t + RBI_spr + RBI_aut_win + POP_t * ACORN + POP_t * RBI_spr + POP_t * RBI_aut_win	9	-10122.2	1.6	0.18
4 ACORN + POP_t + RBI_spr + RBI_aut_win + POP_t * RBI_spr	7	-10124.3	1.7	0.16
5 ACORN + POP_t + RBI_spr + RBI_aut_win + POP_t * RBI_spr + POP_t * RBI_aut_win	8	-10124.2	3.6	0.06
6 ACORN + POP_t + RBI_spr + POP_t * ACORN	6	-10129.4	9.9	0.003

d.f. indicates degrees of freedom; LogLik denotes maximum log likelihood; Δ AIC is the difference in Akaike criterion scores between the top and the actual model and w represents AIC weights. ACORN = Acorn yield index; POP_t = population traits; RBI_spr = RBI during spring and RBI_aut_win = RBI during autumn and winter.

Pearson correlation showed a high correlation ($r=0.96$, $p<0.001$) between model predictions and mean right antlers gross score by year. Acorn yield and *RBI during spring* showed a positive relationship with gross antler score while Population traits showed a negative relationship (Table 4.5.2). Population traits was the variable explaining the highest amount of variance, followed by Acorn yield and, to a lesser extent, *RBI during spring*.

Table 4.5.2 Summary of the best-fitting linear model describing the factors affecting antler size (gross score) in Iberian red deer from south-central Spain, 2002-2011.

Response and explanatory variables	Estimate $\pm$ Standard error	t value	P-value	R squared	Individual Fraction
<i>Left antler gross score</i>					
Acorn yield	10.1 $\pm$ 1.0	10.0	<0.001	0.03	0.03
Population traits	-1.4 $\pm$ 0.1	-9.9	<0.001	0.06	0.05
RBI_spr	7.1 $\pm$ 1.2	5.9	<0.001	0.05	0.02
ACORN*POP_t	1.8 $\pm$ 0.5	3.6	<0.001		
RBI_spr* POP_t	-1.6 $\pm$ 0.5	-3.5	<0.001		

AdjR2 left = 0.12. Adjusted R squared is the proportion of variance explained by the model selected. POP_t, population traits; ACORN, Acorn yield index; RBI_spr, RBI during spring. Model estimates and standard errors are presented for each variable and interaction. All variables were centered on their mean. P-values indicate statistical significance. R squared was calculated based on the proportion of the total variance explained by each variable (without interactions) and the independent fraction as the individual variance explained by each variable that is not explained by any other variable.

Based on the age structure of males each year and the calculated mean antler size by age, we observed a maximum increase of 6.4% in antler gross score (from a score of 106 in 2003 to 113 in 2008; Supplementary material Table S4.5.3) due to age structure, while the total predicted increase of gross score due to the variable Population traits was 13.2%. This indicates that age structure alone cannot explain the total effect produced by the variable Population traits, most likely changes in population density explained the rest of the variance (6.8%). During years with highest acorn crop (controlling for the rest of the variables), mean antler gross score was ca. 13% greater than in years with poor acorn crops. This is a very strong effect because the total variation on mean antler score during

the study period was 24 %. The effect size of *RBI during spring*, when controlling for the rest of the variables, showed approximately a 7% antler score difference between the year with maximum and minimum plant productivity.

Finally, the interaction between Population traits and Acorn yield suggested a greater negative effect of a poor acorn crop when density was high or age structure was younger (Fig. 4.5.3a). On the other hand, the interaction between *RBI during spring* and Population traits revealed only a positive effect of *RBI* on antler size when density was medium or low and/or age structure was balanced or older (Fig. 4.5.3b).

Figure 4.5.3 Predicted antler gross score of Iberian red deer from south-central Spain (2002-2011) as a result of the interactive effect between: (a) Acorn yield and population traits and (b) RBI during spring and population traits. Both interactions are represented at three different levels of population density and/or age structure.

DISCUSSION

Our study highlights the strong role that seed masting, *RBI during spring* and population traits (deer density and age structure) play on cast antler size of red deer at the population level. The influence of both population density (Clutton-Brock & Albon, 1989; Schmidt et al., 2001; Kruuk et al., 2002) and spring nutrition (Gaspar-López et al., 2010) on antler development has been previously

reported. However, to the best of our knowledge, no study has shown an effect of acorn crop on red deer antler development (but see for white-tailed deer: Wentworth et al., 1992), even though acorns have been previously described as a crucial source of high-quality food for deer during autumn and winter in oak-dominated systems (Picard et al., 1991; San Miguel et al., 2000; Azorit et al., 2012). In addition, we demonstrated that changes in male age structure can significantly affect the mean size of cast antlers at the population level. Consequently, although cast antlers may represent a useful tool to monitor the effect of key environmental conditions (e.g., food availability per capita) on antler attributes, we recommend not to use cast antlers unless reliable data on age structure are available given the strong influence of age structure on population antler size.

Our study revealed that two main food resources influenced inter-annual variation of antler size: 1) acorn production, and 2) spring plant productivity. In addition, the absence of an effect of autumn-winter RBI on antler size was only partial because we could not test its potential influence during non-mast years (the two years with lowest acorn crop coincided with the driest autumn and winter recorded; Figs. 4.5.2d, f). Nevertheless, we were able to conclude that during years of medium and high acorn crop there was no significant influence of autumn/winter plant productivity, suggesting a greater effect of seed mast over the autumn-winter green forage on antler size. However, the chemical composition of acorns is low in essential elements required for antler development (proteins and minerals; Landete-Castillejos et al., 2012) compared to that of autumn/winter grasses (Rodríguez-Estévez et al., 2007). Hence, we suggest that acorn production does not have a direct effect on antler development by providing the necessary nutrients for their formation but, instead, may have an indirect effect by helping males build up body fat reserves rapidly after the mating effort (Azorit et al., 2012), enabling them to later concentrate in alternative, more specific nutrients for antler formation.

In addition, our predictor “population traits” accounted for two highly correlated variables (density and age structure) and, thus, we could not discriminate the effect of each variable in the models. However, an alternative approach based on antler data of harvested males helped elucidate that the total observed variation in antler size could not be due to the sole effect of age structure. Age structure explained ca. half of the variation in antler scores, which leads to the conclusion that the rest of the variation was likely due to changes in population density. Nevertheless, the strong influence of high population density on antler size has been widely described (Schmidt et al., 2001; Azorit et al., 2002; Kruuk et al., 2002) because: 1) it limits per capita food intake of high quality and, hence, resources available for antler growth (Mysterud et al., 2005) and 2) it produces a negative delayed effect on adult body size due to poor early life nutrition (Landete-Castillejos et al., 2005b; Gómez et al., 2006; Ceacero et al., 2010; Dryden, 2016).

Finally, the significant interacting effects among the variable population trait (density and age structure) and the two variables used as proxy of food resources (acorn crop and *RBI during spring*) could help to elucidate two points: 1) during mast years there was little or no effect of the variable population trait on antler size while a greater effect was observed during non-mast years, and 2) during years of higher spring plant productivity the variable population traits had a positive effect on antler size but no significant effect occurred during unfavorable years. Nevertheless, further studies should control for both population trait variables to clarify the relative importance of population density and age structure in the observed interactions with acorn crop and *RBI during spring*.

Finally, our results have important management implications particularly in the current context of increasingly higher deer populations (Côté et al., 2004; San Miguel, Perea, & Fernández, 2010; Perea et al., 2014). First, high deer densities decrease per capita resource availability leading to high intraspecific competition

for food, which, in environments with high variation of resources, favors larger inter-annual fluctuations of antler size. Second, high deer densities also limit oak recruitment (Perea & Gil, 2014; López-Sánchez et al., 2016), which reduces the abundance of mature oak trees and thus overall acorn crop. Hence, we recommend reducing deer densities in Mediterranean environments to the estimated optimal for these environments (below 20 to 25 deer/km²; Soriguer et al., 1994) to ensure oak recruitment and improve antler size of unsupplemented red deer populations.

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Supplementary material

Supplementary tables

Table S4.5.1. Modified gross score of red deer antle (based on the International Formulae for the Measurement and Evaluation of Trophies of the CIC)

	Measurements	Identical pair	Factor	Points
Measurements				
1. Length of the main beam	 cm		x 0.5	
2. Length of the brow tine	 cm		x 0.25	
3. Length of the tray tine	 cm		x 0.25	
4. Circumference of coronet	 cm	x 2	x 1	
5. Circumference of lower beam	 cm	x 2	x 1	
6. Circumference of upper beam	 cm	x 2	x 1	
Weight (dry) of antlers	 kg	x 2	x 2	
Number of tine ends	1 tine end >2 cm = 1 point	x 2	x 1	
Beauty points				
Bay tines	0 – 1 points	x 2	x 1	
Crow tines	0 – 10 points	x 2	x 1	
			Total Gross score	

Study 5

Differentiation between fresh and old antlers was based on color wear, the presence of skin, hair or blood under the burr and the experience of the rangers. Antler measurements were made by the same person, following the Trophy Evaluation System — TES © of the International Council for Game and Wildlife Conservation (CIC).

We measured to the nearest centimeter (1) the length of main beam, (2) length of brow tine, (3) length of tray tine, (4) circumference of coronet, (5) circumference of lower beam, (6) circumference of upper beam, the dry weight of antlers in kilograms and the number of tine ends. Only bay and crown tines beauty points were included. The inside span could not be measured and the two subjective measurements (color and pearling) were not included in the formula. Because we only used one cast antler per animal (left or right) in the analyses, we calculated the gross score as if the pair was identical.

Table S4.5.2 Changes in age structure for the Iberian red deer male population subset (≥ 2 years old) during the study period.

Season	Mean age $\pm$ SD	Median age	% of males ≥ 4 years	% of males ≥ 6 years
2001-2002	4.2 $\pm$ 2.5	3	49.2	21.6
2002-2003	3.9 $\pm$ 2.2	3	43.8	18.3
2003-2004	3.9 $\pm$ 2.1	3	46.6	17.6
2004-2005	4.1 $\pm$ 2.1	4	55.7	20.2
2005-2006	4.1 $\pm$ 2.0	4	52.5	23.9
2006-2007	4.5 $\pm$ 2.2	4	59.1	30.5
2007-2008	4.7 $\pm$ 2.4	4	60.7	34.5
2008-2009	4.6 $\pm$ 2.4	4	57.4	30.8
2009-2010	4.6 $\pm$ 2.5	4	55.8	30.4
2010-2011	4.7 $\pm$ 2.8	4	57.2	30.9
Correlation with density:	-0.82**	-0.91***	-0.88***	-0.82**

** ($p < 0.01$); *** ($p < 0.001$)

Table S4.5.3 Assessment of the impact of male age structure on antler gross score between the year with the lowest proportion of older males (2003 - 2004) and the year with the highest (2007 - 2008). Age structure was calculated as the percentage of males of each age.

Male Age (year)	Mean Gross Score by age	Age Structure Year 2003	Age Structure Year 2008
2	84	0.337	0.222
3	108	0.225	0.172
4	113	0.147	0.144
5	117	0.108	0.117
6	130	0.061	0.109
7	131	0.034	0.094
8	137	0.039	0.050
9	136	0.020	0.050
10	139	0.007	0.029
11	133	0.007	0.010
12	138	0.012	0.002
13	141	0.002	0.000
14	129	0.000	0.000
Population mean gross score		106.27	113.11

Supplementary figures

Figure S4.5.1 Retrospective estimation of the population structure for males ≥ 2 years old.

Figure S4.5.2 Averaged antler gross score of males harvested during “Monterías” before and after the study period (1997; density 36 ind/km² and 2012-2013 density 26 ind/km²). Calculated according to the modification of the CIC formulae in Table S4.5.1 with the exception of antler weights that were not recorded for hunted males.

Study 6: Peláez, M., Sanuy, I., Peral, J. C., Álvarez Esteban J. L., Lavín, S., Serrano, E., & Perea, R. (in preparation)

Early life antlers and body size as reliable indicators of adult performance in roe-deer Mediterranean populations

Abstract

Recent research has challenged the idea that cervid antlers are such costly sexual traits as no significant trade-offs have been found between early life allocation to antler growth and future body development in temperate environments. Thus, previous studies based on antlers during their growth (velvet), have shown that early life antler investment is an honest signal of future phenotypic quality. However, studies on fully-formed clean antlers are needed to avoid possible bias caused by the adjustment of antler measurements to the median date of capture of individuals. In addition, further research is needed in other regions such as the Mediterranean Europe as trade-offs may be more easily detected at the edge of a species distribution range.

Here, we studied a semi-captive population of European roe deer (*Capreolus capreolus*) at its southern distribution (Valsemana research station, Spain), with a distinct sub-Mediterranean climate. First, we described the age-class-specific variation in antler size and body measurements and studied the magnitude of senescence in antler size (i.e., the decline in antler size between adult and senescent stage). To do so, we used 1162 measurements of 488 different roe deer individuals captured during the 2003-2017 period. Second, based on 146 individuals measured two or more times during their lifetime, we aimed to elucidate what body measurements (size, mass or antler size of fawns and yearlings) would better predict future male adult quality in terms of trophy and body mass. Finally, we investigated whether allocation to secondary sexual traits during early-life affected body size or antler development during adulthood.

We found that the magnitude of senescence in antler size was lower in Mediterranean populations compared to other northern populations, which agrees with the idea of a latitudinal increase in senescence rate. In addition, the variables that better predicted body mass during adulthood were body mass as yearling but also body size as 6 months of age. Antler score between 10-18 months resulted the best indicator of antler size during the adult stage. Finally,

similarly to other northern temperate populations of roe deer, we concluded that there is no negative effect of a high investment on antler during early life on adult antler size or body mass. Therefore, even at its southern distribution and under Mediterranean environments, roe deer allocation to antlers during early life was an honest signal of high adult performance. We also highlight that the use of clean antlers provides more robust and reliable results because any transformation or prediction on the future antler size may introduce bias in the analysis.

INTRODUCTION

The principle of allocation (Cody, 1966) states that, as organisms live in resource-limited environments, individuals have to distribute the energy gain between different biological processes such as growth, reproduction and survival (Williams, 1966a). Thus, trade-offs explain that any extra energy allocated to one vital function can only occur at the expense of another (Cody, 1966; Williams, 1966a; Gadgil & Bossert, 1970). These trade-offs have been crucial for the development of life-history theory (Stearns, 1989), which states that organisms should maximize their fitness (i.e. their success passing their genes to the next generation) by allocating resources to different biological processes according to environmental conditions. Therefore, for widespread species, such as cervids, we can expect the trade-offs between different vital functions to vary across their distribution range.

One of the most important energy demanding times in the life cycle of male cervids is the formation of secondary sexual traits such as antlers (Goss, 1970 on Cervidae; Van Ballenberghe, 1982 on moose *Alces alces*; Vanpé et al., 2007 on roe deer *Capreolus capreolus*). Antlers are costly to produce and maintain as they are grown and shed yearly and, thus, they require the allocation of large amounts of nutrients and energy (Goss, 1970; Hyvärinen et al., 1977 on red deer *Cervus elaphus*; Geist & Bromley, 1978 on Cervidae; Mohen et al., 1999 on moose; Vanpé et al., 2007 on roe deer). However, while cervid antlers have been widely studied in northern regions, more information is needed to understand the individual and

environmental factors that influence antler growth in Mediterranean environments (but see Gaspar-López et al., 2010; Gómez et al., 2012; Peláez et al., 2018; Cappelli et al., 2020) as trade-offs may be more easily detected at the edge of a species range of distribution (Kawecki, 2008).

Many factors have been described to influence antler development such as genetics, age, yearly environmental conditions and early-life conditions. Antler formation requires the partial demineralization of the skeleton and thus any factor affecting growth at early life, such as the date of birth (Albon et al., 2009; Schmidt et al., 2001 on red deer) or mother condition at birth (Guinness et al., 1978 on red deer; Layne, 2005 on reindeer (*Rangifer tarandus*), can affect antler development later in life (see Clutton-Brock, 1981 on cervids; Pélabon & van Breukelen, 1998). In addition, antler size might vary depending on the access to high-quality food (Schmidt et al., 2001; on red deer; Simard et al., 2014; Quebedeaux et al., 2019 on white-tailed deer *Odocoileus virginianus*) and therefore, antler size has also been described as condition dependent (see Mysterud et al., 2005 on red deer; Simard et al., 2014 on white-tailed deer). Therefore, many studies show an allometric relationship between antler development and body measurements (e.g. body mass, size, and condition) at the beginning of the antler growth (Hyvärinen, Kay, & Hamilton, 1977; Goss, 1983; Vanpé et al., 2007; Gaspar-López et al., 2010) as only high quality males can afford the cost of developing above average antlers (Zahavi, 1975; Andersson, 1986; Pélabon & van Breukelen, 1998). Finally, antler size is also strongly related to age as adults or prime-age males develop larger antlers than fawns and yearlings with the same body mass or body size but also larger than senescent males, which indicates that reproductive effort peaks during prime-age ('mating strategy-effort' hypothesis: Yoccoz et al., 2002). In this line, a recent study performed on different roe deer populations detected an increasing trend in the magnitude of senescence on antler size (i.e. the decline in antler size between adult and senescent stage) with increasing winter severity, suggesting that senescence was more accentuated in northern locations than in southern locations (Vanpé et al., 2007). Furthermore, the allometric relationship between antler size

and body mass for senescent males was steeper for the northern populations as there was a disproportional effect on antler allocation between light and heavy senescent roe deer (Vanpé et al., 2007). However, this study was performed based on only three different populations (two in France and one in Sweden) and therefore needs further support. Mediterranean environments are suitable to test this hypothesis as they are characterized by mild winters and long summer droughts, which represent the period of limiting resources. Indeed, female red deer increase body weight during autumn and the beginning of winter, contrary to their northern counterparts (Martínez-Jauregui et al., 2009; Rodríguez-Hidalgo et al., 2010). Therefore, in Mediterranean environments, we could expect a lower magnitude of senescence on antler size as environmental conditions during antler development (i.e. autumn and winter) are more favorable.

Finally, although most cervid males do not successfully mate until late in life (i.e. 3-4 years old for roe deer: Vanpé et al., 2009; 5-7 years for red deer: Clutton-Brock & Albon, 1989), they develop antlers during the first year of life (at the age of 8-10 months) while they are also experiencing an extraordinary body growth. Thus, it was hypothesized that a trade-off between early-life antler growth and body growth may result from the allocation of resources competing functions (i.e. reproduction and growth; Kotiaho, 2001). However, the studies carried out so far on cervids showed the opposite results. For example, red deer antler growth at prime age (4-9 years) did not affect antler development later in life (i.e. senescence; Lemaître et al., 2014). Similarly, for roe deer, the greater the absolute or relative investment in antlers during early life (i.e. first two years of life), the larger the antlers during late prime age (4-8 years; Lemaître et al., 2018). Therefore, both studies challenged the idea that deer antlers are such costly sexual traits (Lemaître et al., 2014; Lemaître et al., 2018). Conversely, they support the assertion that antlers are an honest signal of phenotypic quality (Vanpé et al., 2007). However, the study performed on roe deer based its premises and results on data collected during the antler growth (i.e. when they are in velvet; not fully formed or clean), and afterwards they standardized these values to the median

date of capture (8th of February; Lemaître et al., 2018). This approach may introduce bias in the analysis due to, for instance, inter-individual variation in antler phenology (Clements et al., 2010) or intra- and inter-annual variation in resources during the antler growth period.

In Mediterranean environments, little is known about the long lasting effects of early-life investment on antlers. This type of studies involves the capture and recapture of individuals during their lifetime, which is difficult to achieve for wild populations. Here, we used data on 488 known-age semi-captive roe deer from NW Spain during the period 2003-2017. We used fully-formed antlers before casting (October-November) to ensure we are recording actual antler size. We tested the following hypotheses and predictions:

H1. At population level, we expect:

- a) heavier and larger individuals of a given age to develop greater antler sizes, similarly to other European roe deer populations.
- b) the magnitude of senescence in antler size in Mediterranean populations to be less accentuated than in northern populations, according to Vanpé et al. (2007).

H2. At individual level, we assessed which fawn and yearling body measurements best predicted antler size or body mass during adulthood. We hypothesized that:

- a) fawn antlers (those growing at the age of 8-10 months) would act as an honest signal for adult phenotypic quality (Lemaître et al., 2018). Therefore, those 10-18 months old individuals with greater antlers would have above-average antler size throughout their prime age (2-7 years).
- b) the greater the fawn and the yearling body mass and body size, the higher the body mass during adulthood

H3. Finally, we hypothesize that if fawns invest more on antlers in relation to their body mass or body size (relative allocation to antlers), they would have greater antler size later in life (Lemaître et al., 2018) and higher body mass. Therefore, we do not predict any negative consequence on antler size during adulthood as a result of a strong allocation to the antlers as fawns.

MATERIALS AND METHODS

Study area

The Valsemana roe deer research station is located in north-western Spain (42° 46' N- 5° 15' O), in the province of León (Fig. 4.6.1A). This research station was created by the regional government in 1978 for two main purposes: 1) as a recovery facility to attend injured and snow-trapped roe deer and 2) as a breeding facility to repopulate low density areas or areas where roe deer had disappeared. In addition, in 2003, research on roe deer became a new main objective, adjusting and reorganizing the previous facilities to record information on roe deer at individual level. All roe deer living in Valsemana came from, or descended from, individuals captured or rescued in the nearby areas (i.e. from the province of León).

The research station comprises a fenced area of 856 hectares, where roe deer are kept in semi-captivity within 15 independent enclosures ranging between 3 and 45 ha (Fig. 4.6.1B). The vegetation is characterized by a mosaic of open grasslands, oak woodlands (*Quercus pyrenaica*) and mixed pine forests (*Pinus sylvestris* and *Pinus pinaster*), with an understory composed by species such as those belonging to the genera *Adenocarpus*, *Cytisus* or *Genista*. The natural vegetation was the main source of food for the specie, although eventually and, for manage purposes, they had access to supplementary food. The climate is sub-Mediterranean characterized by a 2-3 months of summer drought (mid-Jun to mid-September). During the period of study (2003-2017), mean annual temperature was 11.1 °C with mild to hot summers (mean temperature of July 19.8 °C) and cooler winters (mean temperature of January 3.3 °C; Spanish Meteorological State Agency; AEMET; Station of León, Virgen del Camino, Id. 2661; www.opendata.aemet.es). The mean annual rainfall was 886.8 mm with a high inter-annual variation (from 637 mm in 2009 to 1281 mm in 2014; Station of Puerto San Isidro, León, Id. 2661; www.opendata.aemet.es).

Figure 4.6.1 A) The Valsemana roe deer research station is located in the Leon province, NW Spain, B) network of enclosures with natural vegetation, C) observation station installed inside one enclosure and D) release of a male roe deer after the captures to assess health status and record individual measurements.

Study species

Compared to other northern-hemisphere cervid species, roe deer are small in size (male weight between 20-24 kg) and display low sexual dimorphism, with males around 10 % larger than females (Andersen, Duncan, & Linnell, 1998). Roe deer are income breeders, which have low fat reserves and therefore body mass or size has been described as a better proxy for phenotypic quality than body condition (Toïgo, et al., 2006). According to their mating system, males are not strongly polygynous and display a resource defense strategy to monopolize females and thus they are territorial (Vanpé, 2007). Antler formation occurs between November and March as they are long day breeders and male territorial period extends from early spring till late summer (Sempéré & Boissin, 1981).

Finally, their relative investment on antlers is small compared to other cervid species (antler weight relative to body weight is 2.53-4.91% for red deer but only 0.92-1.52 % for roe deer; Huxley, 1931).

Data collection

Roe deer living in the Valsemana research station were captured with drive-nets

every year during October and November from 2003 to 2017. Once captured, specialized veterinarians assessed health status, provided vaccinations and took body and antler measurements as they were captured yearly just before males cast their antlers. Almost all individuals were captured during their first year of life (at approximately 6 months old) and were marked with an ear-tag for individual identification on subsequent years. Therefore, for the same individual, there was yearly information on: 1) *Body mass*: autumn live weight of the animal measured to the nearest 0.5 kg, 2) *Body size*: length from the tip of the nose to the base of the tail (nose-tail length) and shoulder height, both measured to the nearest cm, 3) *Age*: calculated as the difference between the year of birth and the year of capture and 4) *Antler measurements*: such as main beam length measured on the external side, brow and back tines length and burr circumference, all to the nearest mm. Unlike previous studies that measured antlers while they were still in velvet or growing (Vanpé et al., 2007; Lemaître et al., 2018), we measured antlers when they were fully formed and hard. However, as individuals were captured during October and November, for any given age, antlers measurements corresponded to the previous growing season as antlers grow between December and March.

Variables calculation

Age class: Each male was classified into five age classes according to their age at capture: fawns (6 months), yearlings (18 months), adults (2-7 years) and senescents (8-13 years). Grouping criteria followed that of Vanpé et al. (2007) and Lemaître et al., (2018). However, note that fawns complete their first full antlers

at the age of 10 months but body measurements are taken at the age of 6 months. Therefore, for each fawn, the antler value would be the one measured in the next capture (when they are yearlings, age of 18 months). We made this adjustment because, for example, fawn body measurement at 6 months of age would influence the size of antlers formed between 8-10 months of age, which were measured during the subsequent captures of October-November before they were cast. The same applies also for the other age classes.

Body size score was calculated by performing a principal component analysis (PCA) using the nose-tail length and the shoulder height as variables (n=1140 males). Then, the first axis (92.79% of variance) was used as a proxy for body size. For adult and senescent stages, we calculated the median body scores across all repeated measurements of the same individuals since we detected some variation in adult body size and assumed that it was due to a measurement error as body size does not greatly change during adulthood.

Antler Score was calculated based on the different measurements taken from the left and right antlers (n=669 males). If one male had a missing (already casted) or broken antler, we used the measurement of the other intact antler. Thus, we performed a principal component analysis (PCA) and used the first axis (77.86 % of variance) as a proxy for antler score (Simard et al., 2014). To validate our proxy, we compared it with other common metrics used in other studies (i.e. ln-transformed Antlers length in mm, Vanpé et al., 2007) and also with the antler length in mm (Lemaître, et al., 2018; see Supplementary material, Fig. S4.6.1A).

Statistical Analysis

To assess age-specific differences in antler and body measurements at population level (H1), we used a database of 488 different roe deer individuals, with a total of 1162 capture times from year 2003 to 2017. However, antler and body measurements were not always available for all individuals and years. For example, antler size could only be assessed 568 times while shoulder height was assessed a total of 1145 times (See Table 4.6.1 for a summary of sample sizes).

Therefore, for each subset of data and age class, we calculated: 1) mean antler length, 2) mean body mass, 3) shoulder height and 4) mean nose-tail length. Then, we performed linear models to assess if differences antler and body measurements among age classes were significant (H1a). For this purpose, we used the 'lm' function from the stats package and also performed a pairwise comparison of means between age classes by using the 'lsmeans' function from the homonymous package (R Core Team, 2018). In addition, we estimated the magnitude of senescence in antler size for our Mediterranean population (H1b), calculated as the percentage of reduction of antler length (log-transformed) between adult to senescent stage. We used this transformation to be able to compare results of our Mediterranean population with other northern populations (as in Vanpé et al., 2007).

Intra-individual analyses were performed based on a database of a total of 146 individuals that were captured at least two times throughout their lifetime, one time as fawns or yearlings and the other, as adults. The mean number of measures per individual was 4.17 ± 1.93 (mean $\pm$ SD; range 2–9; n= 609 captures and recaptures). However, similarly, not all body measurements were assessed for each individual on each capture [see sample sizes (N) in Table 4.6.2]. Therefore, to assess which fawn and yearling body measurements best predicted antler size during adulthood (H2a), we firstly calculated for each individual its *median adult antler score* by using all measurements available between the ages of two to seven years. Then, we performed five different linear models all of them using *median adult antler score* as response variable and one of the following predictors: 1) *fawn body mass at 6 months*, 2) *fawn body size score at 6 months*, 3) *yearling body mass at 18 months*, 4) *yearling body size score at 18 months* and 5) *antler score between 10-18 months* (first antlers). We included on each linear model a weighting parameter to give proportionally more importance to individuals with higher number of measurements during adulthood. Similarly, to test H2b, we used *median adult body mass* as response variable and the same five abovementioned predictors. We also included a weighting parameter based on the number of body mass measurements

taken during adulthood for each individual. In addition, for each response variable, we compared the adjusted R-square of each regression in order to elucidate which early-life body variable was the best predictor for *median antler score* and *body mass*.

Finally, also based on our individual database (n=146 individuals), we assessed the effects of fawn relative allocation to antlers in the observed antler score and body mass during adulthood (H3). Firstly, in order to calculate *fawn allocation to antlers* (i.e. relative to body mass and body size score), we used the residuals of the two linear regression performed between the response variable *antler score between 10-18 months* and the predictors *fawn body mass* and *body size score at 6 months* (see Lemaître et al., 2018). Afterwards, we performed four linear models to elucidate the relationship between *median adult antler score* and *median adult body mass* (response variables) and *fawn allocation to antlers relative to body mass* and *fawn allocation to antlers relative to body size* (predictors). Similarly, these linear models had a weighting parameter proportional to the number of repeated measurements during the adult stage. All analyses were performed by using R software (version R 3.5.1; R Core Team, 2018).

RESULTS

At population level, we detected differences in body measurements between males of different age classes, with measurements increasing from fawns to yearlings and peaking at adult stage (Table 4.6.1). Furthermore, body measurements were significantly different between most age groups. We detected significantly different antler length between all age classes with the exception of yearling and senescent group for which the differences were not significant ($t = -1.026$, $p = 0.734$; Supplementary material Fig. S4.6.3; Table S4.6.2).

Table 4.6.1 Age-specific variation on body measurements for the population studied (mean $\pm$ SD). Antler length refers to the year antlers were formed and not to the year of measurement (i.e. fawns antler length corresponds to the set of antlers they formed between 8-10 months of age, which were measured during the captures of the subsequent October-November). N indicates the sample size used to calculate each mean.

Age Class	Antler length (mm)	Body mass (kg)	Shoulder height (cm)	Nose-tail length (cm)
Fawns (6 months)	121 $\pm$ 34 (n=211)	14.4 $\pm$ 2.3 (n=288)	62 $\pm$ 4 (n=407)	98 $\pm$ 7 (n=405)
Yearlings (18 months)	212 $\pm$ 27 (n=124)	24.1 $\pm$ 2.6 (n=216)	70 $\pm$ 4 (n=320)	116 $\pm$ 5 (n=303)
Adults (2-7 years)	222 $\pm$ 22 (n=212)	27.1 $\pm$ 2.7 (n=288)	71 $\pm$ 3 (n=377)	120 $\pm$ 3 (n=355)
Senescents (>8 years)	205 $\pm$ 23 (n=21)	25.6 $\pm$ 2.4 (n=26)	72 $\pm$ 2 (n=41)	121 $\pm$ 3 (n=42)
Total	(n= 568)	(n= 820)	(n=1145)	(n= 1105)

Body mass was significantly different among all age classes and showed an increase from fawns till the adult stage and a slight decrease for the senescent group (Supplementary material Table S4.6.2; Fig. S4.6.2). Shoulder height and Nose-tail were significantly different between fawns, yearlings and adults but not between adults and senescents ($t=-0.511$, $p=0.957$ and $t=0.471$, $p=0.966$, respectively) as body size (bone structure) does not vary substantially once attained the adult stage (Supplementary material Fig. S4.6.2).

As predicted in H1a, we found a positive relationship between body measurements during October-November (body mass and body size) and subsequent antler score (Fig. 4.6.1; Supplementary material Table S4.6.3). Only the senescent group showed a non-significant increase in antler size with increasing body mass ($t=0.48$, $p=0.633$, $n=14$) or body size ($t=1.38$, $p=0.167$, $n=21$; Supplementary material Table S4.6.3). Furthermore, the differences in the slope was non-significant among most age classes (Table S4.6.4). The only group that had overall higher slopes was the fawn group for which the relationship between body mass and antler score was steeper than that of adults and also the relationship between fawn body size score and antler was significantly steeper than that of yearlings. In addition, the reduction of antler length from adult to

Study 6

senescent stage (H1b) was 1.48 % in Valsemana (from 5.40 to 5.32 log-transformed scale; see Supplementary material Fig. S4.6.3).

Figure 4.6.1 Relationship between body measurements during October and November (A: Body mass and B: Body size score) and subsequent antler score while controlling by age class.

At intra individual level, the variable that best predicted *median adult antler score* (H2a) was *antler score at 10-18 months* (Table 4.6.2).

Table 4.6.2 Results of the different models performed to assess the relationship between *median adult antler score* and *median adult body mass* (response variables; H2) and five different fawn and yearling body measurements (predictors: *fawn body mass*, *fawn body size score*, *antler score between 10-18 months*, *yearling body mass* and *yearling body size score*). N indicates sample size used on each model.

Predictors	Response variable							
	<i>Median adult antler score (H2a)</i>				<i>Median adult body mass (H2b)</i>			
	Estimate $\pm$ std	P- value	R- adj	N	Estimate $\pm$ std	P- value	R- adj	N
Fawn body mass at 6 months	0.15 $\pm$ 0.08	0.06	0.06	47	0.51 $\pm$ 0.13	<0.001	0.18	66
Fawn body size score at 6 months	0.35 $\pm$ 0.18	0.06	0.04	63	1.54 $\pm$ 0.30	<0.001	0.21	95
Antler score between 10-18 months	0.44 $\pm$ 0.10	<0.001	0.22	60	0.86 $\pm$ 0.19	<0.001	0.19	81
Yearling body mass at 18 months	0.14 $\pm$ 0.05	0.01	0.08	65	0.58 $\pm$ 0.07	<0.001	0.43	94
Yearling body size score at 18 months	0.26 $\pm$ 0.19	0.18	0.01	75	1.89 $\pm$ 0.27	<0.001	0.28	116

The positive relationship between both variables was significant ($t=4.16$, $p<0.001$) and the model explained 22% of the total variance (Fig. 4.6.2A-B). *Yearling body mass* also had a significantly positive relationship with *median adult antler score* although the total variance explained by this predictor was smaller (8%). The other predictors (*fawn body mass*, *fawn body size score* and *yearling body size*) had a positive but non-significant relationship with *median adult antler score* (Table 4.6.2; Fig. S4.6.4).

In addition, the variable that best predicted *median adult body mass* was *yearling body mass* (H2b), which explained 43% of the total variance (Table 4.6.2; Fig. 4.6.2C-D). The rest of variables also had a significantly positive relationship with *median adult body mass*. Interestingly, for fawns, *body size score* correlated better than *body mass* with *median adult body mass* (21% of the total variance; Table 4.6.2; Fig. S4.6.5).

Figure 4.6.2 A) Relationship between *antler score at 10-18 months* and *median adult antler score* ($n= 60$ different individuals). B) Age-specific variation on antler score for 60 different individuals. Color indicates antler score between 10-18 months. C) Relationship between *yearling body mass* and *median adult body mass* ($n=94$). D) Age-specific variation on body mass for 94 different individuals. Color indicates body mass at 18 months of age (1.5 years).

Finally, according to H3, we found a positive and significant relationship between *median adult antler score* and *allocation to antler as fawns*, both, relative to fawns body mass ($t=2.757$, $p=0.009$, $R\text{-adj}=0.13$) and relative to fawns body size ($t=3.626$, $p<0.001$, $R\text{-adj}=0.18$; Fig. 4.6.3A, B). Similarly, a positive relationship between *median adult body mass* and *allocation to antler as fawns* was found. However, in this case, the relationship was significant for the *allocation relative to fawn body mass* ($t=2.64$, $p=0.011$, $R\text{-adj}=0.10$), but not for the *allocation relative to fawn body size* ($t=1.122$, $p=0.265$, $R\text{-adj}=0.003$; Fig. 4.6.3C, D).

Figure 4.6.3 Relationship between the *allocation to antlers as fawns* (relative to body size and body mass) and *median adult antler score* or *median adult body mass*. Dot size represents the number of repeated measurements taken during the adult stage.

DISCUSSION

Overall, body and antler measurements of roe deer in Valsemana were similar to those reported in other studies carried out in Mediterranean environments (Horcajada-Sánchez & Barja, 2016; Cappelli et al., 2020) and in central Europe (Pélabon & van Breukelen, 1998; Vanpé et al., 2007; Lemaître et al., 2018), but smaller than those reported for more northern populations such as the Scandinavian peninsula (Danilkin, 1996). This trend in male antlers and body measurements agreed with the trend reported for female body mass across the latitudinal gradient (Flajšman et al., 2018), which showed no differences between females from southern and central Europe (between 46° and 56° N) but found an increasing trend in body mass above 56 °N.

Similar to most cervid species (Saether & Haagenrud, 1985 on moose; Mysterud et al., 2005 on red deer; Vanpé et al., 2007 on roe deer), our Mediterranean roe deer population showed significant differences in antler size between most age classes, increasing from fawns to yearlings, peaking at prime-age and decreasing again at senescent stage to a similar size to that of yearlings. Furthermore, similar to another southern population (Chizé, south-western France), senescence in antler size in Valsemana was less pronounced than in more northern locations such as Trois Fontaines (northern France) or Bogesund (Sweden; Vanpé et al., 2007), which agrees with our hypothesis H1b. Therefore, the reduction of antler length from adult to senescent stage was only 1.48 % in Valsemana, 6.1% in Chizé but 23.2% and 46.0% in Trois Fontaines and Bogesund respectively (compare Fig. S4.6.3 of this manuscript with Fig. 2 of Vanpé et al., 2007). Therefore, our results agree with previous studies suggesting that the strength of winter severity may produce a trend across the latitudinal gradient in the senescence rate of secondary sexual traits (Gaillard et al., 1993a; Vanpé et al., 2007), being the magnitude of senescence less marked in locations with milder winters such as the Mediterranean environment.

In our Mediterranean population, we found a positive relationship between body measurements during October-November (body mass and body size) and subsequent antler score. This relationship was steeper for fawns compared to yearlings and adults, which indicates that the pattern in the allocation to antlers between light and heavy fawns is highly contrasting. Furthermore, our study also supports the hypothesis that, in southern populations, the relationship between senescent body mass and antler size is not as steep as in other northern populations (Vanpé et al., 2007). However, this relationship was non-significant for our study probably because sample size on senescents ($n=14$) was small and therefore these results should be taken with caution. Nevertheless, further research performed on other populations at different latitudes should confirm this trend in senescence rate as the number of populations studied so far is still low ($n=4$).

At individual level, yearling body mass (measured at 18 months) seemed to be the best predictor of future adult body mass, although all the other predictors (yearling body size, fawn body mass and body size at 6 months and antler size between 10-18 months) also had a significant relationship with adult body mass. Previous studies on roe deer described antler size at 8 months of age as a good predictor of body mass during adulthood (Lemaître et al., 2018) but for our Mediterranean population, we demonstrated that body size at 6 months can also be a good predictor of body mass during adulthood. Nevertheless, even though we confirmed that the latter can be used as an early-life predictor of adult body mass, we have also found a high measurement error when assessing body size, partly because of the difficulties of fully immobilize alive individuals to measure them (see Materials and Methods section). Therefore, we emphasize that body mass is probably more accurate measurement than body size, but recommend the use of body size at 6 months in the cases when the assessment of weight is not possible. In addition, antler size at early life (between 10-18 months) was the best predictor for antler size during adulthood. Yearling body mass also had a significant relationship with antler size during adulthood but the

strength of this correlation was smaller for our population. Therefore, the assertion that antler size (between 10-18 months) acts as an honest signal of future adult phenotypic quality (Lemaître et al., 2018) also applied for our Mediterranean population. Farmers and game managers focused on roe deer production of meat or trophy in Mediterranean environments should perform the phenotypic selection of individuals at the yearling stage removing individuals with low antler size between 10-18 months to select for high antler size during adulthood and high body mass as yearling to select for high adult body mass.

Finally, this study confirmed the results observed by Lemaître et al. (2018), that fawns showing a high allocation to antlers also displayed greater antlers during adulthood. However, it is important to note that our antler measurements were taken on males that had fully-formed clean antlers. Previous studies were based on standardized antler measures, since data was collected from January to March, when roe deer are still growing their antlers and a correction had to be made to take into account date of capture (Vanpé et al., 2007; Lemaître et al., 2018). We believe that the use of clean antlers data provide more robust results because any transformation or prediction on the future antler size may introduce bias in the analysis due to the inter-individual variation in antler phenology (Clements et al., 2010). Furthermore, we believe body measurements should be taken ideally before casting or at the beginning of antler growth as some studies have demonstrated that body mass at the start of antler growth has a great influence on subsequent antler growth (Gaspar-López et al., 2010 on red deer).

As a conclusion, our work provides support to Lemaître et al. (2014) hypothesis who first put into question that sexual characters are so highly-priced (Clutton-Brock et al., 1979), specially for a low dimorphic cervid, since we have seen that early life antler investment does not negatively affect adult performance, even at the edge of the species distribution range. Therefore, our results indicate that a high allocation during early life is an honest signal of high adult performance in Mediterranean environments.

Acknowledgments

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Supplementary material

Supplementary tables

Table S4.6.1 Descriptive statistics (mean and SD) for different antler metrics recorded for different age classes of male roe deer.

Age class	Antler score (mean $\pm$ SD)	Antler length (mm $\pm$ SD)	Ln[Antler length] (mm $\pm$ SD)	Sample size (N)
Fawns (10-18 months)	-2.45 $\pm$ 1.21	121 $\pm$ 34	4.74 $\pm$ 0.39	211
Yearlings (22-30 months)	1.23 $\pm$ 1.50	212 $\pm$ 27	5.35 $\pm$ 0.14	124
Adults (2-7 years)	2.18 $\pm$ 1.31	222 $\pm$ 22	5.40 $\pm$ 0.10	212
Senescents (>8 years)	1.45 $\pm$ 1.20	205 $\pm$ 23	5.32 $\pm$ 0.12	21

Table S4.6.2 Pairwise comparison of mean between body measurements for different age classes.

Pairwise comparison	Antler length (mm)		Body mass (kg)		Shoulder height (cm)		Nose-tail length (cm)	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Fawns-Yearlings	-28.906	<.0001	-42.823	<.0001	-27.380	<.0001	-44.689	<.0001
Fawns -Adults	37.630	<.0001	60.284	<.0001	35.696	<.0001	57.772	<.0001
Fawns-Senescents	-13.236	<.0001	-21.780	<.0001	-15.410	<.0001	-23.722	<.0001
Yearlings-Adults	3.436	0.004	12.989	<.0001	6.905	<.0001	10.965	<.0001
Yearlings-Senescents	-1.026	0.734	2.917	0.019	3.563	0.0022	4.398	0.0001
Adults-Senescents	2.756	0.031	2.752	0.031	-0.511	0.9566	0.471	0.9655

Table S4.6.3 Model describing the relationship between the predictors *Body mass* and *Body size* during October and November and antler score the subsequent year (formed between November and March) for each age class.

Age class	Body mass				Body size			
	Estimate $\pm$ std	t- value	P- value	N	Estimate $\pm$ std	t- value	P- value	N
Fawns	0.26 $\pm$ 0.04	5.61	<0.001	145	0.92 $\pm$ 0.11	38.55	<0.001	207
Yearlings	0.16 $\pm$ 0.05	3.24	0.001	94	0.50 $\pm$ 0.16	3.21	0.001	120
Adults	0.14 $\pm$ 0.03	4.16	<0.001	175	0.79 $\pm$ 0.18	4.30	<0.001	209
Senescents	0.07 $\pm$ 0.16	0.48	0.633	14	1.04 $\pm$ 0.75	1.38	0.167	21

Table S4.6.4 Significance variation among slopes between age classes of the model describing the relationship the predictors *Body mass* and *Body size* during October and November and antler score the subsequent year (formed between November and March) for each age class.

Pairwise comparison	Body mass (kg)		Body size	
	t-value	p-value	t-value	p-value
Fawns-Yearlings	-1.565	0.118	-2.216	0.0271
Fawns -Adults	2.126	0.034	-0.584	0.559
Fawns-Senescents	-1.173	0.242	0.162	0.871
Yearlings-Adults	0.289	0.772	1.217	0.224
Yearlings-Senescents	-0.517	0.606	0.705	0.481
Adults-Senescents	-0.421	0.674	0.320	0.749

Supplementary figures

Figure S4.6.1 A) Relationship between “Antler Score” and two common metrics used in other studies; Antler length (left y-axis) and $\ln$ -transformed Antler length (right y-axis). Antler length was measured along the exterior part of the main beam, from the coronet till the end of the top tine. B) Correspondence between our “Body size Score” and Nose to tail measurement (cm). C) Relationship between “Body size Score” and Shoulder height (cm). Blue solid lines represent model prediction using “loess smoother” method (A) or linear models (B, C).

Figure S4.6.2 Age-specific variation on body measurements for the population studied. Blue solid lines represent model prediction using “loess smoother” method. Antler length refer to the age the individual had when formed it and not the age at measurement.

Figure S4.6.3 Differences of mean ln-transformed antler size (in mm) between yearlings (YG; $n=212$), prime-age males (PA; 124), and senescent males (SN; $n=21$) for Valsemana. Estimates are based on raw data measurements at population levels. Error bars represent standard errors of the mean.

Figure S4.6.4 Results of the models performed to assess which fawn or yearling body measurements best predicted antler size during adulthood (H1): A) *fawn body mass*, B) *fawn body size score*, C) *yearling body mass* or C) *yearling body size score*. Point size represent the number of repeated measurements during adulthood.

Figure S4.6.5 Results of the models performed to assess which fawn or yearling body measurements best predicted body mass during adulthood (H1): A) *fawn body mass*, B) *fawn body size score*, C) *antler score between 10-18 months*, D) *yearling body size score*. Point size represent the number of repeated measurements during adulthood.

5 GENERAL DISCUSSION

Throughout this thesis, we have studied the effects of environmental conditions on the reproduction of two widespread deer species, the red deer and the roe deer. Here, we addressed a knowledge gap regarding two different ecosystems that constitute environmental extremes at the distribution range of both species, the Mediterranean environment and the Alpine region. Furthermore, these habitats make both species particularly vulnerable in the context of climate change where an intensification of drought episodes has been forecasted for Mediterranean environments (IPCC, 2007) and further advances in plant phenology are predicted for Alpine environments (IPCC, 2013).

In this section, our main findings will be discussed in an integrative way following the three main parts established in the objectives and hypotheses section:

I Environmental variation and female reproductive timing in the context of climate change

Here, we studied the key factors that lead to variation in the timing of breeding for both studied species. This knowledge is crucial in the current context of global change to be able to predict possible responses of wild populations to different climate scenarios.

According to female breeding timing, we have observed an opposite response between both species to the variation of environmental conditions. While many factors interplayed to drive reproduction timing of red deer in Mediterranean environments, roe deer seemed to display little phenotypic plasticity to rapidly adjust parturition timing to match environmental changes in Alpine environments.

Study 1 provided novel data on the variation of red deer conception dates, as most research on breeding phenology of large herbivores paid greater attention to variation of parturition dates in response to environmental conditions (but see Moyes et al., 2011). Therefore, this dissertation contributed to the existing body of knowledge by studying conception timing, a key event in reproductive phenology that has a large impact on birth timing. Our results showed that inter-annual variation in red deer conception dates was high in Mediterranean environments, quickly responding to different population level factors (density), climatic factors (spring rainfall and end of summer drought) and individual level factors (age and body mass).

Conversely, we found that roe deer in Alpine environments had limited ability to track temporal changes in plant phenology by rapidly adjusting parturition dates (**Study 3**). Hence, even though there was a high inter-annual variability on the timing of the onset of spring plant growth and flowering dates and a clear advance through time of these parameters, roe deer birth timing remained comparatively stable through time. This produced an increasing mismatch between parturition time and the peak of resource availability. Furthermore, the faster rate of advance of plant phenology compared to parturition dates was also supported by the measurements of vegetation height at marking sites, which increased throughout the study period.

However, despite the increasing mismatch observed between plant phenology and parturition date, we did find a consistent but small advance toward earlier parturition dates across all scales and most elevation ranges. Previous studies performed on a lowland forested area of France suggested a lack of phenotypic plasticity on parturition dates (Plard et al., 2014a). This study, however, suggests that, although very slowly and at population level, roe deer parturition timing changed in the direction of advancing peak resource availability. This is an important biologically meaningful finding for the species, which at least in highly heterogeneous environments, is showing some degree of response to

climate change. We interpreted this result as a possible effect of migration or dispersion movements towards higher elevations. Thus, instead of rescheduling breeding timing *in situ* to counteract the phenological mismatch via phenotypic plasticity or microevolution, we suggest that roe deer could have tracked appropriate conditions in space by moving towards higher elevations (i.e. behavioral plasticity). However, more research is needed to understand the mechanisms that led to the small response observed along the elevational gradient.

Overall, red deer and roe deer appear to be at opposite ends of the spectrum according to the response of breeding phenology to climate change. Thus, red deer responded more plastically to environmental changes than roe deer, which seems to be, so far, the studied ungulate species with least plasticity (see **Study 3** for a comparison among different ungulates).

In addition, this research provides new insights into the response of red deer to environmental changes in Mediterranean environments (**Study 1**). Previous findings on the Island of Rum, Scotland, described that red deer advanced breeding phenology as a response to climate change (Moyes et al., 2011). There, the advancement of spring plant phenology decreased the length and severity of winter (the limiting season) and increased spring-summer resource availability. Hence, red deer females increased body condition giving birth earlier through time, keeping in synchrony with the earlier spring phenology. However, in Mediterranean environments, climate change is predicted to increase drought severity, increasing the length and intensity of the limiting season, the summer. Therefore, longer drought periods would produce a higher depletion of body fat reserves for lactating females and thus, would lead to delayed and less synchronous conception dates the following season. If gestation length cannot compensate the observed delay on parturition date (Scott et al., 2008; Clements et al., 2011), it is expected that climate change in Mediterranean environments would produce an increasing mismatch between plant phenology and birth

timing, having detrimental consequences on calf survival (Clutton-Brock et al., 1987) and hind reproductive success the following season (Clutton-Brock et al., 1983). Therefore, in Mediterranean environments the plastic phenotypic response observed on red deer conception dates may turn out to be non-adaptive in the context of climate change (Ghalambor et al., 2007; Chevin & Hoffmann, 2017).

Finally, the little plasticity displayed by roe deer birth timing to counteract the rapid changes imposed by climate change does not mean that they do not have the ability to match optimal environmental condition across latitudes, given sufficient evolutionary time for selection to operate, or across elevations, if they can freely track resources at small spatial scales (behavioural plasticity). In fact, in our **Study 2**, we described in detail how roe deer displayed later parturition dates with increasing latitude and elevation. Furthermore, we demonstrated that the rate of change in roe deer parturition timing was similar with both 120 meters increase in altitude and one-degree increase in latitude, which agrees with the Bioclimatic Law (Hopkins, 1938). Interestingly, roe deer were able to match parturition dates with the peak of plant phenology across both gradients even though the spatial scales were very different (i.e. 3000 km between the northern- and the southernmost populations but only 300 km between the highest and lowest populations). Therefore, roe deer were able to adapt timing of parturition to the high variability of environmental conditions generated by the elevational gradient, even though this variation occurred over short geographical distances. However, we were unable to disentangle the potential mechanisms that could account for the response along the elevational gradient and we can only hypothesize that this response might be partly explained by migratory movements toward higher elevations (behavioural plasticity; Cagnacci et al., 2011). Therefore, further research is needed to understand the relative effect of evolutionary adaptation and phenotypic/behavioural plasticity on the response of populations to environmental variation along the elevational gradient.

II Effects of environmental variation on female litter size

In this dissertation, we shed some light on the geographical variation of litter size for a large herbivore by providing novel empirical data on elevational clines in roe deer litter size (**Study 4**). Interestingly, we observed an increase in roe deer litter size with latitude but a decreasing trend with elevation. This is the first time to observe this opposite relationship for a large mammal although this situation (higher clutch sizes at higher latitudes and lower clutches at higher elevations) occurred with substantial consistency across bird species (see Hille & Cooper, 2015), creating what is known as fecundity gradient paradox (Pincheira-Donoso & Hunt, 2017).

Unfortunately, we could not elucidate which adaptive mechanisms (phenotypic plasticity, local adaptation or both) were predominantly involved in the adaptation of large herbivores to each environmental gradient as we lacked the necessary individual-level information on female attributes to answer this question. Thus, we could only hypothesize the potential causes that led to the observed trend. The major assumption made in this study was that clutch/litter size is mainly driven by female body mass (Williams, 1966b). We studied the relationship between female body mass across both gradients and concluded that while female body mass increased dramatically at the highest latitudes (Flajšman et al., 2018), body mass did not significantly increase along the elevational gradient in Switzerland. We posed two, non-mutually excluding hypotheses. On the one hand, if body size changes may occur at a scale smaller than that at which individuals move (through migration or dispersion), we hypothesize that gene flow could constrain selection for an increased body mass along the elevational

gradient, similarly to the constraint hypothesized for parturition date (**Study 2**). On the other hand, an increase in body size may not necessarily help endure the harsh winter conditions, as they can simply move towards lower elevations (i.e. valley areas). This behavioural plasticity, also hypothesized to partly explain the results obtained along the elevational gradient in **Study 2 and 3**, would allow them to optimize the energy metabolism efficiently without an increase in body size, producing an indirect constraint in litter size selection. However, the observed lack of trend in body size would only explain a lack of trend in litter size but not a decrease. We speculated that without a favorable scenario for body mass selection along the elevational gradient, the decrease of litter size at higher elevations reported in our **Study 4** may be the result of a plastic response of females to the decrease in the growing season length and delayed parturition dates (**Study 2**).

Further studies should investigate this topic on other widespread polytocous cervids such as white-tailed deer or mule deer and also on other ungulates such as wild boar, for which an increase in litter size with latitude was demonstrated recently (Bywater et al., 2010). These results are important to improve the understanding of the relationships between geographical clines and mammalian life-history traits and also to be able to predict population dynamics of large mammals.

III Environmental and individual factors affecting male secondary sexual traits

In this final section, we analyzed the variation of red deer (**Study 5**) and roe deer (**Study 6**) antler size in Mediterranean environments.

Interestingly, as we studied the effect of environmental variation on reproductive traits for the female and male section of the same red deer population (**Study 1** and **Study 5**), both studies complemented each other and thus, they can be viewed as inter-related pieces of the same puzzle.

One of the most important factors influencing antler size variation in Mediterranean environments was our variable representing population traits (i.e. density and male age structure). Both population traits variables were highly correlated as the high densities at the beginning of the study were the result of previous trophy hunting management with little population control over females, which also led to a female-biased sex ratio and a young-male age structure (i.e. trophy hunting selects older males). Throughout the study period, management measurements were implemented to decrease density, balance sex ratios and create a more natural male age structure, which produced a constant increase of antler size at population level (**Study 5**) but also a steady advance in conception dates (**Study 1**).

In addition, our study highlights the strong role that food availability played on both male antler size (**Study 5**) and conception dates. In fact, Mediterranean environments are characterized by a high inter-annual variation in resource availability caused by the irregular precipitations (different spring plant productivity and summer drought length) and the irregular mast-seeding events

(acorn production). Unfortunately, for the female study, we did not have the data on acorn production. However, we speculated that the variable end of summer rains might be linked to mast production as September rains are an important factor for acorn maturation (García-Mozo et al., 2012). Nevertheless, we observed that red deer antler size at population level displayed a high inter-annual variation (**Study 5**), similar to the high phenotypic plasticity observed for female conception dates (**Study 1**). This strong inter-annual variation is what we expected from capital breeders such as red deer living in a highly variable environment such as the Mediterranean region.

Finally, a major result is that for both, females and males, we found an interactive effect between population traits and the variables accounting for resource availability. These results indicated that, in Mediterranean environments, the negative impact of the high variability in food resources was reduced when density was low, sex ratio was balanced and population had a natural age structure. Therefore, as drought is predicted to intensify in Mediterranean environments (IPCC, 2007), maintaining population traits at optimal levels may be a good management strategy to help red deer cope with the effects of climate change.

Lastly, in our **Study 6**, we tested whether there was a trade-off between early life investment on antlers and future adult performance, as fawns also need to heavily allocate resources to body growth. Here, we provided further evidence, that for a Mediterranean roe deer population, early-life (absolute and relative) antler investment is an honest signal for antler size during adulthood in line with more temperate populations (Lemaître et al., 2018). Furthermore, we found that those yearlings with greater antler size, also had higher body mass and body condition as adults. This result was firstly observed in France, where yearlings showing a high allocation to antlers also displayed longer antlers during adulthood (Lemaître et al., 2018). However, it is important to note that our antler measurements were taken on males that had fully formed clean antlers. Previous studies were based on standardized antler measures, since data was collected while

antlers were still growing, and a correction had to be made to take into account date of capture (Vanpé et al., 2007; Lemaître et al., 2018). We believe that the use of clean antlers data provides more robust results as any transformation or prediction on the future antler size could introduce bias on the analysis. This bias might be even more accentuated as antler growth phenology is known to vary among individuals (Clements et al., 2010).

Nevertheless, our work gives support to Lemaître et al. (2014) hypothesis, which puts into question that sexual characters, even for the highly competitive red deer males, are so highly-priced. Therefore, we demonstrated that yearling antler size is a good indicator of adult performance also in Mediterranean environments.

Finally, further research should focus on the consequences of a high allocation to secondary sexual traits during early life for the highly dimorphic red deer in Mediterranean environments. Although previous research found no negative consequences on a more northern population (Lemaître et al., 2014), we believe that trade-offs for this species, which has high energy expenditure in intra-sexual competition, may be more easily detected at the edge of its range of distribution

Implications for management and conservation

The questions addressed and the results obtained in the 6 studies of this thesis have numerous implications for management and conservation of both species, and probably for other cervid species. Here, we just outline a few of them that might be useful for farmers, managers and decision-makers. Further studies would be advisable to confirm that some of the management recommendations are viable not only ecologically but also socioeconomically.

- If the predicted future drought intensification materializes in Mediterranean environments (IPCC, 2007), it could produce a misalignment between parturition timing and resources availability. Thus, dry years could cause a higher investment of females on reproduction and thus, produce later conception dates the following year. Similarly, for males, low resource availability during spring due to drought would lead to high intraspecific competition for food, which may negatively affect antler size. In this context, we recommend lowering population densities to reduce the negative impact of drought and thus, increase per capita resource availability. This management measure, along with a compensation of population sex ratio and male age structure, would also lower inter-annual fluctuations on both conception dates and antler sizes.
- In alpine environments, earlier plant phenology as a consequence of climate change may cause a further misalignment between parturition date and the onset of plant phenology. Apart from the negative demographic consequences of a phenological mismatch (Plard et al., 2014a), fawns at highest elevations will be also exposed to higher mortality risk due to mowing (Jarnemo, 2002), as they are born increasingly near to the time of

hay cutting. To avoid an increasing fawn mortality resulting from farming operations, management measures should be taken such as altering mowing patterns (i.e. reducing speed, starting from the center outwards, leaving small patches without cropping), searching for fawns and carrying them away (i.e. dog searches or UAV-based system; Israel, 2011) or installing deterrent devices before the mowing season to coerce them to move to another bed site area (Steen et al., 2012).

- Furthermore, in Alpine environments, roe deer should be able to perform their migratory or dispersal movements to track resources in space. Therefore, mitigation strategies should be implemented in fragmented landscapes, where fencing or infrastructures prevent them from tracking the most suitable environment for each time of the year.
- Cast antlers represent a cost-effective and non-invasive method to collect large amount of data as sample size and representativeness are not limited by hunting quotas or methods. However, as deer antler size varies dramatically with age, the absence of control for changes in male age structure could lead to inferential error. Therefore, although cast antlers could reflect the condition of males at population level, we do not recommend their use unless reliable data on age structure is available.
- Acorn crop resulted in an important factor determining red deer antler size in Mediterranean environments. Conservation and restoration of oak-dominated systems, by ensuring natural regeneration of oaks and their acorn production, would benefit red deer performance.
- Farmers and game managers focused on roe deer production for trophy hunting should perform the phenotypic selection of individuals (e.g., big individuals with large antlers) at the age of yearlings. Antler and body measurements should be taken during autumn before antler cast. Furthermore, fawns with above average weight at the age of six months can already be selected as high quality males.

6 CONCLUSIONS

1. Red deer and roe deer appear to be at opposite ends of the spectrum according to the response of breeding phenology to the rapid changes produced by climate change, with red deer responding more plastically to environmental changes and roe deer displaying little plasticity.

2. The longer drought periods predicted as a consequence of climate change may lead to delayed and less synchronous conception dates for red deer in Mediterranean environments, creating a potential mismatch between birth date and plant phenology. Roe deer displayed little plasticity to rapidly adjust parturition timing to match the increasingly advanced plant phenology of Alpine environments.

3. In evolutionary terms, roe deer were able to adjust birth timing to match the period of peak energy demand of females along both, the latitudinal and the elevational gradients.

4. Roe deer litter size increased with latitude but decreased with elevation. This constitutes what is known as the fecundity gradient paradox that has been observed with substantial consistency across bird species but this is the first study reporting the occurrence of this paradox in a large mammal.

5. In Mediterranean environments, population traits and the inter-annual variation in food availability were the most important factors explaining the variation of male antler size and female conception dates in red deer. Lowering population density, balancing sex ratio and creating a balanced male age structure may be suitable management practices to lower inter-annual fluctuations of both traits and to mitigate the negative effects of climate change.

6. Early-life absolute and relative investment on roe deer antler size is a reliable indicator of adult performance in Mediterranean environments.

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- Zhao, Q. K. (1994). Birth timing shift with altitude and its ecological implication for *Macaca thibetana* at Mt Emei. *Oecologia Montana*, 3, 24–26.

8 Other scientific outcomes during the pre-doctoral training

Other publications

Peláez, M., Dirzo, R., Fernandes, G. W., & Perea, R. (2019). Nurse plant size and biotic stress determine quantity and quality of plant facilitation in oak savannas. *Forest Ecology and Management*, 437, 435-442.

López-Sánchez, A., **Peláez, M., Dirzo, R., Fernandes, G. W., Seminatore, M., & Perea, R.** (2019). Spatio-temporal variation of biotic and abiotic stress agents determines seedling survival in assisted oak regeneration. *Journal of Applied Ecology*, 56(12), 2663-2674.

Alonso-Martínez, L., Ibañez-Álvarez, M., Brolly, M., Burnside, N. G., Calleja, J. A., **Peláez, M., ... & Serrano, E.** (2020). Remote mapping of foodscapes using sUAS and a low cost BG-NIR sensor. *Science of the Total Environment*, 718, 137357.

International research stays

University Claude Bernard Lyon I. (France). 01/04/2017- 30/06/2017
Laboratoire de biométrie et biologie évolutive. (3 months)
Supervisor: Jean Michel Gaillard.

Laval University (Canada). Department of Biology. 01/02/2016 - 30/04/2016
Supervisor: Steeve Côté. (3 months)

Stanford University (USA). Department of Biology. 01/03/2015 - 31/08/2015
Supervisor: Rodolfo Dirzo. (3 months)

Participation in research projects

HEREGE: Herbivoría y regeneración natural en sistemas mediterráneos dominados por ungulados: Implicaciones para una gestión sostenible ante el cambio global. Organismo Autónomo de Parques Nacionales. IP: Ramón Perea.

INCREMENTO: Análisis global de incremento poblacional de ungulados silvestres sobre la integridad de los ecosistemas. Ministerio de Ciencia e Innovación. Universidades. IP: Ramón Perea, Mónica Eva González and Emmanuel Serrano.

BIOSTRESS: Linking biotic and abiotic stress into the net outcome of plant interactions. European Commission. IP: Ramón Perea.

AEQUILIBRIUM: Estudio de la predación del Águila real (*Aquila chrysaetos*) sobre las poblaciones de corzo (*Capreolus capreolus*) del interior de la península ibérica durante el periodo reproductor. Asociación del Corzo Español. Co-IP: Alfonso San Miguel, Ramón Perea and Marta Peláez.

ASESORÍA TÉCNICA sobre posibles zonas de translocación de ejemplares de cabra montés y su relación con la capacidad de carga en el Parque Nacional de la Sierra de Guadarrama. Grupo Tragsa. IP: Alfonso San Miguel; Participantes: Aida López, Marta Peláez, Irene Moreno y Ramón Perea.

ASESORÍA TÉCNICA sobre selección de indicadores ambientales y poblacionales para la elaboración de un plan de gestión de la cabra montés en el Parque Nacional de la Sierra de Guadarrama. Consejería de Medio Ambiente, Ordenación del Territorio y Sostenibilidad. Comunidad de Madrid. IP: Ramón Perea; Participantes: Aida López, Marta Peláez, Pablo Refoyo, y Alfonso San Miguel.

Scientific meetings and conferences

- **XIV MEDECOS & XIII AEET** meeting. Sevilla, Spain. (01 - 04 February 2017). Oral presentation.
- **VIII Reunião de Ungulados Silvestres Ibéricos RUSI**, Arcos de Valdevez, Portugal (15-16 September 2017). Poster presentation. **Awarded first prize.**
- **SEEEE** International Congress of the Spanish Society of Ethology and Evolutionary Ecology. Mieres, Spain. (04-08 September 2018). Oral presentation.
- **RUSI IX**. Capileira, Spain (4-6 October 2018). Poster presentation.
- **XXI Congreso SOCEPA**. Pontevedra, Spain (3-5 July 2019). Abstract co-author.
- The International Forum on Tiger and Leopard transboundary conversation. Harbin, China. (28-29 July 2019). Abstract co-author.
- **XXIV International Congress FeMeSPRum**, León, Spain. (26-28 September 2019). Abstract co-author.
- **RUSI X**, Lousã, Portugal (18-19 October 2019). Oral presentation.

University Teaching Experience

Teaching assistant

120 hours at the Universidad Politécnica de Madrid throughout the 2015-2018 academic years:

- Gestión de Especies Protegidas. Grado en Ingeniería del Medio Natural (45 hours).
- Pascicultura y Sistemas Agroforestales. Grado de Ingeniería Forestal. (75 hours).

Assistant advisor for final degree projects

- Daniel Gamba Caravantes. 2017. Universidad Politécnica de Madrid.
- Pablo Iglesias Casero. 2017. Universidad de Castilla-La Mancha.

- Elena García Lobato. 2018. Universidad Politécnica de Madrid.
- Paula Rodríguez Sanz. 2019. Universidad Politécnica de Madrid.
- Andrea Escribano Nieto. 2019. Universidad Politécnica de Madrid.
- Carlos Delgado Lara. 2020. Universidad Politécnica de Madrid.

Assistant advisor for Master thesis

- Isabel Sanuy Latorre. 2019. Universitat Autònoma de Barcelona.
- José Martel Serrano. 2020. Universidad Politécnica de Madrid.

Workshops

- “Casos de estudio de análisis de R”. Doctorado en Investigación Forestal Avanzada (4 hours)
- “Utilización de drones en la gestión del territorio”. Organismo Autónomo Parques Nacionales. Ministerio para la Transición Ecológica. (2 hours)

Innovative educational projects

- Magnificando la docencia a través del teléfono inteligente: empleo de minilupas y herramientas TIC en asignaturas de ámbito agroforestal. IP: Ramón Perea

Others

Scientific reviewer for: *Journal of Animal Ecology*, *European Journal of Wildlife Research* and *Galemys*.

Workshop organization “Wild Ungulates Management in National Parks”. 17-19 of June 2019, CENEAM, Valsaín, Spain. 33 participants.