Multidisciplinary approach to elucidate aspects involved in the decline of farmland birds

Nekazal inguruneko hegaztien gainbeheran eragina duten faktoreak argitzeko diziplina anitzeko azterketa

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Universidad del País Vasco
Euskal Herriko Unibertsitatea

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A thesis submitted by
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for the degree of Doctor of Philosophy,
under the supervision of
Dr. Benjamín Juan Gómez Moliner and **Dra. Beatriz Arroyo López**

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¿Qué es una tesis Doctoral?

Una tesis es mucho más que un trabajo original de investigación. Una tesis es una experiencia vital única e intensa. Buena o mala dependiendo de diversos factores, muchos de ellos ajenos al doctorando. Una tesis es una aventura muy exigente anímicamente y donde la buena compañía es fundamental.

En mi caso, estos últimos cinco años han sido un intenso, apasionante y a la vez estresante proceso de aprendizaje. Proceso durante el cual he tenido la suerte de contar con la mejor compañía que uno pudiera imaginar, tanto en el ámbito profesional, trabajando bajo la supervisión de investigadoras e investigadores de gran calidad humana e intelectual, como en el ámbito personal. Gracias a todas vosotras esta tesis es lo que es y puedo decir que mi etapa como doctorando ha sido muy enriquecedora y positiva a nivel personal.

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Contents

PREFACE

This thesis is divided into seven chapters.

The general framework is given within the introduction (**Chapter 1**). First the study species, farmland birds, are presented and, secondly, the factors limiting their populations are described, highlighting the gaps that still exist in this field.

The specific goals of the thesis are gathered in **Chapter 2**.

Chapter 3 is subdivided into three papers (Paper I-III) and assesses the relationship between diverse measures of agricultural intensification and farmland birds.

In **Chapter 4** the possible relationship between massive releases of farm-reared birds for hunting purposes and the strong population decline of an endangered bird is assessed. This is summarized in Paper IV.

Chapter 5, is subdivided into three papers (Paper V-VII), deals with the molecular assessment of these birds' diet and endoparasites.

Chapter 6 is a Synthesis of the thesis.

Chapter 7 lists the main conclusions that can be drawn from this PhD thesis.

Finally the **Other scientific contributions** section includes other scientific works derived from the PhD formation period and published on SCI journals but not directly related to this thesis' framework.

From all articles of this thesis, two have already been published in SCI journals, four are currently under revision and one is in preparation to be submitted to an SCI journal:

Chapter	Paper	Status
3	**Cabodevilla, X.** *et al.* Irrigation drives declines in farmland bird communities.	Under a second review
3	**Cabodevilla, X.** *et al.* (2021). Vineyard modernization drives changes in bird and mammal occurrence in vineyard plots in dry farmland. *Agriculture, Ecosystems & Environment*, 315, 107448. https://doi.org/10.1016/j.agee.2021.107448	Published
3	**Cabodevilla, X.** *et al.* Farmland composition and farming practices explain spatio-temporal variations in red-legged partridge density in central Spain.	Under a second review
4	**Cabodevilla, X.** *et al.* (2020). Are population changes of endangered little bustards associated with releases of red-legged partridges for hunting? A large-scale study from central Spain. *European Journal of Wildlife Research*, 66(2), 1-10. https://doi.org/10.1007/s10344-020-1366-3	Published
5	**Cabodevilla, X.** *et al.* (2020). Simultaneous analysis of the intestinal parasites and diet through eDNA metabarcoding. *Authorea*. May 12, 2020. https://doi.org/10.22541/au.158531783.33894277/v2	Under review
5	**Cabodevilla, X.** *et al.* Metabarcoding insights onto the diet and trophic diversity of six declining farmland birds.	Under review
5	**Cabodevilla, X.** *et al.* A semi-quantitative approximation to *Blastocystis* spp. infections in farmland birds: very high parasitic load in protected Bustards.	In preparation

HITZAURREA

Tesi hau zazpi kapitulutan banatzen da.

Sarreran (**1. kapitulua**) gaiaren ikuspegi orokorra eskaintzen da. Lehenik eta behin, ikertu beharreko espezieak, nekazal inguruneko hegaztiak, aurkezten dira, eta, bigarrenik, haien populazioak mugatzen dituzten faktoreak deskribatzen dira, oraindik jorratu gabe dauden arloak nabarmenduz.

Tesiaren helburu espezifikoak **2. kapituluan** jasotzen dira.

3. kapitulua hiru artikuluz osatuta dago (I.-III. artikuluak), eta nekazaritzaren intentsifikazio-neurrien eta nekazal inguruneko hegaztien arteko erlazioa ikertzen du.

4. kapituluan, helburu zinegetikoak dituzten haztegietan hazitako hegaztien askatze masiboen eta arriskuan dagoen hegazti baten populazio-gainbehera handiaren arteko erlazioa aztertzen da. Hori IV. artikuluan laburbiltzen da.

5. kapitulua hiru artikulutan banatzen da (V.-VII. artikuluak), eta hegazti horien dietaren eta endoparasitoen ebaluazio molekularrari buruz datza.

6. kapitulua tesiaren sintesia da.

7. kapituluak doktorego-tesi honetatik atera daitezkeen ondorio nagusiak zerrendatzen ditu.

Azkenik, **Beste ekarpen zientifiko batzuk** atalean, doktoregoaren prestakuntza-alditik eratorritako eta SCI aldizkarietan argitaratutako doktoregaiaren beste lan zientifiko batzuk aurkezten dira, tesi honen esparruarekin lotura zuzenik ez daukatenak.

Tesi hau osatzen duten artikuluetatik, bi SCI aldizkarietan argitaratu dira, lau errebizio prozesuan daude eta bat SCI aldizkari batera bidaltzeko prestatzen ari gara:

Kapitulua	Artikulua	Egoera
3	**Cabodevilla, X.** *et al.* Irrigation drives declines in farmland bird communities.	Errebizio prozesuan
3	**Cabodevilla, X.** *et al.* (2021). Vineyard modernization drives changes in bird and mammal occurrence in vineyard plots in dry farmland. *Agriculture, Ecosystems & Environment*, 315, 107448. https://doi.org/10.1016/j.agee.2021.107448	Argitaratua
3	**Cabodevilla, X.** *et al.* Farmland composition and farming practices explain spatio-temporal variations in red-legged partridge density in central Spain.	Errebizio prozesuan
4	**Cabodevilla, X.** *et al.* (2020). Are population changes of endangered little bustards associated with releases of red-legged partridges for hunting? A large-scale study from central Spain. *European Journal of Wildlife Research*, 66(2), 1-10. https://doi.org/10.1007/s10344-020-1366-3	Argitaratua
5	**Cabodevilla, X.** *et al.* (2020). Simultaneous analysis of the intestinal parasites and diet through eDNA metabarcoding. *Authorea*. May 12, 2020. https://doi.org/10.22541/au.158531783.33894277/v2	Errebizio prozesuan
5	**Cabodevilla, X.** *et al.* Metabarcoding insights onto the diet and trophic diversity of six declining farmland birds.	Errebizio prozesuan
5	**Cabodevilla, X.** *et al.* A semi-quantitative approximation to *Blastocystis* spp. infections in farmland birds: very high parasitic load in protected Bustards.	Prestatzen

1

INTRODUCTION

Introduction

1. Farmland birds

The term "Farmland birds" is used to refer to those bird species that are strongly adapted to, and rely on, extensive and heterogeneous agricultural environments, where they breed and find trophic resources throughout the year (Benton *et al.*, 2003). However, agroecosystems are not natural, but human-made semi-natural environments that did not exist millennia ago (Zeder, 2008). Thus, farmland is not the native environment for these species, which probably inhabited open landscapes with natural grasslands or shrubland ecosystems time ago, and with the emergence and expansion of agriculture, they adapted to these new environments.

Agriculture slowly spread together with human settlements (Zeder, 2008), opening gaps in the forest masses and providing new semi-natural open lands (Marquer *et al.*, 2017), an environment where certain birds found suitable breeding or wintering habitats (Tucker and Heath, 1994; Tucker and Evans, 1997; Robinson *et al.*, 2001). Agricultural development and its slow expansion over the millennia is likely to have favoured these species. Although they are called farmland birds, most of these species probably do not rely on agriculture itself but on the landscape associated with extensive agriculture, which provides a suitable substitute to their original grasslands (sometimes richer in terms of nutrients than grasslands themselves).

Nowadays, agricultural environments are widely spread, to the point that a large part of the European landscape is strongly linked to agriculture (Krebs *et al.*, 1999) and they have become highly relevant for conservation. These environments hold more than 50% of bird species in the European Union (EU; Burfield, 2005). However, farmland biodiversity is strongly threatened since the middle of the last century, when agricultural systems started a marked qualitative change and a process of mechanisation and production intensification (Matson *et al.*, 1997). Since then, agriculture is undergoing a strong change, with large implications for landscape composition and biodiversity (Matson *et al.*, 1997; Pain and Pienkowski, 1997; Stoate *et al.*, 2001; Benton *et al.*, 2002), which in the short term involve important limiting factors to farmland birds' populations (Pain and Pienkowski, 1997; Donald *et al.*, 2001; Benton *et al.*, 2003). Most of these species are currently in sharp decline (Burfield, 2005; Donald *et al.*, 2006), and as a result of this, the European farmland holds 55% of European bird species listed in the IUCN Red List (Burfield, 2005; Donald *et al.*, 2006).

2. Populations' limiting factors

The size of bird populations depends on several factors, intrinsic ones (demographic) and extrinsic ones (external factors that influence populations; Newton, 1998, 2007). Population trends are conditioned by intrinsic factors (the rates of births and deaths, immigration and emigration) and depend on the balance between inputs (births + immigrants) and losses (deaths + emigrants; Newton, 1998). Demographic studies (including population modelling) provide information on which demographic variables (fecundity, survival, net emigration) are influencing most strongly population trends at a given time. However, they do not necessarily provide information on the causes of these trends. On the other hand, studies on how intrinsic factors are modulated by the environmental extrinsic factors (such as, trophic resources, availability of nest sites, or natural enemies; Newton, 1998, 2007) provide knowledge of how these factors affect the populations, which is also crucial for their conservation. Trophic resources, nesting sites, or natural enemies can be key factors in the population dynamics of species (Newton, 1998).

Each of these environmental extrinsic factors is highly relevant and therefore needs to be studied in detail to properly understand the reasons driving a population decline. Many studies have shown that the reduction of trophic resources has clear and direct effects on the population dynamics of species (van Balen, 1980; Jansson *et al.*, 1981; Watson *et al.*, 1992; Martin, 1995; Oro *et al.*, 2004; Siriwardena *et al.*, 2008), even if this reduction occurs only for a relatively short period of the year if this creates a bottleneck (Jansson *et al.*, 1981). Thus, populations require a sufficient supply of food throughout the year. The limitation of suitable nesting areas *per se* is also known to lead to significant interference with population dynamics (Martin, 1993; Newton, 1994; Martin, 1995; Wiebe, 2011). This is especially relevant for bird species that require special places to put their nests, such as cavity-nesters, cliffs-nesters, or ground-nesters (Newton, 1994; Martin, 1995; Wiebe, 2011). Besides the resources, natural enemies are also very relevant to population dynamics, whether they are predators, parasites, or competitors (Cody, 1974; Martin, 1995; Redpath *et al.*, 2006; Cresswell, 2011). Although in different ways, all three natural enemies are capable of affecting population dynamics. Predators impact population dynamics mostly through the direct removal of individuals (Cresswell, 2011). Parasites often lead to a reduction in fecundity (Redpath *et al.*, 2006). And competitors limit the available resources, such as food and nesting sites (Cody, 1974).

Moreover, populations may be simultaneously constrained by more than one factor, and factors may interact with each other, increasing (or decreasing) their effect on the populations (Newton, 1998). For example, a smaller area of suitable nesting habitat is likely to imply a higher risk of predation, as nests will be easier to find (Pescador and Peris, 2001). There can be many different interactions between environmental factors (Martin and Roper, 1988; Newton, 2004; Newton, 2007) and anthropogenic modifications of the environment can enhance these interactions (Ford *et al.*, 2001; Newton, 2004; Innes *et al.*, 2010). In many cases, humans (or human activities) are the reason for changes in trophic resources, the availability of nesting sites, or increased pressure from natural enemies (Ford *et al.*, 2001; Newton, 2004). Besides, humans also are the origin of other (anthropogenic) risk factors (Loss *et al.*, 2015) such as hunting, power lines, wind farms, or the use of toxic substances (medicines or agrochemicals).

Regarding farmland bird species, agriculture intensification seems the main driver of their population dynamics (Donald *et al.*, 2001), and this may be related to direct demographic effects, limiting environmental factors, and interactions between them. For example, early harvesting or extensive use of agrochemicals can be a direct cause of mortality for farmland bird species (Wilson *et al.*, 2005; Rodríguez-Estival *et al.*, 2010; Grüebler *et al.*, 2015; Ortiz-Santaliestra *et al.*, 2020). In addition, the reduction in the amount and distribution of suitable habitats (trophic or nesting resources) may be limiting for these species populations (Donald *et al.*, 2001; Newton, 2004). And, even, the reduction of those resources could lead to a higher incidence of predation and parasitism, as bird populations are more concentrated (Whittingham and Evans, 2004; Mustin *et al.*, 2018).

3. Factors probably affecting farmland bird population dynamics related to agriculture intensification

As mentioned above, since the middle of the XX century, many farmland ecosystems have been strongly modified, modernised and intensified, in a race to increase farmland productivity in response to a growing demand for food (Matson *et al.*, 1997; Foley *et al.*, 2005; Tilman *et al.*, 2011; Kastner *et al.*, 2012). In contrast to the slow transformation of the natural vegetation into an agricultural environment throughout millennia, farmland is now being intensified very rapidly, leading to major habitat changes in just a few decades (Matson *et al.*, 1997; Pain and Pienkowski, 1997). Such rapid changes in the landscape hardly give species time to adapt.

The intensification involves changes in the landscape (e.g. through the increase in field size for allowing more efficient mechanization, the disappearance of field boundaries, and increased monoculture), in land-uses (as some crops have been promoted over others), and in agricultural practices within land-uses (e.g. mechanization, use of pesticides, fertilizers or irrigation; Matson *et al.*, 1997; Pain and Pienkowski, 1997). These changes are directly or indirectly associated with the most important limiting factors for bird populations (Donald *et al.*, 2001; Newton, 2004).

For example, changes at landscape and land-use level may reduce nesting sites, trophic resources, as well as increase the pressure of natural enemies (Donald *et al.*, 2001; Newton, 2004). A major consequence of farmland intensification is the homogenization of the landscape and at the same time the reduction of the percentage of non-farmland habitats (natural vegetation) and fallow lands (Traba and Morales, 2019; Tarjuelo *et al.*, 2020). Natural vegetation and fallow lands are highly suitable environments for farmland birds, providing foraging, mating, and nesting habitats (Vickery *et al.*, 2002; Vickery *et al.*, 2009; Mcmahon *et al.*, 2010; Tarjuelo *et al.*, 2020). Thus, intensification reduces nesting habitats for many farmland species (Whittingham and Evans, 2004). This reduction in nesting habitats leads to a concentration of breeding birds and an increased risk of nest predation (Pescador and Peris, 2001; Whittingham and Evans, 2004), especially for ground-nesting species. Homogenization also affects the seasonality of trophic resources, as the number of weed species is reduced and therefore birds depend almost exclusively on crops, increasing competition (Whittingham and Evans, 2004).

On the other hand, changes in agricultural practices may have direct effects on the species demography, as the massive use of agrochemicals may affect both fecundity and survival (Lopez-Antia *et al.*, 2016; Fernández-Vizcaíno *et al.*, 2020; Ortiz-Santaliestra *et al.*, 2020; Lopez-Antia *et al.*, 2021). It has been shown experimentally that the coated cereal seeds sowed in central Spain (Lopez-Antia *et al.*, 2016) disrupt reproduction and reduce red-legged partridge productivity by half (Fernández-Vizcaíno *et al.*, 2020; Lopez-Antia *et al.*, 2021) and therefore have the potential to cause rapid population declines. Moreover, when fertilizers (mostly nitrates) are applied via the irrigation systems (fertigation), their concentration in water is very high, exceeding by 10 times the recommended limit for public safety (Rodríguez-Estival *et al.*, 2010) and may expose birds to a toxic dose of nitrates, with associated adverse effects on health (Ley 1986; Rodríguez-Estival *et al.*, 2010). Harvest mechanization may also be an important cause of mortality and breeding failure, especially for ground-nesting birds (Wilson *et al.*, 2005; Grüebler *et al.*, 2015; Santangeli *et al.*, 2015). Besides, some agricultural practices such as the use of agrochemicals affect weed and arthropod abundance, and so the trophic resources of farmland (Wilson *et al.*, 1999; Storkey *et al.*, 2012; Andreasen *et al.*, 2018; Raven and Wagner, 2021).

4. Other anthropic factors probably affecting farmland bird population dynamics (hunting intensification)

Most farmland habitats in western Europe, or at least in Spain, are home to many birds and medium-sized mammals that are regularly hunted (e.g. partridges or rabbits). Farmland habitats are therefore associated with small game hunting in many areas. Therefore, in addition to the agriculture intensification, farmland birds (including non-game species) may be also influenced by hunting activities or game management (Mustin *et al.*, 2018). Hunting has a direct impact on populations of farmland game bird species, in some cases killing more than half a million or even several million specimens a year in Spain (Andueza *et al.*, 2018). This is the case of species such as song thrush (*Turdus philomelos*), red-legged partridge (*Alectoris rufa*), wood pigeon (*Columba palumbus*), common quail (*Coturnix coturnix*), or European turtle dove (*Streptopelia turtur*; Andueza *et al.*, 2018). However, hunting activity can have much wider implications within farmland. For example, hunting activities have shown to affect non-game farmland species through the disturbance associated with the noise of gunshots or dogs (Casas *et al.*, 2009; Tarjuelo *et al.*, 2015). Effects can due not only to hunting itself but also to all the management measures that are carried out in the hunting estates to favour this activity (Mustin *et al.*, 2018).

In that sense, as other farmland birds, many important farmland game bird species are in sharp decline due to agricultural intensification, which has led to an intensification of game management, aiming to sustain the hunting activity. In this line, most hunting estates in Spain focused on small game carry out certain game management activities aimed to improve populations of game species (Ríos-Saldaña, 2010). Hunting management intensification implies an increase in the use of measures such as the provision of food and water, the provision of game crops (crops planted specifically for the game that are not harvested), the improvement of habitats (e.g. by providing more shelter), predator control, and the release of farm-reared animals (Draycott *et al.*, 2008; Fletcher *et al.*, 2010; Smith *et al.*, 2010; Mustin *et al.*, 2018; Aebischer, 2019).

Some of those practices have been proved beneficial for wild game bird populations (Mustin *et al.*, 2018), such as the provision of game crops, the provision of food and water, and legal predator control. Among those, the provision of game crops and legal predator control could be also beneficial for many other species (Mustin *et al.*, 2018), including threatened farmland bird species. Fox control intensity is associated with a higher abundance of non-granivorous steppe bird populations, suggesting a positive effect of this activity (Estrada *et al.*, 2015). The provision of food and water likewise may have a positive effect on non-game species populations, and increasing density of feeders is associated with higher numbers of granivorous steppe birds (Estrada *et al.*, 2015). However, this practice could also be detrimental, as it may concentrate populations and thus favour the transmission of parasites and predation (Mustin *et al.*, 2018). On the other hand, the massive releases of farm-reared birds carried out in many hunting states have been shown to have detrimental effects on wild populations of the target species (Millán *et al.*, 2004; Villanúa *et al.*, 2012; Casas *et al.*, 2012; Mustin *et al.*, 2018), but it is not clear their impact on non-game species as some studies showed negative effect and other positives (Mustin *et al.*, 2018). However, different studies seem to indicate a risk for non-game birds due to the introduction of new parasites through releases (Villanúa *et al.*, 2007; Díaz-Sánchez *et al.*, 2012).

5. Some of the most relevant knowledge gaps related to farmland birds' population dynamics

Although the negative impact of agricultural intensification on farmland birds is well known, the effect of some intensification measures on these birds has not yet been adequately studied. It is not enough to know that agricultural intensification is detrimental to farmland birds, but it is necessary to take a deep understanding of the limiting factors that each intensification measure involves. This information is crucial to plan a bird-friendly agricultural modernisation that can meet the production needs of our times. Among those less studied measures are the implementation of irrigation in arable land and the modernization of vineyards, two widespread measures that are still expanding rapidly nowadays (Matson *et al.*, 1997; FAO, 2011; Ruiz-Pulpón, 2013; MAPAMA, 2017). Implementation of irrigation involves major changes in the environment (including changes in cultivated crops or changes in landscape structure) and has significantly increased since the mid-20th century (Matson *et al.*, 1997). Today, irrigated farmland covers more than 300 million hectares worldwide (FAO, 2011), and even so, its effects on farmland wildlife have been little studied (Brotons *et al.*, 2004; Giralt *et al.*, 2021). The modernisation of vineyards also involves major changes in vines' structure and management (Salguero, 2010; Montero-García *et al.*, 2017), and the modern trellis vineyards have undergone a large expansion in recent decades in Spain. However, although its expansion has been encouraged and funded by the European Union (CE- 1493/1999; Ruiz-Pulpón, 2013), it is probably one of the least studied changes in agricultural environments to date, at least in Spain. It is, therefore, necessary to continue gathering information that can be used to improve regulation of agricultural intensification, making it more ecological and bird-friendly, so that it is profitable, but not detrimental to farmland bird conservation.

In addition, considering that agricultural intensification has a direct impact on the ecosystem's trophic resources and their availability (Wilson *et al.*, 1999; Benton *et al.*, 2002; 2003; Andreasen *et al.*, 2018; Fonderflick *et al.*, 2020; Hallman *et al.*, 2014), the knowledge of the trophic ecology of these species takes on special relevance. However, in many cases, we still lack detailed information on their diet. The study of feeding ecology is essential to understand the biological and ecological requirements of species and may help to understand the impact of some intensification measures that reduce trophic resources (Martin, 1987). Additionally, and although most of these species are in sharp decline and probably under continuous environmental stress, mainly due to changes in agriculture (Donald *et al.*, 2001), very little is known about the health status of their populations and the parasites that affect them, information that is highly relevant to understand integrally the factors driving decline on these species and for proper management and conservation of these populations and environments. Among the parasites affecting wildlife, protists are probably the least studied ones. In particular, the incidence and importance of *Blastocystis* spp. in wild birds is completely unknown. *Blastocystis* spp. is a strict anaerobic protist parasite, with zoonotic capacity, that can be found in the intestinal tract of many different animals including humans (Tan, 2008), and which pathogenic capacity increases in immunosuppressed hosts (Chandramathi *et al.*, 2014). Thus, its importance on threatened farmland bird species might be highly relevant for the population dynamics of these species. Regarding both diet and parasites, non-invasive samples, such as faecal samples, are useful, as they can be obtained efficiently during routine fieldwork and have the potential to simultaneously provide information on diet, gut microbiota and intestinal parasites. Molecular methods have expanded the utility of faecal samples by allowing the analysis of these aspects of species ecology from a new perspective. As a result, the use of DNA-based approaches in trophic ecology and parasitology has grown during the last years (e.g., Pompanon *et al.*, 2012; Shokralla *et al.*, 2012; Bass *et al.*, 2015), especially due to the advent of high-throughput sequencing (HTS), leading to the development of metabarcoding (Taberlet *et al.*, 2012). A new approach that largely lacks the identification biases of traditional methods and allows for much more accurate identification.

Regarding hunting intensification, more research on the possible effect of releases on threatened non-game farmland species is needed. This hunting intensification measure has been identified as negative for wild populations of released game species (Tompkins *et al.*, 2000; Villanúa *et al.*, 2006; Blanco-Aguiar *et al.*, 2008; Casas *et al.*, 2012), but there is very little information on its effect on non-game species (Mustin *et al.*, 2018). In particular, in Spain, the release of farm-reared red-legged partridges is very relevant, with several millions of those birds released annually (Caro *et al.*, 2014). Thus, understanding how the intensity of these massive releases can affect sympatric threatened bird species is crucial for developing appropriate regulations and management measures.

Besides releases of farm-reared birds, the current status of the red-legged partridge is quite worrying. This is a traditionally important game species in Spain, with a high ecologically and socio-economically relevance, whose natural populations inhabit mainly the Iberian Peninsula (Blanco-Aguiar, *et al.*, 2004) and have strongly declined in the second half of the 20th century (Blanco-Aguiar, 2007). This decline has been suggested to be primarily associated with changes in farmland, including loss of habitat heterogeneity, earlier cereal harvest, and use of agrochemicals, although high hunting pressure may also have contributed (Blanco-Aguiar, 2007). However, the magnitude of this decline (at least in recent years) has been contested by hunting sectors (RFEC, 2020) and no large-scale study has assessed whether abundance or trends of red-legged partridge are related to farmland composition or management. This should be evaluated rigorously and in detail, as it is a controversial species with great political and media relevance both in Spain and in Europe, even more since BirdLife International proposed to reclassify it from Least Concern to Vulnerable, which would have affected the hunting of the species.

As mentioned above, there are still many aspects that may be involved in the decline of farmland birds and have not yet been accurately assessed. Thus, this thesis is focused on elucidating the importance of some of those matters on these species' population dynamics.

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1

SARRERA

Sarrera

1. Nekazal inguruneko hegaztiak

"Nekazal inguruneko hegaztiak" terminoa nekazaritza estentsiboan eta nekazal ingurune heterogeneoetan bizi eta horien menpe diren hegaztiak izendatzeko erabiltzen da. Ingurune horretan, hegazti horiek ugaltzen dira, eta, urte osoan zehar, baliabide trofikoak aurkitzen dituzte (Benton *et al.*, 2003). Hala ere, nekazaritza-ekosistemak ez dira naturalak, gizakiak sortutako ingurune erdi-naturalak baizik (Zeder, 2008). Horrela, nekazaritza-lurrak ez dira espezie horien jatorrizko ingurunea; ziurrenik, hegazti horiek larre naturalak edo sastrakadiak barne zituzten paisaia irekietan bizi izan ziren aspaldi, eta nekazaritzaren agerpenarekin eta hedapenarekin batera ingurune berri horietara egokitu ziren.

Nekazaritza poliki-poliki zabaldu zen giza asentamenduekin batera (Zeder, 2008), basoetan tarteak irekiz eta ingurumen erdi-natural berri bat sortuz (Marquer *et al.*, 2017), non hegazti batzuek ugaltzeko edota negua igarotzeko habitat egokiak aurkitu zituzten (Tucker eta Heath, 1994; Tucker eta Evans, 1997; Robinson *et al.*, 2001). Litekeena da milaka urtetan zehar gertatutako nekazaritzaren garapen eta hedapen geldoek mesede egin izana espezie horiei. Nahiz eta nekazal inguruneko hegaztiak deitu, espezie horietako gehienak ziurrenik ez daude nekazaritzaren mende, baizik eta nekazaritza estentsiboarekin lotutako paisaiaren mende; izan ere, ekosistema hori haien jatorrizko larreen ordezko egokia da (batzuetan, elikadurari dagokionez, larreak berak baino aberatsagoak ere bai).

Gaur egun, nekazal inguruneak oso hedatuta daude; Europako paisaiaren zati handi bat nekazaritzarekin oso lotuta dago (Krebs *et al.*, 1999), eta, hortaz, garrantzi handia hartu dute kontserbaziorako. Ingurune horietan, Europar Batasuneko hegazti-espezieen % 50 baino gehiago aurkitzen dira (EU; Burfield, 2005). Hala ere, joan den mendearen erdialdetik nekazaritza-lurren biodibertsitatea oso mehatxatuta dago, hain zuzen, nekazaritza-sistemak aldaketa kualitatibo nabarmena pairatzen hasi zirenetik, mekanizazioa eta intentsifikazioa barne (Matson *et al.*, 1997). Ordutik, nekazaritza aldaketa handia jasaten ari da, eta paisaian eta biodibertsitatearen konposizioan inplikazio handiak ditu (Matson *et al.*, 1997; Pain eta Pienkowski, 1997; Stoate *et al.*, 2001; Benton *et al.*, 2002), eta, hortaz, nekazal inguruneko hegaztien populazioentzat faktore mugatzaile garrantzitsuak bilakatu dira (Pain eta

Pienkowski, 1997; Donald *et al.*, 2001). Gaur egun, espezie horietako gehienak gainbeheran daude (Burfield, 2005; Donald *et al.*, 2006), eta, ondorioz, Europako nekazaritza-lurretan, IUCNren Zerrenda Gorriko Europako hegazti-espezieen % 55 daude (Burfield, 2005; Donald *et al.*, 2006).

2. Populazioen faktore mugatzaileak

Hegazti-populazioen tamaina hainbat faktoreren araberakoa da: intrintsekoak (demografikoak) eta estrintsekoak (populazioetan eragina duten kanpo-faktoreak; Newton, 1998, 2007). Populazioen joerak faktore intrintsekoek baldintzatzen dituzte (jaiotza- eta heriotza-tasak, immigrazioa eta emigrazioa), eta sarreren (jaiotzak + etorkinak) eta galeren (heriotzak + emigranteak; Newton, 1998) arteko orekaren mende daude. Ikerketa demografikoek (populazioen modelizazioa barne) une jakin batean populazioen joeran eragin handiena duten aldagai demografikoei (ugalkortasuna, biziraupena, emigrazioa) buruzko informazioa ematen dute. Hala ere, ez dute nahitaez joera horien kausei buruzko informaziorik ematen. Bestalde, inguruneko faktore estrintsekoek faktore intrintsekoak nola modulatzen dituzten aztertzen duten ikerketek (adibidez, baliabide trofikoek, habiak egiteko tokien eskuragarritasunak edo etsai naturalek; Newton, 1998, 2007) faktore horiek populazioetan duten eraginari buruzko informazioa ematen dute, eta hori ere funtsezkoa da populazio horiek kontserbatzeko. Baliabide trofikoak, habiak egiteko lekuak edo etsai naturalak funtsezko faktoreak izan daitezke espezieen populazio-dinamikan (Newton, 1998).

Inguruneko faktore estrintseko horietako bakoitza oso garrantzitsua da eta, beraz, xehetasunez ikertu beharrekoa, populazioaren gainbehera bultzatzen duten arrazoiak behar bezala ulertzeko. Ikerketa askok frogatu dute baliabide trofikoak murrizteak espezieen populazio-dinamikan ondorio argi eta zuzenak dituela (Van Balen, 1980; Jansson *et al.*, 1981; Watson *et al.*, 1992; Martin, 1995; Oro *et al.*, 2004; Siriwardena *et al.*, 2008), baita murrizketa hori urtearen nahiko epe laburrean gertatzen bada ere, horrek botila-lepo bat sortzen badu (Jansson, 1981). Beraz, populazioek elikagai-hornidura nahikoa behar dute urte osoan zehar. Jakina da, halaber, habiak egiteko eremu egokiak mugatuak izateak eragin handia duela populazioen dinamikan (Martin, 1993; Newton, 1994; Martin, 1995; Wiebe, 2011). Hori bereziki garrantzitsua da habiak jartzeko leku bereziak behar dituzten hegazti-espezieentzat, hala nola barrunbeetan, labarretan edo lurrean habiak egiten dituztenentzat (Newton, 1994; Martin, 1995; Wiebe, 2011). Baliabideez gain, etsai naturalak ere oso garrantzitsuak dira populazioen dinamikarako: harrapariak, parasitoak eta lehiakideak (Cody, 1974; Martin, 1995; Redpath *et al.*, 2006; Cresswell, 2011). Modu ezberdinean bada ere, hiru etsai naturalak populazioen dinamikari eragiteko gai dira. Harrapariek populazioaren dinamikari zuzenean eragiten diote, batez ere banakoak zuzenean akabatuz (Cresswell, 2011). Parasitoek ugalkortasuna murriztea eragiten dute (Redpath *et al.*, 2006). Eta lehiakideek eskura dituzten baliabideak mugatzen dituzte, hala nola elikagaia eta habiak jartzeko lekuak (Cody, 1974).

Gainera, faktore batek baino gehiagok aldi berean mugatu ditzakete populazioak, eta faktoreek elkarren artean eragin dezakete, populazioetan duten eragina handituz (edo murriztuz) (Newton, 1998). Adibidez, litekeena da habiak egiteko habitat egokiaren azalera txikitzerakoan harraparitza-arriskua handitzea, habiak errazago aurkituko baitira (Pescador eta Peris, 2001). Interakzio ezberdin asko egon daitezke ingurumen-faktoreen artean (Martin eta Roper, 1988; Newton, 2004; Newton, 2007), eta ingurunean gertatutako aldaketa antropogenikoek interakzio horiek indartu ditzakete (Ford *et al.*, 2001; Newton, 2004; Innes *et al.*, 2010). Kasu askotan, gizakiak (edo giza jarduerak) dira baliabide trofikoetan, habiak egiteko tokien eskuragarritasunean edo etsai naturalen presioan izandako aldaketen arrazoia (Ford *et al.*, 2001; Newton, 2004). Gainera, gizakiak badira beste arrisku-faktore (antropogeniko) batzuen jatorria ere (Loss *et al.*, 2015), hala nola ehiza, linea elektrikoak, parke eolikoak edo substantzia toxikoen erabilera (sendagaiak edo agrokimikoak).

Nekazal inguruneko hegazti-espezieei dagokienez, badirudi nekazaritzaren intentsifikazioa dela populazio-dinamikaren bultzatzaile nagusia (Donald *et al.*, 2001), eta horrek zerikusia izan dezake eragin demografiko zuzenekin, ingurumen-faktore mugatzaileekin eta haien arteko interakzioekin. Adibidez, uztaren biltze goiztiarra edo agrokimikoen erabilera estentsiboa espezie horien hilkortasuna handitzearen kausa izan daitezke (Wilson *et al.*, 2005; Rodriguez-Estival *et al.*, 2010; Grüebler *et al.*, 2015; Ortiz-Santaliestra *et al.*, 2020). Gainera, habitat egokien kopurua eta banaketa murriztea (baliabide trofikoak edo habiak egiteko lekuak) mugatzailea izan daiteke espezie horien populazioentzat (Donald *et al.*, 2001; Newton, 2004). Eta, halaber, baliabide horiek murrizteak harraparitzaren eta parasitismoaren eragin handiagoa ekar lezake, hegazti-populazioak kontzentratuago baitaude (Whittingham eta Evans, 2004; Mustin *et al.*, 2018).

3. Nekazal inguruneko hegazti-populazioen dinamikari eragin diezaioketen nekazaritzaren intentsifikazioarekin loturiko faktoreak

Lehen aipatu bezala, XX. mendearen erdialdetik aurrera, nekazaritza-lurrak erabat aldatu, modernizatu eta intentsifikatu dira, nekazaritza-lurren produktibitatea handitzeko lasterketa batean, elikagaien eskari gero eta handiagoari erantzuteko (Matson *et al.*, 1997; Foley *et al.*, 2005; Tilman *et al.*, 2011; Kastner *et al.*, 2012). Landaredi naturaletik nekazaritza-ingurune baterako eraldatze geldoa (milaka urtetan zehar) ez bezala, gaur egun nekazaritza-lurrak oso azkar ari dira intentsifikatzen, eta horrek habitatean aldaketa handiak eragiten ditu hamarkada gutxitan (Matson *et al.*, 1997; Pain eta Pienkowski, 1997). Paisaian gertatutako aldaketa hain azkarrek ez diete espezieei egokitzeko denborarik ematen.

Nekazaritzaren intentsifikazioak hainbat aldaketa dakartza: paisaian (adibidez, sailen tamaina handituz mekanizazio eraginkorragoa ahalbidetzeko, sailen mugak desagerraraziz, eta monolaborantzak areagotuz), lurraren erabileretan (labore batzuk beste batzuen gainetik sustatu baitira) eta erabilitako nekazaritza-praktiketan (adibidez, mekanizazioa, pestiziden, ongarrien edo ureztatzearen erabilera; Matson *et al.*, 1997; Pain eta Pienkowski, 1997). Aldaketa horiek, zuzenean edo zeharka, lotuta daude hegazti-populazioentzako mugatzaile diren faktore garrantzitsuenekin (Donald *et al.*, 2001; Newton, 2004).

Adibidez, paisaian eta lurraren erabileran gertatzen diren aldaketek habiak egiteko lekuak eta baliabide trofikoak murriztu ditzakete, bai eta etsai naturalen presioa handitu ere (Donald *et al.*, 2001; Newton, 2004). Nekazaritza-lurren intentsifikazioaren ondorio nagusietako bat paisaiaren homogeneizazioa da, eta, aldi berean, habitat naturalen (landaredi naturala) eta lugorrien (Traba eta Morales, 2019; Tarjuelo *et al.*, 2020) murriztea. Landaredi naturala eta lugorriak oso ingurune egokiak dira nekazal inguruneko hegaztientzat, jana, ugaltzeko toki egokiak eta habia egiteko leku aproposak aurkitzen baitituzte (Vickery *et al.*, 2002; Vickery *et al.*, 2009; McMahon *et al.*, 2010; Tarjuelo *et al.*, 2020). Hortaz, intentsifikazioak habiak egiteko habitatak murrizten ditu nekazal inguruneko espezie askorentzat (Whittingham eta Evans, 2004). Habiak egiteko habitat egokien murrizketa horrek hegazti ugaltzaileak kontzentraziora daramatza, eta, beraz, habien gaineko harraparitza-arriskua handitzen da (Pescador eta Peris, 2001; Whittingham eta Evans, 2004), bereziki lurrean habia egiten duten espezieentzat. Homogeneizazioak baliabide trofikoen urtarokotasunari ere eragiten dio; izan ere, sasi eta belar txar espezieen kopurua murrizten da, eta, ondorioz, hegaztien elikagaia laboreetara murrizten da ia erabat, eta horrek lehia areagotzen du (Whittingham eta Evans, 2004).

Bestalde, nekazaritza-praktiketan izandako aldaketek eragin zuzena izan dezakete espezieen demografian; izan ere, agrokimikoen erabilera masiboak ugalkortasunean eta biziraupenean eragina izan dezake (López-Antia *et al.*, 2016; Fernández-Vizcaíno *et al.*, 2020; Ortiz-Santaliestra *et al.*, 2020; López-Antia *et al.*, 2021). Esperimentalki frogatu da Espainiako erdialdean ereiten diren zerealen hazi blindatuek (López-Antia *et al.*, 2016) ugalketa eteten dutela eta eper gorriaren produktibitatea erdira murrizten dutela (Fernández-Vizcaíno *et al.*, 2020; López-Antia *et al.*, 2021), eta, beraz, populazioaren jaitsiera azkarra eragiteko ahalmena dutela. Gainera, ongarriak (gehienak nitratoak) ureztatze-sistemen bidez (fertirrigazioa) aplikatzen direnean, uretan duten kontzentrazioa oso handia da, segurtasun publikorako gomendatutako muga baino 10 aldiz handiagoa (Rodriguez-Estival *et al.*, 2010), eta, hortaz, nitratoen dosi toxiko baten eraginpean jar ditzake ur hori edaten duten hegaztiak, eta osasunean ondorio kaltegarriak eragin (Ley, 1986; Rodríguez-Estival *et al.*, 2010). Uztaren mekanizazioa ere heriotza-tasaren eta ugalketa-porrotaren kausa garrantzitsua izan daiteke, batez ere lurrean habia egiten duten hegaztientzat (Wilson *et al.*, 2005; Grüebler *et al.*, 2015; Santangeli *et al.*, 2015). Gainera, nekazaritza-praktika batzuek, hala nola agrokimikoen erabilerak, belar txarren eta artropodoen ugaritasunari eragiten diote, eta, beraz, nekazaritza-lurren baliabide trofikoei (Wilson *et al.*, 1999; Storkey *et al.*, 2012; Andreasen *et al.*, 2018; Raven eta Wagner, 2021).

4. Nekazal inguruneko hegazti-populazioen dinamikari eragin diezaieketen bestelako faktore antropikoak (ehizaren intentsifikazioa)

Mendebaldeko Europan, edo Espainian behintzat, nekazaritza-lurren habitat gehienak tamaina ertaineko hegazti eta ugaztun espezie zinegetiko askoren gotorleku dira (adibidez, eperrak edo untxiak). Beraz, nekazal inguruneak ehiza xeheari lotuta daude eremu askotan. Hortaz, nekazaritzaren intentsifikazioaz gain, ehiza-jarduerek edo ehizaren kudeaketak nekazal inguruneko hegaztietan (zinegetikoak ez diren espezieak barne) ere eragina izan dezakete (Mustin *et al.*, 2018). Ehizak eragin zuzena du nekazal inguruneko hegazti zinegetikoetan; izan ere, Espainian urtero espezie horietako batzuen milioi erdi edo zenbait milioi banako hiltzen dira (Andueza *et al.*, 2018). Adibidez, birigarroa (*Turdus philomelos*), eper gorria (*Alectoris rufa*), pagausoa (*Columba palumbus*), galeperra (*Coturnix coturnix*) edo usapala (*Streptopelia turtur*; Andueza *et al.*, 2018). Hala ere, ehiza-jarduerak ondorio askoz zabalagoak izan ditzake nekazaritza-lurretan. Esate baterako, ehiza-jarduerek nekazaritza-lurretako espezie ez-zinegetikoei eragiten dietela frogatu da, tiroen edo txakurren zaratarekin lotutako eragozpenengatik (Casas *et al.*, 2009; Tarjuelo *et al.*, 2015). Ondorio horiek, ehiza-jarduera beraren ondorio ez ezik, ehiza-barrutietan ehiza-jarduera bermatzeko gauzatzen diren kudeaketa-neurri guztien ondorio ere izan daitezke (Mustin *et al.*, 2018).

Ildo horretan, nekazal inguruneko beste hegazti batzuk bezala, nekazal inguruneko hegazti zinegetiko garrantzitsu asko gainbeheran daude, nekazaritza-intentsifikazioaren ondorioz, eta horrek ehiza-kudeaketa intentsifikatzea ekarri du, ehiza-jarduerari eusteko helburuarekin. Horregatik, ehiza xeheko Espainiako ehiza-barruti gehienek kudeaketa-jarduera ezberdinak egiten dituzte espezie zinegetikoen populazioak hobetzeko (Rios-Saldaña, 2010). Ehizaren kudeaketa intentsifikatzeak honako neurri hauen erabilera areagotzea dakar: elikagaien eta uraren hornidura, ehiza-laboreen hornidura (espezie zinegetikoei laguntzeko uztatzen ez diren laboreak), habitatak hobetzea (adibidez, babes handiagoa emanez), harrapariak kontrolatzea eta haztegietan hazitako animaliak askatzea (Draycott *et al.*, 2008; Fletcher *et al.*, 2010; Smith *et al.*, 2010; Mustin *et al.*, 2018; Aebischer, 2019).

Neurri horietako batzuk basa-hegazti zinegetikoen populazioentzat onuragarriak direla frogatu da (Mustin *et al.*, 2018), hala nola ehiza-laboreen hornidura, elikagaien eta uraren hornidura eta harraparien legezko kontrola. Horien artean, ehiza-laboreen hornidura eta harraparien legezko kontrola onuragarriak izan litezke beste espezie askorentzat ere (Mustin *et al.*, 2018), baita arriskuan dauden nekazal inguruneko hegazti-espezieentzat ere. Azerien kontrolaren intentsitatea lotuta dago estepako hegazti ez-graniboroen populazioaren ugaritasunarekin, eta horrek neurri horren eragin positiboa iradokitzen du (Estrada *et al.*, 2015). Elikagaien eta uraren hornidurak eragin positiboa izan dezake zinegetikoak ez diren espezieen populazioetan ere; izan ere, janlekuen dentsitatea handitzea estepako hegazti kopuru handiagoarekin lotu da (Estrada *et al.*, 2015). Hala ere, praktika hori kaltegarria ere izan liteke, populazioak kontzentratu baitaitezke eta, hortaz, parasitoen transmisioa eta harraparitza erraztu (Mustin *et al.*, 2018). Bestalde, frogatu da ehiza-barruti askotan egiten diren haztegietan hazitako hegaztien askatze masiboek ondorio kaltegarriak dituztela helburu diren basa-hegazti zinegetikoen populazioentzat (Millán *et al.*, 2004; Villanúa *et al.*, 2012; Casas *et al.*, 2012; Mustin *et al.*, 2018), baina ez dago argi zinegetikoak ez diren espezieetan duten eragina; izan ere, zenbait ikerlanetan eragin negatiboa deskribatu zen (Mustin *et al.*, 2018). Hala ere, ikerlan batzuek adierazten dutenez, badirudi zinegetikoak ez diren espezieetan eragina izan dezaketela, askatzeen bidez basa-populazioetan parasito berriak sartu direlako (Villanúa *et al.*, 2007; Díaz-Sánchez *et al.*, 2012).

5. Nekazal inguruneko hegaztien populazio-dinamikarekin lotutako ezagutza-hutsune garrantzitsuenetako batzuk

Nekazaritzaren intentsifikazioak nekazal inguruneko hegaztietan duen eragin negatiboa ezaguna den arren, intentsifikazio-neurri batzuek hegazti horietan duten eragina oraindik ez da behar bezala aztertu. Nekazaritzaren intentsifikazioa nekazal inguruneko hegaztientzat kaltegarria dela jakitea ez da nahikoa; intentsifikazio-neurri bakoitzak horientzat dakartzan faktore mugatzaileak ondo ulertu behar dira. Informazio hori funtsezkoa da hegaztiak errespetatzen dituen eta, aldi berean, gure garaiko ekoizpen-beharrak ase ahal dituen nekazaritzaren modernizazioa planifikatzeko. Gutxien aztertu diren neurri horien artean, nekazaritza-lurretan ureztapena ezartzea eta mahastien modernizatzea daude. Bi neurri horiek oso hedatuta daude, eta gaur egun azkar hedatzen jarraitzen dute (Matson *et al.*, 1997; FAO, 2011; Ruiz-Pulpón, 2013; MAPAMA, 2017). Ureztapena ezartzeak aldaketa handiak dakartza ingurumenean (laboreetan edo paisaiaren egituran aldaketak barne), eta nabarmen areagotu da XX. mendearen erdialdetik (Matson *et al.*, 1997). Gaur egun, ureztatuko nekazaritza-lurrek 300 milioi hektarea baino gehiago hartzen dituzte mundu osoan zehar (FAO, 2011), eta, hala ere, horrek nekazal inguruneko animalietan dituen eraginak gutxi aztertu dira (Brotons *et al.*, 2004; Giralt *et al.*, 2021). Mahastien modernizazioak aldaketa handiak ekarri ditu mahastien egituran eta horien maneiuan (Salguero, 2010; Montero-García *et al.*, 2017), eta, Espainian, beso-sistemako mahasti berriak asko hedatu dira azken hamarkadetan. Hala ere, horien hedapena Europar Batasunak sustatu eta finantzatu badu ere (EE- 1493/1999;

Ruiz-Pulpón, 2013), ziurrenik orain arte gutxien aztertu den nekazaritza-inguruneen aldaketetako bat da, Espainian behintzat. Beraz, beharrezkoa da nekazaritza-intentsifikazioaren arauketa hobetzeko balioko duen informazioa biltzen jarraitzea, intentsifikazioa ekologikoagoa eta hegaztiekiko errespetagarriagoa egiten lagundu baitezake, nekazaritza errentagarria izan dadin eta, era berean, nekazal inguruneko hegaztientzat ez-kaltegarria.

Gainera, nekazaritzaren intentsifikazioak nekazaritza-ekosistemen baliabide trofikoetan eta horien eskuragarritasunean eragin zuzena duela kontuan hartuta (Wilson *et al.*, 1999; Benton *et al.*, 2002; 2003; Andreasen *et al.*, 2018; Fonderflick *et al.*, 2020; Hallman *et al.*, 2014), garrantzi handia du nekazal inguruneko hegaztien ekologia trofikoa ezagutzeak. Hala ere, kasu askotan, oraindik ez dugu espezie horien dietari buruzko informazio zehatzik. Elikaduraren ikerketa funtsezkoa da espezieen eskakizun biologikoak eta ekologikoak ulertzeko, eta baliabide trofikoak murrizten dituzten intentsifikazio-neurri batzuen eragina ulertzen lagun dezake (Martin, 1987). Gainera, espezie horietako gehienak gainbeheran dauden arren, eta, ziurrenik, nekazaritzan izandako aldaketen ondorioz ingurumen-estres etengabea jasan behar duten arren (Donald *et al.*, 2001), haien populazioen osasun-egoerari eta haiei eragiten dieten parasitoei buruz oso gutxi ezagutzen da. Informazio hori oso garrantzitsua da espezie horien gainbehera bultzatzen duten faktoreak ikuspegi orokor batetik ulertzeko, eta espezie horien populazioak eta horien ekosistema behar bezala kudeatzeko eta kontserbatzeko. Faunari eragiten dioten parasitoen artean, protistak dira gutxien aztertu direnak. Bereziki, erabat ezezaguna da *Blastocystis* spp.-k hegazti basatietan duen eragina eta garrantzia. *Blastocystis* spp. animalia ezberdin askoren hestean aurki daitekeen (gizakia barne) ahalmen zoonotikoa duen parasito protista anaerobiko zorrotza da (Tan, 2008), zeinaren ahalmen patogenikoa handitu egiten den banako immunodeprimituetan (Chandramathi *et al.*, 2014). Beraz, arriskuan dauden nekazal inguruneko hegazti-espezieen populazio-dinamikan eragina izan dezake. Dieta zein parasitoen ikerketari dagokienez, lagin ez-inbaditzaileak, hala nola gorotzak, oso erabilgarriak dira; izan ere, erraz lortzen dira ohiko landa-lana egiterakoan, eta dietari, hesteetako mikrobiotari eta hesteetako parasitoei buruzko informazioa aldi berean emateko ahalmena dute. Bestalde, metodo molekularrek gorotz-laginen erabilgarritasuna asko zabaldu dute, espezieen ekologiaren alderdi horiek ikuspegi berri batetik aztertzea ahalbidetu baitute. Honen ondorioz, ekologia trofikoan eta parasitologian, DNAn oinarritutako tekniken erabilerak gora egin du azken urteetan (adibidez, Pompanon *et al.*, 2012; Shokralla *et al.*, 2012; Bass *et al.*, 2015), batez ere errendimendu handiko sekuentziazioa (HTS) agertu zenetik, zeinak metabarcoding-a garatzea ahalbidetu duen (Taberlet *et al.*, 2012). Ikuspegi berri horrek, neurri handi batean, ez du metodo tradizionalak dituen identifikatzeko alborapen-arazorik, eta identifikazio askoz zehatzagoa ahalbidetzen du.

Ehizaren intentsifikazioari dagokionez, beharrezkoa da askatzeek arriskuan dauden espezie ez-zinegetikoetan izan dezaketen eraginak gehiago ikertzea. Ehizaren intentsifikazio neurri hori negatibotzat jo da askatutako espezie zinegetikoen basa-populazioentzat (Tompkins *et al.*, 2000; Villanúa *et al.*, 2006; Blanco-Aguiar *et al.*, 2008; Casas *et al.*, 2012), baina zinegetikoak ez diren espezieekiko izan dezakeen eraginari buruz oso informazio gutxi dago (Mustin *et al.*, 2018). Espainian bereziki, haztegietan hazitako eper gorrien askatzea oso garrantzitsua da, urtero horrelako zenbait milioi banako askatzen baitira (Caro *et al.*, 2014). Hortaz, askatze masibo horien intentsitateak arriskuan dauden hegazti espezieei nola eragin diezaiekeen ulertzea funtsezkoa da, espezie horien populazioentzako onuragarriak diren kudeaketa-neurri egokiak garatzeko.

Haztegietan hazitako hegaztien askatzeak alde batera utzita, eper gorriaren egoera nahiko kezkagarria da. Espainian tradizionalki espezie zinegetiko oso garrantzitsua da, ekologikoki nahiz sozioekonomikoki. Espezie honen populazio naturalak Iberiar Penintsulan bizi dira nagusiki (Blanco-Aguiar, *et al.*, 2004), eta XX. mendearen bigarren erdian zehar nabarmen murriztu ziren (Blanco-Aguiar, 2007). Gainbehera hori batez ere nekazaritza-lurretan gertatutako aldaketekin lotuta dagoela iradoki da, habitataren heterogeneotasunaren galera, zerealen uzta aurreratzea eta agrokimikoak erabiltzea barne, eta ehizaren presio handiak gainbehera areagotu ahal izan dezake (Blanco-Aguiar, 2007). Hala ere, ehiza-sektoreek eztabaidan jarri dute beherakada horren garrantzia (gutxienez azken urteetan) (RFEC, 2020), eta ez dago eskala handiko ikerketarik ebaluatu duenik eper gorriaren ugaritasuna edo joera lotuta ote dagoen nekazaritza-lurren antolakuntza edo kudeaketarekin. Hori zorroztasunez eta xehetasunez ebaluatu beharreko gauza litzateke; izan ere, bai Espainian, bai Europan garrantzi politiko eta mediatiko handia duen espeziea da, are gehiago BirdLife Internationalek proposatu zuenetik espeziea, arrisku txikiko kategorian sailkatuta zegoena, kaltebera kategoriara birsailkatzea, zeinak espeziearen ehizari eragingo liokeen.

Lehen aipatu bezala, oraindik ere ez dira zehaztasunez ebaluatu nekazal inguruneko hegaztien gainbeheran eragina izan dezaketen hainbat alderdi. Horregatik, alderdi horietako batzuek espezie horien populazio-dinamikan duten garrantzia argitzea da tesi honen helburua.

2

AIMS OF THE THESIS

Aims of the thesis

The main objective of this thesis is to shed light upon some understudied issues related to the intensification of agricultural environments that might help to explain the decline of farmland birds, in order to provide information and recommendations relevant to the conservation of these species. We address these questions from a multidisciplinary approach, combining molecular techniques and complex statistical models, both in a Frequentist and Bayesian framework. The specific goals are to:

1. **Asses the consequences of the implementation of irrigation on farmland birds**

2. **Investigate the implications of vineyard modernisation for farmland birds**

3. **Determine the correlation between habitat composition and farming practices and red-legged partridge abundance**

4. **Estimate the population trend of red-legged partridge and its relationship with habitat composition**

5. **Provide detailed information on the releases of farm-reared red-legged partridges in central Spain during the last decade**

6. **Assess whether there is a relation between little bustard population trend and the releases of farm-reared red-legged partridges**

7. **Evaluate different molecular markers and propose a barcode suitable for the study by metabarcoding of diet and parasites in birds**

8. **Improve the understanding of the diet of six declining farmland birds**

9. **Shed light upon the incidence of parasites affecting the health status of farmland birds, focusing on *Blastocystis* spp**

10. **Investigate the incidence of zoonotic subtypes of the parasite *Blastocystis* spp. in farmland birds**

2

TESIAREN HELBURUAK

Tesiaren helburuak

Tesi honen helburu nagusia da nekazal inguruneko hegaztien gainbehera azaltzen lagun dezaketen nekazaritza-inguruneen intentsifikazioarekin zerikusia duten gutxi ikertutako alderdi batzuk ikertzea, espezie horien kontserbaziorako informazio eta gomendio garrantzitsuak eman ahal izateko. Alderdi horiei diziplina anitzeko ikuspegitik heldu diegu, teknika molekularrak eta eredu estatistiko konplexuak konbinatuz, bai ikuspegi frekuentista, bai bayesiarra erabiliz. Honako hauek dira helburu espezifikoak:

1. **Ureztapena ezartzeak nekazal inguruneko hegaztietan dituen ondorioak ebaluatzea**

2. **Mahastien modernizatzeak nekazal inguruneko hegaztientzat dituen ondorioak ikertzea**

3. **Habitataren konposizioaren eta nekazaritza-praktiken eta eper gorriaren ugaritasunaren arteko erlazioa zehaztea**

4. **Eper gorriaren populazio-joerak habitataren osaerarekin duen lotura zehaztea**

5. **Azken hamarkadan, Espainiako erdialdean egin diren haztegietan hazitako eper gorrien askatzeei buruzko informazio zehatza ematea**

6. **Basoilo txikiaren populazio-joeraren eta haztegietan hazitako eper gorrien askatzeen artean ea loturarik dagoen ebaluatzea**

7. **Hegaztien dieta eta parasitoak metabarcoding bidez aztertzeko markatzaile molekular ezberdinak ebaluatzea eta barcode egoki bat proposatzea**

8. **Gainbeheran dauden nekazal inguruneko sei hegaztien dietaren ulermena hobetzea**

9. **Nekazal inguruneko hegaztien osasun-egoerari eragiten dioten parasitoen intzidentziari buruzko informazioa lortzea, *Blastocystis* spp.-n arreta jarriz**

10. ***Blastocystis* spp. parasitoaren subtipo zoonotikoek nekazal inguruneko hegaztietan duten intzidentzia ikertzea**

3

EFFECTS OF LAND-USES AND FARMING PRACTICES

PAPER I

Irrigation drives declines in farmland bird communities

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Agriculture, Ecosystems & Environment (Under review)

Irrigation drives declines in farmland bird communities

Abstract

Assessing the effects of agricultural intensification on biodiversity is critical for developing effective management plans for farmland conservation. One factor that has not yet been thoroughly studied is the impact of irrigation on wildlife, despite significant increases in the surface area of irrigated farmland since the mid-twentieth century. Irrigated farmland now cover over 300 million hectares worldwide. Here, we evaluate the impact of irrigation on bird species occurrence patterns using a BACI (Before-After Control-Impact) design. Our study occurs in a 100 km^2 area with rain-fed agriculture in the Mediterranean region of northern Spain. We analysed a 13-year dataset comprised of the 47 most common bird species in the region using a multi-species hierarchical occurrence model. We examined how the implementation of irrigation altered the local bird community, identifying which species were negatively (or positively) impacted by changes to the local ecosystem. Irrigation had an overall negative impact on the bird community, with occurrence rates of most species (55.3%) decreasing and only a small fraction (10.6%) increasing after the onset of irrigation, leading to an overall reduction in site-level species richness. Irrigation was most detrimental for farmland birds (including steppe birds, which are of high conservation concern), but also for forest birds, shrubland birds, and non-specialist species that occur frequently in rain-fed agricultural environments. The fact that only a few species responded positively to irrigation suggests that in the long-term irrigation may lead to substantial negative changes in local bird communities, with less diversity and a lack of ecologically-important farmland species. The negative impact of irrigation on bird occurrences is likely due to the loss of nesting and foraging habitat arising from shifts in crops and/or loss of fallow lands. Irrigation schemes should thus be implemented carefully, avoiding areas with high species richness or high densities of endangered species. In cases where irrigation cannot be avoided, promoting diverse agrosystems, avoiding monocultures, and including interspersed rain-fed crops and fallow lands may help to mitigate negative effects on local bird communities and their ecosystems.

Keywords

Agricultural intensification; BACI design; biodiversity conservation; bird community; multi-species occupancy model; steppe birds

1. Introduction

Anthropogenic activities cause multiple impacts on natural and human-dominated landscapes, which can have direct and indirect effects on wildlife (Pimm *et al.*, 1995; Hooke *et al.*, 2012). Agriculture is widespread throughout the world and greatly changes habitat, nutrient loads, and water, and thus, local and regional biodiversity (Matson *et al.*, 1997; Pain and Pienkowski, 1997; Benton *et al.*, 2003). Within the last several decades, agriculture has intensified through increases in land conversion rates, average plot sizes, mechanization and use of high-yield crop varieties, irrigation, fertilizers, and pesticides (Matson *et al.*, 1997). Together, these agricultural intensification measures can have detrimental effects on local and regional plant and animal population abundances and species richness (Pain and Pienkowski, 1997; Stoate *et al.*, 2001; Benton *et al.*, 2002; Donald *et al.*, 2006; Storkey *et al.*, 2012). However, the relative effect of each of these measures can be difficult to tease apart as the effects are often interacting.

The implementation and widespread use of irrigation, in particular, has been an important advancement for agricultural productivity (Alauddin and Quiggin, 2008), but also significantly alters local ecosystems (Baldock *et al.*, 2000; Stoate *et al.*, 2001). Irrigated farmland surface has increased dramatically in the last 50 years (Matson *et al.*, 1997; Alauddin and Quiggin, 2008), covering more than 300 million hectares worldwide (FAO, 2011). This, combined with the increased use of fertilisers, has greatly boosted crop yields (Matson *et al.*, 1997) and has generally rendered unnecessary the maintenance of fallows (Baldock *et al.*, 1994). However, fallow farmland provides critical habitat for many wildlife species in human-dominated landscapes (Denys and Tscharntke, 2002; Schmidt and Tscharntke, 2005; Vickery *et al.*, 2004; Ng *et al.*, 2017; Traba and Morales, 2019). Additionally, when fertilisers are distributed through irrigation schemes (fertigation), nitrate concentration can reach dangerous levels for those wildlife that drink irrigated water (Rodríguez-Estival *et al.*, 2010). Irrigation also influences soil properties through salinization and waterlogging (Matson *et al.*, 1997; Baldock *et al.*, 2000), which can further affect wildlife species distribution and abundance patterns.

In Mediterranean biomes, the implementation of irrigation has led mainly to the loss of rain-fed cereal farming systems (Bignal and McCracken, 1996; Baldock *et al.*, 2000; Caraveli, 2000). These systems, which include a significant proportion of fallow land in annual crop rotations, provide habitat that is of great importance for farmland bird species (Brotons *et al.*, 2004; Sanderson *et al.*, 2013), many of which select this semi-natural habitat for nesting (Benton *et al.*, 2003, Traba and Morales, 2019), including threatened steppe bird species (Sanderson *et al.*, 2013; García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020). Over the last several decades, irrigation in the Mediterranean region has significantly expanded. For example, 1.4 million hectares of agricultural land have been converted to irrigation in Spain alone since 1973 (Baldock *et al.*, 1994). Concurrently, the proportion of arable land left fallow declined from >50% in 1973 to <40% in 1990 (Baldock *et al.*, 1994), and has further declined by another 16-40% in just the last two decades (Traba and Morales, 2019). Similar trends have been documented in Greece, Italy, and Portugal (Caraveli, 2000), suggesting that the adoption of irrigation methods is leading to widespread changes in Mediterranean landscapes.

Despite its importance to agricultural production, irrigation can cause significant changes to surrounding environments (Baldock *et al.*, 2000). The shift from rain-fed farmland to irrigated farmland, and loss of associated semi-natural ecosystems, can have a direct impact on biodiversity, harming some species while benefiting others (Baraibar *et al.*, 2009; González-Estébanez *et al.*, 2011; Andrey *et al.*, 2014; Pérez - Fuertes *et al.*, 2015). Even for farmland birds, one of the groups most affected by agricultural intensification (Donald *et al.*, 2001; Brennan and Kuvlesky, 2005; Donald *et al.*, 2006), the effects of irrigation vary, with some species responding negatively and others adapting well. Steppe birds and other medium-size birds tend to avoid irrigated land (Brotons *et al.*, 2004; De Frutos *et al.*, 2015), while other species (e.g. harriers *Circus* spp and storks *Ciconia ciconia*) are known to use irrigated farmland (Cardador *et al.*, 2011; De Frutos *et al.*, 2015; Torres-Orozco *et al.*, 2016). As compared to rain-fed farmland, irrigated lands can have large quantities of certain prey types (e.g. voles; Jareño *et al.*, 2015) and/or denser vegetation, which some species prefer for breeding (Cardador *et al.*, 2011; Torres-Orozco *et al.*, 2016). However, the limited research on bird responses to irrigation has focused on a few individual species, and there has yet to be an analysis on the broader community-level effects of irrigation that evaluates how both farmland and non-agricultural species respond to the onset of irrigation. Such information is critical to developing conservation management plans for bird communities, especially as the implementation of irrigation is projected to continue increasing (Matson *et al.*, 1997; Alauddin and Quiggin, 2008; FAO, 2011).

Here, we estimate the impact of irrigation on species occurrence patterns across a bird community using a BACI (Before-After Control-Impact; Popescu *et al.*, 2012) design. We examine the effect of implementing irrigation on 47 of the most common species in a Mediterranean region in northern Spain using a 13-year dataset. The study design includes three different contexts: sampling locations in which irrigation was initiated after the first two years of sampling (n=9), sampling locations in which irrigation was initiated after ten years of sampling (n=8), and sampling locations in which irrigation was never applied (n=2). We hypothesized that the implementation of irrigation would have strong effects across the avian community, with negative impacts on many or most of the farmland species. However, we also expected that some species may have responded positively to the changes brought on by irrigation. We thus expected structural changes within the bird community, potentially having consequences for conservation and management.

2. Materials and methods

2.1. Study area and data collection

Our study was conducted at 19 sampling locations across a 100 km^2 area (UTM 30TWN91; Fig. 1) in mid-western Navarra, a province in the north of Spain. The region is characterized by a dry temperate Mediterranean climate, with average annual temperatures of 13.4ºC and rainfall of 560mm. Land-use is dominated primarily by rain-fed (i.e. non-irrigated) agriculture with native vegetation restricted to hillsides and ravines. Although barley, wheat, and vines are the most common crops, there is large variation in the crops produced each year.

Sampling was conducted by a single observer (D.V.) within the Common Bird Census Monitoring Program coordinated by SEO/Birdlife (SACRE), using their standardized design and point-count survey protocols (Escandell, 2012). Sampling locations were chosen based on road and path availability, with a minimum distance of 1 km, and an aim to cover all habitats, and were thus not random. All 19 sampling locations (**Fig. 1**) were located in a rain-fed agricultural environment during 2007, the first year of sampling. The 19 locations were sampled twice annually from 2007 to 2019 (except 2012 for logistical reasons), with the first sampling period occurring between 15 April - 15 May and the second between 15 May - 15 June. Surveys were always carried out during the early morning. During each point-count survey, all birds detected (heard and/or seen) within a five-minute period were recorded, there were no distance limits in the observations.

All 19 sampling locations experienced natural rain conditions between 2007-2008 (two years of sampling). In 2009, a new irrigation system was permanently installed in the eastern side of the survey area, such that nine of the sampling locations were irrigated from 2009 onward (**Fig. 1**). Another irrigation system was permanently installed in 2017 in the western side of the survey area, which led to an additional eight sampling locations being irrigated from 2017 onward (**Fig. 1**; **Table A1**, Appendix A). Thus, only two sampling locations experienced no irrigation throughout the entire duration of the survey. Under irrigation, the traditional rain-fed crops (barley, wheat, and vines) transitioned to new crops (mostly maize and some irrigated vines). In this area, maize is sown in late April, so at the time of the surveys the ground is bare or maize is only beginning to grow (not higher than 30-cm).

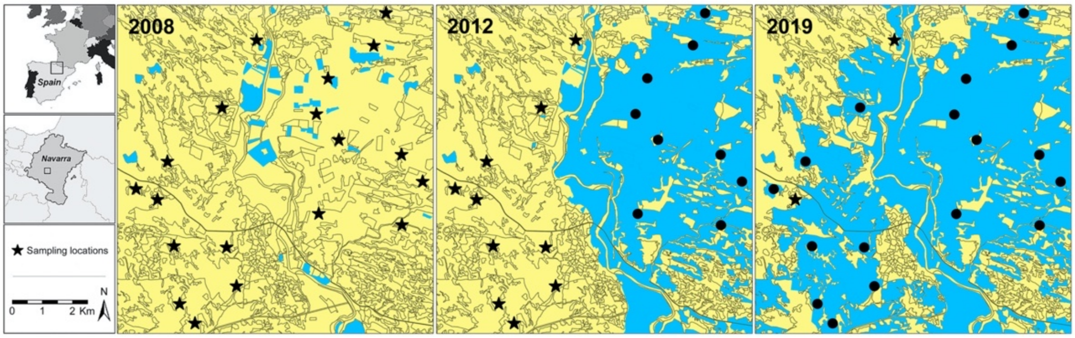

Figure 1. Geographic location of the study area and sampling locations (black stars when non irrigated and black dots when irrigated) at three different times over the course of the study: 2008 (left), 2012 (middle), and 2019 (right). Irrigated surfaces are shown in blue while rain-fed surfaces are in yellow. At the beginning of the study (2008) all sampling locations were in rain-fed farmland habitats. The different panels show the expansion of irrigation over time (https://idena.navarra.es/Portal/ Descargar).

Table 1. A list of species included in our analysis, including their habitat classification and the estimated probability that their occurrence probabilities were lower after the implementation of irrigation. Bold indicates species with > 95%.

English name	Scientific name	Habitat preference	Number of detections	$P[\alpha1_i - \alpha0_i < 0]$
Little bustard	*Tetrax tetrax*	Farmland	28	**1.000**
Red-legged partridge	*Alectoris rufa*		79	**0.975**
Common quail	*Coturnix coturnix*		53	0.767
Greater short-toed lark	*Calandrella brachydactyla*		39	**1.000**
Crested lark	*Galerida cristata*		229	0.910
Calandra lark	*Melanocorypha calandra*		50	0.636
Corn bunting	*Emberiza calandra*		252	0.905
Black-eared wheatear	*Oenanthe hispanica*		43	**0.989**
Zitting cisticola	*Cisticola juncidis*		72	0.508
European goldfinch	*Carduelis carduelis*		134	0.036
Eurasian hoopoe	*Upupa epops*		85	**1.000**
European bee-eater	*Merops apiaster*		62	**0.994**
European turtle-dove	*Streptopelia turtur*		52	0.586
Little owl	*Athene noctua*		28	**0.984**
Common kestrel	*Falco tinnunculus*		67	0.940
Thekla's lark	*Galerida theklae*	Shrubland	57	0.782
Tawny pipit	*Anthus campestris*		107	**0.975**
Woodchat shrike	*Lanius senator*		20	**0.968**
Iberian grey shrike	*Lanius meridionalis*		23	0.877
Dartford warbler	*Sylvia undata*		53	0.251
Subalpine warbler	*Sylvia cantillans*		67	0.839
Sardinian warbler	*Sylvia melanocephala*		50	0.055
European stonechat	*Saxicola rubicola*		64	0.559
Melodious warbler	*Hippolais polyglotta*		26	0.808
Red-billed chough	*Pyrrhocorax pyrrhocorax*	Rocky habitats	38	0.413
Common wood pigeon	*Columba palumbus*	Forest habitats	22	0.746
Common chaffinch	*Fringilla coelebs*		30	**0.975**
Great tit	*Parus major*		23	0.903
Mallard	*Anas platyrhynchos*	Water habitats	57	0.629
White wagtail	*Motacilla alba*		55	0.193
Common house martin	*Delichon urbicum*	Urban habitats	23	0.234
Common swift	*Apus apus*		52	0.532
Common linnet	*Linaria cannabina*	Non-specialists	153	0.300
European serin	*Serinus serinus*		51	0.920
European greenfinch	*Chloris chloris*		47	0.424
Cirl bunting	*Emberiza cirlus*		51	0.492
House sparrow	*Passer domesticus*		81	0.936
Barn swallow	*Hirundo rustica*		46	0.245
Common nightingale	*Luscinia megarhynchos*		53	**0.984**
Common blackbird	*Turdus merula*		69	0.477
Spotless starling	*Sturnus unicolor*		69	0.864
Common cuckoo	*Cuculus canorus*		26	0.376
Magpie	*Pica pica*		36	**1.000**
Carrion crow	*Corvus corone*		46	**0.995**
Western marsh harrier	*Circus aeruginosus*		34	0.571
Black kite	*Milvus migrans*		23	**0.965**
Common buzzard	*Buteo buteo*		45	0.546

We detected a total of 120 species during the surveys, but only included species with $\geqslant$20 detections, leaving 47 species for analysis (**Table 1**). We classified species into groups by their habitat preference during the breeding period: wetland, urban, shrubland, grassland, rocky habitats, agriculture (farmland), and forest (SEO/BirdLife, 2019). We considered those species with a clear preference for a single specific habitat as a "habitat specialist", while species that were known to occur equally frequently in two or more habitats, or that were not attributed to a particular habitat, were classified as "non-specialists" (**Table 1**). All analyses using species groups were conducted post-hoc. Among the 15 farmland bird species, the little bustard and the greater short-toed lark are considered steppe birds, a species group of high conservation concern (Tucker and Evans, 1997). Both species are listed as vulnerable in Spain (Madroño *et al.*, 2004) with little bustard categorized as vulnerable in Europe (BirdLife International, 2015) and threatened with extinction in Navarra (Decreto Foral 254/2019).

2.2. Statistical model

We estimated species occurrence probabilities, including the effect of irrigation, using a multi-species occurrence model (Dorazio and Royle, 2005, Zipkin *et al.*, 2009) modified to accommodate a BACI design (Popescu *et al.*, 2012). The BACI design allowed us to examine the effects of irrigation on individual species occurrence probabilities and localized species richness (Russell *et al.*, 2009; Popescu *et al.*, 2012; Russell *et al.*, 2015). We accounted for imperfect detection using the replicate sampling occasions within years, which allowed us to separate a nondetection from a true absence (Mackenzie *et al.*, 2002). We thus assumed closure between the two sampling periods within a year (no change in species occurrence status at sampling locations for the two months in a breeding season) but that species occurrence status could change annually.

We denoted the occurrence state (i.e. presence-absence) as $Z_{i,j,t}$=1, if species i occurred at site j in year t. We modelled $Z_{i,j,t}$ as a Bernoulli random process such that $Z_{i,j,t}$ ~ Bern($\psi_{i,j,t}$) where $\psi_{i,j,t}$ is the probability that species i occurred at site j in the year t. We modelled $\psi_{i,j,t}$ with covariates using a logit link function:

$$logit\left(\psi_{i,j,t}\right) = \alpha0_i \cdot \left(1 - irr_{j,t}\right) + \alpha1_i \cdot irr_{j,t} + \alpha2_i \cdot arable_j + year_{i,t} + site_j$$

The species-specific intercept was estimated based on a site irrigation status: $\alpha 0_i$ is the intercept for species occurrence in the years before a site was irrigated ($irr_{j,t}$ = 0), while $\alpha 1_i$ is the intercept in the years after a site was irrigated ($irr_{j,t}$ = 1). This approach allowed us to estimate the effect of irrigation on baseline species occurrence (i.e., as $\alpha 1_i$ - $\alpha 0_i$; Popescu *et al.*, 2012; Kéry and Royle, 2020). We included a species-specific effect of habitat ($\alpha 2_i$) in which $arable_j$ is the standardized percent of arable surface (cereal fields) within a 100m buffer at each sampling location (mean = 77%, range 1 – 100), calculated using QGIS software (QGIS Development Team, 2018) and the 2012 Navarra's land-use map (https://idena.navarra.es/Portal/Descargar). We verified that there was no variation in the proportion of arable land between years using the 2019 Navarre land-use map. We also incorporated a year by species random effect ($year_{i,t}$) and a site-level random effect ($site_j$) to account for unexplained variation across time and space.

We summarized the point count data in an array, $Y_{i,j,t,k}$, in which $Y_{i,j,t,k} = 1$ denoted a detection of species i (1, 2, ..., 47) at site j (1, 2, ..., 19) during year t (1, 2, ..., 12) on replicate visit k (1 or 2). If a species was not detected, then $Y_{i,j,t,k} = 0$. We modelled the detection-nondetection data by assuming that species detection was dependent upon the latent occurrence state of each species at each sampling location in each year: $Y_{i,j,t,k} \sim \text{Bern}\,(Z_{i,j,t} \cdot p_{i,j,t,k})$. The parameter $p_{i,j,t,k}$ is the probability of detecting species i at site j in year t during survey replicate k, conditional on species i being present. Bird species detection probabilities are strongly affected by the sampling hour and date (Schmidt *et al.*, 2013). To account for this variation, we included both hour and date as covariates on $p_{i,j,t,k}$ using a logit link function:

$$logit\left(p_{i,j,t,k}\right) = \beta 0_i + \beta 1_i \cdot hour_{i,j,t,k} + \beta 2_i \cdot date_{i,j,t,k} + \beta 3_i \cdot date^2_{i,j,t,k}$$

$\beta 0_i$ is the intercept for species *i*. $\beta 1_i$ is the effect of sampling hour, and the parameters $\beta 2_i$ and $\beta 3_i$ are the linear and quadratic effects of sampling date on the detection of species *i*. Hour was included as a linear effect because sites were only sampled during the morning while date includes both linear and squared effects because species detection probabilities are expected to vary and peak at different times within the breeding season (Slagsvold, 1977). Both hour and date were standardized to have a mean of 0 and standard deviation of 1.

The species models were linked by assuming that the intercept and covariate effects in both the occurrence and detection models ($\alpha 0$–$\alpha 2$ and $\beta 0$–$\beta 3$) were random effects, drawn from a community-level distribution (Kéry and Royle, 2015). For example, we assumed that the species-specific effect of arable land on occurrence probability ($\alpha 2_i$) was drawn from a community-level distribution whose mean and variance were also estimated: $\alpha 2_i \sim \text{dnorm}(\mu_{\alpha 2}, \sigma^2_{\alpha 2})$. We estimated group-level mean occurrence probability, change in occurrence probabilities, and species richness post-hoc. We used a Bayesian approach for inference, carried out using JAGS (Plummer 2003) and R (*jagsUI* R package; Kellner, 2016; R Core Team, 2019). We included uninformative prior distributions for the community-level parameters (see model code at Appendix B) and evaluated convergence by visually inspecting the trace plots of monitored parameters and with the Gelman and Rubin convergence diagnostic (*R-hat* statistic value < 1.1; Gelman and Rubin, 1992; Gelman and Shirley, 2011).

3. Results

Irrigation had a negative effect on mean species occurrence (i.e. $\mu_{\alpha 1}$ - $\mu_{\alpha 0}$), although the 95% Credible Interval (CI) was large and overlapped zero (mean = -0.96, -3.03 to 0.95 95% CI; negative proportion of distribution: 0.70), revealing high variability among bird species in the community (**Table 1**, **Fig. 2**). Of the 47 species included in our analysis, irrigation had a strong negative effect on occurrence of 10 species (95% CI did not overlap zero) and a moderate negative effect on 16 species (50% CI did not overlap zero but 95% CI did). Another 16 species showed no response to irrigation (50% CI contained zero) while five species showed a moderately positive effect to irrigation (50% CI did not overlap zero but 95% CI did). No species had a strong positive response to irrigation (Fig. 2). Irrigation also had a negative impact on species richness (**Fig. 3**) in both the eastern sampling locations after irrigation was implemented in 2009 (from 25.1 species pre-irrigation to 19.2 post-irrigation, on average) and the western sampling locations after implementation in 2017 (from 29.2 species to 22.7 on average).

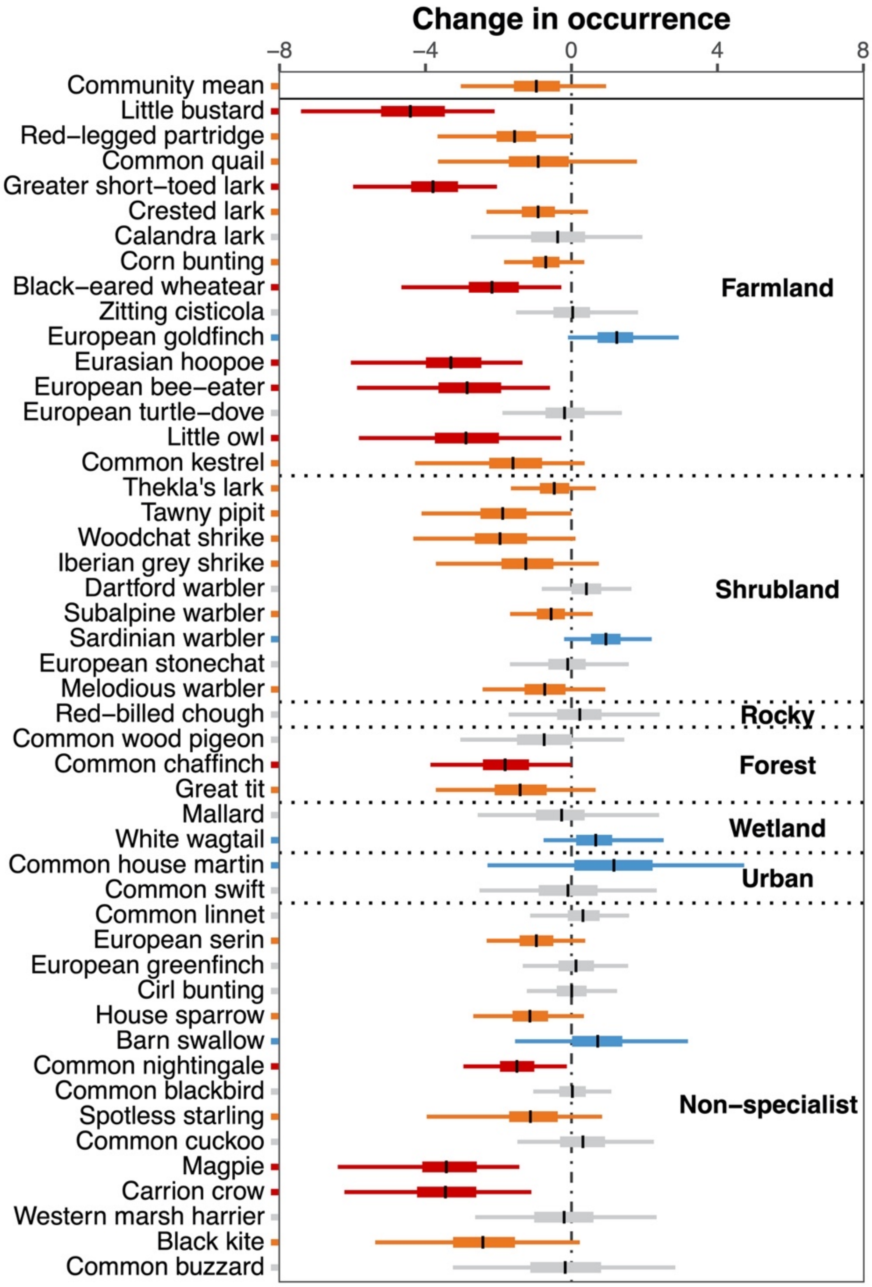

Figure 2. Change in mean species' occurrence ($\alpha 1_i$ - $\alpha 0_i$) after irrigation for all 47 species included in our analysis. Species are organized by habitat classification: farmland, shrubland, rocky habitat, forest habitat, wetland habitats, urban habitat and non-specialist. The short vertical black lines show the mean change (across MCMC iterations), the boxes show the 50% credible intervals (CI) and the horizontal lines delineate the 95% CI. Light grey indicates no change in species occurrence probability, orange (negative effect) and blue (positive effect) indicate that the 50% CI does not overlap zero but an overlap of 95% CI with zero, and red (negative effect) shows that the 95% CI does not overlap zero.

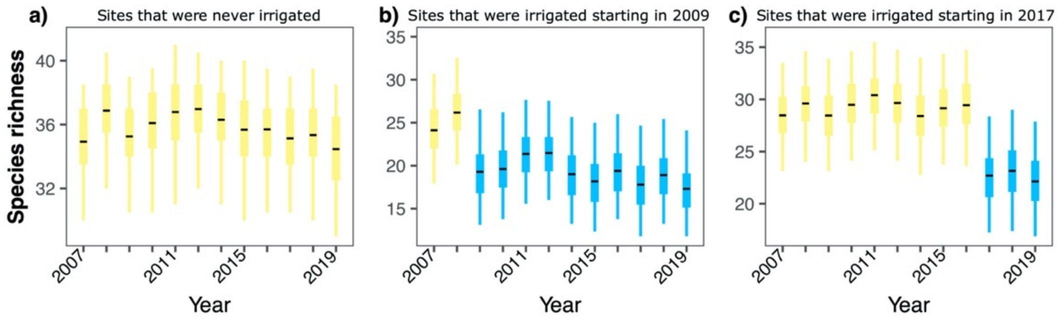

Figure 3. Mean site-level species richness at: a) sampling locations that were never irrigated (control; n=2); b) sampling locations in the eastern side of the survey area where irrigation was initiated in 2009 (n=9); c) sampling locations in the western side of the survey area where irrigation was initiated in 2017 (n=8). Yellow shows species richness in years in which sampling locations were not irrigated, while blue shows richness in years during which sampling locations were irrigated. The short horizontal black lines indicate the means, the boxes show 50% credible intervals (CI) and the vertical lines the 95% CI. No data were collected in 2012 and thus that year is not plotted in any of the panels.

Many species groups were negatively impacted by irrigation. The mean effect of irrigation was especially negative for farmland habitat specialists (mean = -1.63, -3.86 to 0.29 95% CI; negative proportion of distribution: 0.82). Irrigation had a strong negative effect on the occurrence of 6 of the 15 farmland species (**Fig. 2**), which led to a reduction in farmland species richness (from 11.1 species to 7.8 on average). In particular, four experienced >50% reductions in mean occurrence probability after irrigation was implemented (**Fig. A1**, Appendix A): little bustard (from mean occurrence probabilities of 0.80 to 0.21), greater short-toed lark (from 0.53 to 0.09), Eurasian hoopoe (from 0.87 to 0.42) and black-eared wheatear (from 0.70 to 0.32). Irrigation also had a generally negative effect on shrubland specialists, forest specialists, and non-specialist species (negative proportion of mean distribution: 0.68, 0.88 and 0.67 respectively; **Table 1** and **Fig. 2**). The occurrence probabilities of six of the nine shrubland specialists, two of the three forest specialists and seven of the 15 non-specialists were at least moderately reduced (50% CI did not overlap zero; **Table 1** and **Fig. 2**). As a consequence, these groups all suffered reductions in point-level richness: from 3.5 to 2.5 species (shrubland), from 1.4 to 1.1 species (forest), and from 8.3 to 6.3 species (non-specialist). Interestingly, five species experienced a somewhat positive effect of irrigation (**Fig. 2**) and these species did not belong to a single specialist group. One farmland bird (European goldfinch; from mean occurrence probabilities of 0.56 to 0.76), one shrubland bird (Sardinian warbler; from 0.10 to 0.22), one wetland bird (white wagtail; from 0.36 to 0.54), one urban bird (common house martin; from 0.49 to 0.71) and one non-specialist species (barn swallow; from 0.60 to 0.70), all showed a clear increase in their occurrence probability after the onset of irrigation (**Fig. A1**, Appendix A).

Species-level occurrence probabilities were also strongly affected by arable land surface (community mean = -0.27, -1.55 to 0.91 95% CI). Overall, the amount of arable land cover had a positive effect on the occurrence probability of steppe birds, larks and six other species, while it had a negative effect on the occurrence probability of most other species (21 species; **Fig. A2**, Appendix A). Detection probabilities (mean detection = 0.27) were also variable across species, with mean detection ranging from 0.09 for black kite to 0.69 for corn bunting. Across all species, detection probability was fairly consistent throughout the morning (**Fig. A3**, Appendix A), as some species decreased in detection with time since sunrise (e.g. common quail, european turtle-dove, common nightingale), while others increased (e.g. little owl, common kestrel, iberian grey shrike). Detection probability varied for most species by survey date, with average peak detection estimated to be around late May (**Fig. A3**, Appendix A).

4. Discussion

Our results demonstrate a clear effect of irrigation on bird community structure, at least in the Mediterranean landscapes, with the majority of species (55%) showing a negative response in occurrence probability and only a few (11%) showing a positive response. This led to an overall reduction in site-level species richness. Moreover, among species groups, farmland birds, many of which are ground-nesters (Benton *et al.*, 2003, Traba and Morales, 2019), were impacted most significantly, including negative effects on two steppe birds of high conservation value (Madroño *et al.*, 2004; BirdLife International, 2015; Decreto Foral 254/2019). Other species groups, including shrubland species, forest species and non-specialists, were also negatively impacted by irrigation, suggesting that the effects of irrigation may be more widespread than previously thought. The striking changes in bird species occurrence probabilities reveal that irrigation may lead to fundamentally different bird communities at local scales, with communities in irrigated landscapes being more homogenous.

The implementation of irrigation schemes not only leads to increased water within local landscapes, but also results in the use of alternative crop species, increase of monocropping, and the loss of fallow lands, all of which may be driving changes in local bird communities. In our study, farmland birds were most affected by irrigation, likely because irrigation has direct impact on their nesting habitat (i.e. the availability of cereal fields and fallow lands; Benton *et al.*, 2003, Traba and Morales, 2019). In fact, many farmland bird species avoid maize fields (Laiolo, 2005), which is frequently grown as an alternative to wheat and barley in irrigated arable land in Spain. In addition, irrigation usually leads to more intensive farming and a more homogeneous landscape that may be detrimental to farmland bird populations (Baldock *et al.*, 1994; Delgado and Moreira, 2000; Vickery *et al.*, 2004; Traba and Morales, 2019). The occurrence probability of some shrubland and forest birds as well as non-specialist species also decreased, although these species groups are unlikely to breed within or around the crops themselves. Although the mechanism(s) driving changes within these species groups is less clear, irrigation may reduce the attractiveness of agricultural fields for foraging for certain species, either through a decrease in food abundance or food availability through changes in vegetation structure. On the other hand, some species may find increased resources in irrigated lands. Of the five species showing positive responses to irrigation, four of them are insectivorous (white wagtail, common house martin, barn swallow, and Sardinian warbler). These species may have found higher densities of insects in the irrigated landscapes (Keiser *et al.*, 2005; Jaleta *et al.*, 2013). However, despite potential positive changes for some species, the overall effect of irrigation was a loss of species richness. Thus, the direct and indirect effects of irrigation had a primarily negative effect on the local bird community, beyond only the farmland bird species that are typically considered.

Given the high potential for negative impacts on wildlife communities, irrigation schemes should explicitly evaluate approaches to mitigate biodiversity changes, especially for those species that are threatened. Ensuring the conservation of endangered farmland species requires a combination of management and policy actions to offset or limit the impact of widespread implementation of advanced irrigation strategies. Irrigation schemes should be implemented carefully, at least avoiding the European Union’s designated Special Protection Areas or areas with similar conservation value in non-European countries. Traditional rain-fed agriculture with fallow lands should also be valued and actively encouraged as these systems offer unique habitat on which many bird species rely. Ongoing discussions focused on making the European Common Agricultural Policy (CAP) greener should explicitly consider such approaches. When irrigation is implemented, maintaining some rain-fed crops and fallow lands, varying the crops used within agrosystems, and avoiding monocultures can lead to a heterogeneous landscape with resources for a range of species (Berg, 2002; Benton *et al.*, 2003; Siriwardena *et al.*, 2012). Fallow land has been identified as critical for farmland bird conservation (Sanz-Pérez *et al.*, 2019) because it provides foraging, mating, and nesting habitat for a number of species (Mcmahon *et al.*, 2010; Schmidt *et al.*, 2017; Traba and Morales, 2019; Tarjuelo *et al.*, 2020). A mosaic structured landscape, in which fallow lands are dispersed within irrigated fields, is thus likely to reduce some of the negative impact on the bird community, although further research is needed on this and the specific mechanisms causing changes.

Our results provide evidence that the negative effect of irrigation on bird communities goes beyond farmland species, reaching many other bird groups. The implementation of irrigation is increasing worldwide in a race to increase farmland productivity (FAO, 2011). While irrigation can have a positive impact on crop production, it can simultaneously have negative consequences for local bird communities, which may impact the biodiversity of other taxa and ecological processes more generally. Agricultural policies must ensure that food production is compatible with species conservation and the protection of local ecosystem services.

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Supplementary material

Appendix A

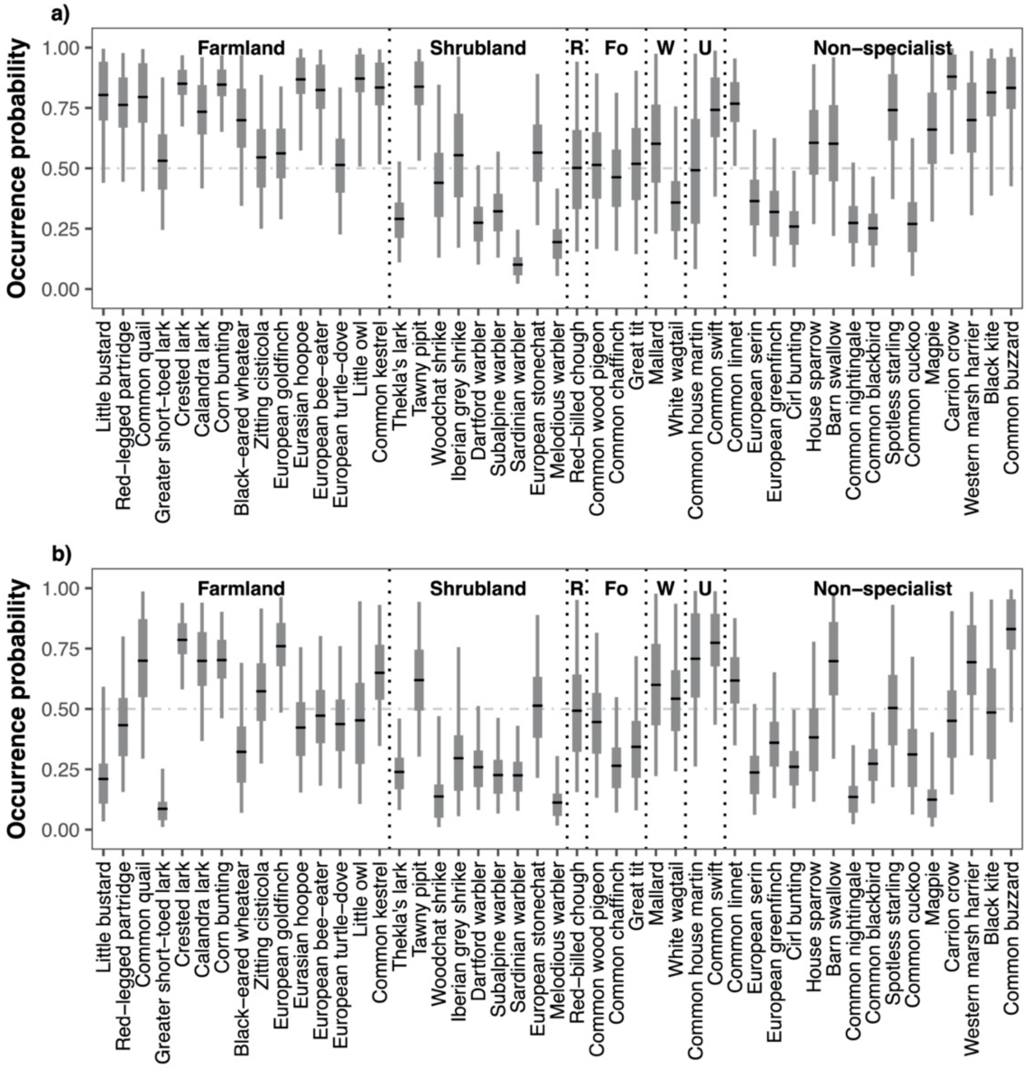

Figure A1. Species-level occurrence probabilities: a) before irrigation (excluding control sampling locations); and b) after irrigation was implemented. Species are organized by habitat classification: farmland, shrubland, (R) rocky habitat specialist species, (Fo) forest habitat specialist species, (W) wetland habitats specialist species, (U) urban habitat specialist species, and non-specialist. The short horizontal black lines show means, the boxes show 50% credible intervals (CI) and the vertical lines show the 95% CI.

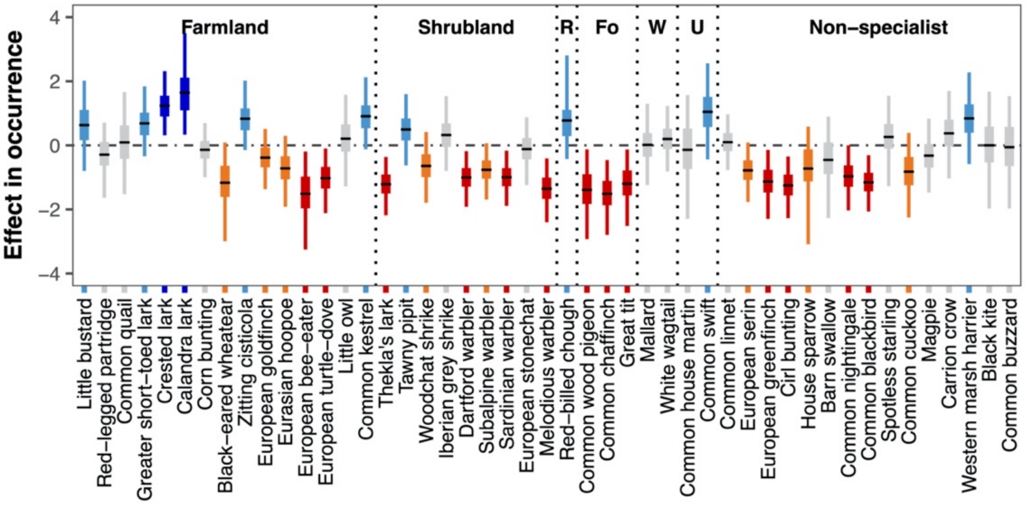

Figure A2. Effect of arable land surface on species occurrence probabilities organized by habitat classification: farmland, shurbland, (R) rocky habitat specialist species, (Fo) forest habitat specialist species, (W) wetland habitats specialist species, (U) urban habitat specialist species, and non-specialist. The short horizontal black lines show the mean value (across MCMC iterations), the boxes show the 50% credible intervals (CI), and the vertical lines delineate the 95% CI. Light grey indicates no effect of arable land on occurrence probability, orange (negative effect) and blue (positive effect) indicate that the 50% CI does not overlap zero but the 95% CI does overlap zero, and red (negative effect) and dark blue (positive effect) indicate that the 95% CI does not overlap zero.

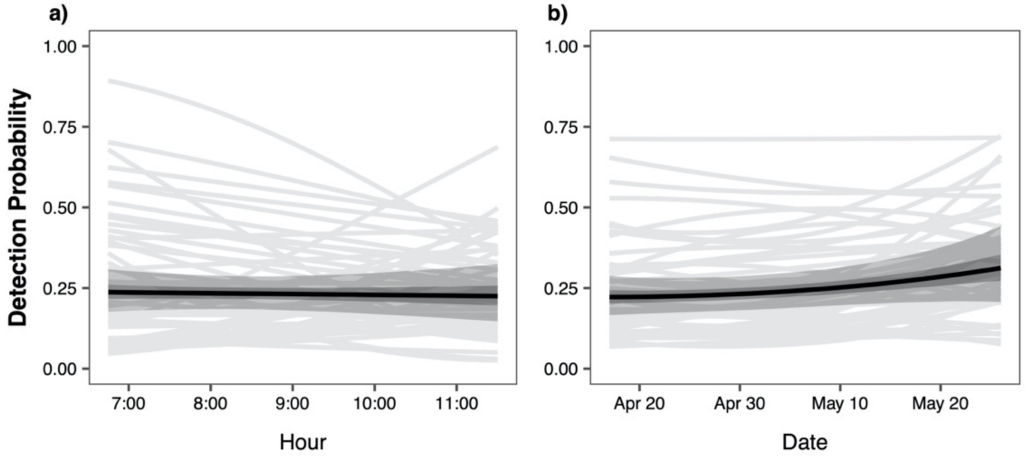

Figure A3. Detection probability by: a) hour; and b) date. The black lines show the mean values across all species analysed (with shaded 50% and 95% credible intervals). Light grey lines show the detection probabilities of each species included in the analysis.

Table A1. Number of irrigated and non-irrigated points sampled each year during the survey period.

Years	N. of Years	Non irrigated	Irrigated
2007-2008	2	19	0
2009-2016	7	10	9
2017-2019	3	2	17

Appendix B

JAGS model code: Multi-species hierarchical occurrence model with a BACI design.

```
model {

# Subscripts:
# i = Species
# j = Site
# k = Visit
# t = Year

# Specify priors
 mu.a.before ~ dnorm(0, 0.37)
 mu.a.after ~ dnorm(0, 0.37)

 mu.a2 ~ dnorm(0, 0.1)
 mu.b0 ~ dnorm(0, 0.1)
 mu.b1 ~ dnorm(0, 0.1)
 mu.b2 ~ dnorm(0, 0.1)
 mu.b3 ~ dnorm(0, 0.1)

 tau.a.spp ~ dgamma(0.1,0.1)
 tau.a2 ~ dgamma(0.1,0.1)

 tau.b0 ~ dgamma(0.1,0.1)
 tau.b1 ~ dgamma(0.1,0.1)
 tau.b2 ~ dgamma(0.1,0.1)
 tau.b3 ~ dgamma(0.1,0.1)
 tau.psi.site ~ dgamma(0.1,0.1)
 tau.psi.year ~ dgamma(0.1,0.1)

 for (j in 1:nsite){
  psi.site[j] ~ dnorm(0, tau.psi.site)
  }

 for (i in 1:nsp){
  a[1,i] ~ dnorm(mu.a.before, tau.a.spp)
  a[2,i] ~ dnorm(mu.a.after, tau.a.spp)

  a2[i] ~ dnorm(mu.a2, tau.a2)
```

```
   b0[i] ~ dnorm(mu.b0, tau.b0)
   b1[i] ~ dnorm(mu.b1, tau.b1)
   b2[i] ~ dnorm(mu.b2, tau.b2)
   b3[i] ~ dnorm(mu.b3, tau.b3)
   for (t in 1:nyear){
    psi.year[t,i] ~ dnorm(0, tau.psi.year)
    }

# Ecological submodel: Define state conditional on parameters
   for (j in 1:nsite){
    for (t in 1:nyear){
     logit(psi[i,j,t]) <- a[irrigation[j,t]+1,i] + a2[i]*arable[j] + psi.year[t,i] + psi.site[j]
     z[i,j,t] ~ dbern(psi[i,j,t])

# Observation model
      for (k in 1:nrep){
       muy[i,j,t,k] <- z[i,j,t]*p[i,j,t,k]
logit(p[i,j,t,k]) <- b0[i] + b1[i]*hour[i,j,t,k] + b2[i]*date[i,j,t,k] + b3[i]*(date[i,j,t,k]^2)
       y[i,j,t,k] ~ dbern(muy[i,j,t,k])
       } #k
      } #t
     } #j
    } #i

# Effect of irrigation per specie
  for(i in 1:nsp){
  effect.a.sp[i] <- a[2,i] – a[1,i]
  }
# Species richness at each site and year
  for (j in 1:nsite){
    for (t in 1:nyear){
      Nsite[j,t] <- sum(z[j,t,])
    }
  }
}
```

PAPER II

Vineyard modernization drives changes in bird and mammal occurrence in vineyard plots in dry farmland

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Vineyard modernization drives changes in bird and mammal occurrence in vineyard plots in dry farmland

Abstract

The fast intensification of agriculture affects many systems. In recent decades, traditional vineyards have been rapidly converted to trellis vineyards in Spain (the country with the largest vineyard surface worldwide) in an attempt to reduce harvesting costs and increase vine productivity. The implications of this modernization of vineyards on farmland biodiversity are still largely unknown. We studied 52 vineyards (26 traditional and 26 trellis) from June to September in south-western Spain, aiming to describe the effect of the modernization of gobelet-shape traditional vineyards on vine structure and their management, as well as its effect on species´ occurrence in vineyard plots. We applied hierarchical occurrence models to assess the occurrence probability of 10 bird species and two mammal species in traditional and trellis vineyard plots. Vineyard modernization involved taller vines (connected by metallic guide wires and poles by rows), greater distance between vine rows, bigger plot sizes, the systematic implementation of irrigation, and an application of fertilizers through the watering system (fertigation) in a third of studied vineyards. Other agrochemical treatments seemed to be equally used in both vineyard types. Due to the use of herbicides and frequent tilling, both types of vineyards showed low natural vegetation groundcover. Vineyard modernization had clear effects on fauna biodiversity in vineyard plots, with a higher occurrence in trellis vineyards of some species (rufous-tailed scrub-robin, European goldfinch, red-legged partridge, and house sparrow) and a higher occurrence of other species (European rabbit and European greenfinch) in traditional ones. Thus, vineyard modernization can drive a change in the community of birds and mammals that inhabit them. In addition, vineyard use by some species (particularly ground-dwelling ones) was strongly determined by the presence of arable land adjacent to the vineyard. These species may therefore use vineyards during summer because they provide water and/or better cover than harvested crops. Animals attracted to vineyards for water could be exposed to toxic doses of nitrates that are routinely applied through the watering system in a third of these. To maximize benefits for biodiversity, it would be advisable to manage modernization schemes in order to maintain landscape heterogeneity, with vineyards of both types combined with other agricultural systems, as well as higher proportions of natural vegetation surface cover.

Keywords

Agricultural intensification; European rabbit; farmland birds; fertigation; hierarchical occupancy model; red-legged partridge

1. Introduction

Traditional farming creates semi-natural ecosystems on which many animal species rely (Bignal and McCracken, 2000; Morelli *et al.*, 2014; Palacín and Alonso, 2018). However, since the middle of the last century, many of these ecosystems have been strongly modified, through modernization and intensification, in a race to increase farmland productivity in response to a growing demand for food (Matson *et al.*, 1997; Foley *et al.*, 2005; Tilman *et al.*, 2011; Kastner *et al.*, 2012). This modification has increased habitat homogeneity (arising from larger plots to allow mechanization, the expansion of crop monoculture, and the loss of non-productive habitats in the farmland matrix), as well as the use of irrigation, fertilizers, and pesticides (López-Antía *et al.*, 2013; Hallmann *et al.*, 2014). In most cases, these changes have driven strong population declines of animal species that inhabit agricultural ecosystems (Matson *et al.*, 1997; Pain and Pienkowski, 1997; Sala *et al.*, 2000; Benton *et al.*, 2002; Benton *et al.*, 2003). Thus, it is essential to understand how agricultural modernization affects biodiversity, in order to propose appropriate compensatory measures if necessary. Currently, the impact of agricultural intensification has been studied mainly in cereal farming systems (Donald *et al.*, 2001; Benton *et al.*, 2002; Benton *et al.*, 2003; José-María *et al.*, 2010; López-Antía *et al.*, 2013; Ortiz-Santaliestra *et al.*, 2020), while in other agricultural systems, such as vineyards, the implications of their modernization are not well-studied (e.g. Arlettaz *et al.*, 2012; Casas *et al.*, 2020).

Vineyards have been an important agricultural crop in the Mediterranean region for centuries (Harris, 2013, McGovern, 2019). There are currently 3.2 million hectares of vineyards in the European Union (45% of the world's total area of vineyard), of which 74.1% are grown in Spain, France, and Italy (Eurostat, 2017). In Spain, currently the country with the largest vineyard surface worldwide, non-irrigated (rain-fed) gobelet shape vines have been traditionally grown. However, vineyards in Spain are undergoing a deep restructuring and intensification process, with the implementation of trellis vineyards being encouraged and funded by the European Union (CE- 1493/1999) to reduce harvesting costs and increase productivity (Ruiz-Pulpón, 2013; MAPAMA, 2017). This has led to a large conversion of traditionally managed vineyards in Spain to trellis vineyards in recent decades (Ruiz-Pulpón, 2013; Montero-García *et al.*, 2017). This conversion is associated with, first, a structural change, shifting from the horizontal shape in the traditional vineyards with branches and leaves covering the ground to the vertical shape in trellis vineyards where vines are taller and connected by metallic guide wires and poles, and where there is more distance between rows to allow for mechanized harvest. Additionally, this transformation is usually accompanied by implementation of irrigation and larger plots (Salguero, 2010; Torquati *et al.*, 2015).

There is however scant evidence on the effects of this agricultural shift on wildlife communities using vineyard plots, despite the large agricultural area covered by these (Eurostat, 2017) and the potential effects that changing crop structure or management could have on wildlife occurrence (Matson *et al.*, 1997; Donald *et al.*, 2002; Benton *et al.*, 2003). The impact of modernization of traditional (gobelet shape) to trellis vineyards has been assessed on very few species, and has been shown to be detrimental for the great bustard (*Otis tarda* Linnæus, 1758; Casas *et al.*, 2020) and the woodlark (*Lullula arborea* Linnæus, 1758; Arlettaz *et al.*, 2012). Because of their vertical structure, trellis vineyards are likely avoided by some ground-dwelling open-landscape species such as great bustard (Casas *et al.*, 2020), little bustard (*Tetrax tetrax* Linnæus, 1758), or sandgrouse species (*Pterocles alchata* Linnæus, 1766 and *Pterocles orientalis* Linnæus, 1758). However, this same structure might be more attractive for perching species such as rufous-tailed scrub-robin (*Cercotrichas galactotes* Temminck, 1820) or shrikes (*Lanius meridionalis* Temminck, 1820 and *Lanius senator* Linnæus, 1758). Moreover, if trellis vineyards are irrigated, this water resource could be also attractive for many birds or mammals during the dry Mediterranean summer (Borralho *et al.*, 1998). To better understand the consequences of vineyard modernization on farmland biodiversity, it is necessary to evaluate occurrence differences between traditional and trellis vineyards in a wide range of species. The use of vineyards by farmland bird and mammal species may be influenced by the habitat surrounding the vineyard plot. Other crops such as cereals may provide necessary resources for feeding or nesting that are not available in vineyards, while vineyards (both traditional and trellised) adjacent to arable land could provide a refuge for various farmland species, especially during summer after harvest, when stubbles lack sufficient cover (Borralho *et al.*, 1998).

Here we evaluate the use of vineyards by a typical wildlife community of south-western Spain, in relation to the vineyard type. For this purpose, we surveyed 26 traditional and 26 trellis vineyards, compared the vine structure and the management practices conducted in these. Further, we used the wildlife surveys and hierarchical occurrence models to evaluate the occurrence patterns of the most common birds and mammals in each vineyard type, including species of conservation concern, such as the rufous-tailed scrub-robin (*Cercotrichas galactotes*) and game species of high socio-economic value, such as red-legged partridge (*Alectoris rufa* Linnæus, 1758), European rabbit (*Oryctolagus cuniculus* Linnæus, 1758) or Iberian hare (*Lepus granatensis* Rosenhaeur, 1856). We expected that structural and management differences between the two types of vineyards would lead to a differential use by bird and mammal species, but that differences would not be homogeneous among species (depending, for example, on whether they are ground-dwelling or perching), and may be influenced by the surrounding environment. We discuss the implications of the findings for farmland biodiversity conservation.

2. Materials and methods

2.1. Study area and data collection

The study was conducted in Feria, Almendralejo and Villafranca de los Barros, three municipalities close to each other located in the province of Badajoz, Extremadura, south-western Spain (38º 35' 22" N; 6º 26' 48" W). The region is characterized by a Mediterranean-continental climate, with average annual temperatures of 16ºC, annual rainfall of less than 600mm, warm and dry summers (mean temperature averages 36ºC during the hottest month), and almost 100 days of frost per year. Vineyards (both traditional and trellis) and olive groves are the main crops in the region, with significant amounts of dry cereal crops in some areas. Almond trees, pastures, and fallow land occurred in lower proportions.

In 2009, wildlife surveys were regularly carried out in 52 vineyards (26 traditional and 26 trellis) from June to September. Surveyed vineyards were selected amongst those available in the study area relative to two criteria: i) adjacency to a road that was perpendicular to the direction of the vineyard rows to facilitate observations; and ii) nonadjacency to another selected vineyard. Overall vineyard selection was, therefore, not random, although among those fitting the previous criteria, surveyed plots were selected haphazardly. We conducted line-transect surveys (Buckland, 2006) by car at each vineyard (along the vineyard edge) during our study period by driving at low speed (<10 km/h) on the road or dirt track adjacent to the vineyard. We systematically stopped to briefly scan between each pair of rows to check for birds of mammals with binoculars. Stop duration slightly varied depending on the number of detections and time needed to identify and record sightings. Each vineyard was sampled four times, once per month on the last weekend of the month. Surveys were carried out early (8:00 am – 11:30 am) or late in the day (6:00 pm – 9:00 pm), with a rotational schedule to ensure that different vineyards were visited at different times.

We recorded all observed birds and mammals in each survey. Additionally, we recorded the sampling date, as well as the start time and duration of the survey in each vineyard. We estimated the mean survey speed by dividing the time spent in each survey by the length of the vineyard side surveyed. For each sampled vineyard, we also recorded the following variables: plot size (in hectares), height of the guide wires in trellis vineyards (cm), height of the vine's lowest branch (cm), distance between vine rows (m), and percentage of ground area covered by vegetation (i.e. weeds or grassy natural vegetation). This percentage was estimated at each visit and averaged across visits for analyses. We did not measure total height of the vine. Finally, we also noted presence/absence of arable land (either cereal plots or fallow land, which are part of the cereal cycle in the area) adjacent to the vineyard. When there was no arable land adjacent to the vineyards, these were mostly surrounded by other vineyards and olive groves.

Additionally, we interviewed farmers owning the sampled vineyards during autumn 2009. We only interviewed a subset of owners (13 traditional and 13 trellis vineyards), as it was not possible to identify and contact all owners. We collected information on vineyard management practices in these interviews, in particular: 1) use of irrigation, 2) use of fertigation (i.e., the application of nitrates through the watering system), 3) number of labour days per year (including any human action within the vineyard), 4) number of tillage applications, 5) number of shoot thinnings, 6) moth treatments, 7) sulphur treatments and 8) herbicide treatments per year.

A total of 27 species (25 birds and 2 mammals; **Table B1**, Appendix B) were detected during our surveys. For the statistical analyses, we only used species with $\geqslant 5$ detections, and therefore used a subsample of 10 bird species and 2 mammal species. These species (ordered from most to least detections) were: crested lark (*Galerida cristata* Linnæus, 1758; n = 63 detections), European rabbit (*Oryctolagus cuniculus*;

n = 26), Iberian hare (*Lepus granatensis*; n = 26), red-legged partridge (*Alectoris rufa*; n = 18), rufous-tailed scrub-robin (*Cercotrichas galactotes*; n = 13), European goldfinch (*Carduelis carduelis* Linnæus, 1758; n = 12), European stonechat (*Saxicola rubicola* Linnæus, 1766; n = 11), house sparrow (*Passer domesticus* Linnæus, 1758; n = 10), corn bunting (*Emberiza calandra* Linnæus, 1758; n = 7), European greenfinch (*Chloris chloris* Linnæus, 1758; n = 6), common linnet (*Linaria cannabina* Linnæus, 1758; n = 5) and common blackbird (*Turdus merula* Linnæus, 1758; n = 5). We classified bird species as specialists, either ground-dwelling species or perching species, or as generalists. Crested lark and red-legged partridges were classified as ground-dwelling since they are almost all the time on the ground; common blackbird and common linnet were classified as generalist species, which spend a lot of time on the ground but frequently perch; rufous–tailed scrub–robin, European goldfinch, European stonechat, house sparrow, corn bunting, and European greenfinch were classified as perching species, since they spend most of the time on perches.

2.2. Statistical analyses

2.2.1. Vineyard structure and management differences

To examine the differences in site-level characteristics relative to the structure and management practices among the two vineyard types, we fit generalised linear models (GLM) to the observed site-level characteristic data using a Bayesian framework (*stan_glm* function of *rstanarm* package in R; Gabry and Goodrich, 2018). We fit GLMs with gamma error and an inverse link function, for the response variables “height of the lowest branch”, “distance between vine rows” and “vineyard plot size”. We fit GLMs with a binomial error and logit link, for the response variables “use of irrigation”, “use of fertigation” and “vineyard ground vegetation”. For the latter, we included a double column response variable (percentage ground surface with vegetation, percentage without vegetation). We fit GLMs with a Poisson error and logarithmic link, for the following response variables: “number of labour days”, “number of tillage applications”, “shoot thinning applications”, “moth treatments”, “sulfide treatments”, and “herbicide treatments” per year. Type of vineyard (traditional or trellis) was included as the sole explanatory variable in all models. The *stan_glm* function performs Bayesian estimation via a Markov chain Monte Carlo process. This fits four Markov chains with 2000 iterations each and 1000 interactions per chain are burned as a warm-up process. We evaluated the convergence of Markov chains by visually inspecting the trace plots of monitored parameters and using Gelman Rubin *R-hat* statistics (values < 1.1; Gelman and Rubin, 1992; Gelman and Shirley, 2011). We used the *modelbased* package in R (Makowski *et al.*, 2019) to obtain, through our model, the mean values of response variables and their 95% and 50% credible intervals.

2.2.2. Hierarchical occurrence models

Occurrence models can be for a single species (single-species occurrence model; Mackenzie *et al.*, 2002) or can be modelled in a unified analytical framework for multiple species (multi-species occurrence model; Dorazio and Royle, 2005, Zipkin *et al.*, 2009). Both types of models incorporate detection biases during the survey process by estimating a probability of detection (p) through the use of replicate observations. This allows for an unbiased estimate of the probability of whether a species is present or absent at a site (ψ, Mackenzie *et al.*, 2002) – our response variable of interest. We accounted for imperfect detection using the replicate sampling occasions within the summer, which allowed us to separate a non-detection from a true absence. Thus, we assumed closure within the sampling period (no change in species occurrence status at sampling locations from June to September). This is supported by biological information on the species: most are sedentary, and for the few migratory ones, the flux of migration takes place in October, after our study period. Thus, we can be confident that the occupancy state of the observed species did not change throughout the study period.

To estimate the effect of the vineyard type (traditional vs trellis), we fit our data to a hierarchical multi-species occurrence model to assess the occurrence probabilities of each bird species or to single-species occurrence models to assess the occurrence probabilities of the two mammal species (rabbit and hare; see model codes in Appendix A).

For the multi-species model, we denoted the occurrence state (i.e. presence-absence) as $Z_{i,j} = 1$, if species i occurred at site j. We modelled $Z_{i,j}$ as a Bernoulli random process such that $Z_{i,j} \sim \mathrm{Bern}(\psi_{i,j})$ where $\psi_{i,j}$ is the probability that species i occurred at site j. We modelled $\psi_{i,j}$ with covariates using a logit link function:

$$logit(\psi_{i,j}) = \alpha0_i + \alpha1_i \cdot vineyardtype_j + \alpha2_i \cdot arableland_j + \alpha3_i \cdot size_j$$

The intercept parameter, $\alpha0_i$, is estimated for each species i. We included a species-specific effect of the vineyard type (0 when traditional vineyard; 1 when trellis vineyard), the presence/absence of arable land around the vineyard, and vineyard plot size ($\alpha1_i$ – $\alpha3_i$). The presence of arable land was included as an indicator of landscape variability around the plot, which may influence use of a given vineyard plot. Plot size was included as it may also influence species occurrence (Haddad *et al.*, 2015). Vineyard plot size was standardized to have a mean of 0 and standard deviation of 1. We did not include more structural and management variables since the inclusion of more variables in the ecological sub-model led to convergence problems. Moreover, there was high collinearity among management variables, and among management variables and vineyard type (as shown in Fig. 1). Thus, we decided to simplify the model by keeping vineyard type as a variable, to the detriment of other possible variables, although we are aware that in this way many of the effects could be masked and this was taken into account within the discussion.

We summarized the point count data in an array, $Y_{i,j,k}$, in which $Y_{i,j,k} = 1$ denoted at least one detection of species i (1, 2, ..., 11) at vineyard j (1, 2, ..., 52) on replicate visit k (1, 2, 3, 4). If a species was not detected during that sampling event, then $Y_{i,j,k} = 0$. We modelled the detection-nondetection data assuming that species detection was dependent upon the latent occurrence state of each species at each sampling location: $Y_{i,j,k} \sim \text{Bern}(Z_{i,j} \cdot p_{i,j,k})$. The parameter $p_{i,j,k}$ is the probability of detecting species i at site j during replicate visit k, conditional on species i being present ($Z_{i,j} = 1$). Bird species detection probabilities may be strongly affected by the sampling hour and the sampling date, as behaviour may vary with temperature changes (and other unexplained variables) throughout the day and throughout the summer (Schmidt *et al.*, 2013). Detection probabilities may also vary with sampling speed (length of vineyard / duration of survey, 'velocity'). To account for this variation, we included hour, date, and velocity as covariates on $p_{i,j,k}$ using a logit link function:

$$logit(p_{i,j,k}) = \beta0_i + \beta1_i \cdot hour_{j,k} + \beta2_i \cdot hour_{j,k}^2 + \beta3_i \cdot date_{j,k} + \beta4_i \cdot date_{j,k}^2 + \beta5_i \cdot velocity_{j,k}$$

The intercept parameter, $\beta0_i$, is estimated for each species i. The parameters $\beta1_i$ and $\beta2_i$ are the linear and quadratic effects of sampling hour, the parameters $\beta3_i$ and $\beta4_i$ are the linear and quadratic effects of sampling date (using Julian calendar) and the parameter $\beta5_i$ is the effects of sampling velocity on the detection of species *i*. All three variables, hour, date, and sampling velocity, were standardized to have a mean of 0 and standard deviation of 1.

The multi-species models were linked by assuming that the intercept and covariate effects in both the occurrence and detection models ($\alpha0$–$\alpha3$ and $\beta0$–$\beta5$) were random effects, drawn from a community-level distribution (Kéry and Royle, 2015). For example, we assumed that the species-specific effect of vineyard type on occurrence probability ($\alpha1_i$) was drawn from a community-level distribution whose mean and variance were also estimated: $\alpha1_i \sim \text{dnorm}(\mu_{\alpha1}, \sigma^2_{\alpha1})$.

For modelling occurrence probabilities of the two mammals, we fit the sampling data to hierarchical single-species occurrence models similar to the above multi-species model. We used single-species occurrence models because two species are not enough to estimate species-specific random effects, and thus no community-level mean and variance are estimated. We modelled ψ_j and $p_{j,k}$ using a logit link function, as above, and included the same covariates and parameters, although here they were modelled for a single species.

$$logit(\psi_j) = \alpha0 + \alpha1 \cdot vineyardtype_j + \alpha2 \cdot arableland_j + \alpha3 \cdot size_j$$

$$logit(p_{j,k}) = \beta0 + \beta1 \cdot hour_{j,k} + \beta2 \cdot hour^2_{j,k} + \beta3 \cdot date_{j,k} + \beta4 \cdot date^2_{j,k} + \beta5 \cdot velocity_{j,k}$$

We modelled Z_j as a Bernoulli random process such that $Z_j \sim$ Bern ψ_j, where ψ_j is now the probability that the species occurred at site *j*. Here we summarized the point count data in an array, $Y_{j,k}$, in which $Y_{j,k} = 1$ denoted at least one detection of the species at vineyard *j* (1, 2, ..., 52) on replicate visit *k* (1, 2, 3, 4). If the species was not detected, then $Y_{j,k} = 0$. We assumed that the species detection was dependent upon the latent occurrence state of the species at each sampling location: $Y_{j,k} \sim$ Bern $(Z_j \cdot p_{j,k})$. The parameter $p_{j,k}$ is now the probability of detecting the species at site *j* during replicate visit *k*, conditional on the species being present.

We used a Bayesian approach for inference, carried out using JAGS (Plummer, 2003) and R (*jagsUI* R package; Kellner, 2016; R Core Team, 2019). We ran three parallel chains with 70000 iterations and a burn-in of 50000 iterations each and thinned by 4 for a total of 15000 iterations for the posterior. We included uninformative prior distributions for parameters. We evaluated convergence by visually inspecting the trace plots of monitored parameters and with the Gelman and Rubin convergence diagnostic (*R-hat* statistic value < 1.1; Gelman and Rubin, 1992; Gelman and Shirley, 2011). To simplify the reading of the results we considered a non-overlapping 95% Credible Interval (CI) as a strong effect/difference, a non-overlapping 50% CI but overlapping 95% CI as a weak effect/difference, and overlapping 50% CI as no effect/difference.

3. Results

3.1. Structural and management differences between traditional and trellis vineyards

A main structural difference between types of vineyards was vine height. Although we did not specifically measure this, in trellis vines, the height was conditioned by the height of the guide wires (in our case this height was 109cm; SD = 17cm), which allows vines to grow vertically. In contrast, gobelet-vines usually grow less than half a meter over the lowest branches. In addition, the trellis vines had a height of the lowest branch that was twice that of the traditional vineyards (Trellis mean = 52cm, 45–61 CI and traditional mean = 27cm, 23–32 CI; strong difference; **Fig. 1a**). The distance between rows was generally higher in trellis vineyards (Trellis mean = 3.19m, 2.91–3.49 CI and traditional mean = 2.85m, 2.60–3.12 CI; weak differences; **Fig. 1b**) and the plot size was also larger (Trellis mean = 4.49ha, 3.40–5.92 CI and traditional mean = 2.55ha, 1.94–3.34 CI; strong difference; **Fig. 1c**). The proportion of vineyard ground covered by vegetation was very low in both types of vineyards and slightly higher in trellis vineyards (Trellis mean = 0.12, 0.11–0.14 CI) than in traditional vineyards (mean= 0.09, 0.08–0.10 CI; strong difference; **Fig. 1d**).

Management practices and their frequency also differed considerably among vineyard types (**Fig. 1**). Trellis vineyards were consistently irrigated in summer (mean = 0.97, 0.85–1.00 CI) and a third of them used fertigation, adding fertilizers (P, K, N, and Na sulfate according to the growers) to the irrigation water (mean = 0.37, 0.13–0.64 CI). Traditional vineyards were very rarely irrigated (0.10, 0.01–0.29 CI; **Fig. 1e** and **1f**) and never fertilized during summer. The number of labor days per year was also considerably higher in trellis vineyards (26 days, 23–29 CI) than in traditional vineyards (16, 14–19 CI; **Fig. 1g**). Moth treatments were moderately more frequent in trellis vineyards (Trellis mean = 0.82 and traditional mean = 0.35, weak differences; **Fig. 1j**) while tilling and shoot thinning frequency seemed to be higher in traditional ones (Tilling: trellis mean = 3.46 and traditional mean = 4.39; Shoot thinning: trellis mean = 0.47 and traditional mean = 1.09, weak differences; **Fig. 1h** and **1i**). By contrast, no differences were found in the application frequency of sulfide and herbicide treatments (**Fig. 1k** and **1l**). When herbicides were used, regardless of the vineyard type, vines were treated with Glyphosate.

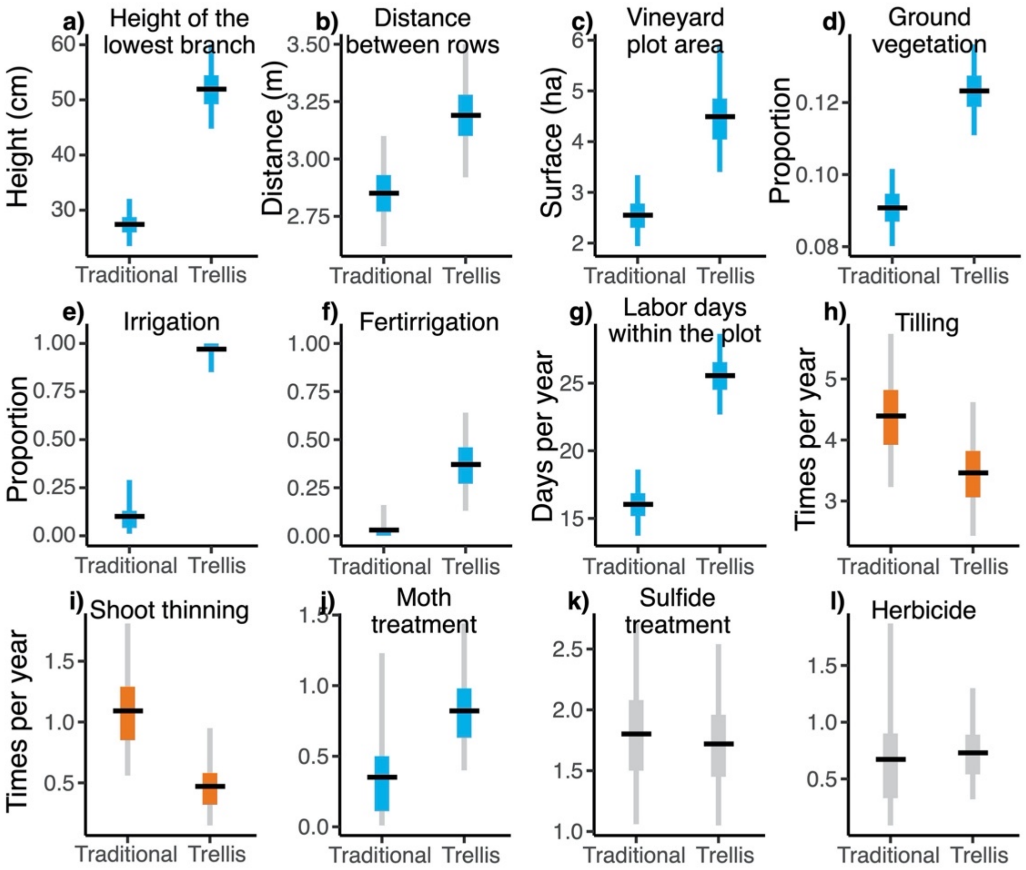

Figure 1. Differences in structure and management practices between traditional and trellis vineyards. The horizontal short black lines show the mean (across all MCMC iterations), the boxes show the 50% credible intervals (CI) and the vertical lines delineate the 95% CI. Light grey indicates no differences, orange indicates higher values in traditional vineyards and blue indicates higher values in trellis vineyards. Orange or blue boxes with grey vertical line indicate weak differences.

3.2. Species occurrence in traditional and trellis vineyards

Hierarchical occurrence models revealed that 5 bird species (red-legged partridge, rufous-tailed scrub-robin, European goldfinch, house sparrow, and common blackbird) were more likely to occur in trellis vineyards than in traditional vineyards (weak effect), while one bird (European greenfinch) and a mammal species (European rabbit) occurred more frequently in traditional than in trellis vineyards (weak effect; **Fig. 2**). Among the six perching species, three thus showed preference for trellis vineyards, one for traditional vineyards, and two were equally present in both types of vineyards. Among the two ground-dwelling bird species, red-legged partridges showed higher occurrence in trellis vineyards while crested larks were equally present in both types of vineyards.

Red-legged partridge and European rabbit occurrence probabilities were greater when vineyards were adjacent to arable land (strong effect; **Fig. 2**). Common linnet and common blackbird occurrence probabilities were also greater in vineyards adjacent to arable land, although this effect was moderate. Thus, species showing higher occurrence in vineyards adjacent to arable land were mostly (three out of four) ground-dwelling or generalist species. In contrast, European goldfinch, European greenfinch, and corn bunting (all perching species) showed a preference for vineyards not adjacent to arable land (weak effect).

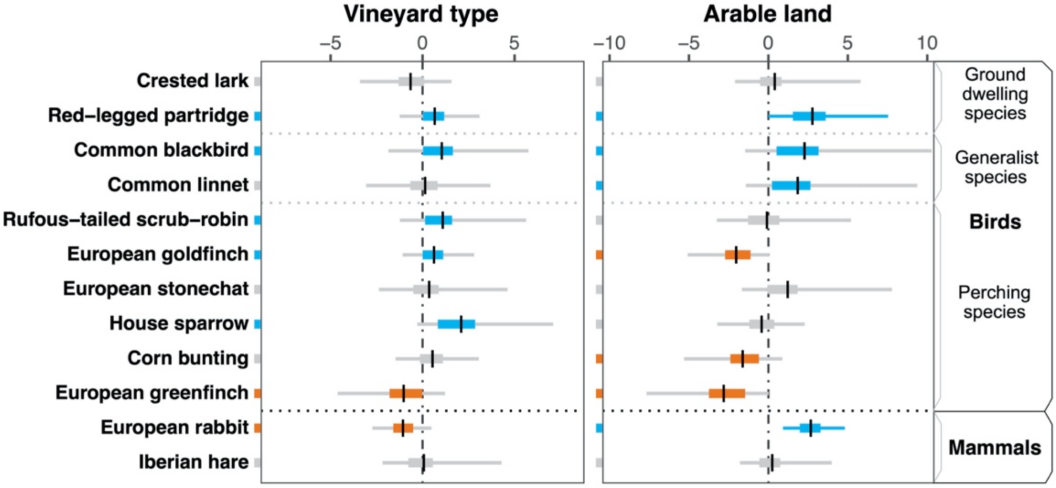

Figure 2. Effects of vineyard type (left) and presence of arable land adjacent to the vineyard (right) on species occurrence (top rows: bird species; bottom rows: mammals). The vertical short black lines show the mean change (across all MCMC iterations), the boxes show the 50% credible intervals (CI) and the horizontal lines delineate the 95% CI. Light grey indicates no differences, orange indicates a negative effect of trellis vineyard management relative to traditional management and a negative effect of the presence of arable land adjacent to vineyard, while blue indicates a positive effect of trellis vineyard management relative to traditional management and a positive effect of the presence of arable land adjacent to vineyard. Orange or blue boxes with grey vertical line indicate weak effects.

Vineyard plot size also had a positive effect on the occurrence of all bird species but did not affect European rabbit or Iberian hare occurrence (**Fig. B1**, Appendix B).

Bird species detection probability averaged 0.18 (ranging from 0.06 -common blackbird- to 0.37 -crested lark). The two mammal species showed higher detection probabilities (0.51 and 0.28 for the European rabbit for Iberian hare, respectively). Detection probability was affected by time, date, and survey speed, and the effect of these variables varied between species (see **Fig. B2**, Appendix B for more details).

4. Discussion

The intensification of agriculture drives important changes in farmland environments, shaping landscape structure and affecting their resources (Matson *et al.*, 1997). Our results demonstrate this modernization led to changes in both vineyard structure (more vertical) and management practices (mostly in relation to irrigation). Further, we found that species occurrence differed between traditional and trellis vineyards, although the preference for one or another vineyard type depended on the species. Specifically, we found that half of the bird species showed a preference for trellis vineyards, and one bird and one mammal species showed a preference for traditional vineyards. Bird species, regardless of their vineyard type preference, occurred more frequently on larger vineyards.

Trellis vineyards were larger than traditional ones, so the higher occurrence of certain species in trellis vineyards could be due to plot size. However, species preferring trellis vineyards were mostly (4 out of 5) perching or generalist species, suggesting that their higher occurrence in these vineyards reflected a real preference related to the availability of taller vegetation and perches. For example, it has been described that rufous-tailed scrub-robins usually select vineyards as breeding habitat (Seoane, 2005), that males prefer tall perches from which to defend their territory (Alvarez, 1997), and that their breeding success was positively associated with the height of their nest over the ground (Tabib *et al.*, 2016). On the other hand, we also found that neither of the two ground-dwelling bird species, crested lark and red-legged partridge, avoided trellis vineyards. The occurrence of red-legged partridges was actually greater in these. The preference for trellis vineyards by the latter in summer could relate to their greater provision of shade to protect from the heat during the warmest times of the day, as well as a water resource throughout the summer. Recent evidence from GPS-tracked red-legged partridges in central Spain has indeed shown that, during summer months, birds use dry arable crops (stubbles and other crops) during the first and last hours of the day, but select vineyards during midday, possibly looking for shade, cover or water (Mougeot *et al.*, unpublished results). The unlimited water resource provided by the irrigated trellis vineyards could be an important attractive factor for the red-legged partridge, whose summer distribution was shown to be conditioned by the presence of nearby water sources (Borralho *et al.*, 1998; Reino *et al.*, 2016).

We cannot discard that differences in use between trellis and traditional vineyards could also be related to the other variables found to differ between both vineyard types, such as the height of the lowest branch, the distance between rows, number of labor days, frequency of tilling, shoot thinning or moth treatments, or presence of ground vegetation. However, some of those variables had only weak differences among vine types, or the effect size was relatively small (e.g. proportion of ground vegetation was 0.09 and 0.12 in traditional and trellis vineyards, respectively) and, therefore, may be biologically meaningless.

Regardless of their type, the use of vineyards was modulated by their surrounding environment for certain species. Some of the monitored species, especially those that spend much of their time on the ground (ground-dwelling and generalist birds, as well as European rabbit), used vineyards mostly when these were near to arable land, avoiding large areas covered by woody crops. This suggested that vineyards were not the most suitable habitats for these species (e.g. Borralho *et al.*, 1998; Buenestado *et al.*, 2008), but that they were used during summer when cereal crops had been harvested and stubble provided very limited vegetation cover to protect from direct sunlight or predators (Borralho *et al.*, 1998; Buenestado *et al.*, 2008; Sumozas, 2009). Species from dry arable farmland might then use vineyards as a refuge. In contrast, other species, such as European goldfinch, European greenfinch, or corn bunting (all three perching species), prefered vineyards not adjacent to arable land (i.e., surrounded by other vineyards or olive groves), indicating that landscapes dominated by woody crops may be favored by those species, either for the structure or the bare ground available in those habitats.

Our study also showed that a main difference in management between traditional and trellis vineyards was the use of irrigation. In arid ecosystems such as the farmland of SW Spain, water is a main limiting factor during summer, and vineyard modernization is almost systematically associated with the installation of surface tubes for drip irrigation (present in 97% of studied trellis vineyards but only 10% of traditional ones). Drip irrigation provides water straight to the vines, therefore saving water. Additionally, this system allows the application of fertilizers, mostly nitrates, via the irrigation system (fertigation), a practice conducted in 37% of irrigated vineyards. Water pollution due to the excess application of nitrates is a widespread problem in agricultural areas (Stoate *et al.*, 2001; Wright and Welbourn, 2002). More than half of the nitrates applied to crops are not assimilated by plants but incorporated in other compartments of the ecosystem or water reserves (Crews and Peoples, 2004). The application of nitrates to vines through the watering systems reduces the amount of applied fertilizers, but their concentration in drip irrigation is very high (10 times the recommended limit for public safety; Rodríguez-Estival *et al.*, 2010), and poses a serious threat to animals drinking this water. Several studies have shown toxic effects of nitrate ingestion on birds (e.g. Ley, 1986; Rodríguez-Estival *et al.*, 2010). This practice, applied in vineyards during summer when natural water sources are scarce, could be especially dangerous to wildlife that may be attracted by this toxic water resource (Rodríguez-Estival *et al.*, 2010) and might convert trellis vineyards into an ecological trap. This should be investigated in future studies and, if confirmed, mitigation measures should be applied to reduce fertigation water consumption. Possible measures include burying the irrigation tubes, providing natural alternative clean water resources, or using bird deterrent measures when fertigation treatments are applied.

The use of other agrochemicals did not vary greatly between vineyard types: moth treatments were more frequent in trellis vineyards, but sulfide and herbicide treatments were applied with a similar frequency in both vineyard types. The type of herbicide used (glyphosate) was also similar across vineyard types. Herbicide applications and frequent tillage occurred in both types of vineyards, in order to leave the ground bare of weeds. The reduced natural vegetation cover in both types of vineyards may affect the availability of food for many wildlife species and may provide insufficient cover for some ground-nesting birds. This could be the reason for the overall poor bird biodiversity observed in vineyards (Potts, 1980; Casas and Viñuela, 2010; Salguero, 2010; Arlettaz *et al.*, 2012). Moreover, strong pressure on ground vegetation could be detrimental for vines because natural vegetation supports natural enemies and its removal may increase pest densities (Altieri *et al.*, 2005; Fiedler *et al.*, 2008; Sáenz-Romo *et al.*, 2019).

Overall, our results indicate that keeping both vineyard types in a mosaic agricultural environment can enrich the structure of the landscape, favoring overall bird diversity. However, trellis vineyards are avoided by ground-dwelling threatened species such as great bustards (Casas *et al.*, 2020), little bustards or sandgrouse (Tarjuelo *et al.*, 2020), so large surface increases of this land-use could adversely affect these species. In addition, farmland areas dominated by vineyards (monocropping) may have reduced species diversity compared with typical farmland mosaics, although this should be further assessed in future studies. To increase the ecological value of vineyards, biodiversity-friendly vineyard management should be promoted, which could also be economically beneficial as it may reduce pest density and the use of phytosanitary treatments (Fiedler *et al.*, 2008; Sáenz-Romo *et al.*, 2019). For this, it might be advisable to maintain landscape heterogeneity, as well as promote the maintenance of natural vegetation cover on soil and field boundaries (Casas and Viñuela, 2010; Salguero, 2010). Regarding ground-nesting birds, tilling the vineyards during the nesting months (April-May-June) could also significantly negatively impact their breeding success (Casas and Viñuela, 2010). Here we have only evaluated the use of vineyards in summer, but it would be necessary to assess the impact of their modernization at other times of the year. For instance, a high tilling frequency probably implies a reduced trophic availability during the winter in both vineyard types. Our study design did not allow us to distinguish the effect of each factor (structure, irrigation, vegetation cover, and shading) on species′ preference for one type of vineyard. Future studies could specifically assess these, investigate to what extent irrigation attracts birds and other wildlife from dry farmland to trellis vineyards, and evaluate the risk of exposure to toxic doses of nitrates during fertigation.

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Supplementary material

Appendix A

JAGS model code: Multi-species hierarchical occurrence model.

```
model {

# Subscripts:
# i = Species
# j = Site
# k = Visit

# Specify priors
  mu.a0 ~ dnorm(0, 0.37)
  mu.a1 ~ dnorm(0, 0.1)
  mu.a2 ~ dnorm(0, 0.1)
  mu.a3 ~ dnorm(0, 0.1)
  mu.b0 ~ dnorm(0, 0.37)
  mu.b1 ~ dnorm(0, 0.1)
  mu.b2 ~ dnorm(0, 0.1)
  mu.b3 ~ dnorm(0, 0.1)
  mu.b4 ~ dnorm(0, 0.1)
  mu.b5 ~ dnorm(0, 0.1)

  tau.a0 ~ dgamma(0.1,0.1)
  tau.a1 ~ dgamma(0.1,0.1)
  tau.a2 ~ dgamma(0.1,0.1)
  tau.a3 ~ dgamma(0.1,0.1)
  tau.b0 ~ dgamma(0.1,0.1)
  tau.b1 ~ dgamma(0.1,0.1)
  tau.b2 ~ dgamma(0.1,0.1)
  tau.b3 ~ dgamma(0.1,0.1)
  tau.b4 ~ dgamma(0.1,0.1)
  tau.b5 ~ dgamma(0.1,0.1)

  for (i in 1:nsp){
    a0[i] ~ dnorm(mu.a0, tau.a0)
    a1[i] ~ dnorm(mu.a1, tau.a1)
    a2[i] ~ dnorm(mu.a2, tau.a2)
    a3[i] ~ dnorm(mu.a3, tau.a3)
    b0[i] ~ dnorm(mu.b0, tau.b0)
    b1[i] ~ dnorm(mu.b1, tau.b1)
    b2[i] ~ dnorm(mu.b2, tau.b2)
```

```
    b3[i] ~ dnorm(mu.b3, tau.b3)
    b4[i] ~ dnorm(mu.b4, tau.b4)
    b5[i] ~ dnorm(mu.b5, tau.b5)

# Ecological submodel: Define state conditional on parameters
    for (j in 1:nsite){
      logit(psi[j,i]) <- a0[i] + a1[i]*arable[j] + a2[i]*size[j] +
      a3[i]*type[j]
      z[j,i] ~ dbern(psi[j,i])

# Observation model
      for (k in 1:nrep){
        muy[j,k,i] <- z[j,i]*p[j,k,i]
        logit(p[j,k,i]) <- b0[i] + b1[i]*hour[j,k] + b2[i]*(hour[j,k]^2) +
        b3[i]*date[j,k] + b4[i]*(date[j,k]^2) + b5[i]*velocity[j,k]
        y[j,k,i] ~ dbern(muy[j,k,i])
        } #k
    } #j
  } #i
}
```

JAGS model code: Single-species hierarchical occurrence model.

```
model {

# Subscripts:
# j = Site
# k = Visit

# Specify priors
  a0 ~ dnorm(0, 0.37)
  a1 ~ dnorm(0, 0.1)
  a2 ~ dnorm(0, 0.1)
  a3 ~ dnorm(0, 0.1)
  b0 ~ dnorm(0, 0.37)
  b1 ~ dnorm(0, 0.1)
  b2 ~ dnorm(0, 0.1)
  b3 ~ dnorm(0, 0.1)
  b4 ~ dnorm(0, 0.1)
  b5 ~ dnorm(0, 0.1)

# Ecological submodel: Define state conditional on parameters
     for (j in 1:nsite){
       logit(psi[j]) <- a0 + a1*arable[j] + a2*size[j] +
       a3*type[j]
       z[j] ~ dbern(psi[j])

# Observation model
       for (k in 1:nrep){
         muy[j,k] <- z[j]*p[j,k]
         logit(p[j,k]) <- b0 + b1*hour[j,k] + b2*(hour[j,k]^2) +
         b3*date[j,k] + b4*(date[j,k]^2) + b5*velocity[j,k]
         y[j,k] ~ dbern(muy[j,k])
       } #k
     } #j
}
```

Appendix B

Table B1. Number of detections by species and vineyard type. In grey those species that were observed at least 5 times.

Species	Scientific name	Group	Trellis	Traditional	Total
European rabbit	*Oryctolagus cuniculus*	Mammal	10	16	26
Iberian hare	*Lepus granatensis*	Mammal	12	14	26
Crested lark	*Galerida cristata*	Bird	21	42	63
Red–legged partridge	*Alectoris rufa*	Bird	13	5	18
Rufous–tailed scrub–robin	*Cercotrichas galactotes*	Bird	9	4	13
European goldfinch	*Carduelis carduelis*	Bird	8	4	12
European stonechat	*Saxicola rubicola*	Bird	6	5	11
House sparrow	*Passer domesticus*	Bird	10	0	10
Corn bunting	*Emberiza calandra*	Bird	6	1	7
European greenfinch	*Chloris chloris*	Bird	1	5	6
Common linnet	*Linaria cannabina*	Bird	3	2	5
Common blackbird	*Turdus merula*	Bird	4	1	5
Northern wheatear	*Oenanthe oenanthe*	Bird	3	1	4
Common buzzard	*Buteo buteo*	Bird	0	3	3
Eurasian stone-curlew	*Burhinus oedicnemus*	Bird	1	1	2
Woodchat shrike	*Lanius senator*	Bird	2	0	2
Iberian grey shrike	*Lanius meridionalis*	Bird	1	1	2
Azure-winged magpie	*Cyanopica cyanus*	Bird	1	1	2
Eurasian collared dove	*Streptopelia decaocto*	Bird	1	1	2
Eurasian magpie	*Pica pica*	Bird	1	1	2
Eurasian hoopoe	*Upupa epops*	Bird	1	0	1
Great tit	*Parus major*	Bird	0	1	1
Common raven	*Corvus corax*	Bird	1	0	1
Spotless starling	*Sturnus unicolor*	Bird	1	0	1
Pin-tailed sandgrouse	*Pterocles alchata*	Bird	0	1	1
White wagtail	*Motacilla alba*	Bird	1	0	1

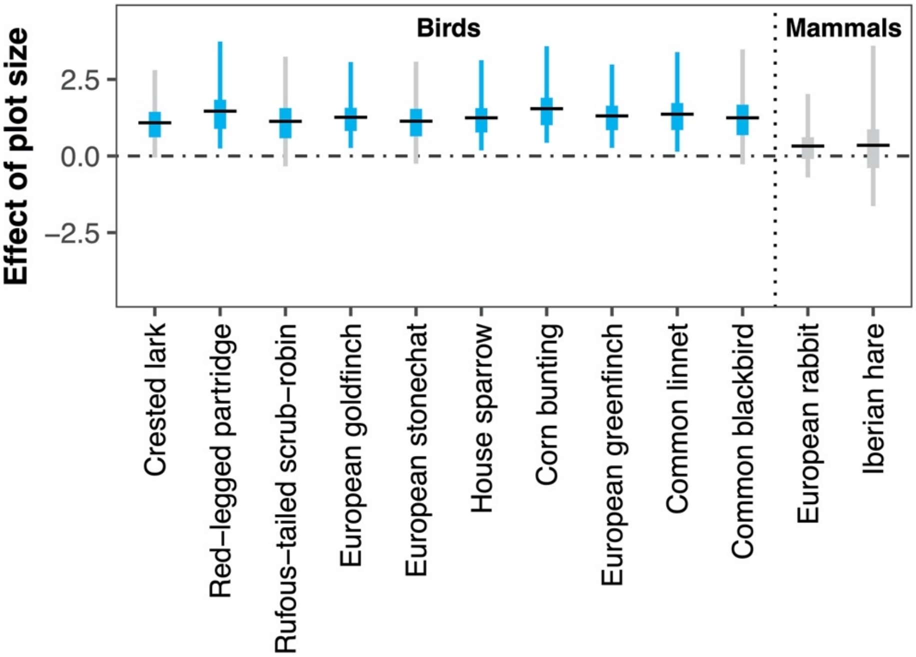

Figure B1. Effect of vineyard size on the species-level mean occurrence (birds on the right, mammals on the left). The horizontal short black lines show the mean change (across all MCMC iterations), the boxes show the 50% credible intervals (CI) and the vertical lines delineate the 95% CI. Light grey indicates no differences and blue indicates positive effect of plot size. When boxes are blue but the vertical lines are grey, indicates that 50% CI did not overlap zero but 95% CI did.

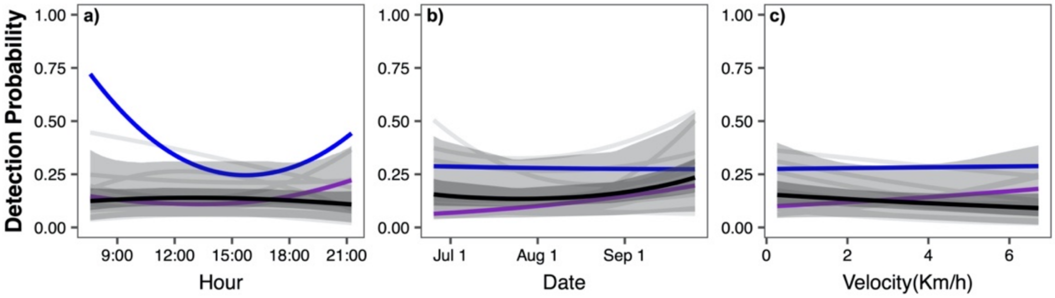

Figure B2. Detection probability by: a) hour; b) date; and c) car velocity. The black lines show the mean values across bird species analysed (with shaded 50% and 95% credible intervals). Light grey lines show the detection probabilities of each bird species included in the analysis, blue line show the detection probabilities of European rabbit and purple line show the detection probabilities of Iberian hare.

PAPER III

Farmland composition and farming practices explain spatio-temporal variations in red-legged partridge density in central Spain

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Science of the Total Environment (Under review)

Farmland composition and farming practices explain spatio-temporal variations in red-legged partridge density in central Spain

Abstract

Many farmland bird populations are declining, and their negative trends are often associated with changes in land-use or farming practices, including the use of agrochemicals. The red-legged partridge is a Mediterranean farmland game species of high socio-economic importance whose populations are thought to have declined sharply since the mid-20th century associated with farmland changes. However, no large-scale studies have tested whether abundance or trends of red-legged partridge are related to farmland composition or management.

We used hierarchical distance sampling models to estimate red-legged partridge abundance in 2010 in central Spain (Castilla-La Mancha), a main European population stronghold of this species. We studied associations between red-legged partridge density and land-uses (including variation in management: irrigated crops or organic farming). We also assessed regional abundance variation over seven years (2010-2017) and its relationship with changes in land-use.

Our results show that red-legged partridge abundance increased with the availability of natural vegetation and traditional rain-fed vineyards, but decreased with increasing proportions of tree crops and irrigated vineyards; the latter association was less pronounced in areas sensitive to nitrate contamination in water, where the amount of fertilizers applied in farmland and use of certain farming practices is more strictly regulated. These results support the idea that increases in intensive vineyards are detrimental to the red-legged partridge. We also report a strong population decline of red-legged partridge in the region, with a 51% abundance reduction in seven years. This decline was steeper in areas where more natural vegetation had been lost and where ecological tree crops had increased.

Overall, our results indicate that changes in land-use (type of crop, or the destruction of natural vegetation in farmland) and farming practices (e.g. use of irrigation in certain crops, use of nitrates) have important impacts on this farmland bird, affecting both spatial distribution and population dynamics.

Keywords

Abundance; *Alectoris rufa*; agriculture intensification; hierarchical distance sampling; land-use; management

1. Introduction

After millennia of slow agricultural expansion, a large part of the European landscape is currently dedicated to agriculture (Krebs *et al.*, 1999). Many terrestrial bird species have adapted and rely on this semi-natural environment, finding suitable breeding or wintering habitats in it (Tucker and Heath, 1994; Tucker and Evans, 1997; Robinson *et al.*, 2001). However, the Common Agricultural Policy (CAP), a milestone for European agriculture, has been a turning point for farmland biodiversity (Donald *et al.*, 2002). The deep and quick modernization of European agricultural landscapes after the CAP, with a marked agricultural intensification in a short time (Matson *et al.*, 1997; Pain and Pienkowski, 1997), has driven strong declines of many farmland bird species (Donald *et al.*, 2001; Benton *et al.*, 2002; Donald *et al.*, 2002; Storkey *et al.*, 2012). Farmland intensification has led to changes in land-use (as some crops have been promoted over others) and the landscape (e.g. through the increase in field size for allowing more efficient mechanization, the disappearance of field boundaries, and increased monoculture), but also to changes in agricultural practices within land-uses (e.g. mechanization, use of pesticides, fertilizers or irrigation), all of which have had direct or indirect effects on wildlife (Matson *et al.*, 1997; Pain and Pienkowski, 1997).

Land-use and landscape changes may alter the extent of suitable areas for birds, associated with changes in the vegetation structure or food abundance (Benton *et al.*, 2002; Wilson *et al.*, 2005; Storkey *et al.*, 2012). Within farmland, landscape heterogeneity usually increases habitat suitability for birds, as this increases the likelihood of finding adequate resources for a wide range of species throughout the year (Benton *et al.*, 2003; Siriwardena *et al.*, 2012). Additionally, the importance of non-farmland habitats (natural habitats) and fallow lands within the farmland matrix has also been highlighted for many birds, providing foraging, mating, and nesting habitats (Vickery *et al.*, 2002; Vickery *et al.*, 2009; Mcmahon *et al.*, 2010; Tarjuelo *et al.*, 2020).

Farming practices, such as mechanization, change in harvest schedules, or use of chemical inputs, may also have important effects on wildlife (Fry, 1995; Matson *et al.*, 1997; Mineau and Whiteside, 2013; Stanton *et al.*, 2018), even when land-use does not change. Examples of this are the comparison of traditional (gobelet-shaped) vs trellis irrigated vineyards in Spain (Salguero, 2012; Cabodevilla *et al.*, 2021), organic vs traditional crops (Solomou and Sfougaris, 2011; Myers *et al.*, 2019), or the use of coated seeds in annual crops (Prosser and Hart, 2005; Lopez-Antia *et al.*, 2016, 2021). Farmland practices may also have impacts on the quality of the environment. For example, excessive use of nitrates as fertilizers may alter the quality of surface and underground waters, subsequently impacting vegetation or fauna using those areas (Rodríguez-Estival *et al.*, 2010; Britton *et al.*, 2017; Chen *et al.*, 2019; Paredes *et al.*, 2020).

The red-legged partridge (*Alectoris rufa*) is a common species of the rain-fed farmland of south-western Europe, whose natural populations inhabit mainly the Iberian Peninsula (Blanco-Aguiar, *et al.*, 2004). The species has a strong socio-economic and ecological importance: it is a main small gamebird species (Andueza *et al.*, 2018), and an important trophic resource for many Iberian predators (Calderón, 1977), including threatened species such as Bonelli's eagle (Aquila fasciata) and Imperial eagle (Aquila adalberti). Red-legged partridge populations strongly declined in the second half of the 20th century (Blanco-Aguiar, 2007), and the decline may have continued in recent decades (BirdLife International, 2018). Declines have been suggested to be primarily associated with changes in farmland, including loss of habitat heterogeneity, earlier cereal harvest, and use of agrochemicals, although high hunting pressure has also contributed (Blanco-Aguiar, 2007). However, the magnitude of this decline (at least in recent years) has been contested from hunting sectors (RFEC, 2020) and no large-scale study has assessed whether abundance or trends of red-legged partridge are related to farmland composition or management, which could have important implications when designing management measures to promote the species conservation status.

Here we used hierarchical distance sampling models to estimate red-legged partridge density variation in space and time over seven years (2010-2017) in Castilla-La Mancha, a large and important Spanish region for the species (Blanco-Aguiar *et al.*, 2003; Blanco-Aguiar *et al.*, 2004). First, we assessed the relationship between land-use composition in the farmland environment and spatial variations in abundance. In particular, we tested whether red-legged partridge abundance varied with the availability of natural (non-farmed) habitats or with farming practices (e.g. irrigated vs non-irrigated crops, organic farming, or limitations to the use of fertilizers in areas sensitive to nitrate contamination in water). Second, we assessed abundance trends over seven years and investigated if population changes can also be explained by habitat changes. We discuss the implications of our results for the species´ conservation, management, and hunting sustainability.

2. Materials and methods

2.1. Study species and area

The red-legged partridge is a habitat generalist, which inhabits natural habitats as well as farmland mosaics, but is more abundant in areas dominated by arable land (Blanco-Aguiar, 2007). Habitat changes over time have strongly affected how red-legged partridge use different habitats (Delibes-Mateos *et al.*, 2012). In the mid-twentieth century the areas favorable to red-legged partridge were strongly associated with natural vegetation in mountainous areas, mostly scattered scrubland and open evergreen oak forests with underlying grasslands. However, in recent decades, the habitats in those mountainous areas have been abandoned or modified, and turned into dense scrublands or woodlands, which are unsuitable to partridges. Nowadays, the most favorable areas for red-legged partridge are within croplands (Delibes-Mateos *et al.*, 2012), making them much more likely to be affected by agricultural intensification.

Many aspects associated with agricultural intensification are considered a threat to red-legged partridge. For example, abundance and survival are lower in more homogeneous farmland areas with fewer field boundaries (Buenestado *et al.*, 2009), high nest losses are observed due to earlier harvest of cereal (Casas and Viñuela, 2010), and coated seeds have been shown to have negative effects on the species (Lopez-Antia *et al.*, 2016, 2021; Lennon *et al.*, 2020). Nitrates absorbed through drinking water have also been shown to have negative effects on the species (Rodríguez-Estival *et al.*, 2010), so areas where fertigation in vineyards is widespread can potentially be negative for red-legged partridge. Many studies conducted on captive birds have shown that red-legged partridge survival and reproduction are adversely affected by agrochemicals currently used in modern agriculture (e.g. Fernández-Vizcaíno *et al.*, 2020; Ortiz-Santaliestra *et al.*, 2021), although fewer studies have quantified exposure levels in wild red-legged partridges (e.g. Lopez-Antia, 2016) and none have quantified the extent of wild red-legged partridge population declines and whether these are linked to proxies of agrochemical exposure. Recognition of habitat change as a cause of decline has led hunters to demand changes in agricultural policies to promote the species´ recovery, and to link hunting and habitat management. In this regard, hunters are developing specific projects, such as the RUFA project (https://www.fundacionartemisan.com/investigacion/proyecto-rufa/; promoted by Fundacion Artemisan), focused on improving farmland habitat quality to promote populations of the species and sustainable hunting.

We conducted this study in Castilla-La Mancha (**Fig. A1**, Appendix A), a region that holds one of the population strongholds of the red-legged partridge in Spain and Europe (Blanco-Aguiar *et al.*, 2003; Blanco-Aguiar *et al.*, 2004). It is also one of the Spanish regions in which red-legged partridge hunting is most important (Blanco-Aguiar *et al.*, 2003). Castilla-La Mancha (79,463 km^2) is administratively divided into five provinces and is sparsely populated (25.74 per/km^2). This region is mostly a plateau, with a mean elevation of 696 m.a.s.l. (meters above sea level) and around 80% of its surface is below 1000 m.a.s.l., although there are also some important mountainous areas reaching 1800 m.a.s.l. (Pons-Giner *et al.*, 2011). Regarding agriculture, 46% of the surface is cultivated (around 37,000 km^2), with rain-fed cereal (11,759 km^2), vineyards (4,652 km^2), and olive trees (4,437 km^2) as the most important crops (ESYRCE, 2020). 23.5% of the arable land (8,724 km^2) is kept as fallow annually, as part of the farming cycle. Regarding natural habitats, forests cover 38% of the region's surface (30,049 km^2) and grasslands 6.5% (ESYRCE, 2020).

2.2. Partridge data

Field data was collected by regional wardens as part of a monitoring program of red-legged partridge breeding phenology (Arroyo and Guzman, unpublished results). Observations took place from 2010 to 2017. A large number of transects were sampled the first year throughout the region (n = 170; **Fig. A1a**, Appendix A), although 49 of them had to be discarded due to a lack of information on transect length or observation distances. In subsequent years, a much smaller number of transects was monitored (28–36 transects per year). Transects sampled in 2011-2017 were not necessarily the same each year. For the study of trends, we only considered those transects sampled at least twice between 2010 and 2017 (n = 48; **Fig. A1b**, Appendix A).

The transects (ca. 14.5 km in length, range 3.2 km to 39.8 km) were driven by car at slow speed (<30 km/hr), stopping each time that a partridge or a group of partridges was observed. For each sampling in each transect, date, start, and finishing time were noted. For each observation, the number of partridges, their age group (i.e. adult or chick), and the distance to the transect line (estimated visually) were noted. Samplings in each transect were aimed to be repeated weekly or every 10 days from mid-April to late-July, although variations occurred among transects due to logistic constraints (with an average of 10 visits per transect and year, range 1 to 16). Samplings occurred throughout the day, although most frequently during early morning or early evening. In this study, we only considered counts of adult red-legged partridges.

2.3. Land-use and environmental data

We used the Spanish Land Cover Information System (SIOSE, 2011) to describe land-use composition in each transect in 2010. The SIOSE database is spatially very accurate and provides detailed information about the distributions of various types of land cover, including information on whether crops are irrigated or not. There are four available versions of SIOSE, and we used SIOSE 2011 as the closest temporally to partridge data obtained in 2010.

We calculated for each transect the proportion of each land-use as follows. SIOSE data was set on a polygon layer. A polygon might have a unique value (e.g. herbaceous crops) or be a combination of habitats with their respective percentages into the polygon (e.g. 75% herbaceous crops, 25% olive groves). We obtained a polygon layer for each of the habitats of interest for the red-legged partridge (shrubland; pastures; forests; urban areas; irrigated and rain-fed vineyards, irrigated and rain-fed arable lands, and irrigated and rain-fed tree crops, see Appendix B) whatever its percentage into the polygon. We created a buffer of 200 meters on each side of the transect line (200m being the distance above which partridges were not detected). We obtained the proportion of each habitat type into the buffered transects applying the corresponding correction related to the percentage (i.e. if a polygon of 100 ha had 75% of herbaceous crops, we considered 75 ha of herbaceous crops in that polygon).

As red-legged partridge uses field margins for breeding and refuge (Casas and Viñuela, 2010), we also calculated an index of field margins for each transect. For this, we intersected the polygon layer of SIOSE land-use types with the buffered transects. In each transect, the index was calculated as the sum of perimeters obtained within the intersection divided by the area of the transect buffer. All these spatial analyses were performed in R (R Core Team, 2018) with the packages raster (Hijmans, 2017) and dplyr (Wickham *et al.*, 2018).

Additionally, we used QGIS software (QGIS Development Team, 2018) to calculate for each transect the proportion of organic vineyards, organic fallows/pastures, organic annual crops, and organic tree crops. These were estimated based on geographic information on crops with organic certification in the year 2011, provided by the regional government (Junta de Comunidades de Castilla-La Mancha, JCCM). This information was available as a list with an ID for the plots where these crops existed. We used the Geographic Information System for Agricultural Plots (SIGPAC) from 2007 (the closest available to 2011) to link those plot IDs to specific plots, and thus create shapefiles for the surface area of organic crops, which were used to estimate the proportion area with organic crops within each transect buffer.

Each transect was also categorized as being within or outside an area sensitive to nitrate contamination in water (hereafter nitrate sensitivity), based on the distribution of vulnerable zones to nitrate contamination for the years 2008-2011 (MITECO, 2013). We considered each transect as “nitrate sensitive” if it was partially or totally inside a nitrate-contamination risk area; and “non-sensitive”, if it was entirely outside a nitrate-contamination risk area. Nitrate sensitive areas (i.e. declared as vulnerable to nitrate contamination in water) have additional restrictions regarding the amount of nitrate that can be applied in farmland (lower than in non-sensitive areas), as well as the type of farming practices that can be implemented in order to reduce nitrate inputs (Order of 07/02/2011, JCCM).

Furthermore, we calculated climatic and topographic variables for each transect (Appendix B). These variables were raster layers with an original resolution of ~1 km^2 (Agencia Estatal de Meteorología, 2011; US Geological Survey, 1996). From these, we created raster layers with higher resolution (100 m x 100 m) maintaining the original values and obtained a mean value in each of the transect buffers.

For assessing habitat changes for the analyses of partridge trends (see below), the only available update of SIOSE was for 2014. We considered three years as too short a temporal distance to identify changes. For assessing changes in land-use, therefore, we used Corine Land Cover, which has less information (e.g. it does not allow separating between irrigated and non-irrigated crops, and it provides a single land-use type for each polygon, which may underestimate the availability of certain land-uses in areas with a combination of habitats), but was available for 2006, 2012 and 2018. We used the Accounting Layers (https://www.eea.europa.eu/data-and-maps/data/corine-land-cover-accounting-layers), which allow direct comparison of land-use estimation across years, and thus calculation of changes. We calculated (using the R software) the proportional difference in each land-use between 2006 and 2018 to maximize the identification of land-use changes with time. We estimated changes in the following land-uses: arable land (CLC code 211), heterogeneous agricultural areas (areas covered by a diversity of crops in small plots, CLC code 242, or areas of farmland interspersed with small patches of natural vegetation, CLC code 243), vineyards (CLC code 221), tree crops (olive trees and fruit trees, CLC codes 222 or 223), pastures (grasslands or grazed pastures, CLC codes 321 or 231), shrubs (transitional woodland-shrub, CLC code 324, and sclerophyllous vegetation, CLC code 323) and urban areas (CLC code 11; see Appendix F). We also estimated changes in organic crops (organic vineyard, organic fallow and pastures, organic annual crops, organic tree crops) using data from plots with organic accreditation in 2011 and 2015 (provided by JCCM). We created shapefiles of distribution of organic crops as described above, although for data of the year 2015 we used the SIGPAC database from 2018 (as it was closest to 2015). We estimated changes in the proportion covered by those organic crops within each transect buffer using QGIS software (QGIS Development Team, 2018).

2.4. Analyses of red-legged partridge abundance and habitat

For analyses of correlates of spatial variation in abundance, we only used the 2010 data, when the sample size was largest (n = 121 transects; **Fig. A1a**, Appendix A). We performed a hierarchical distance-sampling model to estimate partridge abundance in each transect. Specifically, we fitted the generalized distance sampling model of Chandler *et al.* (2011) using the function *gdistsamp* from the R package *unmarked* (Fiske and Chandler, 2011) that extends the distance sampling model of

Royle *et al.* (2004) to estimate the probability of being available for detection, and also allows the use of the negative binomial distribution to model abundance. Hierarchical models (base on repeated surveys) simultaneously account for both spatial variations in abundance and heterogeneity in detection probability (Sillett *et al.*, 2012; Kéry and Royle, 2015). Thus, parameters are hierarchically structured, and variations at each level (i.e. abundance and detection) can be directly modeled as functions of covariates (Chandler *et al.*, 2011). Using the replicas we can also estimate the probability *phi* of the individuals being available for detection, relaxing the distance sampling assumption that at distance 0 of the transect line, all individuals are detected ($g(0) = 1$). We assumed closure within each sampling period (i.e. no change in adult abundance within transects from mid-April to late-July). We truncated our data by observation distances (maximum of 150 m) to favor a proper fit of distance sampling models. By doing so, we excluded 0.34% of the observations. We performed three steps in the model selection process: 1) null models to select between Poisson or the negative binomial distribution, and to estimate the detection function; 2) to the best model identified in the previous step, we added variables to the detection parameter; 3) to the best model identified in the previous step, we added variables potentially affecting abundance and selected the best models. In all cases, the best models were selected using AIC comparisons. Combinations of tested models are provided in Appendix C. In the third step, we obtained 10 models that had AIC values within two points of the best model, so we considered all these 11 models as equally adequate. We used a parametric bootstrap to test the goodness of fit (GoF) of the best model. We simulated 1000 data sets from this model and used error sums-of-squares, chi-square, and Freeman–Tukey fit statistics to quantify the fit of the model to the data sets (Kéry and Royle, 2015). We report the estimates of these models in Appendix D.

Candidate variables affecting detection were the following: hour (categorical variable; morning, midday/afternoon, evening), date (continuous variable starting 1 = April), quadratic term of date (to allow for non-linear seasonal detection variation), habitat structure (open, close and intermediate), and the interactions between hour and date and between hour and quadratic term of date (see Appendix C). Hour categories were defined as follows: morning was considered before 12:00, midday/afternoon was considered between 12:00 and 18:00, and evening was considered after 18:00. Habitat structure was derived from the above-mentioned habitat variables as follows: open = proportion of closed habitats (forest, scrub and buildings) < 10%, intermediate = proportion of closed habitats > 10% < 30%, close = proportion of closed habitats > 30%. Variables affecting detection were selected based on previous work on the study species (Jakob *et al.*, (2014).

Candidate variables affecting abundance included a pool of 37 climatic, topographic, and habitat variables that theoretically could affect partridge abundance (Appendix B), as well as nitrate sensitivity (risk of nitrate contamination in water). Variables were standardized for analyses. We performed a variance inflation factor (VIF) with all the variables to check for collinearity, removing sequentially the variable with the highest VIF until the set of variables had a VIF lower than 3 (Zuur *et al.*, 2010). We performed this procedure separately for environmental (climate and topography) and habitat variables. Afterward, we checked the VIF of the remaining variables altogether. The whole set of environmental variables is detailed in Appendix B, as well as the final set selected for modeling. VIF values were calculated with the function *multicol* from R package *fuzzySim* (Barbosa, 2015).

We plotted the predicted effect of each variable on detection and abundance based on model averaging, using the *predict* function of *unmarked* R package on a *fitList* object containing the 11 best models (Appendix C). In addition, we used the function *ranef* of *unmarked* R package to estimate posterior abundances from the best model (mod20). This function uses empirical Bayes methods. We then used these posterior abundances to calculate the density of partridges in each transect (red-legged partridge/ha per transect) and plotted them in a map to show the spatial distribution of red-legged partridge densities (red-legged partridge/ha) in the study region.

2.5. Analyses for partridge trends and habitat changes

These analyses were carried out using only those transects that had been monitored at least two years between 2010 and 2017 (n = 48). We first estimated the red-legged partridge abundance in each transect and year with hierarchical distance-sampling models (one model per sampling year) using the function *gdistsamp* from the R package *unmarked* (Fiske and Chandler, 2011). We applied the same model selected above, i.e. with negative binomial distribution and a Hazard rate detection function. We also truncated our data by observation distances (maximum of 150 m) to favor a proper fit of distance sampling models. As we had not found temporary migration in the abundance model (2010 full model, phi = 1), i.e. no variation in population sizes between the beginning and the end of the temporary replications, here we assume no temporary migration. In each model, we also added the same variables described above to explain the detection parameter (hour, date, quadratic term of date, habitat structure, and the interactions between hour and date and between hour and quadratic term of date). We used a parametric bootstrap to test the GoF of the model for each year. We simulated 1000 data sets from each of our models and used error sums-of-squares, chi-square, and Freeman–Tukey fit statistics to quantify the fit of each model to the data sets (Kéry and Royle, 2015). All models had quite good fits to the data (Appendix G), with a *c-hat* between 1.2 and 1.9. We used the function *ranef* of *unmarked* R package to estimate posterior distributions of the abundance. As above, these posterior abundances were used to calculate the density of partridges in each transect (red-legged partridge/ha per transect).

To assess population change during our study period, we initially carried out a Generalized Linear Mixed Model (GLMM, R package *lme4*; Bates, *et al.*, 2015), with log(density) in each transect and year as a response variable (gaussian distribution), year as a categorical covariate, and "transect identity" as a random term affecting the intercept. We used a Tukey HSD post hoc test for comparisons between 2010 and 2017 abundances (*lsmeans* R package; Russell, 2016). Subsequently, we built GLMMs to assess whether habitat change affected temporal trends in density, with log(density) as the response variable, "transect identity" as a random term, year and year^2 (to account for the non-linear trends found, see results) as continuous variables, and two-term interactions between year and each habitat-change variable. As above, we first assessed whether habitat-change variables were collinear by performing a variance inflation factor (VIF) with all the habitat-change variables, removing sequentially the variable with the highest VIF until the set of variables had a VIF lower than three (Zuur *et al.*, 2010). In this process, we removed "change in arable land" and "change in mosaic farmland areas". All other habitat-change variables were retained (Appendix F). Models, therefore, included changes in tree crops, vineyards, shrubland, pastures, buildings, organic annual crops, organic tree crops, organic fallow and pastures, and organic vineyard. We used the *dredge* function of the *MuMIn* R package (Barton, 2020) to identify the best model (within the subset that included year and year^2 as explanatory terms) according to AIC values.

3. Results

3.1. Partridge abundance and habitat

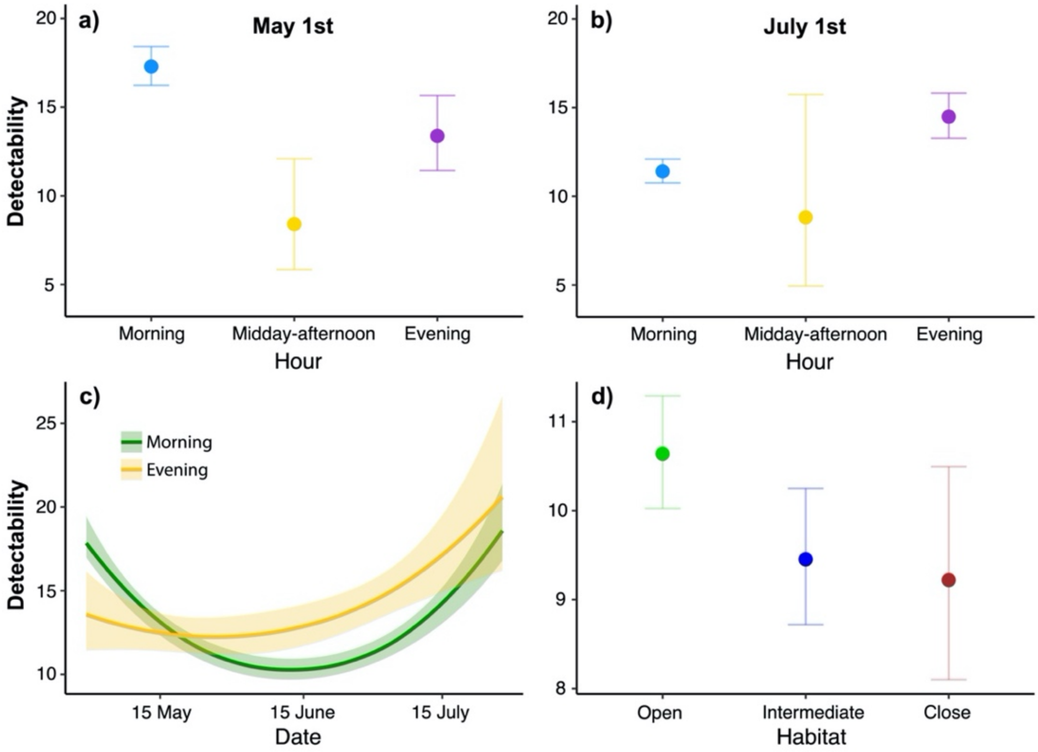

Figure 1. Red-legged partridge's detectability (based on models average of best models in **Table D1**, Appendix D) in relation to the time of day (a and b), date (c), and habitat openness (d). See estimates and statistical significance of the best 11 models with an AIC value within 2 points in **Table D1**, Appendix D. The effects of hour and date are represented for open habitat and the effect of habitat is represented for morning and considering mean values for date. Dark line/dots represent the mean effect and the shaded area and whiskers the 95% confidence intervals.

Detectability of red-legged partridge varied quadratically throughout the season (**Table 1**; **Table D1**, Appendix D), being higher at the beginning of the survey period (April) and in summer (July), but lowest in late May and June (**Fig. 1c**). Time of day had also an important effect on detectability (**Table 1**; **Table D1**, Appendix D), which was overall higher during morning and evening than during midday and early afternoon (**Fig. 1a**; **Fig. 1b**). Moreover, as shown by the statistical significance of their interaction, the effect of time of day varied throughout the season: at the beginning of the sampling season (April and early May), detectability was higher in the morning whereas from June onwards detectability was higher in the evening (**Fig.**

1a; **Fig. 1b**; **Fig. 1c**). Finally, detectability varied with habitat structure and was higher in open habitats (**Fig. 1b**; **Table 1**; **Table D1**, Appendix D).

Table 1. Estimates (±SE) and parameter statistics of the best model (mod20) on variations in red-legged partridge abundance. Variable codes as in Appendix B.

Detection	**Estimate**	**SE**	***Z*-ratio**	***P***
Intercept	2.365	±0.03	78.02	**<0.001**
Hour Midday	-0.028	±0.17	-0.17	0.87
Hour Evening	0.144	±0.05	3.03	**<0.01**
Date	-0.134	±0.01	-10.83	**<0.001**
Date2	0.135	±0.01	11.69	**<0.001**
Habitat_I	-0.117	±0.03	-3.48	**<0.001**
Habitat_C	-0.141	±0.06	-2.32	**<0.05**
Midday:Date	0.142	±0.12	1.2	0.23
Evening:Date	0.168	±0.03	4.84	**<0.001**
Midday:Date2	-0.226	±0.13	-1.77	0.08
Evening:Date2	-0.072	±0.03	-2.41	**<0.05**
Abundance	**Estimate**	**SE**	***Z*-ratio**	***P***
Intercept	-2.442	±0.13	-19	**<0.001**
PAut	-0.555	±0.10	-5.41	**<0.001**
TnWin	0.359	±0.09	3.92	**<0.001**
RfVine	0.178	±0.09	1.96	**<0.05**
IVine	-0.429	±0.17	-2.48	**<0.05**
Past	0.189	±0.10	1.87	0.06
RfTreeC	-0.177	±0.09	-2.03	**<0.05**
Nitrate	0.159	±0.17	0.93	0.35
IVine:nitrate	0.334	±0.19	1.77	0.08

Density in transects ranged from 0.06 to 60.64 partridges per km^2 (mean = 11.79 red-legged partridge/km^2; 0.12 red-legged partridge/ha), being heterogeneously distributed throughout the region (**Fig. 2**). The best model explaining variations in red-legged partridge abundance included seven explanatory variables (**Table 1**): abundance was higher in areas with higher winter temperature and lower autumn rainfall, was higher where the proportion of pastures and the proportion of traditional rain-fed vineyards was higher, but where the proportion of irrigated vineyards and rain-fed tree crops was smaller (**Fig. 3**). This model also included the interaction between the proportion of irrigated vineyards and the nitrate sensitivity category (**Fig. 4**). The latter showed that the negative association between red-legged partridge abundance and the proportion of irrigated vineyards was less pronounced in nitrate sensitive areas, where the use of nitrates is more restricted (**Fig. 4**). This model had a good fit to the data (Appendix E), with a *c-hat* of 1.31, providing accurate abundance estimations. Ten other models had AIC values within 2 points of the best model (**Table D2**, Appendix D). The climatic variables entered in all of them, as well as rain-fed and irrigated vineyards. Differences with the best model included an additional inclusion of shrubland, organic tree crops and irrigated arable land in certain models (**Fig. 3**). According to these models, red-legged partridge abundance was higher where the proportion of shrubland was higher and where the proportion of organic tree crops was lower. Red-legged partridge abundance also appeared to be negatively related to the proportion of irrigated arable land, although this association was weak and not significant (**Fig. 3f**; **Fig. 3h**; **Fig. 3i**; **Table D2**, Appendix D).

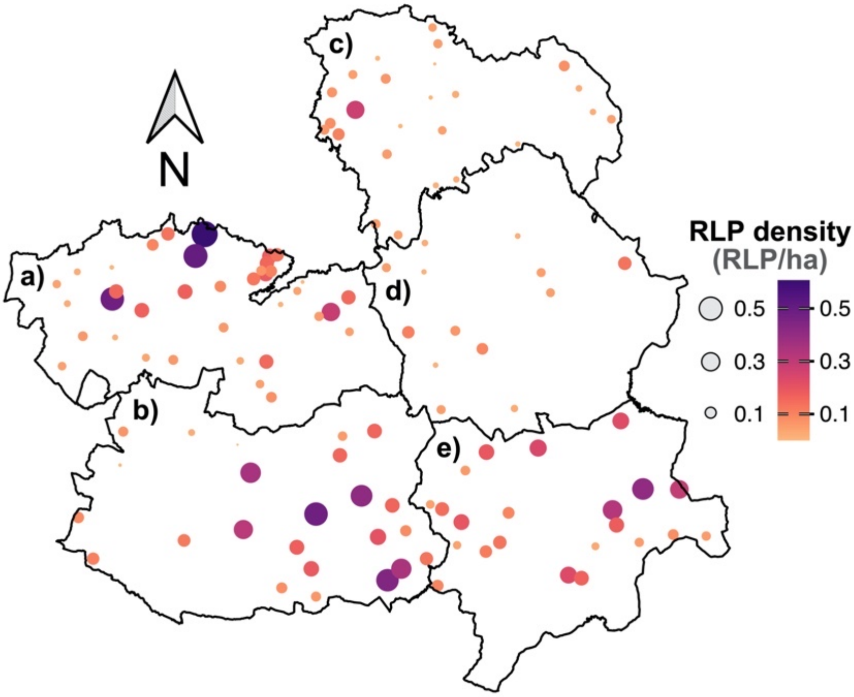

Figure 2. Spatial distribution of Red-legged partridge's densities (red-legged partridge/ha; simplify as RLP/ha) in the studied region. Densities obtained from the best model (mod20) using the ranef function. Letters represent the 5 provinces of the region: a) Toledo, b) Ciudad Real, c) Guadalajara, d) Cuenca, e) Albacete. Darker colors and larger circles indicate higher densities.

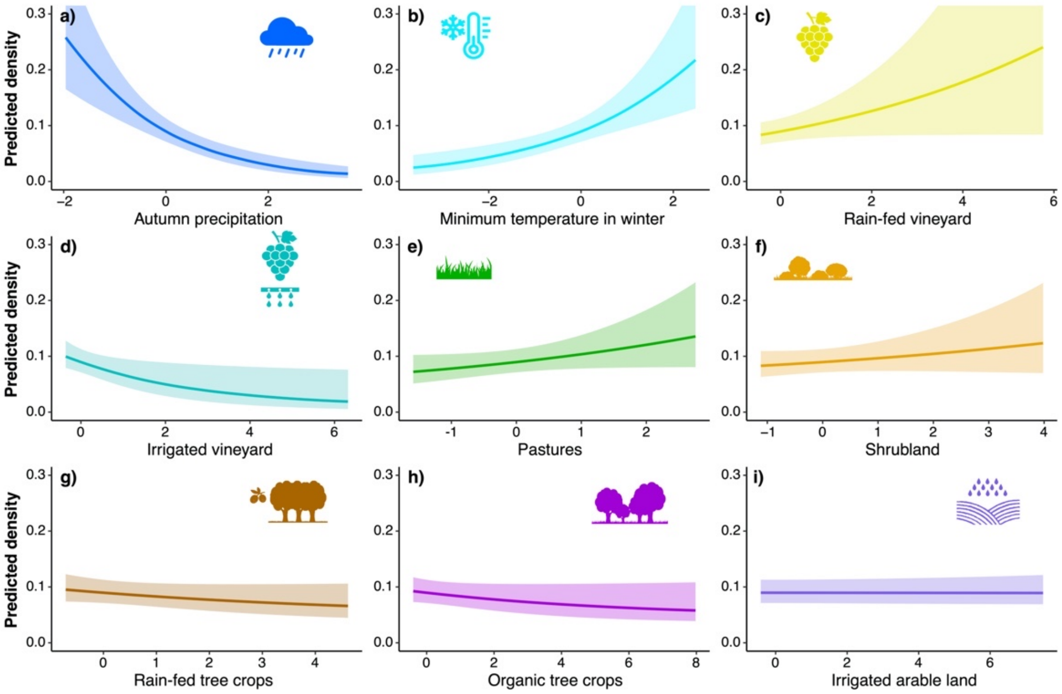

Figure 3. Red-legged partridge densities (red-legged partridge/ha) in relation to climate and land-use variables based on model averaging (best models presented in **Table D2**, Appendix D. The effect of each variable is represented considering mean values for the other variables and for non-sensitive nitrate areas. Dark lines represent the mean effect and the shaded areas their 95% confidence intervals.

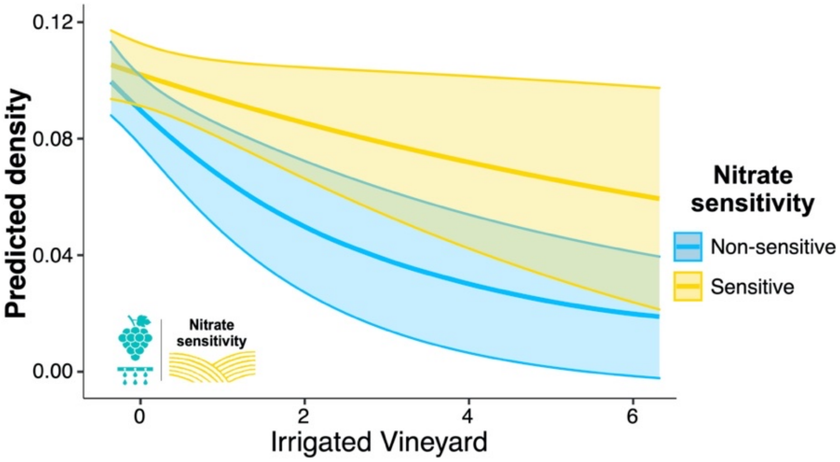

Figure 4. Relationships between red-legged partridge density (red-legged partridge/ha) and the proportion of irrigated vineyards in nitrate-sensitive areas (yellow) and non-sensitive areas (blue). This effect is based on model averaging (best models in **Table D2**, Appendix D) and represented considering mean values for each other climatic and habitat variables. Dark lines represent the mean effect and the shaded areas their 95% confidence intervals. Note that the extent of the y axis differs from that of **Figure 4**.

3.2. Partridge abundance trends

Red-legged partridge density significantly differed between years (GLMM with year included as a categorical factor; F7,210 = 18.95, P < 0.001). Overall, red-legged partridge density decreased throughout the study period (**Fig. 5**), with a mean density in 2017 that was 51% lower than that of 2010 (Tukey HSD test t210 = 5.21, P < 0.001; considering the extremes from the 95% CI, this decline ranged from -8% to -74%). This decline was not linear but occurred mainly in the middle of the study period, between 2012 and 2014 (**Fig. 5**).

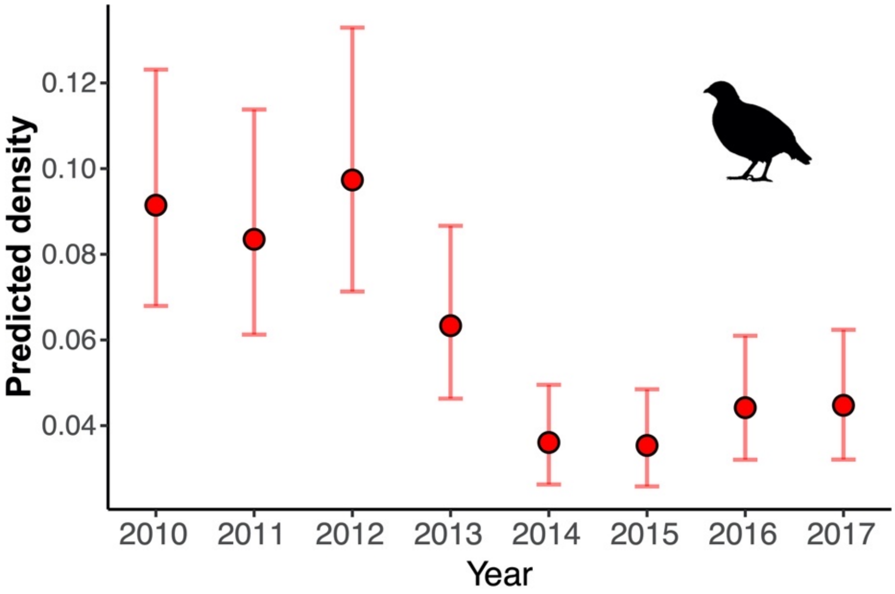

Figure 5. Red-legged partridge density (red-legged partridge/ha) variation over the studied period: density estimation by year (dots) and its confidence intervals (whiskers) according to a GLMM model with year as a categorical explanatory variable.

The best model explaining red-legged partridge trend variations included year (as a continuous variable), its quadratic term, and the interaction between year and two explanatory habitat-change variables: change in pastures and change in organic tree crops (**Table 2**). The interactions showed that partridge abundance declined more strongly where more pastures had been lost, and where organic tree crops had increased most (**Fig. 6**).

Table 2. Estimates (±SE) and statistic parameters of the best model on the effect of habitat on red-legged partridge abundance trends. Variable codes as in Appendix F.

Model	AIC		Intercept	Year	Year^2	YR:OrgTreeChg	YR:PastChg
Mod140	576.1	Estimate	-2.304	-0.221	0.015	-0.023	0.025
		(±SE)	(±0.15)	(±0.05)	(±0.01)	(±0.01)	(±0.01)
		Df	1, 69	1, 217	1, 217	1, 254	1, 245
		F	245.7	17.4	3.8	16.2	18.4
		P	**<0.001**	**<0.001**	0.05	**<0.001**	**<0.001**

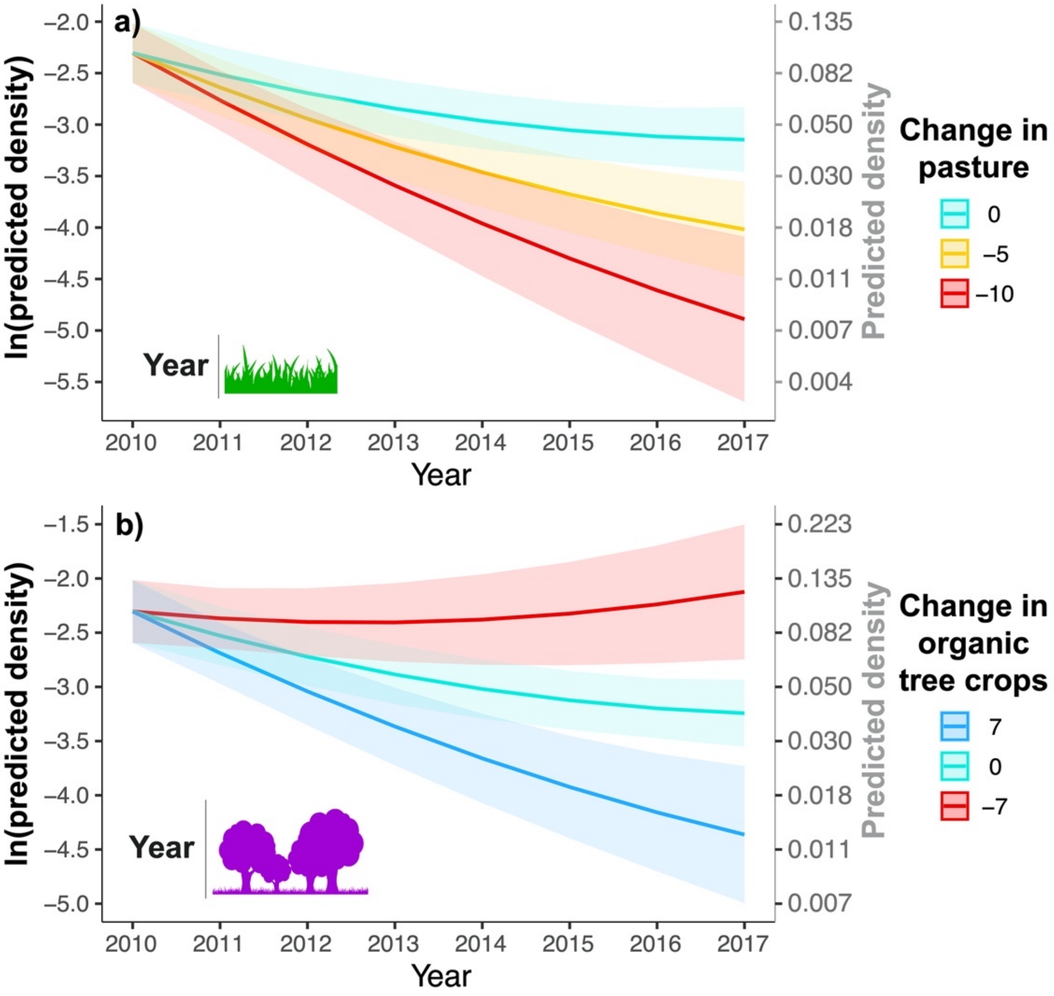

Figure 6. Effect of changes on the surface covered by (a) pasture and (b) organic tree crops on red-legged partridge trends (from the model in **Table 2**). Predicted densities are given in red-legged partridge/ha. Change values are given as a proportional decrease or increase in their percentage. The dark line represents the mean effect and the shaded area the 95% confidence intervals.

4. Discussion

Our study showed that variation in red-legged partridge abundance was strongly dependent on farmland composition and management after taking climate effects into account. Specifically, it showed that density was positively related to the extent of natural vegetation and traditional rain-fed vineyards, but negatively related to the extent of irrigated vineyards and tree crops. In addition, our results confirmed that wild red-legged partridge populations in Castilla-La Mancha, one of the most important regions for the species, have markedly declined (-51%) between 2010 and 2017. We further showed that these negative trends were modulated by land-use changes.

Consistent with previous works (Buenestado *et al.*, 2008; Casas and Viñuela, 2010), we found that agrosystems that include a higher proportion of natural vegetation (shrub and particularly pastures) support higher red-legged partridge abundance. This emphasizes the necessity of maintaining natural vegetation patches within farmland landscapes, which provide a suitable habitat for nesting, feeding, and protection, and for the conservation of farmland biodiversity more generally (Perkins *et al.*, 2002; Vickery *et al.*, 2002; Casas and Viñuela, 2010; Mcmahon *et al.*, 2010; Tarjuelo *et al.*, 2020). Additionally, certain elements of agrosystems were also associated with higher abundance, such as traditional rain-fed vineyards. Vineyards have great importance for red-legged partridge populations, since they provide shade and protective cover, especially after cereal harvest (that takes place between late May and mid-June in this region) when cereal crops (stubbles) do not offer any cover (Buenestado *et al.*, 2008; Sumozas, 2009). GPS-tracking studies have indeed recently shown that red-legged partridge uses vineyards more often in summer and during midday, the hottest hours (Mougeot *et al.*, unpublished results), looking for shade, cover for protection or water. On the opposite, tree crops (including olive groves) do not seem to be suitable habitats for red-legged partridges in the region. This result is in line with the findings by Buenestado *et al.*, (2008) in southern and central Spain, but contrasts with others studies from southern Spain and Portugal, where red-legged partridge seemed to benefit from olive groves (Borralho *et al.*, 1999, 2000; Delibes-Mateos *et al.*, 2012). These differences may be related to the ground management of those tree crops, as within the studied region olive groves are often intensively managed, with mechanical tillage leaving the ground bare between trees, and with the use of fertigation in irrigated groves (Moncunill, 2013). Moreover, an agricultural landscape dominated by tree crops could be detrimental to the species. In any case, these inconsistencies should be addressed and clarified in future studies, but we suggest they might be mostly due to management differences.

In line with the above, and beyond the importance of specific land-uses, we found that red-legged partridge abundances were conditioned by the way some crops were managed. Thus, even if red-legged partridge abundances increased with the availability of traditional rain-fed vineyards, the relationship was the opposite if considering irrigated vineyards. Irrigated vineyards in Spain are almost always trellis-vineyards (Salguero, 2012; Cabodevilla *et al.*, 2021), and thus their physiognomy and structure are different from that of traditional vineyards. The more vertical structure and wider open space between rows may provide less cover for red-legged partridge, but more shade and more visibility under the vines. A study at the plot level showed that the probability of red-legged partridge occurrence, over the summer, was higher in trellis vineyards than in traditional vineyards (Cabodevilla *et al.*, 2021). Thus, the variation in abundance may not be directly related to the different structure of these irrigated crops, but directly to the provision of water. Red-legged partridge depends on water resources during the summer and might be attracted to irrigated vineyards to drink (Borralho *et al.*, 1998; Cabodevilla *et al.*, 2021). The application of fertilizers through the irrigation water is a common practice (fertigation is applied in at least a third of modern, irrigated vineyards; Cabodevilla *et al.*, 2021). Irrigation by dripping allows to provide water straight to the vines and therefore to save water in arid farmland. When fertilizers (mostly nitrates) are applied via the irrigation systems their concentration in water is very high, exceeding by 10 times the recommended limit for public safety (Rodríguez-Estival *et al.*, 2010) and may expose birds like red-legged partridge to a toxic dose of nitrates, with associated adverse effects on health (Ley 1986; Rodríguez-Estival *et al.*, 2010). Our results showed that a greater proportion of irrigated vineyards was associated with reduced red-legged partridge abundance at the landscape level and that this association was stronger in non-sensitive areas to nitrate contamination, where there are no limitations regarding the amount of fertilizer that can be applied to irrigation water (Order of 07/02/2011, JCCM). These patterns are consistent with a negative effect of nitrate exposure in intensive vineyards on red-legged partridge populations, and with experimental studies that showed adverse effects on nitrate water consumption on bird physiology and health (Rodríguez-Estival *et al.*, 2010).

Other types of management did not seem to affect red-legged partridge abundance. For example, we found no evidence that the availability of organically-grown crops was reflected in higher red-legged partridge densities. However, the relative importance of organic crops, in terms of percentage of agricultural surface, was very small (4.6% on average), and may not have been sufficient for us to detect significant associations. These results should be verified in future studies focused on the effect of organic crops (e.g. Moreau *et al.* 2021).

Beyond the spatial relationships with land-use observed in 2010, we also found a marked temporal variation in abundance, with a 51% decline of red-legged partridge abundance between 2017 and 2010 (8% – 74% considering best-case and worst-case scenarios). This is of particular concern given that this region holds one of the largest wild populations of red-legged partridge in the world (Blanco-Aguiar *et al.*, 2003; Blanco-Aguiar *et al.*, 2004) and is likely to be a good indicator of the red-legged partridge populations status globally. Our results also showed that the red-legged partridge population decline was not linear throughout the study period, but mainly occurred in two years (2013 and 2014) with periods of relative stability before and after. This suggests that the population may not have the capacity to recover from stochastic events leading to a few bad years in terms of poor survival or bad reproduction.

In line with the results on abundance, red-legged partridge trends were modulated by land-use changes: declines were more marked where the proportion of pasture (natural vegetation) had declined most, and where tree crops had increased most. As mentioned above, natural vegetation within the farmland landscape is extremely important for red-legged partridge and its reduction was associated with steeper declines. Probably the decrease in pastures implies a reduction of trophic resources and suitable breeding habitat for this species (Vickery *et al.*, 2002; Vickery *et al.*, 2009; Casas and Viñuela, 2010; Mcmahon *et al.*, 2010). Regarding the effect of organic tree crops, this could be indicative of the availability of any tree crop. This relationship could be the result of the loss of suitable habitat for the species, as areas dominated by tree crops seem to be negative for the species. A limitation on the amount of tree crops has been identified as a conservation measure within protected farmland areas for steppe birds (JCCM, 2017), and these results support this measure. The extent of the observed decline (-51% in seven years) is also consistent with knowledge on red-legged partridge exposure to current use agrochemicals, in particular to triazole fungicides that are routinely applied as cereal seed treatment during sowing in central Spain (Lopez-Antia *et al.*, 2016). Exposure to these fungicides during late winter has been shown by experiment to disrupt reproduction and reduce red-legged partridge productivity by half (Fernández-Vizcaíno *et al.*, 2020; Lopez-Antia *et al.*, 2021) and therefore have the potential to cause rapid population declines like those reported in this study.

The observed population decline of red-legged partridge confirms the delicate situation of this game bird species (BirdLife International, 2020) and has strong implications for its management. In Spain, millions of red-legged partridges (including both wild and farm-reared ones) are hunted each year (Andueza *et al.*, 2018) and red-legged partridge hunting generates a large economic turnover, of more than a billion euros annually (Garrido, 2012). Thus, this is not only a conservation issue for red-legged partridge but also a socio-economic issue. Even if hunting may not be the main cause of the decline, the observed decline highlight the need to adjust hunting pressure to the species abundance and dynamics in order to prevent additive effects. Scientists, public institutions, farmers, and hunting federations should work together to solve the red-legged partridge conservation issue. Currently, many hunting estates invest important efforts trying to increase the red-legged partridge populations in their estates for hunting purposes (Arroyo *et al.*, 2012; Arroyo *et al.*, 2017). They implement some measures potentially useful for wild red-legged partridge population recovery, such as predator control, the provision of supplementary food, water, and game crops (crops planted specifically for game that are not harvested), which might also be beneficial to other threatened species (Smith *et al.*, 2010; Estrada *et al.*, 2015; Cabodevilla *et al.*, 2020). But many of these estates also conduct massive releases of farm-reared red-legged partridges (Caro *et al.*, 2014; Cabodevilla *et al.*, 2020), which may be detrimental to wild populations (Villanúa *et al.*, 2008; Casas *et al.*, 2012; Díaz-Sánchez *et al.*, 2012) and can even increase the hunting pressure on the declining wild stocks (Casas *et al.*, 2016).

The observed marked population decline, which has been sometimes questioned (e.g. RFEC, 2020), highlights the need for a continued monitoring of this species. In that respect, our results also provided useful information regarding the species detectability that can be used to optimize future monitoring programs. Our results indicate that the best time to perform red-legged partridge surveys (within the time frame of our study) is in late April/early May or in July. The low detectability observed from mid-May to early July is probably due to both incubation behavior and the higher vegetation in crops at that time. In any case, this period should be avoided for assessing abundance (although it is an important time to perform assessments of hatching and thus breeding phenology, Guzmán *et al.*, 2020). Regarding the sampling hour, morning monitoring seemed to be more efficient in early spring (Jakob *et al.*, 2014), whereas detectability was higher in the hours before sunset in July, perhaps because of a higher activity of birds after the very hot afternoons. This should be taken into account when designing monitoring programs, although decisions would depend on whether sampling is planned for a specific time of the year or in a longitudinal pattern throughout the breeding season, like this one. One way or another, it will always be advisable to account for detectability during the modeling process (Jakob *et al.*, 2014; Kellner and Swihart, 2014) in order to obtain more reliable information on red-legged partridge abundance.

In conclusion, this study shows that farmland composition and farming practices explain variation in the abundance and trends of the red-legged partridge, an important farmland bird species. The results highlight the value of a mosaic agricultural landscape rich in natural vegetation for generalist farmland birds like red-legged partridges (Berg, 2002; Siriwardena *et al.*, 2012). A landscape capable of providing suitable habitats for breeding and foraging, but also offering a wide variety of plant structures for shelter and protection from predators and summer heat, especially after harvest. Our results also provide new evidence on a potential negative impact of fertigation, showing that the use of this practice might imply serious risks for birds. Besides, the marked population decline of this species reported in the last decade suggests that red-legged partridges might not be currently able to recover from negative effects of stochastic events. These results reflect the delicate situation of this species of strong socioeconomic and ecological importance and the fundamental role of agriculture in the population dynamics of farmland birds. Thus, to address the conservation of the red-legged partridge it seems necessary to rethink agricultural management, avoiding intensification and an excessive use of agrochemicals, restricting the expansion of tree crops, and promoting patches of natural or semi-natural vegetation.

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Supplementary material

Appendix A

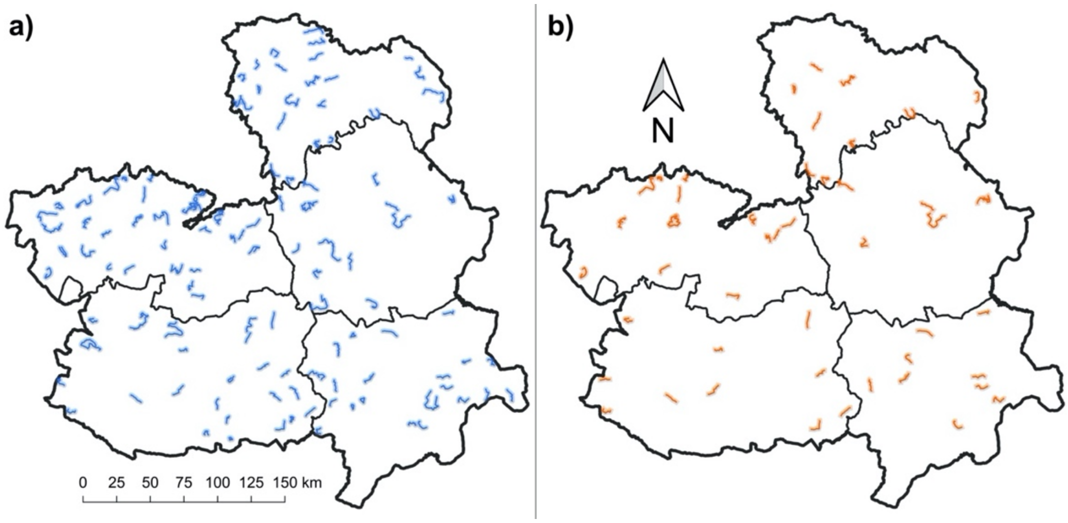

Figure A1. Map of the Castilla-La Mancha region showing the locations of transects used for the analyses of: a) red-legged partridge abundance in 2010 (n=121 transects; blue lines) and b) red-legged partridge density trends (n = 48 transects repeated at least twice between 2010 and 2017; orange lines).

Appendix B

Climatic, topographic, and habitat variables considered in the abundance model.

Highlighted in bold are those variables with VIF < 3 that were finally included in the abundance level of the model, as stated in Methods. The column named “Order” denotes the order by which variables were excluded with the VIF procedure until VIF < 3. The VIF procedure was performed separately for environmental (climate and topography) and habitat variables. VIF values of excluded variables are those that the variable had before being removed from the set of variables. VIF values in bold are those obtained with the final set of selected variables. VIF values were calculated with standardized variables. The variable “Nitrate sensitivity” was not considered in VIF as it was categorical.

Type of variable	Variable name	Code	VIF	Order
Final set selected (climate[2] and habitat[1,4,5] variables)	**Rain-fed tree crops (Olive groves and other woody crops)**	**RfTreeC**	**2.79**	
	Days with maximum temperature ≥25ºC in summer	**DTx25Sum**	**2.67**	
	Annual temperature range (=TJul - TJan) (ºC)	**TRan**	**2.34**	
	Shrubland	**Shrub**	**2.16**	
	Organic tree crops	**OrgTree**	**2.01**	
	Pastures	**Past**	**1.88**	
	Forests	**Forest**	**1.87**	
	Organic annual crops	**OrgAnnu**	**1.75**	
	Field margin density	**FMargin**	**1.72**	
	Total precipitation in autumn	**PAut**	**1.71**	
	Organic fallow and pastures	**OrgPast**	**1.70**	
	Rain-fed Vineyard	**RfVine**	**1.69**	
	Minimum temperature in winter (ºC)	**TnWin**	**1.68**	
	Irrigated Vineyard	**IVine**	**1.60**	
	Irrigated Arable land	**IArable**	**1.40**	
	Buildings	**Build**	**1.22**	
	Irrigated tree crops (Olive groves and other tree crops; almond or pistachio)	**ITreeC**	**1.17**	
	Nitrate sensitivity	**Nitrate**		
Habitat[1,4]	Rain-fed Herbaceous crops	RfArable	55.45	1
	Organic vineyard	OrgVine	3.16	2
Climate[2] and Topography[3]	Mean temperature in winter	TWin	3634.76	1
	Maximum temperature in summer	TxSum	1182.71	2
	Mean temperature in summer	TSum	906.84	3
	Mean temperature in spring	TSpr	585.49	4
	Mean temperature in autumn	TAut	420.91	5
	Maximum temperature in autumn	TxAut	131.37	6
	Maximum temperature in spring	TxSpr	67.05	7
	Maximum temperature in July	TxJul	42.21	8
	Days with maximum temperature ≥25ºC	DTx25	35.71	9
	Total precipitation in winter	PWin	13.45	10
	Maximum temperature in winter	TxWin	12.60	11
	Days with maximum temperature ≥25ºC in autumn	DTx25Aut	10.04	12
	Total precipitation in spring	PSpr	8.34	13
	Total precipitation in summer	PSum	6.64	14
	Days with maximum temperature ≥25ºC in spring	DTx25Spr	5.45	15
	Mean annual actual evapotranspiration (mm)	AET	4.32	16
	Mean altitude (m)	Alt	3.52	17

Sources: 1 SIOSE (2011), 2 Agencia Estatal de Meteorología (2011), 3 US Geological Survey (1996), 4 JCCM, 5 MITECO

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Appendix C

Candidate models tested during the abundance modelling process. In all tables, models are sorted by AIC. Best models are shaded in grey.

Table C1. Null models.

Abundance distribution	Detection function	AIC
Negative Binomial	Hazard rate	-907.73
Negative Binomial	Exponential	-245.25
Negative Binomial	Half normal	1815.45
Poisson	Hazard rate	1822.37
Poisson	Exponential	2621.83
Poisson	Half normal	4091.66

Table C2. Variables affecting detection (maintaining the negative binomial distribution and the hazard rate).

Hour	Day	Day^2	Hour:Date	$Hour:Date^2$	Habitat	AIC
x	x	x	x	x	x	-1119.19
x	x	x	x		x	-1114.17
x	x	x	x	x		-1105.08
x	x	x	x			-1100.08
x	x	x		x	x	-1098.03
x	x	x			x	-1097.79
x	x	x				-1082.72
	x	x				-1062.8
x	x				x	-960.44
	x				x	-944.65
x	x					-942.32
x					x	-935.73
	x					-924.58
x						-921.48
						-907.73

Table C3. Variables affecting abundance (maintaining the negative binomial distribution, the hazard rate, and detection variables of the best model in step 2). Variable codes as in Appendix B.

Model	AIC	PAut	TRan	TnWin	DTx25Sum	Build	RfVine	RfVine^2	IVine	IVine^2	Shrub	Shrub^2	FMargin	Past	Past^2	IArable	IArable^2	Forest	Forest^2	RfTreeC	RfTreeC^2	ITreeC	ITreeC^2	OrgAnnu	OrgTree	OrgPast	Nitrate	IVine:Nitrate	IHerb:Nitrate	ITreeC:Nitrate	OrgTree:DOlive
mod20	-	x		x			x		x					x						x							x	x			
mod24	-	x		x			x		x		x			x											x		x	x			
mod28	-	x		x			x		x					x											x		x	x			
mod25	-	x		x			x		x		x			x											x						
mod23	-	x		x			x		x		x									x											
mod21	-1152.3	x		x			x		x					x						x							x				
mod26	-1151.65	x		x			x		x		x														x		x	x			
mod27	-	x		x			x		x		x			x						x					x		x	x			
mod29	-	x		x			x		x		x			x													x	x			
mod22	-	x		x			x		x											x							x	x			
mod18	-	x		x			x		x					x		x				x							x	x			
mod17	-	x		x			x		x					x	x	x	x			x							x	x			
mod16	-	x	x	x			x		x					x	x	x	x			x							x	x			
mod19	-	x		x			x		x					x		x				x							x	x	x		
mod15	-	x	x	x			x		x		x			x	x	x	x			x							x	x			
climat	-	x	x	x	x																										
mod13	-	x	x	x			x		x		x			x	x	x	x	x		x					x		x	x			
mod12	-	x	x	x			x		x		x			x	x	x	x	x		x					x	x	x	x			
mod14	-1144.65	x	x	x			x		x		x			x	x	x	x	x		x					x		x	x			x
mod11	-	x	x	x			x		x		x			x	x	x	x	x	x	x					x	x	x	x			
mod10	-	x	x	x			x		x		x			x	x	x	x	x	x	x					x	x	x				
mod9	-1140.3	x	x	x	x		x		x		x			x	x	x	x	x	x	x					x	x	x				
mod7	-	x	x	x	x		x		x	x	x			x	x	x	x	x	x	x					x	x	x				
mod8	-	x	x	x	x		x		x		x			x	x	x		x	x	x					x	x	x				
mod5	-	x	x	x	x		x		x	x	x			x	x	x	x	x	x	x		x			x	x	x				
mod6	-1136.86	x	x	x	x		x		x	x	x			x	x	x	x	x	x	x		x			x	x	x			x	
mod4	-1136.42	x	x	x	x		x		x	x	x			x	x	x	x	x	x	x		x		x	x	x	x				
mod3	-	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x		x		x	x	x	x				
mod2	-	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x		x	x	x	x	x	x				
mod1	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x				
full	-1124.81	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
eco	-1119.28																							x	x	x	x				
null	-																														
habitat	-					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				

Appendix D

Estimates of the best model on red-legged partridge abundance and of the other six models with an AIC value within 2 points of the best model. Variable codes as in Appendix B.

Table D1. Estimates of variables affecting detectability

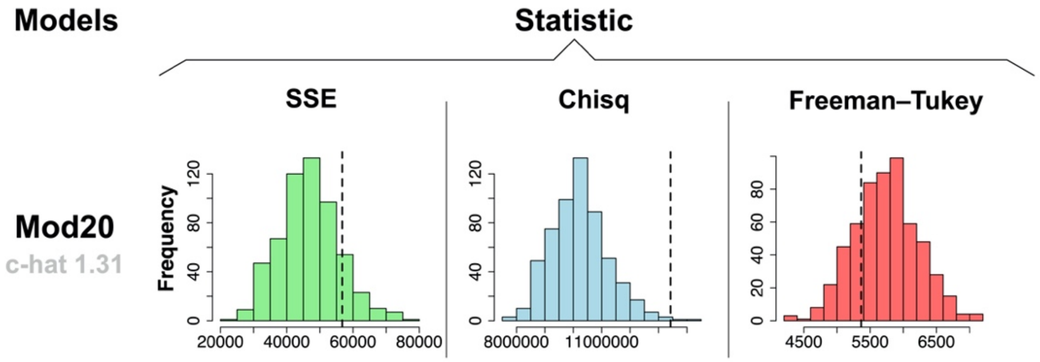
Model	AIC		Intercept	Hour Midday	Hour Evening	Date	Date^2	Habitat_I	Habitat_C	Midday:Date	Evening:Date	Midday:Date^2	Evening:Date^2
Mod20	-1152.986	**Estimate**	2.365	-0.028	0.144	-0.134	0.135	-0.117	-0.141	0.142	0.168	-0.226	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	78.02	-0.17	3.03	-10.83	11.69	-3.48	-2.32	1.20	4.84	-1.77	-2.41
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod24	-1152.967	**Estimate**	2.365	-0.029	0.144	-0.134	0.135	-0.119	-0.146	0.142	0.168	-0.227	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	77.99	-0.17	3.02	-10.85	11.7	-2.37	-3.54	1.19	4.83	-1.76	-2.39
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod28	-1152.815	**Estimate**	2.365	-0.029	0.144	-0.134	0.135	-0.117	-0.146	0.142	0.168	-0.227	-0.072
		(±SE)	(±0.03)	(±0172)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	78.03	-0.17	3.02	-10.84	11.69	-2.22	-3.48	1.20	4.83	-1.77	-2.39
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod25	-1152.655	**Estimate**	2.365	-0.029	0.144	-0.134	0.135	-0.120	-0.146	0.142	0.169	-0.226	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	77.98	-0.17	3.02	-10.86	11.71	-2.37	-3.56	1.19	4.85	-1.76	-2.40
		P	**<0.001**	0.86	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod23	-1152.395	**Estimate**	2.364	-0.029	0.145	-0.134	0.135	-0.118	-0.148	0.142	0.169	-0.226	-0.073
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	77.93	-0.17	3.03	-10.86	11.71	-2.40	-3.50	1.20	4.87	-1.76	-2.42
		P	**<0.001**	0.86	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod21	-1152.3	**Estimate**	2.365	-0.028	0.144	-0.134	0.135	-0.119	-0.141	0.142	0.169	-0.226	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	78.01	-0.17	3.03	-10.84	11.70	-2.32	-3.54	1.20	4.86	-1.77	-2.42
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod26	-1151.65	**Estimate**	2.364	-0.029	0.144	-0.134	0.135	-0.116	-0.144	0.142	0.168	-0.227	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	77.93	-0.17	3.02	-10.86	11.70	-2.34	-3.46	1.20	4.84	-1.77	-2.39
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod27	-1151.553	**Estimate**	2.365	-0.029	0.144	-0.134	0.135	-0.119	-0.148	0.142	0.168	-0.227	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	77.99	-0.17	3.02	-10.85	11.70	-2.40	-3.52	1.19	4.84	-1.77	-2.40
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod29	-1151.477	**Estimate**	2.365	-0.029	0.144	-0.134	0.135	-0.121	-0.148	0.141	0.168	-0.226	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	78.01	-0.17	3.02	-10.82	11.68	-2.41	-3.58	1.19	4.82	-1.76	-2.39
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod22	-1151.107	**Estimate**	2.364	-0.029	0.145	-0.134	0.135	-0.116	-0.138	0.142	0.169	-0.227	-0.073
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	77.96	-0.17	3.04	-10.85	11.70	-2.26	-3.45	1.20	4.87	-1.77	-2.42
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**
Mod18	-1151.021	**Estimate**	2.365	-0.028	0.144	-0.134	0.135	-0.117	-0.142	0.142	0.168	-0.227	-0.072
		(±SE)	(±0.03)	(±0.17)	(±0.05)	(±0.01)	(±0.01)	(±0.03)	(±0.06)	(±0.12)	(±0.03)	(±0.13)	(±0.03)
		Z-ratio	78.02	-0.17	3.03	-10.83	11.69	-2.33	-3.48	1.20	4.84	-1.77	-2.41
		P	**<0.001**	0.87	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.05**	0.23	**<0.001**	0.08	**<0.05**

Table D2. Estimates of variables affecting abundance

Model		Intercept	PAut	TnWin	RfVine	IVine	Shrub	Past	RfTreeC	IArable	OrgTree	Nitrate	IVine:Nitrate
Mod20	**Estimate**	-2.442	-0.555	0.359	0.178	-0.429		0.189	-0.177			0.159	0.334
	(±SE)	(±0.13)	(±0.10)	(±0.09)	(±0.09)	(±0.17)		(±0.10)	(±0.09)			(±0.17)	(±0.19)
	Z-ratio	-19.00	-5.41	3.92	1.96	-2.48		1.87	-2.03			0.93	1.77
	P	**<0.001**	**<0.001**	**<0.001**	**<0.05**	**<0.05**		0.06	**<0.05**			0.35	0.08
Mod24	**Estimate**	-2.465	-0.572	0.359	0.193	-0.443	0.135	0.182			-0.166	0.184	0.399
	(±SE)	(±0.13)	(±0.10)	(±0.09)	(±0.09)	(±0.17)	(±0.09)	(±0.10)			(±0.08)	(±0.17)	(±0.19)
	Z-ratio	-19.44	-5.49	3.93	2.12	-2.58	1.43	1.80			-2.02	1.09	2.08
	P	**<0.001**	**<0.001**	**<0.001**	**<0.05**	**<0.05**	0.15	0.07			**<0.05**	0.27	**<0.05**
Mod28	**Estimate**	-2.461	-0.538	0.348	0.163	-0.402		0.213			-0.170	0.189	0.356
	(±SE)	(±0.13)	(±0.10)	(±0.09)	(±0.09)	(±0.17)		(±0.10)			(±0.08)	(±0.17)	(±0.19)
	Z-ratio	-19.24	-5.23	3.81	1.83	-2.35		2.10			-2.06	1.12	1.87
	P	**<0.001**	**<0.001**	**<0.001**	0.06	**<0.05**		**<0.05**			**<0.05**	0.27	0.06
Mod25	**Estimate**	-2.316	-0.570	0.361	0.163	-0.096	0.115	0.170			-0.154		
	(±SE)	(±0.08)	(±0.10)	(±0.09)	(±0.09)	(±0.08)	(±0.09)	(±0.10)			(±0.08)		
	Z-ratio	-28.58	-5.44	3.99	1.92	-1.15	1.23	1.67			-1.86		
	P	**<0.001**	**<0.001**	**<0.001**	0.06	0.25	0.22	0.10			0.06		
Mod23	**Estimate**	--2.310	-0.521	0.381	0.167	-0.157	0.119		-0.172				
	(±SE)	(±0.08)	(±0.10)	(±0.09)	(±0.09)	(±0.08)	(±0.09)		(±0.09)				
	Z-ratio	-28.29	-5.35	4.19	1.89	-1.89	1.26		-1.95				
	P	**<0.001**	**<0.001**	**<0.001**	0.06	0.06	0.21		0.05				
Mod21	**Estimate**	-2.390	-0.550	0.350	0.142	-0.146		0.179	-0.164			0.128	
	(±SE)	(±0.13)	(±0.10)	(±0.09)	(±0.09)	(±0.08)		(±0.10)	(±0.09)			(±0.18)	
	Z-ratio	-18.37	-5.33	3.81	1.65	-1.72		1.78	-1.87			0.72	
	P	**<0.001**	**<0.001**	**<0.001**	0.10	0.08		0.08	0.06			0.47	
Mod26	**Estimate**	-2.444	-0.501	0.374	0.184	-0.447	0.168				-0.167	0.164	0.383
	(±SE)	(±0.13)	(±0.10)	(±0.09)	(±0.09)	(±0.17)	(±0.10)				(±0.08)	(±0.17)	(±0.19)
	Z-ratio	-19.03	-5.20	4.04	1.96	-2.56	1.77				-2.02	0.96	1.98
	P	**<0.001**	**<0.001**	**<0.001**	**<0.05**	**<0.05**	0.08				**<0.05**	0.34	**<0.05**
Mod27	**Estimate**	-2.455	-0.580	0.365	0.202	-0.459	0.123	0.173	-0.088		-0.113	0.164	0.395
	(±SE)	(±0.13)	(±0.10)	(±0.09)	(±0.09)	(±0.17)	(±0.10)	(±0.10)	(±0.11)		(±0.11)	(±0.17)	(±0.19)
	Z-ratio	-19.27	-5.57	3.98	2.19	-2.65	1.29	1.72	-0.78		-1.05	0.97	2.07
	P	**<0.001**	**<0.001**	**<0.001**	**<0.05**	**<0.01**	0.20	0.09	0.44		0.29	0.33	**<0.05**
Mod29	**Estimate**	-2.466	-0.567	0.350	0.173	-0.413	0.139	0.183				0.213	0.345
	(±SE)	(±0.13)	(±0.11)	(±0.09)	(±0.09)	(±0.17)	(±0.09)	(±0.10)				(±0.17)	(±0.19)
	Z-ratio	-19.26	-5.35	3.83	1.91	-2.39	1.47	1.79				1.26	1.80
	P	**<0.001**	**<0.001**	**<0.001**	0.06	**<0.05**	0.14	0.07				0.21	0.07
Mod22	**Estimate**	-2.371	-0.476	0.363	0.132	-0.166			-0.183			0.109	
	(±SE)	(±0.13)	(±0.09)	(±0.09)	(±0.09)	(±0.08)			(±0.09)			(±0.18)	
	Z-ratio	-17.98	-5.02	3.91	1.48	-1.98			-2.08			0.61	
	P	**<0.001**	**<0.001**	**<0.001**	0.14	**<0.05**			**<0.05**			0.55	
Mod18	**Estimate**	-2.445	-0.555	0.358	0.175	-0.429		0.186	-0.179	-0.016		0.163	0.341
	(±SE)	(±0.13)	(±0.10)	(±0.09)	(±0.09)	(±0.17)		(±0.10)	(±0.09)	(±0.08)		(±0.17)	(±0.19)
	Z-ratio	-18.95	-5.41	3.91	1.91	-2.48		1.81	-2.03	-0.18		0.95	1.76
	P	**<0.001**	**<0.001**	**<0.001**	0.06	**<0.05**		0.07	**<0.05**	0.85		0.34	0.09

Appendix E

Goodness of fit of the best abundance model of 2010



Appendix F

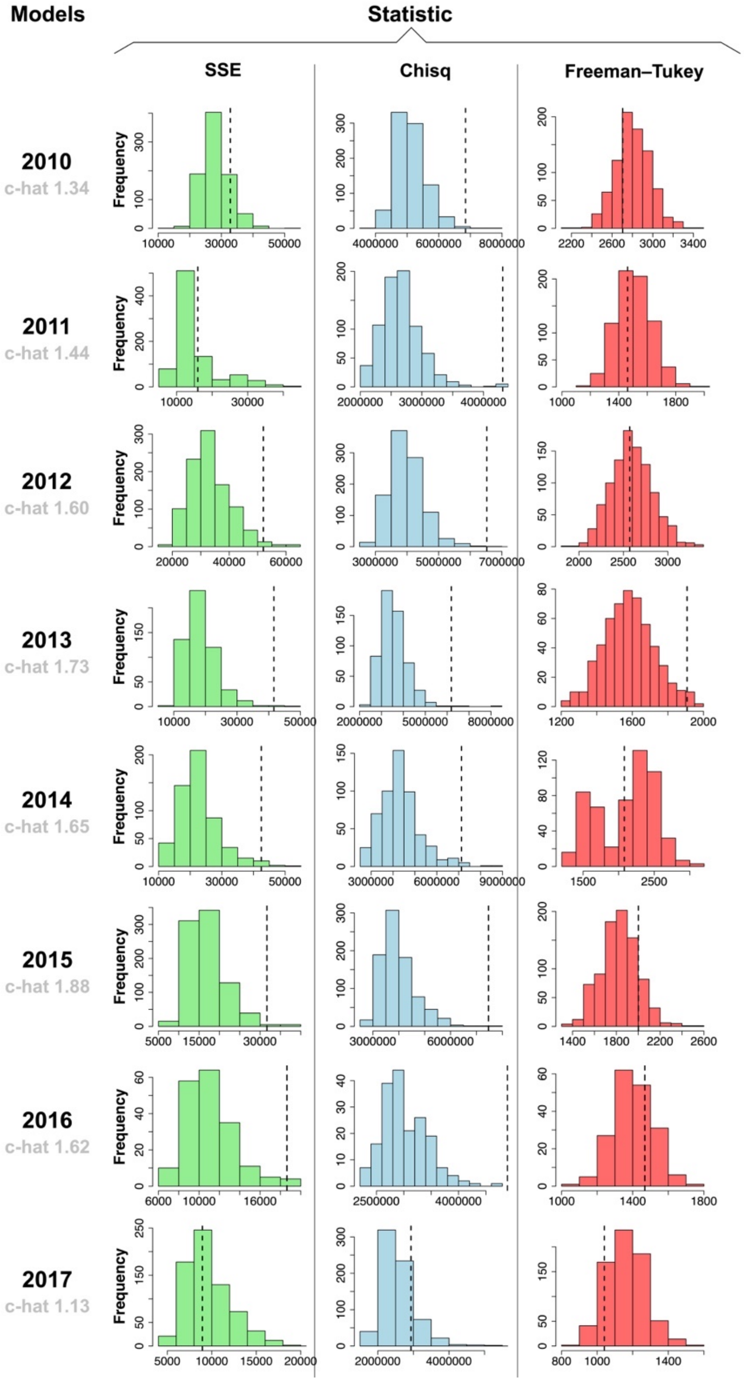
Land-use variables considered in the abundance trend model.

Highlighted in bold are those variables with VIF < 3 that were finally included in the abundance trend models, as stated in Methods. VIF values of excluded variables are those that the variable had before being removed from the set of variables. VIF values in bold are those obtained with the final set of selected variables. VIF values were calculated with standardized variables.

Variable name	CORINE code	Code	VIF
Change in shrublands	**323 + 324**	**ShrubChg**	**1.547**
Change in pastures	**231 + 321**	**PastChg**	**1.527**
Change in vineyards	**221**	**VineChg**	**1.465**
Change in organic vineyards		**OrgVineChg**	**1.342**
Change in tree crops	**222+223**	**TreeChg**	**1.339**
Change in organic fallow and pastures		**OrgPastChg**	**1.309**
Change in organic annual crops		**OrgAnnuChg**	**1.180**
Change in organic tree crops		**OrgTreeChg**	**1.100**
Change in urban areas	**111 + 112 + 121 + 122 + 123 + 124 + 131 + 132 + 133**	**UrbChg**	**1.037**
Change in mosaic farmland areas	243 + 242	MosaicChg	20.680
Change in arable land	211	HerbChg	12.883

Appendix G

Goodness of fit of the models for each year for trends analyses



3

LURZORUAREN ERABILEREN ETA NEKAZARITZA-JARDUEREN ERAGINAK

I. ARTIKULUA

Ureztatzeak nekazal inguruneko hegazti-komunitateen gainbehera eragiten du

- **Laburpena**
- **Sarrera**
- **Eztabaida**

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Ureztatzeak nekazal inguruneko hegazti-komunitateen gainbehera eragiten du

Laburpena

Nekazaritza-lurra kontserbatzeko kudeaketa-plan eraginkorrak egiteko, funtsezkoa da nekazaritzaren intentsifikazioak biodibertsitatean dituen ondorioak ebaluatzea. Ureztatzeak fauna basatian duen eragina ez da oraindik sakonki aztertu, XX. mendearen erdialdetik nekazaritza-lur ureztatuen azalera asko handitu den arren. Gaur egun, lur ureztatuek 300 milioi hektarea baino gehiago hartzen dituzte mundu osoan. Hemen, ureztatzeak hegazti-espezieen agerpenean duen eragina ebaluatu dugu, BACI (Before-After Control-Impact) diseinuko eredu bat erabiliz. Gure ikerlana lehorreko nekazaritza zuen 100 km2-ko eremuan egiten zen, Espainiako iparraldeko eskualde mediterraneo batean. Eskualde horretako 47 hegazti-espezie ohikoenek osatutako 13 urteko datu-multzo bat aztertzen genuen, eta, horretarako, gertaera-eredu multiespezifiko hierarkiko bat erabili genuen. Ureztatze-sistemak tokiko hegaztien komunitatea nola aldatu zuen aztertu genuen, eta tokiko ekosisteman izandako aldaketek espezieen agerpenean zuen eragina identifikatu genuen, bai negatiboa zein positiboa izan. Ureztatzeak oro har hegaztien komunitatean eragin negatiboa izan zuen. Espezie gehienen agerpen-tasak jaitsi egin ziren (% 55,3), eta espezie gutxirentzat, berriz, igo egin zen (% 10,6); horrek, beraz, leku-mailan espezieen aberastasuna murriztea ekarri zuen. Ureztatzea nekazal inguruneko hegaztientzat kaltegarriagoa izan zen (kontserbaziorako interes handiko estepetako hegaztiak barne), baina baita lehorreko nekazaritza-inguruneetan maiz gertatzen diren baso-inguruneko hegaztientzat, sastrakadi-inguruneko hegaztientzat eta espezie ez-espezializatuentzat ere. Erantzun positiboa espezie gutxi batzuek bakarrik izateak iradokitzen du, epe luzera, ureztatzeak tokiko hegazti-komunitateetan aldaketa negatibo nabarmenak eragin ditzakeela, zeinek dibertsitate txikiagoa izango zuen, garrantzi ekologikoko espezieen galerarekin. Ziurrenik, ureztatzeak hegaztien presentzian duen eragin negatiboa lotuta dago laboreak aldatzearekin eta/edo lugorriak galtzearekin, habiak egiteko lekuak eta dietaren eskuragarritasuna murriztea eragiten baitu. Beraz, ureztatze sistema berriak kontu handiz ezarri behar dira, espezie ugari edo espezie mehatxatuen dentsitate handiak dituzten eremuak saihestuz. Ureztatzea ezartzea saihestu ezin den kasuetan, nekazaritza-sistema heterogeneoak sustatzea, monolaborantzak saihesteak eta lehorreko laboreak eta tartekatutako lugorriak mantentzeak lagun dezakete ureztatzearen eragin kaltegarriak arintzen tokiko hegazti-komunitateetan eta horien ekosistemetan

Gako-hitzak

BACI diseinua; biodibertsitatearen kontserbazioa; estepetako hegaztiak; gertaera-eredu multiespezifikoa; hegazti-komunitatea; nekazaritzaren intentsifikazioa

1. Sarrera

Jarduera antropogenikoek paisaia naturaletan eta antropizatuetan inpaktu ugari eragiten dituzte, eta horiek fauna basatian eragin zuzenak eta zeharkakoak izan ditzakete (Pimm *et al*., 1995; Hooke *et al*., 2012). Nekazaritza oso hedatuta dago mundu osoan zehar, eta, neurri handi batean, habitatetan, baliabideetan eta uretan eragina dauka, eta, beraz, baita tokiko biodibertsitatean ere (Matson *et al*., 1997; Pain eta Pienkowski, 1997; Benton *et al*., 2003). Azken hamarkadetan, nekazaritza intentsifikatu egin da, lursailen tamaina handituz, errendimendu handiko laboreak eta mekanizazioa erabiliz eta ureztatze, ongarri eta pestiziden erabilera areagotuz (Matson *et al*., 1997). Oro har, nekazaritzaren intentsifikazio-neurriek ondorio kaltegarriak izan ditzakete tokiko landareen eta animalien populazioen ugaritasunean eta espezieen aberastasunean (Pain eta Pienkowski, 1997; Stoate *et al*., 2001; Benton *et al*., 2002; Donald *et al*., 2006; Storkey *et al*., 2012). Hala ere, neurri horietako bakoitzaren eragin erlatiboa bereiztea zaila izan daiteke, efektuek sarritan elkarri eragiten baitiote.

Ureztapenaren ezarpena, bereziki, aurrerapen handia izan da nekazaritza-produktibitaterako (Alauddin eta Quiggin, 2008), baina tokiko ekosistemak nabarmen aldatzea ere eragiten du (Baldock *et al*., 2000; Stoate *et al*., 2001). Nekazaritza-lur ureztatuen azalera izugarri handitu da azken 50 urteetan (Matson *et al*., 1997; Alauddin eta Quiggin, 2008), eta, gaur egun, 300 milioi hektarea baino gehiago hartu ditu mundu osoan zehar (FAO, 2011). Horrek, ongarrien erabilera handiagotzearekin batera, laboreen errendimendua izugarri bultzatu du (Matson *et al*., 1997), eta, oro har, lugorrien beharra gutxitu egin da (Baldock *et al*., 1994). Hala ere, lugorriek habitat egokia eskaintzen dute paisaia antropizatuetako fauna basati askorentzat (Denys eta Tscharntke, 2002; Schmidt eta Tscharntke, 2005; Vickery *et al*., 2004; Ng *et al*., 2017; Traba eta Morales, 2019). Gainera, ongarriak ureztatzeko sistemen bidez (fertirrigazioa) banatzen direnean, nitratoen kontzentrazioa arriskutsua izan daiteke ureztatzeko ura edaten duten animalientzat (Rodríguez-Estival *et al*., 2010). Ureztatzeak lurzoruaren propietateetan ere eragina izan dezake, gazitzearen eta urpetzearen ondorioz (Matson *et al*., 1997; Baldock *et al*., 2000), eta horrek ere espezie basatien banaketa eta ugaritasunean eragin handia izan dezake.

Mediterraneoko biometan, ureztapena ezartzeak lehorreko zerealen nekazaritza-sistemen murrizketa eragin du nagusiki (Bignal eta McCracken, 1996; Baldock *et al*., 2000; Caraveli, 2000). Sistema horiek lugorri proportzio esanguratsua izaten dute, zeina nekazal inguruneko hegaztientzat habitat aproposa den (Brotons *et al.*, 2004; Sanderson *et al.*, 2013), estepetako hegazti mehatxatuak barne (Sanderson *et al.*, 2013; García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020); horietako batzuek habia egiteko toki aproposa aurkitzen dute habitat erdi-natural honetan (Benton *et al.*, 2003, Traba eta Morales, 2019). Azken hamarkadetan, eskualde mediterraneoko ureztatutako lur-azalera nabarmen hedatu da. Adibidez, Espainian 1973tik 1,4 milioi hektarea berri ureztatu dira (Baldock *et al.*, 1994). Aldi berean, lugorri utzitako lurraren proportzioa >% 50etik <% 40ra jaitsi zen 1973 eta 1990 bitartean (Baldock *et al.*, 1994), eta azkenengo bi hamarkadetan beste % 16-40ko murrizketa jasan du (Traba eta Morales, 2019). Grezia, Italia eta Portugalen, antzeko joerak dokumentatu dira (Caraveli, 2000), eta iradokitzen du ureztatzeak paisaia mediterraneoetan aldaketa handiak eragiten dituela.

Ureztatzeak, nekazaritza-ekoizpenerako oso garrantzitsua bada ere, aldaketa esanguratsuak eragin ditzake nekazaritza-inguruneetan (Baldock *et al.*, 2000). Lehorreko laborantzako lurretan ureztapena ezartzeak eta ekosistema erdi-naturalak galtzeak eragin zuzena izan dezake biodibertsitatean, espezie batzuei kalte eginez eta beste batzuei mesede eginez (Baraibar *et al.*, 2009; González-Estébanez *et al.*, 2011; Andrey *et al.*, 2014; Pérez-Fuertes *et al.*, 2015). Nekazal inguruneko hegaztien kasuan ere, nekazaritzaren intentsifikazioak gehien kaltetzen duen taldean (Donald *et al.*, 2001; Brennan eta Kuvlesky, 2005; Donald *et al.*, 2006), beraz, ureztatzearen ondorioak espeziaren araberakoak dira, espezie batzuek negatiboki erantzuten baitute eta beste batzuk ondo egokitzen baitira. Estepako hegaztiek eta tamaina ertaineko beste hegazti batzuek ureztatze-lurrak saihesteko joera dute (Brotons *et al.*, 2004; De Frutos *et al.*, 2015), eta jakina da beste espezie batzuek (adibidez, mirotzek *Circus* spp. eta zikoinak *Ciconia ciconia*) ureztatutako laboreak erabiltzen dituztela (Cardador *et al.*, 2011; De Frutos *et al.*, 2015; Torres-Orozco *et al.*, 2016). Lehorreko laborantza-lurrekin alderatuta, ureztatutako lurrek harrapakin mota jakin batzuen kantitate handiak izan ditzakete (adibidez, lursaguak; Jareño *et al.*, 2015) eta/edo landaredi trinkoagoa, espezie batzuek habia jartzeko nahiago dutena (Cardador *et al.*, 2011; Torres-Orozco *et al.*, 2016). Hala ere, ureztatzeak hegaztietan duen eragina aztertu duten ikerketak espezie bakarrean edo espezie talde txikietan zentratu dira, eta oraindik ez da ikerketarik egin ureztatzeak komunitate-mailan dituen ondorioak aztertzen duenik, nekazal inguruneko hegaztietan eta nekazaritza-ingurunean aritzen diren beste hegazti batzuetan. Informazio hori funtsezkoa litzateke hegazti-komunitateen kontserbazioa kudeatzeko plan egoki bat garatzeko, bereziki, ureztatutako lurren azalera handitzen jarraitzea aurreikusten delako (Matson *et al.*, 1997; Alauddin eta Quiggin, 2008; FAO, 2011).

Ikerketa honetan, BACI diseinuko eredu bat erabiliz (Before-After Control-Impact; Popescu *et al.*, 2012), ureztatzeak hegazti-komunitate baten espezieen agerpenean duen eragina balioesten dugu. Espainiako iparraldeko eskualde mediterraneo bateko 47 espezie arruntenen gaineko ureztatzearen eragina aztertu dugu, 13 urteko datu-multzoa erabiliz. Ikerketaren diseinuak hiru testuinguru hartzen ditu barne: laginketaren bigarren urtetik aurrera ureztatzen hasi ziren tokiak (n = 9), laginketaren hamargarren urtetik aurrera ureztatzen hasi ziren tokiak (n = 8), eta inoiz ureztatu ez ziren tokiak (n = 2). Gure hipotesia zen ureztatzearen aplikazioak ondorio gogorrak izango zituela hegazti-komunitatean, eta nekazal inguruneko espezie askotan edo gehienetan eragin negatiboak izango zituela. Hala ere, ureztatzeari espezie batzuek positiboki erantzungo ziotela espero genuen. Beraz, hegazti-komunitatean egitura-aldaketak gertatuko zirela espero genuen, eta aldaketa horiek kontserbazioan eta kudeaketan ondorioak izan zitzaketela.

4. Eztabaida

Gure emaitzek erakusten dute ureztatzeak eragin argia duela hegazti-komunitatearen egituran, gutxienez Mediterraneoko inguruneetan; izan ere, espezie gehienen (% 55) agertzeko probabilitatea murriztu egin zen, eta gutxi batzuek (% 11) erantzun positiboa izan zuten. Horrek, leku-mailan, espezieen aberastasunaren murrizketa orokorra ekarri zuen. Gainera, espezie-taldeen artean, nekazal inguruneko hegaztiek, horietako asko lehorreko hegaztiak izanik (Benton *et al.*, 2003, Traba eta Morales, 2019), erantzun negatibo nabarmena erakutsi zuten, eta kontserbazio-balio handiko estepako bi hegaztietan eragina oso negatiboa izan zen (Madroño *et al.*, 2004; BirdLife International, 2015; 254/2019 Foru Dekretua). Beste espezie talde batzuek ere, sastraka-inguruneko espezieak, baso-inguruneko espezieak eta espezie espezializatuak, eragin negatiboa jasan zuten, eta horrek iradokitzen du ureztatzearen ondorioak uste baino orokorragoak izan daitezkeela. Hegazti-espezieen agerpen-probabilitatean aurkitutako aldaketa handiek agerian uzten dute ureztatzeak hegazti-komunitateetan aldaketa garrantzitsuak eragin ditzakeela, ureztatutako nekazaritza-lurretako hegazti-komunitateak homogeneoagoak bilakatuz.

Ureztatze-sistemak ezartzeak, nekazaritza-lurrei ura gehitzea ez ezik, labore alternatiboak erabiltzea, monolaborantzak areagotzea eta lugorriaren murrizketa dakartza, eta horrek guztiak eragina eduki dezake tokiko hegazti-komunitateetan. Ureztatzeak gehien kaltetu zituen espezieak nekazal ingurunekoak izan ziren, seguruenik ureztatzeak habiak egiteko habitatean eragin zuzena duelako (hau da, zereal- eta lugorri-zelaien eskuragarritasunean; Benton *et al.*, 2003, Traba eta Morales, 2019). Izan ere, nekazal inguruneko hegazti espezie askok arto-soroak saihesten dituzte (Laiolo, 2005), eta hori maiz landatzen da Espainiako nekazaritza-lur ureztatuetan, garia eta garagarra landatzearen alternatiba gisa. Gainera, ureztatzeak nekazaritzaren intentsifikazioa areagotzea eta homogeneizazioa ekarri ohi du, nekazal inguruneko hegaztien populazioentzat kaltegarria izan daitekeena (Baldock *et al.*, 1994; Delgado eta Moreira, 2000; Vickery *et al.*, 2004; Traba eta Morales, 2019). Sastraka-inguruneko eta baso-inguruneko zenbait hegaztiren eta espezie ez-espezializatu batzuen agertzeko probabilitatea ere murriztu egin zen, horiek nekazaritza-lurretan habia ez egin arren. Ureztatzeak espezie horietan eragin negatiboa izatearen arrazoia argi ez egon arren, ureztatzeak nekazaritza-inguruneko erakargarritasuna murriztu dezake, bai baliabide trofiko batzuen eskuragarritasuna murriztuz, bai landarediaren egitura aldatuz. Bestalde, beste espezie batzuek lur ureztatuetan baliabide gehiago aurki ditzakete. Ureztatzeari positiboki erantzun zioten bost espezieetatik lau intsektujaleak dira (buztanikara zuria, enara azpizuria, enara arrunta eta txinbo burubeltza). Baliteke espezie horiek intsektu-dentsitate handiagoak aurkitu izana ureztatutako lurretan (Keiser *et al.*, 2005; Jaleta *et al.*, 2013). Hala ere, ureztatzea espezie batzuentzat positiboa izan arren, horren eragin orokorra espezieen aberastasunaren galera izan zen. Horrela, ureztatzearen zuzeneko eta zeharkako ondorioek tokiko hegazti-komunitatean eragin negatiboa izan zuten batez ere, nekazal inguruneko hegazti espezietatik harago.

Fauna-komunitateetan inpaktu negatiboa izateko potentzial handia duenez, ureztatze berriak ezarri nahi direnean, horien eragina arintzeko aukerak ondo ebaluatu behar dira, batez ere arriskuan dauden espezieak kontuan izanik. Arriskuan dauden nekazal inguruneko espezieen kontserbazioa bermatzeko, kudeaketa-ekintzak eta -politikak konbinatzea beharrezkoa da, ureztatzearen eragina konpentsatzeko edo, gutxienez, mugatzeko. Ureztatze-planak kontuz aplikatu behar dira, gutxienez Europar Batasunak izendatutako Hegaztientzako Babes Bereziko Eremuak edo Europatik kanpo antzeko kontserbazio-balioa duten zonaldeak saihestuz. Era berean, lugorria erabiltzen duen lehorreko nekazaritza tradizionala baloratu eta aktiboki sustatu beharko litzateke, sistema horiek hegazti askorentzat habitat paregabea eskaintzen baitute. Hori kontuan eduki behako litzateke Europako Nekazaritza Politika Bateratua (NPB) ekologikoago bihurtzeko egiten ari diren eztabaidetan. Ureztatzea aplikatzen denean, lehorreko laboreak eta lugorriak mantentzeak, labore aldaketak eta monolaborantzak saihesteak hegazti espezie ezberdinentzako baliabideak dituen paisaia heterogeneo bat sustatu dezake (Berg, 2002; Benton *et al.*, 2003; Siriwardena *et al.*, 2012). Lugorria habitat erabakigarria da nekazal inguruneko hegaztien kontserbaziorako (Sanz-Pérez *et al.*, 2019), hainbat espeziek elikagaia, ugal-tokia eta habia egiteko tokia aurkitzen baitute bertan (McMahon *et al.*, 2010; Schmidt *et al.*, 2017; Traba eta Morales, 2019; Tarjuelo *et al.*, 2020). Beraz, litekeena da mosaiko-egitura duen nekazaritza-paisaia batek, lugorriak ureztatze-eremuen artean sakabanatuta daudelarik, ureztatzeak hegaztien komunitatean duen eragin negatiboaren zati bat murriztu ahal izatea. Hala ere, horri buruz eta aldaketak eragiten dituzten mekanismo espezifikoei buruz ikertzen jarraitzea beharrezkoa da.

Gure emaitzek erakusten dute ureztatzeak hegazti-komunitateetan duen eragin negatiboa nekazal inguruneko espezieetatik harago doala. Ureztatzearen inplementazioa areagotzen ari da mundu osoan, labore-lurren produktibitatea handitzeko lasterketa batean (FAO, 2011). Ureztatzeak laboreen ekoizpenean eragin positiboa izan dezakeen arren, aldi berean, ondorio negatiboak izan ditzake tokiko hegazti-komunitateetan, horrek, aldi berean, eragina izan dezake beste taxon batzuen biodibertsitatean eta, oro har, prozesu ekologikoetan. Nekazaritza-politikek bermatu behar dute elikagaien ekoizpena bateragarria dela espezieen kontserbazioarekin eta tokiko ekosistemen zerbitzuen babesarekin.

Esker onak

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II. ARTIKULUA

Mahastien modernizazioak aldaketak sustatzen ditu lehorreko nekazal inguruneko hegazti eta ugaztunen presentzian

- **Laburpena**
- **Sarrera**
- **Eztabaida**

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Mahastien modernizazioak aldaketak sustatzen ditu lehorreko nekazal inguruneko hegazti eta ugaztunen presentzian

Laburpena

Nekazaritzaren intentsifikazio azkarrak eragina dauka labore-sistema askotan. Azken hamarkadetan, Espainian (munduan mahasti gehien dituen herrialdean) mahasti tradizionalen ordez (baso-sistema) beso-sistemako mahastiak oso azkar ari dira zabaltzen, uztaren kostuak murriztu eta mahatsondoaren produktibitatea handitzeko ahaleginean. Baina mahastien modernizazio horrek laborantza-lurren biodibertsitatean dituen ondorioak oso ezezagunak dira oraindik. Ikerketa honetan, ekainetik irailera bitartean, Espainiako hego-mendebaldeko 52 mahasti aztertu genituen (26 tradizionalak eta 26 beso-sistemakoak), baso-sistemako mahasti tradizionalen modernizazioak mahatsondoaren egituran eta maneiuan duen eragina deskribatzeko, bai eta mahastiak erabiltzen dituzten espezieen presentzian duen eragina deskribatzeko ere. Bi mahasti-sistemetan (tradizionaletan eta beso-sistemako mahastietan), mahastietan ohikoak diren animalia-espezieen (10 hegazti-espezie eta bi ugaztun-espezie) gertaera-probabilitatea ikertzeko, gertaera-eredu hierarkikoak erabili genituen. Mahastien modernizazioak ekarri zituen mahatsondo altuagoak (metalezko kablez eta zutoinez ilaraka lotuak), mahatsondo-ilaren arteko distantzia handiagoak, lursailen tamaina handiagoa, ureztatzearen inplementazio sistematikoa eta, aztertutako mahastien herenean, ureztatze-sistemaren bidezko ongarrien aplikazioa (fertirrigazioa). Gainerako tratamendu agrokimikoak bi mahasti-mota horietan berdintsu erabiltzen zirela zirudien. Herbiziden erabileraren eta ohiko laborantzaren ondorioz, bi mahasti-sistemek landare-estaldura natural txikia aurkezten zuten. Mahastien modernizazioak mahasti-sailetako faunaren biodibertsitatean eragin argia izan zuen: zenbait espeziek beso-sistemako mahastietan presentzia altuagoa aurkeztu zuten (buztangora, karnaba, eper gorria eta etxe-txolarrea); beste batzuek, ordea, mahasti tradizionaletan (untxia eta txorru arrunta). Beraz, mahastien modernizazioak bertan bizi diren hegazti eta ugaztunen komunitatea aldatzea bultza dezake. Gainera, zenbait espeziek (batez ere lurrean bizi direnek) mahastiak erabiltzea guztiz loturik zegoen mahastien ondoan laborantza-lurrak egotearekin. Hortaz, espezie horiek udan zehar mahastiak erabil ditzakete ura edota uztatutako laboreek baino estaldura hobea ematen dutelako. Hala ere, ur bila mahastietara gerturatzen diren animaliak nitrato-dosi toxikoak pairatzeko

arriskupean egon daitezke; izan ere, beso-sistemako mahastien herenek fertirrigazioa erabiltzen dute. Biodibertsitatea bultzatzeko, komenigarria litzateke modernizazio-planak paisaiaren heterogeneotasuna bermatzeko kudeatzea, non bi motatako mahastiak beste nekazaritza-sistema batzuekin konbinatu beharko liratekeen, bai eta landaredi naturalaren estaldura-proportzioa handitzea faboratu ere.

Gako-hitzak

Eper gorria; fertirrigazioa; gertaera-eredu hierarkikoa; nekazal inguruetako hegaztiak; nekazaritzaren intentsifikazioa; untxia

1. Sarrera

Nekazaritza tradizionalak animalia-espezie askorentzako onuragarriak diren ekosistema erdi-naturalak sortzen ditu (Bignal eta McCracken, 2000; Morelli *et al.*, 2014; Palacín eta Alonso, 2018). Hala ere, joan den mendearen erdialdetik hona, ekosistema horietako asko asko aldatu dira, modernizatuz eta intentsifikatuz, nekazaritza-lurren produktibitatea handitzeko lasterketa batean eta elikagaien eskari gero eta handiagoari erantzuteko (Matson *et al.*, 1997; Foley *et al.*, 2005; Tilman *et al.*, 2011; Kastner *et al.*, 2012). Aldaketa horiek habitataren homogeneotasuna handitu dute (lursail handiagoak, mekanizazioa, monolaborantzaren hedapena eta lur ez-emankorren desagerpena bermatuz), bai eta ureztatzearen, ongarrien eta pestiziden erabilera ere (López-Antia *et al.*, 2013; Hallmann *et al.*, 2014). Kasu gehienetan, aldaketa horiek beherakada handiak eragin dituzte nekazal inguruneko animalia-espezieen populazioetan (Matson *et al.*, 1997; Pain eta Pienkowski, 1997; Sala *et al.*, 2000; Benton *et al.*, 2002, 2003). Beraz, nekazaritzaren modernizazioak biodibertsitatean duen eragina ulertzea funtsezkoa da, beharrezkoa izanez gero, konpentsazio-neurri egokiak proposatu ahal izateko. Gaur egun, nekazaritzaren intentsifikazioaren eragina batez ere zereal-laboreetako inguruneetan aztertu da (Donald *et al.*, 2001; Benton *et al.*, 2002, 2003; José-María *et al.*, 2010; López-Antia *et al.*, 2013; Ortiz-Santaliestra *et al.*, 2020); beste nekazaritza-sistema batzuetan, berriz, mahastietan adibidez, horren eragina nahiko ezezaguna da (e.g. Arlettaz *et al.*, 2012; Casas *et al.*, 2020).

Mediterraneo aldean, mahastiak nekazaritza-labore garrantzitsuak izan dira mendeetan zehar (Harris, 2013, McGovern, 2019). Gaur egun, Europar Batasunean mahastiek 3,2 milioi hektarea hartzen dituzte (munduko mahasti guztien % 45), eta horietatik % 74,1 Espainian, Frantzian eta Italian daude (Eurostat, 2017). Espainian, gaur egun mundu-mailan mahasti-azalera handiena duen herrialdean, ureztatu gabeko (lehorreko) baso-itxurako mahastiak landu izan dira tradizionalki. Hala ere, Espainiako mahastiak Europar Batasunak sustatu eta finantzatutako (EE-1493/1999) modernizazio- eta intentsifikazio-prozesu sakona jasaten ari dira, bilketa-kostuak murriztu eta produktibitatea handitzeko (Ruiz-Pulpón, 2013; MAPAMA, 2017). Horren ondorioz, azken hamarkadetan, Espainian mahasti tradizional asko eraldatu eta beso-sistemako mahasti bihurtu dira (Ruiz-Pulpón, 2013; Montero-García *et al.*, 2017). Eraldaketa hori, lehenik eta behin, mahatsondoen egitura-aldaketa bati lotuta dago; izan ere, egitura horizontala daukaten mahasti tradizionalak egitura bertikala daukaten beso-sistemako mahasti bihurtu dira; horietan, mahatsondoak altuagoak dira, eta alanbre-gidariz eta metalezko zutoinez lotuta daude; era berean, lerroen arteko distantzia handiagoa da, uzta mekanizatua egin ahal izateko. Gainera, eraldaketa horrekin batera, ureztatzea ezartzen da, eta mahastien tamaina handiagoa izaten ohi da (Salguero, 2010; Torquati *et al*., 2015).

Hala ere, ebidentzia gutxi daude nekazaritzaren aldaketa horrek mahastiak erabiltzen dituzten fauna basatiaren komunitateetan dituen ondorioei buruz, nahiz eta mahastiek estaltzen duten nekazaritza-lurraren azalera handia izan (Eurostat, 2017) eta laboreen egituraren edo laboreen kudeaketen aldaketak fauna basatiaren presentzian ondorio potentzialak izan ditzakeen (Matson *et al*., 1997; Donald *et al*., 2002; Benton *et al*., 2003). Mahasti tradizionalen (baso-formako mahastiak) modernizazioaren eragina oso espezie gutxitan ebaluatu da, eta frogatu da basoilo handiarentzat (*Otis tarda*; Casas *et al*., 2020) eta pirripioarentzat (*Lullula arborea*; Arlettaz *et al*., 2012) kaltegarria dela. Beso-sistemako mahastiek egitura bertikala dutenez, litekeena da lurrean denbora luzea igarotzen duten espezieek mahasti horiek saihestea, hala nola basoilo handiak (Casas *et al*., 2020), basoilo txikiak (*Tetrax tetrax*) edo gangek (*Pterocles alchata* eta *Pterocles orientalis*). Aitzitik, horien egitura erakargarriagoa izan liteke pausatzen diren espezieentzat, esate baterako, buztangora (*Cercotrichas galactotes*) edo antzandobientzat (*Lanius meridionalis* eta *Lanius senator*). Gainera, Mediterraneoko uda lehorrean beso-sistemako mahastiak ureztatzen direnean, baliabide hidriko hori erakargarria izan daiteke hegazti edo ugaztun askorentzat (Borralho *et al*., 1998). Mahastien modernizazioak nekazal inguruneko biodibertsitatean dituen ondorioak hobeto ulertzeko, mahasti tradizionalak eta beso-sistemako mahastiak erabiltzen dituzten espeziak ikertu behar dira. Kontuan hartu behar da, nekazal inguruneko hegazti- eta ugaztun-espezieek egiten duten mahastien erabilera mahastien inguruko habitatak baldintzatu dezakeela. Beste labore batzuek, hala nola zerealek, mahastietan eskuragarri ez dauden baliabide trofikoak edo habiak egiteko lekuak eduki ditzakete; bestalde, laborantza-lurren ondoko mahastiak (tradizionalak zein beso-sistemakoak) nekazal inguruneko zenbait espezierentzako babesleku izan daitezke, bereziki uztaren ondoren, uztatutako laboreek behar besteko estaldurarik ematen ez dutenean (Borralho *et al*., 1998).

Ikerketa honetan, ebaluatu dugu Espainiako hego-mendebaldeko mahastien fauna basatiko komunitate tipiko batek nola erabiltzen dituen mahastiak, mahasti motaren arabera. Horretarako, 26 mahasti tradizional eta beso-sistemako 26 mahasti aztertu genituen, eta mahatsondoaren egitura eta mahatsondoetan egindako kudeaketa-praktikak alderatu genituen. Gainera, fauna-zenbaketak eta gertakari-eredu hierarkikoak erabili ditugu mahasti-mota bakoitzean hegaztien eta ugaztunen gertaeren patroiak ebaluatzeko, kontserbaziorako interesgarriak diren espezieak, hala nola buztangora (*Cercotrichas galactotes*), eta balio sozioekonomiko handiko espezie zinegetikoak, hala nola eper gorria (*Atectoris rufa*), untxia (*Oryctolagus cuniculus*) edo erbia (*Lepus granatensis*) barne. Espero genuen bi mahasti-moten arteko egitura- eta kudeaketa-desberdintasunek hegazti- eta ugaztun-espezieen erabileran eragingo zutela baina mahasti-motaren hautaketa espeziearen araberakoa izango zela, eta hori, aldi berean, mahastien ingurunearen eraginpean egongo zela. Aurkikuntzek nekazal inguruneko biodibertsitatea kontserbatzeko izan ditzaketen ondorioak eztabaidatzen dira.

4. Eztabaida

Nekazaritzaren intentsifikazioak aldaketa handiak bultzatzen ditu nekazaritza-inguruneetan, paisaiaren egitura aldatuz eta bertako baliabideei eraginez (Matson *et al.*, 1997). Gure emaitzek erakusten dute modernizazio horrek aldaketak eragin zituela mahastien egituran (bertikalagoa) eta kudeaketa-praktiketan (batez ere ureztatzeari dagokionez). Gainera, mahasti tradizionaletan eta beso-sistemako mahastietan, espezieen presentzia desberdina dela ikusi genuen, nahiz eta espezieen gertaera-probabilitatea mahasti-motaren araberakoa izan. Hain zuzen ere, hegazti-espezieen erdiek nahiago zituzten beso-sistemako mahastiak, eta hegazti-espezie batek eta ugaztun-espezie batek nahiago zituzten mahasti tradizionalak. Mahasti-mota alde batera utzita, hegazti-espezieak maizago agertu ziren mahasti handienetan.

Beso-sistemako mahastiak tradizionalak baino handiagoak ziren, eta, beraz, horietan aurkitutako espezieen gertaera-probabilitate altuagoa mahastiaren tamainaren ondorio izan liteke. Hala ere, beso-sistemako mahastiak nahiago zituzten espezie gehienak (5etik 4) pausatzen diren espezieak edo espezie generalistak ziren, eta horrek iradokitzen du mahasti horietako mahatsondoen egitura (mahatsondo altuagoak edo pausalekuak izatea) positiboki hautatzen dutela. Esate baterako, deskribatu da buztangorek mahastiak ugalketa-habitat gisa hautatzen dituztela (Seoane, 2005), arrek, haien lurraldea defendatzeko, pausaleku altuak nahiago dituztela (Álvarez, 1997), eta buztangoren ugalketa-arrakasta positiboki erlazionatuta dagoela haien habiak lurrarekiko duen altuerarekin (Tabib *et al.*, 2016). Bestalde, lurrean denbora luzea igarotzen duten bi hegazti espezieek, kutturlio arruntak eta eper gorriak, ez zituzten beso-sistemako mahastiak saihesten. Izan ere, horietan, eper gorriaren presentzia handiagoa izan zen. Udan zehar eper gorriek beso-sistemako mahastiak nahiago izatea izan daiteke horietan ura eta eguneko ordurik beroenetan gerizpea aurkitzen dutelako. Izan ere, Espainiako erdialdean burututako GPS bidezko eper gorrien jarraipenaren arabera, udan eper gorriek lehorreko laboreak (uztondoak eta beste labore batzuk) erabiltzen dituzte eguneko lehen eta azken orduetan, baina eguerdian mahastiak aukeratzen dituzte, beharbada itzal, estaldura edo ur bila (Mougeot *et al.*, argitaratu gabeko emaitzak). Eper gorriarentzat erakarpen-faktore garrantzitsua izan daiteke mahasti ureztatuetan egoten den baliabide hidriko mugagabea; izan ere, ezaguna da, udan, ur-iturrien presentziak baldintzatzen dituela eper gorrien banaketak (Borralho *et al.*, 1998; Reino *et al.*, 2016).

Hala ere, ezin dugu baztertu aurkitutako bi mahasti-moten erabilerari buruzko desberdintasunak beste aldagai batzuekin lotuta egotea, hala nola behe-adarraren altuera, ilaren arteko distantzia, lanegunen kopurua, laborantza-maiztasuna, xirmenduen mozketa-aldiak, sitsak kontrolatzeko tratamendu kopurua, edo mahastien lurreko belar txarren presentzia. Hala ere, aldagai horietako batzuek ezberdintasun txikiak zituzten mahasti-moten artean, edo efektuaren tamaina nahiko txikia zen (adibidez, lurreko belar txarren proportzioa 0,09 zen mahasti tradizionaletan eta 0,12 zen beso-sistemako mahastietan), eta, beraz, ez dute esanahi biologikorik.

Bestalde, zenbait espezierentzat, mahastien erabilera, edozein motatakoak izanda ere, mahastien inguruko laboreen araberakoa zen. Ikertutako espezieetako batzuek, batez ere lurrean denbora luzea igarotzen dutenek (lurrean egoten diren hegaztiak, hegazti generalistak eta untxia), mahastiak laborantza-lurretatik gertu zeudenean erabiltzen zituzten gehien bat, mahastien azalera handiak saihestuz. Horrek iradokitzen du mahastiak ez direla espezie horientzako habitat egokienak (adibidez, Borralho *et al.*, 1998; Buenestado *et al.*, 2008), zeintzuek ziur aski udan, zerealen laboreak uztatu ondoren, uztondoek estaldura oso mugatua ematen dutenean erabiltzen dituzten, eguzki-argitik edo harraparietatik babesteko (Borralho *et al.*, 1998; Buenestado *et al.*, 2008; Sumozas, 2009). Hortaz, laborantza-lur lehorreko espezieek mahastiak babesleku gisa erabil ditzakete. Aitzitik, beste espezie batzuek, hala nola karnabak, txorru arruntak edo gari-berdantzak (esekitzen diren hiru espezie), nahiago izan zituzten laborantza-lurren ondoan ez zeuden mahastiak (hau da, beste mahasti edo olibadi batzuez inguratuak), eta horrek adierazten du zurezko laboreak nagusi diren paisaiek espezie horiei mesede egin diezaieketela, dela egituragatik, dela habitat biluziengatik.

Gure ikerketak, halaber, erakutsi zuen mahasti tradizionalen eta beso-sistemako mahastien arteko ezberdintasun nagusietako bat ureztatzea zela. Espainiako hego-mendebaldeko ekosistema idorretan, udan, ura da faktore mugatzaile nagusiena, eta mahastiaren modernizazioa ia sistematikoki tantakako ureztatze-sistemekin lotuta dago (aztertutako beso-sistemako mahastien % 97tan dago). Tantakako ureztatzeak ura zuzenean mahatsondoetara heltzea ahalbidetzen du, eta, beraz, ura aurrezten da. Gainera, sistema horrek ongarriak ureztatze-urari gehitzeko aukera ematen du (fertirrigazioa), batez ere nitratoak; fertirrigazioa ureztatutako mahastien % 37tan erabiltzen da. Nekazaritza-eremuetan, nitratoen gehiegizko aplikazioaren ondoriozko uraren kutsadura arazo orokorra da (Stoate *et al.*, 2001; Wright eta Welbourn, 2002). Laboreei aplikatutako nitratoen erdia baino gehiago ez dute landareek asimilatzen; aitzitik, ekosistemako beste konpartimentu batzuetara edo ur-erreserbetara heltzen dira (Crews eta Peoples, 2004). Ureztatze-sistemen bidez mahatsondoei nitratoak aplikatzeak aplikatutako ongarrien kopurua murriztu egiten du, baina tantakako ureztatzean izaten den nitrato-kontzentrazioa oso handia da (segurtasun publikorako gomendatutako muga baino 10 aldiz altuagoa; Rodriguez-Estival *et al.*, 2010), eta, hortaz, ur hori edaten duten animalientzat oso arriskutsua da. Zenbait ikerlanek frogatu dute nitratoak irensteak ondorio toxikoak dituela hegaztietan (adibidez, Ley, 1986; Rodriguez-Estival *et al.*, 2010). Mahastietan praktika hori normalean udan aplikatzen da, ur-iturri naturalak urriak direnean, eta, beraz, fauna basatiarentzat erakargarria izan daitekeen garaian. Baliabide hidriko toxiko hori oso arriskutsua izan daiteke (Rodriguez-Estival *et al.*, 2010), eta mahastiak tranpa ekologiko bihur ditzake. Hori ikertu egin beharko litzateke etorkizunean, eta, baieztatuz gero, fertirrigazioko uraren kontsumoa murrizteko neurriak aplikatu beharko lirateke. Neurri posibleen artean honako hauek daude: ureztatzeko hodiak lurperatzea, ur garbiko baliabide naturalak ematea edo hegaztientzako disuasio-neurriak erabiltzea fertirrigazio-tratamenduak aplikatzen direnean.

Ez genuen aurkitu ezberdintasunik beste produktu agrokimiko batzuen erabileran: sitsaren aurkako tratamenduak ohikoagoak izan ziren beso-sistemako mahastietan, baina sulfuroarekin eta herbizidarekin egindako tratamenduak antzeko maiztasunarekin aplikatu ziren bi mahasti-motetan. Erabilitako herbizida-mota (glifosatoa) ere antzekoa izan zen mahasti-moten artean. Bi mahasti-mota horietan, herbiziden aplikazioak egin ziren, eta maiz landu ziren, lurra belar txarrez garbi uzteko. Bi mahasti-motetan aurkitutako lurreko landaredi natural ugaritasun txikiak eragina izan dezake espezie basati askoren elikagaien eskuragarritasunean, eta lurraren estalki pobrea eskasa izan daiteke lurrean habia egiten duten hegazti batzuentzat. Hori izan liteke, oro har, mahastietan behatutako hegaztien biodibertsitate eskasaren arrazoia (Potts, 1980; Casas eta Vinuela, 2010; Salguero, 2010; Arlettaz *et al.*, 2012). Gainera, mahatsondoen lurreko belar txarren kontrako presio handia kaltegarria izan liteke mahatsondoentzat, landaredi naturalak etsai naturalen sostengu baitira eta horien eskasiak izurrien dentsitateak areagotu ditzake (Altieri *et al.*, 2005; Fiedler *et al.*, 2008; Sáenz-Romo *et al.*, 2019).

Oro har, gure emaitzek adierazten dute bi mahasti-mota horiek mosaiko-egiturako nekazaritza-ingurune batean mantentzeak paisaiaren egitura aberastu dezakeela, eta hegaztien aniztasuna, oro har, handitu dezaketela. Hala ere, lurrean bizi diren espezie mehatxatuek beso-sistemako mahastiak saihestu egiten dituzte, hala nola basoilo handiak (Casas *et al.*, 2020), basoilo txikiak edo gangak (Tarjuelo *et al.*, 2020); beraz, beso-sistemako mahastien azalera handitzeak espezie horiei kalte egin liezaieke. Gainera, mahastiak nagusi diren nekazaritza-eremuek (monolaborantza) espezie-aniztasun txikia izan dezakete laborantza-lurretan ohikoak diren mosaiko-egiturako nekazaritza-eremuekin alderatuta, nahiz eta hori etorkizunean zehatzago ebaluatu beharko litzatekeen. Mahastien balio ekologikoa handitzeko, biodibertsitatea errespetatzen duen mahastiaren kudeaketa sustatu beharko litzateke, zeina ekonomikoki ere onuragarria izan litekeen, izurrien dentsitatea eta tratamendu fitosanitarioen erabilera murriztu baititzake (Fiedler *et al.*, 2008; Sáenz-Romo *et al.*, 2019). Horretarako, gomendagarria izan liteke paisaiaren heterogeneotasunari eustea, bai eta lurzoruan eta mahastien mugetan landare-estalki naturala mantentzea ere (Casas eta Vinuela, 2010; Salguero, 2010). Lurrean habia egiten duten hegaztiei dagokienez, habiak egiten diren hilabeteetan (apirila-maiatza-ekaina) mahastien lurra goldatzeak ere eragin kaltegarria izan dezake ugalketa-arrakastan (Casas eta Vinuela, 2010). Ikerketa honetan, udan baino ez dugu ebaluatu mahastien erabilera, baina beharrezkoa izango litzateke urteko beste sasoi batzuetan ere mahastien modernizazioak izan dezakeen eragina ebaluatzea. Adibidez, neguan zehar goldatze-maiztasun handiak bi mahasti-motetan eskuragarritasun trofiko txikiagoa izatea dakar. Gure ikerketaren diseinuak ez zigun ahalbidetu faktore bakoitzak (egitura, ureztatzea, landare-estalkiaren falta eta gerizpea) mahasti-mota bakoitzaren espezieen erabileran duen eragina bereizten. Etorkizunean, faktore horiek bakarka ebaluatu beharko lirateke, eta bereziki ikertu zein neurritan erakartzen dituzten ureztatzeak eta beso-sistemako mahastiek lehorreko hegaztiak eta bestelako fauna; halaber, fertirrigazioak ekartzen duen arrisku erreala ebaluatu beharko litzateke.

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III. ARTIKULUA

Laborantza-lurren konposizioak eta nekazaritza-praktikek Espainiako erdialdeko eper gorriaren dentsitatearen espazio-eta denbora-aldaketak azaltzen dituzte

- **Laburpena**
- **Sarrera**
- **Eztabaida**

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Science of the Total Environment (Errebizio prozesuan)

Laborantza-lurren konposizioak eta nekazaritza-praktikek Espainiako erdialdeko eper gorriaren dentsitatearen espazio- eta denbora-aldaketak azaltzen dituzte

Laburpena

Nekazal inguruneko hegazti-populazio asko gainbeheran daude, eta horien joera negatiboak lotuta egon ohi dira lurzoruaren erabileran edo nekazaritza-jardueretan gertatzen diren aldaketekin, baita produktu agrokimikoen erabilerarekin ere. Eper gorria mediterraneo eremuko garrantzi sozioekonomiko handiko espezie zinegetikoa da, zeinaren populazioak izugarri murriztu diren XX. mendearen erdialdetik, laborantza-lurren aldaketekin lotuta. Hala ere, orain arte ez da egin eper gorriaren ugaritasunak edo joerek laborantza-lurren konposizioarekin edo kudeaketarekin duten loturari buruzko eskala handiko ikerketarik.

Ikerlan honetan, distantzien laginketako eredu hierarkikoak erabili ditugu 2010ean Espainiako erdialdeko (Gaztela-Mantxa) eper gorriaren ugaritasuna zenbatesteko, Europako espezie honetako populazio nagusietako bat baita. Eper gorriaren dentsitatearen eta lurzoruaren erabileren arteko loturak aztertu ditugu (kudeaketaren aldaketa barne: labore ureztatuak edo nekazaritza ekologikoa). Halaber, zazpi urtetan zehar (2010-2017) eskualdeko ugaritasunak izandako aldakuntza ebaluatu dugu, bai eta lurzoruaren erabileran izandako aldaketekin duen lotura ere.

Gure emaitzen arabera, eper gorriaren ugaritasuna handitu egin zen landaredi naturalaren eta lehorreko mahasti tradizionalen eskuragarritasunarekin batera, baina zuhaitz-laboreen eta ureztatutako mahastien proportzioek gora egin ahala, murriztu egin zen; azken erlazio hori ez zen hain nabarmena izan uretako nitratoen kutsadurarekiko sentikorrak diren eremuetan, non nekazaritza-lurretan aplikatutako ongarrien kopurua eta nekazaritza-praktika jakin batzuen erabilera hertsiki araututa dauden. Emaitza horien arabera, mahasti intentsiboen gorakada kaltegarria da eper gorriarentzat. Horrez gain, aurkitu genuen, ikertutako zazpi urteetan zehar, eskualdeko eper gorrien populazioak ugaritasunean % 51ko beherakada pairatu zuela. Gainbehera hori nabarmenagoa izan zen landaredi natural gehien galdu zen eta zuhaitz ekologikoen laborantza handitu zen lekuetan.

Oro har, gure emaitzen arabera, lurzoruaren erabileran (labore-mota edo laborantza-lurretan landaredi naturala suntsitzea) eta nekazaritza-jardueretan (adibidez, labore jakin batzuk ureztatzea, nitratoak erabiltzea) izandako aldaketek eragin handia dute nekazal inguruneko hegazti honetan, eta horrek eragina du espezieak espazioan duen banaketan eta horren populazio-dinamikan.

Gako-hitzak

Alectoris rufa; distantzietako laginketa hierarkikoa; lurzoruaren erabilera; maneiua; nekazaritzaren intentsifikazioa; ugaritasuna

1. Sarrera

Milaka urtetan zehar gertatu den nekazaritzaren hedapen motelaren ostean, Europako paisaiaren zati garrantzitsu bat nekazaritza-lurrez osaturik dago gaur egun (Krebs *et al.*, 1999). Lehorreko hegazti-espezie asko egokitu egin dira ingurune erdi-natural honetara, non ugaltzeko edota negua igarotzeko egokiak diren habitatak aurkitzen dituzten (Tucker eta Heath, 1994; Tucker eta Evans, 1997; Robinson *et al.*, 2001). Hala ere, Nekazaritza Politika Bateratuaren ezarpena (NPB), Europako nekazaritzaren mugarria, inflexio-puntu bat izan zen nekazal inguruneko biodibertsitatearentzat (Donald *et al.*, 2002). NPBren ondorengo Europako nekazaritza-paisaien modernizazio sakon eta azkarrak eta denbora laburrean izandako nekazaritzaren intentsifikazioak (Matson *et al.*, 1997; Pain eta Pienkowski, 1997) nekazal inguruneko hegazti-espezie askoren beherakada handia eragin dute (Donald *et al.*, 2001; Benton *et al.*, 2002; Donald *et al.*, 2002; Storkey *et al.*, 2012). Nekazaritza-lurren intentsifikazioak aldaketak eragin ditu lurraren erabileran (labore batzuk sustatu baitira beste batzuen kaltetan) eta paisaian (adibidez, sailen tamaina handitzea mekanizazio eraginkorragoa ahalbidetzeko, eremuen mugak desagerraraztea eta monokultiboak areagotzea), baina baita nekazaritza-jardueretan ere (adibidez, mekanizazioa, pestizida, ongarri edo ureztatzearen erabilera). Aldaketa horiek guztiek zuzeneko edo zeharkako ondorioak izan dituzte basa-bizitzan (Matson *et al.*, 1997; Pain eta Pienkowski, 1997).

Lurraren erabileran eta paisaian gertatzen diren aldaketek hegaztientzako eremu egokien azalera alda dezakete, landaretzaren egituran edo elikagaien ugaritasunean gertatzen diren aldaketekin lotuta (Benton *et al.*, 2002; Wilson *et al.*, 2005; Storkey *et al.*, 2012). Nekazaritza-lurretan, paisaiaren heterogeneotasunak habitataren egokitasuna handitu ohi du hegaztientzat, aukera handitzen baitu urtean zehar espezie askok baliabide egokiak aurkitzeko (Benton *et al.*, 2003; Siriwardena *et al.*, 2012). Gainera, nekazaritza-jarduerei lotuta ez dauden habitatak (habitat naturalak) eta lugorriak oso garrantzitsuak dira hegazti askorentzat; izan ere, elikagaiak, ugaltze-toki egokiak eta habia egiteko leku aproposak aurkitzen dituzte (Vickery *et al.*, 2002; Vickery *et al.*, 2009; McMahon *et al.*, 2010; Tarjuelo *et al.*, 2020).

Nekazaritza-jarduerek, hala nola mekanizazioak, uzta-egutegien aldaketak edo agrokimikoen erabilerak, basa-bizitzan eragin garrantzitsuak izan ditzakete (Fry, 1995; Matson *et al.*, 1997; Mineau eta Whiteside, 2013; Stanton *et al.*, 2018), baita lurraren erabilera aldatzen ez denean ere. Horren adibide dira Espainian mahasti tradizionalen (basoan) eta beso-sistemako mahastien arteko ezberdintasunak (Salguero, 2012; Cabodevilla *et al.*, 2021), laborantza ekologikoen eta tradizionalen arteko ezberdintasunak (Solomou eta Sfougaris, 2011; Myers *et al.*, 2019), edo urteko laboreetan egiten den hazi blindatuen erabilera (Prosser eta Hart, 2005; López-Antia *et al.*, 2016, 2021). Nekazaritza-jarduerek eragina izan dezakete ingurumenaren kalitatean ere. Adibidez, nitratoak ongarri gisa gehiegi erabiltzeak azaleko eta lurpeko uren kalitatean eragin dezake, eta, ondorioz, eremu horiek erabiltzen dituzten landaredian edo faunan eragina izan dezake (Rodríguez-Estival *et al.*, 2010; Britton *et al.*, 2017; Chen *et al.*, 2019; Paredes *et al.*, 2020).

Eper gorria (*Alectoris rufa*) Europako hego-mendebaldeko lehorreko lurretan bizi den espeziea da, eta haren banako gehienak Iberiar penintsulan bizi dira (Blanco-Aguiar *et al.*, 2004). Espezieak garrantzi sozioekonomiko eta ekologiko handia du: ehiza xeheko espezie nagusietako bat da (Andueza *et al.*, 2018), eta harrapari iberiar askorentzat baliabide trofiko garrantzitsua da (Calderon, 1977), espezie mehatxatuak barne, hala nola aztore-arranoa (*Aquila fasciata*) eta eguzki-arrano iberiarra (*Aquila adalberti*). Eper gorriaren populazioak nabarmen murriztu ziren XX. mendearen bigarren erdian (Blanco-Aguiar, 2007), eta baliteke gainbeherak aurrera jarraitzea azken hamarkadetan (BirdLife International, 2018). Gainbehera, nagusiki, laborantza-lurren aldaketekin lotuta dagoela iradoki da, habitataren heterogeneotasunaren galerarekin, zerealen uzta aurreratzearekin eta agrokimikoen erabilerarekin, nahiz eta ehizaren presio handiak ere lagundu duen (Blanco-Aguiar, 2007). Hala ere, gutxienez azken urteetan, ehiza-sektoreak gainbehera horren garrantziaren kontra agertu dira (RFEC, 2020). Ez dago guztiz argi azken urteetako espeziearen joera, eta orain arte ez da egin eper gorriaren ugaritasunak edo joerek laborantza-lurren konposizioarekin edo kudeaketarekin duten loturari buruzko eskala handiko ikerketarik, eta horrek ondorio garrantzitsuak izan ditzake espeziearen kontserbazio-egoera sustatzeko kudeaketa-neurriak diseinatzerakoan.

Ikerketa honetan, distantzien laginketako eredu hierarkikoak erabili ditugu Gaztela-Mantxan, espeziearentzat garrantzitsua den Espainiako eskualde handi batean (Blanco-Aguiar *et al.*, 2003; Blanco-Aguiar *et al.*, 2004), eper gorriak espazioan eta denboran (zazpi urtez; 2010-2017) izandako dentsitatearen aldaketa kalkulatzeko. Lehenik eta behin, nekazaritza-inguruneko lurzoruaren erabileraren eta eper gorriaren ugaritasunaren aldaketa espazialen arteko erlazioa ebaluatu genuen. Bereziki, aztertu genuen eper gorriaren ugaritasunak zer erlazio duen habitat naturalen eskuragarritasunarekin (landu gabeak) edo nekazaritza-praktikekin (adibidez, laboreen ureztapena, nekazaritza ekologikoa edo ura nitratoz kutsatuta egotekoarekiko sentikorrak diren eremuetan ongarrien erabilerari jarritako mugak). Bigarrenik, zazpi urtean zehar, eper gorrien ugaritasunaren joera ikertu genuen, bai eta ugaritasun-aldaketak habitat-aldaketekin izan dezakeen erlazioa ebaluatu ere. Gure emaitzek espeziearen kontserbazioan, kudeaketan eta ehizaren iraunkortasunean izan ditzaketen inplikazioak eztabaidatzen ditugu.

4. Eztabaida

Gure ikerketak frogatu zuen, klimaren eragina kontuan hartuta, eper gorriaren ugaritasunaren aldaketa, neurri handi batean, laborantza-lurren osaera eta kudeaketaren araberakoa dela. Zehazki, landaretza naturalaren dentsitatearekin eta lehorreko mahasti tradizionalen hedadurarekin positiboki lotuta dagoela erakutsi zuen, baina, ordea, negatiboki lotuta, ureztatutako mahastien eta zuhaitz-laboreen hedadurarekin. Gainera, gure emaitzek egiaztatu zuten Gaztela-Mantxako (espeziearentzat eskualde garrantzitsuenetako bat) eper gorri basatien populazioak, 2010 eta 2017 bitartean, gainbehera nabarmena (-% 51) jasan duela. Bestalde, lurzoruaren erabileraren aldaketek gainbehera horren tamaina modulatzen zutela frogatu zen.

Beste lan batzuekin bat etorriz (Buenestado *et al.*, 2008; Casas eta Viñuela, 2010), aurkitu zen landaredi naturalaren proportzio handiena duten agrosistemek (sastrakadiak eta, bereziki, larreak) eper gorri gehiago jasaten dituztela. Horrek nabarmentzen du nekazaritza-paisaietan landaredi naturaleko eremuak mantendu behar direla, hegaztiek habia egiteko toki egokiak, elikagaiak eta babesa aurkitzen baitituzte eta, oro har, nekazal inguruneko biodibertsitatea kontserbatzeko habitat egokiak baitira (Perkins *et al.*, 2002; Vickery *et al.*, 2002; Casas eta Viñuela, 2010; McMahon *et al.*, 2010; Tarjuelo *et al.*, 2020). Gainera, agrosistemetako beste labore batzuk eper gorrien ugaritasun handiagoarekin ere lotu ziren, lehorreko mahasti tradizionalak kasu. Mahastiek garrantzi handia dute eper gorrien populazioentzat, itzala eta babesa ematen baitute, batez ere zerealen uztaren ondoren (eskualde honetan, maiatzaren amaieratik eta ekainaren erdialdera izaten dena), uztondoek ez baitute estaldurarik eskaintzen (Buenestado *et al.*, 2008; Sumozas, 2009). Izan ere, GPS bidez egindako jarraipen-ikerketek frogatu dutenez, eper gorriek sarriago erabiltzen dituzte mahastiak udan eta eguerdietan, ordurik beroenetan (Mougeot *et al.*, argitaratu gabeko emaitzak), itzal, babes edo ur bila. Aitzitik, badirudi zuhaitz-laboreak (olibadiak barne) ez direla habitat egokiak eskualdeko eper gorrientzat. Emaitza hori bat dator Buenestado *et al.*-ek (2008) Espainiako hegoaldean eta erdialdean egindako aurkikuntzekin, baina Espainiako hegoaldeko eta Portugalgo beste azterlan batzuekin kontrastea egiten du; izan ere, horietan, bazirudien olibondoek onura egiten zietela eper gorriei (Borralho *et al.*, 1999, 2000; Delibes-Mateos *et al.*, 2012). Desberdintasun horiek lotuta egon daitezke zuhaitz-labore horien lurzoruaren erabilerarekin; izan ere, aztertutako eskualdean, olibadiak modu intentsiboan kudeatu ohi dira, zuhaitzen arteko lurra biluzik uzten duen laborantza mekanikoarekin, eta ureztatutako olibadietan fertirrigazioa erabiliz (Moncunill, 2013). Gainera, zuhaitz-laboreak nagusi diren nekazaritza-paisaia kaltegarria izan daiteke espeziearentzat. Nolanahi ere, inkoherentzia horiek aztertu eta argitu beharko lirateke etorkizunean, horiek kudeaketan dauden desberdintasunengatik izan daitezkeela iradokitzen dugun arren.

Aurrekoarekin bat etorriz, eta lurzoruaren erabilera espezifikoen garrantziaz haratago, aurkitu genuen eper gorriaren ugaritasuna labore batzuen kudeaketak baldintzatzen zuela. Horrela, nahiz eta eper gorriaren ugaritasuna lehorreko mahasti tradizionalen eskuragarritasunarekin areagotu, mahasti ureztatuekiko erlazioa kontrakoa zela ikusi zen. Espainian, ureztatutako mahastiak ia beti beso-sistemako mahastiak dira (Salguero, 2012; Cabodevilla *et al.*, 2021); beraz, haien fisonomia eta egitura ez dira lehorreko mahasti tradizionalen modukoak. Egitura bertikalak eta ilaren arteko eremu irekiagoak estaldura txikiagoa eman dezakete eper gorriarentzat, baina, aldi berean, itzal handiagoa eta ikuspen handiagoa mahatsondoen azpian. Lursail-mailako ikerketa batek erakutsi zuenez, udan eper gorria agertzeko probabilitatea handiagoa da beso-sistemako mahastietan tradizionaletan baino (Cabodevilla *et al.*, 2021). Beraz, baliteke eper gorrien ugaritasunaren aldaketak ez izatea zuzeneko loturarik ureztatutako labore horien egitura desberdinarekin, ur-hornidurarekin baizik. Eper gorria ur-baliabideen menpe dago udan, eta, hortaz, ureztatutako mahastietako urek erakar dezakete (Borralho *et al.*, 1998; Cabodevilla *et al.*, 2021). Bestalde, ureztatutako mahasti modernoetan, ongarriak ureztatzeko uraren bidez aplikatzea ohikoa da (fertirrigazioa mahasti horien heren batean aplikatzen da gutxienez; Cabodevilla *et al.*, 2021). Tantakako ureztatzeak mahatsondoei zuzenean ura ematea ahalbidetzen du, eta, beraz, nekazaritza-lur idorretan ura aurreztea. Ongarriak (gehienak nitratoak) ureztatze-sistema horien bidez aplikatzen direnean, uretan duten kontzentrazioa oso handia da, segurtasun publikorako gomendatutako muga baino 10 aldiz handiagoa (Rodriguez-Estival *et al.*, 2010), eta eper gorrien gisako hegaztiak nitrato-dosi toxiko baten eraginpean jar ditzake, eta horien osasunean ondorio kaltegarriak eragin (Ley, 1986; Rodríguez-Estival *et al.*, 2010). Gure emaitzek erakutsi zuten paisaia-mailan ureztatutako mahastien proportzio handiagoa izatea lotuta dagoela eper gorri gutxiago egotearekin, eta lotura hori handiagoa dela nitratoen kutsadurarekiko sentikorrak ez ziren eremuetan, ez baitago mugarik ureztatzeko urari aplika dakiokeen ongarri-kopuruari dagokionez (2011/02/07ko Agindua, JCCM). Patroi horiek koherenteak dira mahasti intentsiboetan izan daitekeen nitratoekiko esposizioak eper gorrien populazioetan izan dezakeen eragin negatiboarekin eta esperimentalki nitratoekin kutsatutako uraren kontsumoak hegaztien fisiologian eta osasunean aurkeztutako ondorio kaltegarriekin (Rodriguez-Estival *et al.*, 2010).

Bestalde, badirudi beste kudeaketa-mota batzuek ez diotela eragiten eper gorriaren ugaritasunari. Adibidez, ez genuen aurkitu labore ekologikoen eskuragarritasunak eper gorrien dentsitate handiagotzea eragiten duelako frogarik. Hala ere, labore ekologikoen garrantzi erlatiboa, nekazaritza-lurren azaleraren ehunekoari dagokionez, oso txikia zen (% 4,6, batez beste), eta agian hori ez da nahikoa izan erlazio esanguratsuak hautemateko. Etorkizunean, emaitza horiek egiaztatu egin beharko lirateke labore ekologikoen eragina ardatz duten ikerketetan (adibidez, Moreau *et al.*, 2021).

2010ean ikusitako lurzoruaren erabileraren eta eper gorrien ugaritasunaren arteko erlaziotik harago, denboran zehar, ugaritasunaren aldaketa ere ikusi egin zen, 2017 eta 2010 artean eper gorriaren ugaritasuna % 51 jaitsi baitzen (% 8 - % 74, kasu onena eta txarrena kontuan hartuta). Eskualde honetan eper gorrien basa-populazio handienetako bat dagoela kontuan izanda (Blanco-Aguiar *et al.*, 2003; Blanco-Aguiar *et al.*, 2004), aurkitutako eper gorrien gainbehera bereziki kezkagarria da; izan ere, litekeena da hori eper gorri basatiekin mundu-mailan gertatzen ari denaren adierazgarri izatea. Gure emaitzek, halaber, erakutsi zuten, ikertutako denboran zehar, eper gorriaren populazioaren gainbehera ez zela lineala izan; izan ere, populazio-tamainaren aldaketa nagusiki bi urtetan gertatu zen (2013 eta 2014), eta horien aurretik eta ondoren egonkortasun erlatiboko aldiak izan ziren. Horrek iradokitzen du, agian, populazioak ez direla gai izango biziraupen edo ugalketa txarreko urte gutxi batzuetara daramaten gertaera estokastikoetatik errekuperatzeko.

Ugaritasunari buruzko emaitzekin bat etorriz, lurzoruaren erabileraren aldaketek eper gorrien joerak modulatu egin zituzten: larreen proportzioa (landaretza naturala) murriztu zenean eta zuhaitz-laboreen proportzioa handitu zenean jaitsierak nabarmenagoak izan ziren. Arestian aipatu bezala, landaretza naturala oso garrantzitsua da eper gorriarentzat, eta horren murrizketa eper gorrien gainbehera nabarmenagoekin lotu zen. Ziurrenik, larreak gutxitzeak espezie horrentzako baliabide trofikoak eta ugalketa-habitat egokiak murriztea ekarriko du (Vickery *et al.*, 2002; Vickery *et al.*, 2009; Casas eta Viñuela, 2010; McMahon *et al.*, 2010). Zuhaitz-labore ekologikoen efektuari dagokionez, edozein zuhaitz-laboreren adierazgarri izan daiteke. Erlazio hori eper gorriarentzat egokia den habitataren galeraren emaitza izan liteke, zuhaitz-laboreak nagusi diren eremuak espeziearentzat negatiboak direla baitirudi. Zuhaitz-laboreen kopurua mugatzea babestutako estepako hegaztientzat onuragarria den kontserbazio-neurri gisa identifikatu da (JCCM, 2017), eta emaitza hauek neurri hori babesten dute. Behatutako gainbeheraren magnitudea (-% 51 zazpi urtean) ere bat dator eper gorriak erabilera arrunteko produktu agrokimikoen eraginpean egotearen eraginei buruzko ezagutzekin, batez ere Espainiako erdialdean ereiten diren triazol fungizidez trataturiko zerealen haziekin elikatzearen eraginarekin (López-Antia *et al.*, 2016). Esperimentalki, frogatu da neguaren amaieran fungizida horiekiko esposizioak ugalketa eteten duela eta eper gorriaren produktibitatea erdira murrizten duela (Fernández-Vizcaíno *et al.*, 2020; López-Antia *et al.*, 2021), eta, beraz, populazioaren gainbehera azkarrak eragiteko ahalmena duela, ikerlan honetan aurkitutakoaren modukoak.

Eper gorrietan behatutako populazioaren gainbeherak hegazti zinegetiko honen egoera larria egiaztatzen du (BirdLife International, 2020), eta horren kudeaketarako ondorio garrantzitsuak ditu. Espainian, urtero milioika eper gorri ehizatzen dira (haztegietan hazitako askatutako eper gorriak barne), (Andueza *et al.*, 2018) eta eper gorriaren ehiza negozio ekonomiko garrantzitsua da gaur egun, urtean mila milioi eurotik gora mugitzen baititu (Garrido, 2012). Beraz, eper gorriaren egoera kontserbazio-arazoa izateaz gain, arazo sozioekonomiko garrantzitsua ere bada. Nahiz eta ehiza ez izan gainbeheraren arrazoi nagusia, behatutako beherakadak agerian uzten du, eragin gehigarriak saihesteko, ehizaren presioa espeziearen ugaritasunera eta dinamikara doitzeko beharra. Zientzialariek, erakunde publikoek, nekazariek eta ehiza-federazioek batera lan egin beharko lukete eper gorriaren kontserbazioaren arazoa konpontzeko. Gaur egun, ehiza-barruti askok eper gorriaren populazioak handitzeko ahalegin handiak egiten dituzte (Arroyo *et al.*, 2012; Arroyo *et al.*, 2017). Barruti horietan eper gorri basatien populazioak berreskuratzeko baliagarriak izan daitezkeen neurri batzuk jarri dituzte martxan, hala nola harraparien kontrola, elikagai osagarrien eta uraren hornikuntza, eta ehiza-laboreen hornikuntza (ehizatzeko berariaz landatutako laboreak, zeinak uztatzen ez diren). Neurri horiek onuragarriak izan daitezke beste espezie mehatxatu batzuentzat ere (Smith *et al.*, 2010; Estrada *et al.*, 2015; Cabodevilla *et al.*, 2020). Baina barruti horietako askok haztegietan hazitako eper gorrien askatze masiboak ere egiten dituzte (Caro *et al.*, 2014; Cabodevilla *et al.*, 2020), eta populazio basatientzat kaltegarriak izan daitezke (Villanúa *et al.*, 2008; Casas *et al.*, 2012; Díaz-Sánchez *et al.*, 2012), eta populazio basatien gaineko ehiza-presioa ere handitu ere (Casas *et al.*, 2016).

Aurkitutako populazioaren gainbehera nabarmenak, batzuetan zalantzan jarri den arren (adibidez, RFEC, 2020), agerian uzten du espezie honen etengabeko jarraipena egin behar dela. Ildo horretan, gure emaitzek eman zuten jarraipen-programak optimizatzeko erabilgarria izan daitekeen espeziearen detektagarritasunari buruzko informazioa ere. Gure emaitzen arabera, eper gorriari buruzko zenbaketak egiteko unerik onena (gure ikerlanaren denbora-esparruaren barruan) apirilaren amaiera/maiatzaren hasiera edo uztaila da. Maiatzaren erdialdetik uztailaren hasierara arte antzemandako detektagarritasun-ahalmen txikia, ziur asko, garai hartan ugaldutako banakoak inkubatzen ari zirelako eta landarediaren eraginez izan zen. Nolanahi ere, epealdi hori saihestu egin beharko litzateke ugaritasuna ebaluatzeko. Hala ere, ugalketa-fenologia aztertzeko, kontuan hartu beharreko garaia da; izan ere, une horretan, eklosioak izaten dira (Guzmán *et al.*, 2020). Laginketa-orduari dagokionez, badirudi udaberriaren hasieran eraginkorragoa zela goizeko jarraipena (Jakob *et al.*, 2014); aldiz, uztailean, detektagarritasuna handiagoa zen eguzkia sartu aurreko orduetan, beharbada, hegaztiek jarduera handiagoa zutelako bero handiko arratsaldeen ondoren. Hori kontuan hartu beharko litzateke jarraipen-programak diseinatzerakoan; hala ere, diseinua laginketaren helburuaren araberakoa izan behar da. Era batera edo bestera, modelizazio-prozesuan detektagarritasuna kontuan hartzea beti izango da gomendagarria (Jakob *et al.*, 2014; Kellner eta Swihart, 2014), eper gorriaren ugaritasunari buruzko informazio fidagarriagoa lortzeko.

Laburbilduz, ikerketa honek erakusten du laborantza-lurren konposizioak eta nekazaritza-praktikek eper gorriaren ugaritasunean eta joeretan izan diren aldaketak azaltzen dituztela; izan ere, hori nekazal inguruneko hegazti-espezie garrantzitsua da.

Emaitzen arabera, landaredi naturalean aberatsa den mosaiko-itxurako nekazaritza-paisaiak balio handia du nekazal inguruneko hegaztientzat, hala nola eper gorriarentzat (Berg, 2002; Siriwardena *et al.*, 2012). Nekazaritza-ingurune bat, non elikagaiak lortzeko eta habiak egiteko habitat egokiak dauden, baita harraparietatik eta berotik babesteko egitura begetalak ere, batez ere uztaren ondoren. Gure emaitzek fertirrigazioak izan dezakeen eragin negatiboari buruzko ebidentzia berriak ematen dituzte, eta erakusten dute praktika hori erabiltzeak hegaztientzat arrisku larriak ekar ditzakeela. Gainera, azken hamarkadan deskribatutako eper gorriaren populazioaren beherakada nabariak iradokitzen du gaur egun espeziea ez dela gertaera estokastikoen ondorio negatiboetatik errekuperatzeko gai izango. Emaitza horiek erakusten dute, batetik, garrantzi sozioekonomiko eta ekologiko handia duen espezie honen egoera delikatua, eta, bestetik, nekazaritzak nekazal inguruneko hegaztien populazio-dinamikan duen funtsezko eginkizuna. Hala, eper gorria kontserbatzeari ekiteko, nekazaritza-kudeaketa birplanteatzeak beharrezkoa dirudi, intentsifikazioa eta agrokimikoen gehiegizko erabilera saihestuz, zuhaitz-laboreen hedapena murriztuz eta landaredi natural edo erdi-naturaleko orbanak sustatuz.

Esker onak

Egileek eskerrak ematen dizkiete Gaztela-Mantxako Komunitateen Batzordeari eper gorrien zenbaketak partekatzeagatik eta zenbaketa horietan parte hartu duten basozainei. Eskerrak, baita ere, Barbara G. Montenegrori (Nekazaritza, Ingurumen eta Landa Garapeneko Saila, Landa Garapeneko ZN; Gaztela-Mantxako Komunitateen Batzordea) labore organikoei buruzko informazioa partekatzeagatik eta Mario Fernández Tizóni informazio hori GIS programan lantzen laguntzeagatik. Ikerlan hau Gaztela-Mantxako Komunitateen Batzordeak eta Eskualde Garapenerako Europako Funtsak (FEDER) finantzatutako AGROPERDIZ (SBPLY/17/180501/000245) proiektuari egindako ekarpena da. Xabier Cabodevillak Eusko Jaurlaritzaren Hezkuntza, Unibertsitate eta Ikerketa Sailaren doktoretza-bekaren babesarekin burutu zuen lana (Erref.: PRE_2018_2_0273).

4

OTHER ANTHROPIC FACTORS

(hunting intensification)

PAPER IV

Are population changes of endangered little bustards associated with releases of red-legged partridges for hunting? A large-scale study from central Spain

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Are population changes of endangered little bustards associated with releases of red-legged partridges for hunting? A large-scale study from central Spain

Abstract

The release of farm-reared game birds for hunting is an increasingly common game management practice. However, releasing could have negative effects on sympatric wild species, for example, through parasite transmission. Here, we document the spatial-temporal patterns and intensity of red-legged partridge releases in the province of Ciudad Real, Spain, over a 15-year period (2002–2016), relating them to local changes in the abundance of little bustards estimated from two surveys carried out in 2005 and 2016. Within the province, > 600,000 red-legged partridges were released annually over at least 20% of the area. Releasing intensity varied between estates and fluctuated over the 15-year study period, probably because of an economic crisis during 2008–2014. Overall, numbers of little bustards dropped by 46% between surveys, the decrease being more marked in the west of the province. Contrary to expectation, the only hunting estates where little bustards did not decrease were those with higher release intensity. This may be a consequence of management measures or other factors that benefit little bustards and are more prevalent on those estates than elsewhere, such as game crop provision, predator control or habitat quality.

Keywords

Alectoris rufa; Farm-reared birds; Game crops; Management; Population decrease; *Tetrax tetrax*

1. Introduction

Hunting and its management have changed markedly throughout human history (Washburn and Lancaster, 2017) and are currently undergoing a process of intensification in parts of Europe. For example, practices like the release of farm-reared game birds with the aim of increasing hunting stock have become increasingly common (Arroyo and Beja, 2002; Aebischer, 2019). In the case of small-game species, this sometimes involves the release of millions of birds (Mondain-Monval and Girard, 2000; Noer *et al.*, 2008; Champagnon *et al.*, 2009; Caro *et al.*, 2014; VKM, 2017; Aebischer, 2019). However, this practice carries many potential problems for the wild populations of released species (Champagnon *et al.*, 2012): changes in behaviour, demography and morphology, dissemination of pathogens or genetic introgression in wild populations (Tompkins *et al.*, 2000; Villanúa *et al.*, 2006; Arroyo and Beja, 2002; Champagnon *et al.*, 2010; Champagnon *et al.*, 2012; Díaz-Fernández *et al.*, 2013). Furthermore, it may also affect sympatric species owing to competition for resources or spread of pathogens (Prenter *et al.*, 2004; Bicknell *et al.*, 2010), although positive effects associated with habitat management have also been reported (Mustin *et al.*, 2018).

The red-legged partridge (*Alectoris rufa*) is one of the game species released for hunting in Europe (Arroyo and Beja, 2002), with millions being released annually in the UK, France and Spain (Tupigny, 1996; Caro *et al.*, 2014; Aebischer, 2019). The red-legged partridge is numerically and socially a very important quarry species in Spain (Andueza *et al.*, 2018), being hunted in 94% of the small-game estates of central Spain (Ríos-Saldaña, 2010). Official data report annual hunting bags of between 2.5 and 4 million red-legged partridges in that country (MAAMA, 2014), a figure that could exceed the wild red-legged partridge breeding population, estimated at 1.7–3.7 million pairs (Blanco-Aguiar *et al.*, 2004). This is only possible through the release of farm-reared red-legged partridges for hunting, which has intensified in Spain in recent decades with several millions of red-legged partridges released annually (Caro *et al.*, 2014).

Releasing as a management practice is controversial (Sokos *et al.*, 2008; Gamborg and Jensen, 2017; Mustin *et al.*, 2018; Avery, 2019), even within the hunting sector (Delibes-Mateos *et al.*, 2014; Delibes-Mateos *et al.*, 2015; Gamborg *et al.*, 2016). In Spain, the main perceived benefit of releases is economic, as it is seen as the only way to maintain commercial hunting (Delibes-Mateos *et al.*, 2015). However, releases (as currently carried out) do not necessarily help the recovery of wild populations, which they can for instance contaminate genetically (Blanco-Aguiar *et al.*, 2008; Casas *et al.*, 2012). Farm-reared red-legged partridges also host a greater quantity and range of pathogens than do wild partridges (Millán *et al.*, 2004; Pagès-Manté *et al.*, 2007), and releases of farmed red-legged partridges can expose wild birds to disease (Millán *et al.*, 2004; Villanúa *et al.*, 2008; Díaz-Sánchez *et al.*, 2012a, b). Importantly, red-legged partridge releases have the potential to affect not only wild partridges but also other sympatric species. For example, the little bustard (*Tetrax tetrax*) is a protected steppe bird characteristic of Spanish cereal farmland areas, whose populations have decreased by 48% in Spain during the last decade (2005–2016; García de la Morena *et al.*, 2018). Villanúa *et al.*, (2007) described the occurrence of a new parasite for this species that apparently originated from released red-legged partridges, which could potentially cause a problem. On the other hand, little bustards are more abundant on hunting estates with high levels of fox control than elsewhere (Estrada *et al.*, 2015), and predator control is more intensive on estates that release high numbers of red-legged partridges (Arroyo *et al.*, 2012).

This study had two main objectives: first, to document the spatio-temporal dynamics of red-legged partridge releases in the Ciudad Real province (central Spain) and second, to assess whether these releases are associated with changes in the local population of the little bustard in this province. As Ciudad Real is one of the Spanish provinces where partridge releasing is numerically important (Ríos-Saldaña, 2010) and is also a stronghold for little bustard in the Iberian Peninsula (García de la Morena *et al.*, 2018; Casas *et al.*, 2019), we compared population changes of little bustards between hunting estates characterised by different levels of releasing intensity.

2. Materials and methods

2.1. Study area

Data were collected in the province of Ciudad Real (19,813 km^2), located on the Spanish southern plateau. It holds one of the highest densities of breeding little bustards in Spain (García de la Morena *et al.*, 2018). It is also one of the most important areas in Spain for hunting red-legged partridges (Blanco-Aguiar *et al.*, 2003). More than 80% of the area of Ciudad Real consists of privately managed hunting estates. In most cases, the owner of the hunting rights is not the same as the owner of the land (Arroyo *et al.*, 2012). A range of management measures are frequently implemented with the aim of improving red-legged partridge hunting, the commonest being the provision of supplementary food (grain) and water, predator control and releases of farm-reared partridges (Arroyo *et al.*, 2012).

2.2. Red-legged partridge releases

A hunting estate wanting to release farm-reared red-legged partridges in Spain needs to have this specified explicitly in its Hunting Technical Plan (a document reassessed by the administration every five years, specifying the hunting intentions for the following years) and must also make an official request to the provincial game office just before the release. We obtained and analysed data from these official requests in Ciudad Real from 2002 to 2016. Our data refer to minimum numbers of released birds, as there may have been hunting estates releasing partridges without complying with the regulation (i.e. without making an official request beforehand). However, we consider that, even if not complete, our data provide a reliable source for estimating spatial and temporal trends of releases, as there is not, in principle, any bias in the type or location of estates providing information.

Technically, the release of farm-reared red-legged partridges is carried out mainly as part of "population reinforcement" and can legally occur only outside the hunting season. However, most releases take place in late summer, usually as close as possible to the opening of the hunting season (Caro *et al.*, 2014). In addition, some hunting estates are legally labelled "intensive" or "commercial". In these intensive hunting estates, there are no legal restrictions on the number or timing of farm-reared red-legged partridge releases, so releases take place throughout the hunting season and usually in very large numbers (Arroyo *et al.*, 2012). In Ciudad Real, around 6% of hunting estates are "intensive" (official data of JCLM).

For each hunting estate requesting a permit to release red-legged partridges to the provincial game office between 2002 and 2016, we collected data on the number of birds released, its area, perimeter, location and whether it was legally labelled as "intensive" or "commercial". With this information, we mapped the distribution of hunting estates that had released partridges between 2002 and 2016 using QGIS (QGIS Development Team 2018).

2.3. Temporal patterns of red-legged partridge releases

We graphically compared trends in the total number of red-legged partridges released each year on intensive and non-intensive hunting estates separately, in the number of hunting estates that released partridges each year by type of hunting estate and in the annual number of birds released per hectare by type of hunting estate.

2.4. Little bustard abundance

Two national little bustard surveys were carried out in Spain during the breeding seasons (April and May) of 2005 and 2016 (García de la Morena *et al.*, 2006; García de la Morena *et al.*, 2018). We used information from these surveys for the province of Ciudad Real. The surveys were carried out in UTM cells of 10 × 10 km (100 km^2) that comprised potentially suitable habitat for little bustard. Each 100 km^2 cell was subdivided into four 25 km^2 squares, sampling only the 25 km^2 square at the southwest of the 100 km^2 square. Within each chosen 25 km^2 square, 20 points were sampled by noting all little bustards heard or seen within a 250 m radius. In 2016, the same sampling design was used in an attempt to resample the same exact points. In total, 48 25 km^2 squares were sampled in both years. Other squares were sampled in only one of the years (either 2005 or 2016). We restricted analyses to sampling points monitored in both surveys (695 points), which represented 69.2% of all points sampled in 2005. We calculated the density of little bustard males within each circle with radius of 250 m (19.61 ha). We considered only males because females were much less detectable (García de la Morena *et al.*, 2006).

2.5. Analysis of change in little bustard density

We examined the change in numbers of little bustards by fitting a generalised linear mixed model (GLMM) with Poisson error and logarithmic link, within a Bayesian framework (*stan_glmer* function of *rstanarm* package in R; Gabry and Goodrich, 2018). Our response variable was the number of male little bustards observed at each point. As explanatory variables, we included year, date (as a categorical variable with six levels, i.e. six periods from early April to late May) and hour (as a categorical variable with 10 levels, 10 periods from sunrise to sunset); we included sampling point identity as a random factor. Hour was included to account for changes in detectability throughout the day (greatest in early morning and late afternoon, lowest at mid-day; García de la Morena *et al.* 2006), and date was included to allow for changes in detectability as bustard behaviour and habitats changed as the season progressed (García de la Morena *et al.*, 2006). We built the model in a Bayesian framework to address convergence issues using a standard GLMM approach (*lme4* package in R; Bates *et al.*, 2015). The *stan_glmer* function performs Bayesian estimation via a Markov chain Monte Carlo process. According to the default settings, this function fits four Markov chains with 2000 iterations each and 1000 interactions per chain are burned as a warm-up process. We evaluated the convergence of Markov chains using Gelman Rubin *R-hat* statistics (values < 1.1). We used the estimate package in R (Makowski *et al.*, 2019) to obtain, through our model, the mean abundance of little bustard per year, its 95% credible intervals and the percentage population change.

To map the spatial variation in little bustard population change between 2005 and 2016, we used the 48 25 km^2 squares sampled in both surveys. Each 25 km^2 square was subdivided into squares measuring 100 by 100 m (1 ha), and to each 1 ha square, we attached a smoothed density of little bustard males in a given survey calculated as (number of individuals within 1 km of the centre point of the square)/(area surveyed within 1 km of the square point) as proposed by Watson *et al.* (2007). For each 1 ha square, we then calculated the natural logarithm of the ratio between male densities in 2016 and 2005 (adding 0.5 to each value to avoid zeros). We plotted those values spatially using QGIS (QGIS Development Team, 2018).

We built another model for checking statistically the spatial distribution of population change. We calculated the natural logarithm of the ratio between male numbers counted in 2016 and 2005 (adding 0.5 to each value to avoid zeros) at each sampling point. First, we checked the variogram for spatial correlation among points (Webster and Oliver, 2007) and found none. We then built GAM models, using *mgcv* R package (Wood, 2003), to assess potential non-linearity in the relationship between population change and longitude or latitude. We found that the relationship with longitude was linear, but that with latitude seemed quadratic. We subsequently built a GLM in R, including as explanatory variables longitude, latitude, latitude squared and the interactions between longitude and the two latitude variables. Longitude and latitude were standardised before analysis using the formula (x – mean(x))/sd(x).

2.6. Relationship between releases and changes in abundance of little bustard

We used only information on red-legged partridge releases carried out in the period between the two national little bustard surveys, from 2005 to 2015 (releases of 2016 were not used because they occurred after the 2016 little bustard survey), and selected all hunting estates that included little bustard survey points in both years (2005 and 2016). Hunting estates were grouped into four categories of release intensity based on the frequency of releases and the number of birds released per ha, which should reflect the probability of restocked birds coming into contact with wild ones (**Fig. A1**, Appendix A). Categories were as follows: (i) no release: hunting estates where no release had officially been done in any of the study years (n = 90, average size = 20.20 km^2); (ii) low-intensity releases: releases occurred in less than 5 of the study years with fewer than 4 partridges per ha (n = 14, average size = 36.18 km^2); (iii) medium-intensity releases: releases occurred in more than five study years, with 4 to 15 partridges per ha (n = 6, average size = 23.63 km^2); (iv) high-intensity releases: releases were made in more than five study years with more than 15 partridges per ha (n = 6, average size = 42.43 km^2). To these, we added a fifth category for the patches of land that included a survey point and were not hunting estates (non-hunting areas, n = 234, average size = 0.3 km^2). The latter were more numerous than hunting estates, but each of them was much smaller in size (as they mainly related to small disjunct areas between hunting estates).

To estimate little bustard abundance in each of those hunting estates (or non-hunting areas) and survey year, we calculated little bustard density at each sampled point as number of males counted per survey area (19.61 ha). Then using QGIS, we determined the total sampled area in each hunting estate (or non-hunting area) as the sum of the areas of the sampled circles that were inside each hunting estate. We estimated the number of male little bustards in the sampled area as [(little bustard density in each circle × area of this buffer inside each estate)]. Finally, we divided the number of little bustards by the total sampled area (in km^2) to estimate the density of little bustards (birds per km^2) in each hunting estate or non-hunting area.

Using these data, we fitted a GLMM model (*nlme* R package; Pinheiro *et al.*, 2019) to ln(little bustard density + 1). To examine spatial effects, we included year, standardised longitude, standardised latitude, the square of standardised latitude and the interactions between longitude and the two latitude variables as explanatory variables, and the ID of hunting areas as a random effect. We included the area sampled on each hunting estate as a weight in the models. We then augmented the model by adding hunting estate category and its interactions with the spatial variables as further explanatory variables. We used Tukey HSD post hoc test for comparisons between estate categories (*lsmeans* R package; Russell, 2016). All analyses were carried out with R v3.5.1 (R Core Team, 2018), and graphics were produced with the package *ggplot2* of R (Wickham, 2016).

3. Results

3.1. Spatial and temporal patterns of red-legged partridge releases

Between 2002 and 2008, the number of hunting estates releasing red-legged partridges doubled, and the overall numbers of red-legged partridges released annually in Ciudad Real increased fourfold (**Fig. 1**). The increase in overall numbers released was due mainly to the intensive hunting estates, which constituted a third of the hunting estates where releasing took place and where the number of birds released per hectare increased eight-fold (**Fig. 1**). By contrast, the lower increase in released birds on non-intensive hunting estates was due to a greater number of estates releasing, not to a rise in the number of released birds per ha (**Fig. 1**).

From 2009 to 2016, the overall number of released red-legged partridges approximately halved, although trends differed according to the type of hunting estate. On the non-intensive hunting estates, this change was again mainly due to a decrease in the number of hunting estates that released, as the number of released red-legged partridges per ha declined only after 2013 (**Fig. 1**). On the intensive hunting estates, the number of released partridges per ha decreased strongly after 2010, while the number of hunting estates releasing was maintained (**Fig. 1**). On these intensive estates, the number of released partridges started to increase again from 2014 (**Fig. 1**). Spatially, releases of red-legged partridges in Ciudad Real were mainly concentrated in the south-eastern part of the province, although large estates with non-intensive releasing were also present in the north and north-east (**Fig. 2**). Non-intensive hunting estates that released red-legged partridges covered an area of 3343.56 km^2, while intensive hunting estates covered 545.98 km^2 . This meant that partridge releases occurred in at least 20% of the area of the Ciudad Real province.

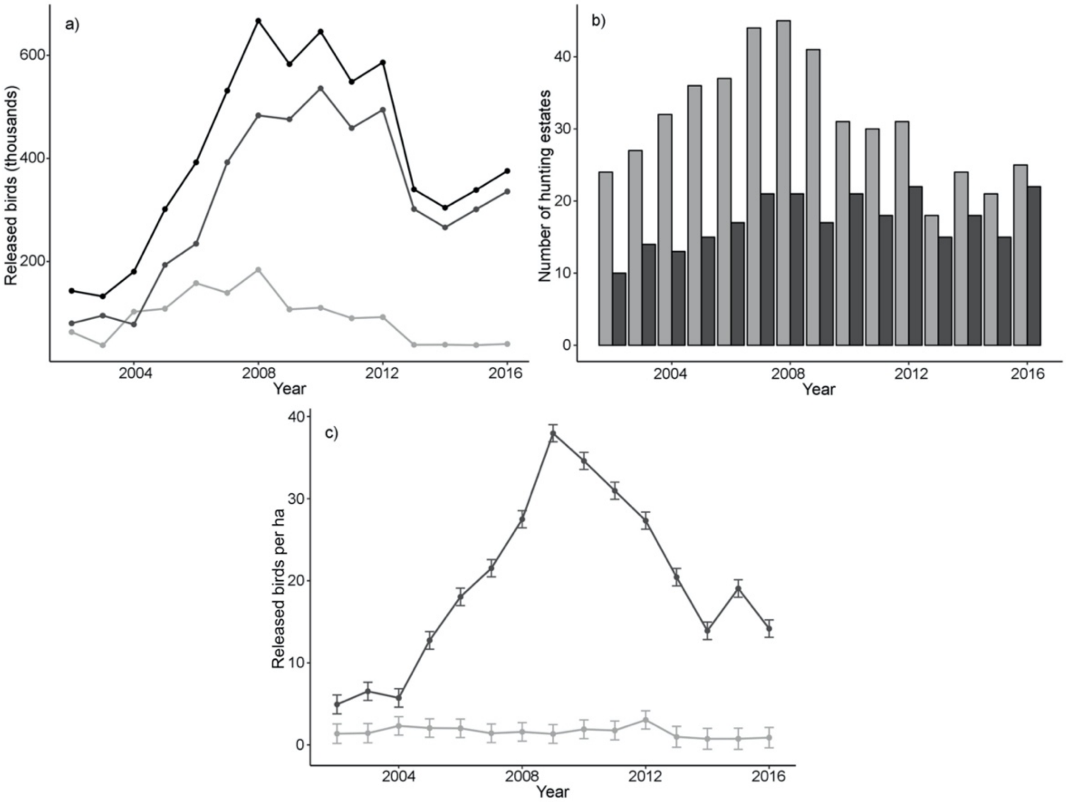

Figure 1. Temporal trends in farm-reared red-legged partridge releases in Ciudad Real, Spain, during 2002–2016. **a)** Total annual number of birds released (in thousands) on all estates (black line), on non-intensive estates (light grey) and on intensive estates (dark grey). **b)** Number of non-intensive (light grey) and intensive (dark grey) hunting estates that released partridges each year. **c)** Annual mean (± SE) number of partridges released per ha on non-intensive (light grey) and intensive (dark grey) hunting estates.

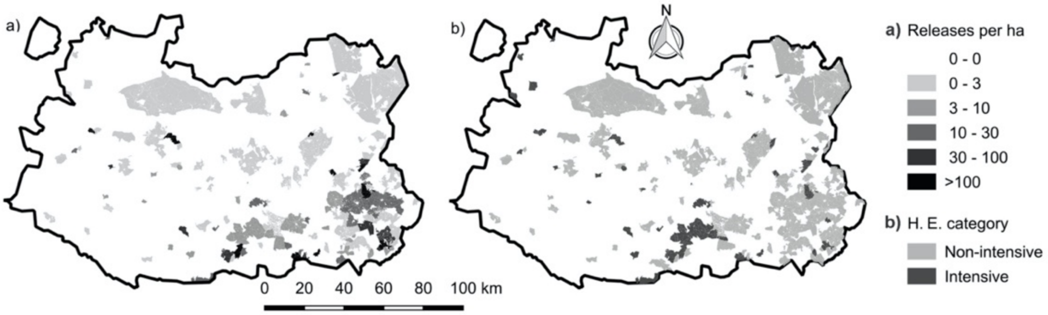

Figure 2. Distribution of red-legged partridge releases in Ciudad Real, Spain. **a)** Total releases per ha (across the 15 study years 2002–2016). **b)** Distribution of hunting estates (H.E.) by category (intensive vs non-intensive).

3.2. Changes in little bustard abundance

We found that little bustard counts varied according to sampling date and sampling hour (**Fig.3**). The number of little bustards counted per point peaked in mid-April, then declined in mid-May, increasing again in late May. Regarding the sampling hour, more birds were counted in the early morning and in the late afternoon than in the middle of the day (**Fig. 3**). In addition, we observed an overall significant decrease between 2005 and 2016 (non-overlapping 95% Bayesian credible intervals) of little bustard counts from 0.413 (95% CI 0.342–0.493) to 0.224 (95% CI 0.177–0.271) birds per point (n = 695 sampled points) in 2005 and 2016 respectively, representing a difference of - 45.8% (95% CI 30.7–60.9%).

Little bustard population change was not homogeneous across the province, and decreases appeared more pronounced in the east (**Fig. 4**). A GLM analysis confirmed this pattern, showing a positive linear relationship between population change and longitude (**Table 1**). The effect of latitude was quadratic, with lower decreases in the north and south of the province (**Table 1**). The interaction between longitude and quadratic latitude was significant, the slope of latitude being more pronounced in the south than in the north and the quadratic effect of longitude being more marked in the east than in the west (**Table 1**).

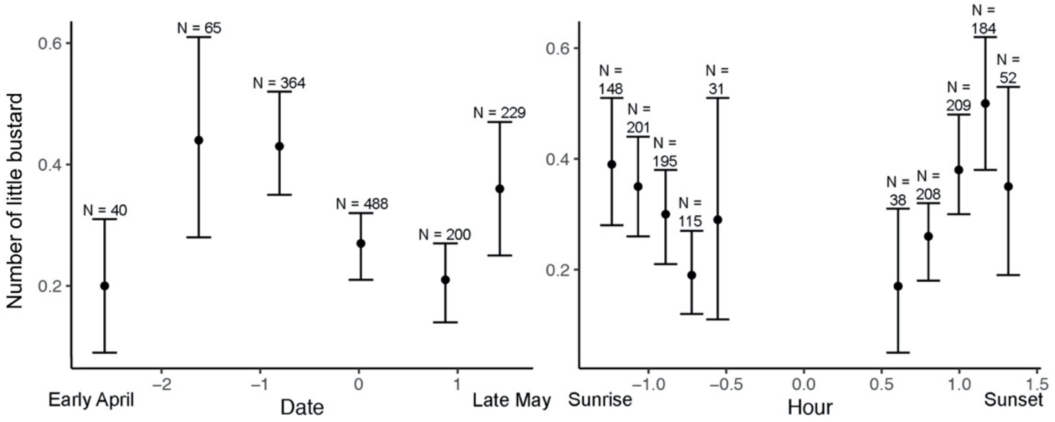

Figure 3. Effect of sampling date and hour on the number of male little bustards detected within 250 m of a sampling point. Estimated number of little bustards with 95% credible intervals for each 10 day-period (left) or each hour (right).

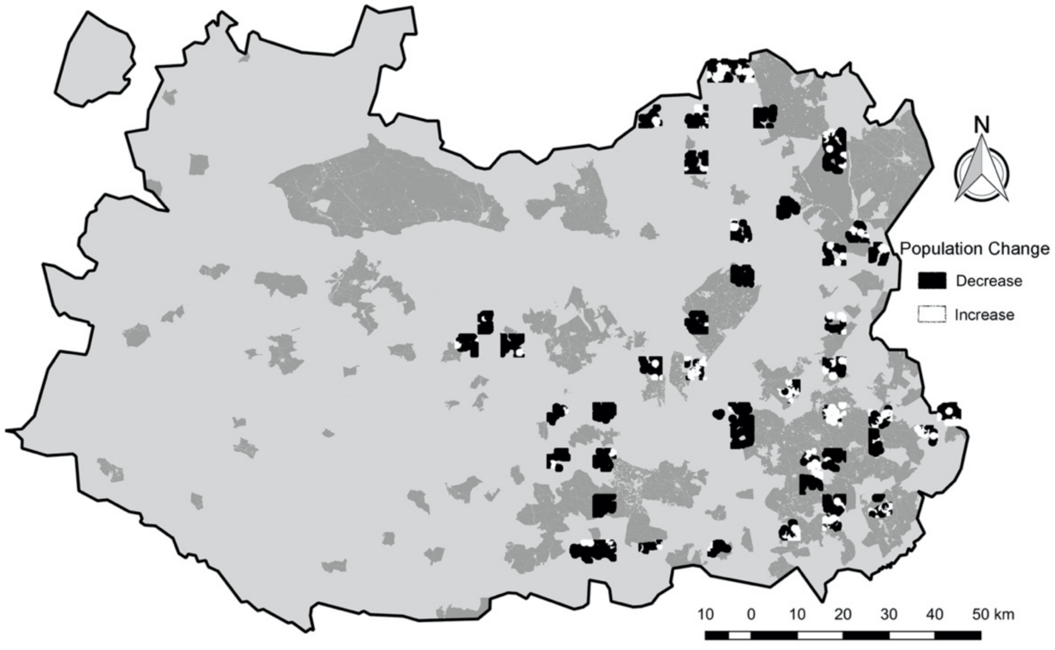

Figure 4. Spatial distribution of little bustard population change during 2005–2016 in CiudadReal, Spain. Decrease is defined as values of the ratio between 2016/2005 abundances smaller than 0, increase as values higher than 0. The hunting estates with red-legged partridge releases are represented in dark grey.

Table 1. Statistical results of GLM test for the spatial analysis of little bustard population change during 2005-2016 in Ciudad Real, Spain.

Variable	LR Chisq	Df	*P*	Estimate	SE
X	23.62	1	< **0.001**	0.09	0.019
Y	2.01	1	0.16	-0.02	0.014
Y2	13.27	1	< **0.001**	0.05	0.012
X*Y	0.10	1	0.75	0.01	0.018
$X*Y^2$	3.71	1	0.05	-0.03	0.017

X = longitude; *Y* = latitude

3.2. Relationship between releases and little bustard population change

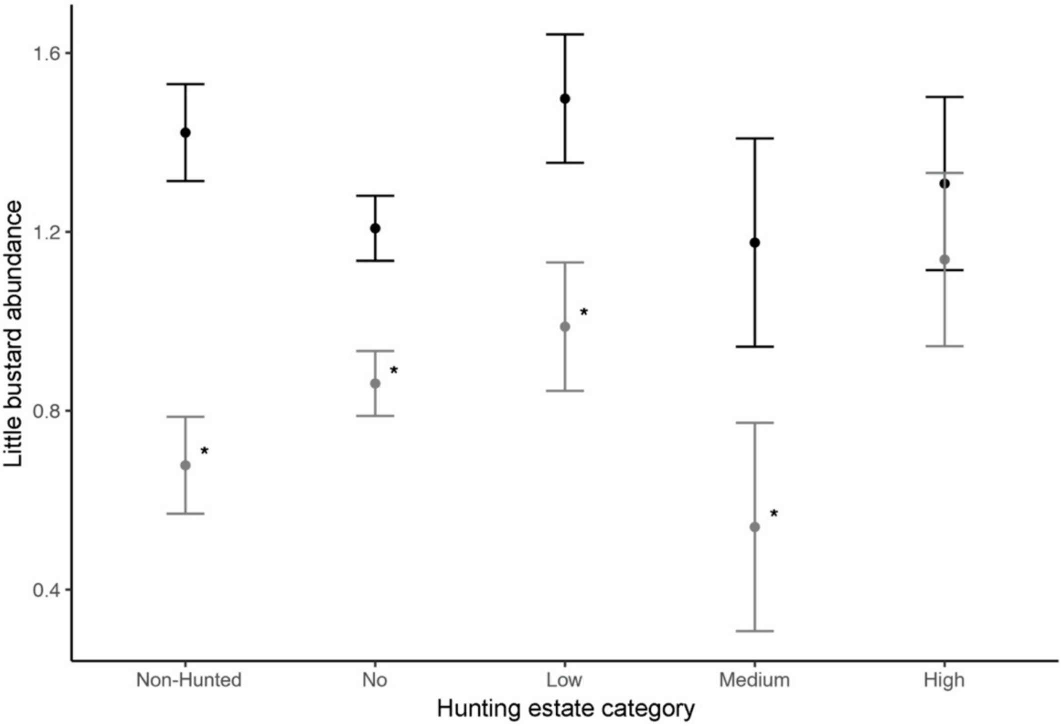

Figure 5. Mean(±SE)abundanceof breeding male little bustards (log-transformed; Ln(N/km^2 + 1)) in each type of hunting estate and survey year (2005, black and 2016, grey). *$P < 0.05$. “Non- hunted” indicates non-hunting areas; “No” indicates hunting estates without releases; “Low”, “Medium” and “High” refer to hunting states with low, medium or high release intensity.

Our GLMM model showed a significant interaction between year and hunting area categories explaining little bustard abundance (X24 = 13.28, P < 0.01), as well as a significant interaction between longitude and latitude squared (X21 = 4.74, P < 0.05). According to our data, little bustard density in 2005 was similar in all types of hunting areas (**Fig. 5**). In 2016, little bustard abundance decreased significantly in all categories except in hunting estates with high intensity of releases (t345 = 1.36, P = 0.94) (**Fig. 5**). The level of decrease was greatest in medium-intensity hunting estates (54%; t345 = 3.32, P < 0.05) and non-hunting areas (52%; t345 = 5.59, P < 0.001) (**Fig. 5**). In low-intensity hunting estates and hunting estates without releases, the level of decrease was less (34%; t345 = 3.77, P < 0.01 and 29%; t345 = 6.54, P < 0.001 respectively).

4. Discussion

4.1. Spatial and temporal patterns of red-legged partridge releases

Official release data in Ciudad Real showed a large increase in the use of this management practice during 2002–2008. This trend reversed after 2008, probably because of the economic crisis (Fernández-Albertos *et al.*, 2013; Caro *et al.*, 2014; Rahman *et al.*, 2017), which seemed to affect differently non-intensive and intensive estates. Some non-intensive estates continued releasing the same number of red-legged partridges, but many others decided to stop releasing. By contrast, all intensive estates continued releasing, but reduced the numbers of partridges per release. Intensive estates are more profitable (Arroyo *et al.*, 2017), potentially more resilient to financial problems, and may have opted for releasing less instead of stopping releases. Non-intensive hunting estates do not increase their profitability through releasing (Arroyo *et al.*, 2017), and hence, when facing economic problems, they may have decided to stop releasing completely. The decrease in the use of this management practice lasted until 2013 on intensive hunting estates. In more recent years, coinciding with the end of the worst years of the economic crisis in Spain, 2008–2013 (Rahman *et al.*, 2017), the number of released red-legged partridges increased again. This shows the importance that these hunting estates give to releases, with the numbers released increasing as soon as the economic climate was again favourable.

According to official data, around 600,000 red-legged partridges were released annually between 2008 and 2012, although these official numbers are probably underestimates. In the province, a total of 575 out of the 1370 (42%) hunting estates had written into their Hunting Technical Plans the intention of releasing partridges (Ríos-Saldaña, 2010), but only 29% of these (n = 165, representing 12% of the total) made an official request in at least one year by 2016. Estates whose Technical Hunting Plans allow them to request releases may (and do) choose not to release in one or more years. However, the discrepancy in figures may also indicate that some estates have not requested the annual authorisation, particularly if they are non-intensive and release small number of birds (see also Caro *et al.*, 2014). In a study that interviewed 51 game managers of non-intensive estates (randomly chosen) from the same area, 35% of them declared having performed releases in the previous years (Arroyo *et al.*, 2012), a percentage more similar to that coming from the Technical Hunting Plans than that arising from the annual official requests to release.

Red-legged partridge releases were concentrated in certain areas, mainly in the south-eastern part of the province. This spatial pattern suggests that the releases done by a given hunting estate may be somehow conditioned by the releases done by neighbouring hunting estates, something that has been suggested in interviews with game managers (Arroyo, unpublished results). In any case, our results indicate that partridge releases are substantial not only in terms of number but also in terms of their spatial extent, occurring on at least 20% of the area of Ciudad Real province.

4.2. Changes in little bustard abundance and its relationship with release intensity

The Spanish little bustard population, the largest in Western Europe (García de la Morena *et al.*, 2006), has suffered a 48% population reduction in 11 years (García de la Morena *et al.*, 2018). These population decreases varied among Spanish regions, with Castilla-La Mancha, which includes the Ciudad Real province, still holding 60% of the Spanish population (García de la Morena *et al.*, 2018). Despite this, our results showed that in Ciudad Real, little bustard population has also paired a strong decline, finding a difference of 46% between the counts of 2005 and 2016. Taking into account the credible interval of this estimate (31–61%), it is similar in magnitude to the value of 37% estimated by García de la Morena *et al.* (2018). Population decreases were more pronounced in the west than in the east of the province, and less pronounced in the north and south than in the longitudinal centre of the province. This spatial distribution could be explained, at least in part, by the social dynamics of the species. As a lekking species with strong conspecific attraction (Jiguet *et al.*, 2000, Morales *et al.*, 2014), the overall decrease may have prompted individuals from resultant low-density areas to move to remaining high-density areas such as the south-east of the province, following the mechanism proposed by Inchausti and Bretagnolle (2005). Whatever the mechanism, it is notable that little bustard populations decreased less (or concentrated themselves) in the south-eastern part of the province, just where the most intensive red-legged partridge releasing was conducted.

This result was also found at the scale of hunting estates categorised in terms of releasing intensity: the only areas where little bustard densities had not significantly changed were on hunting estates with the highest releasing intensity. This suggests that there may be factors that positively affect the little bustard on this type of hunting estate. Such factors may be related to habitat management or other forms of management, such as the provision of food and water or predator control (Draycott *et al.*, 2008; Fletcher *et al.*, 2010; Smith *et al.*, 2010). We did not collect data about habitat quality or quantity, but other studies have shown that some management practices used by hunting estates making frequent releases may benefit other species (Draycott *et al.*, 2008; Estrada *et al.*, 2015). Among those, predator control and the provision of game crops (crops planted specifically for game that are not harvested) are probably the most potentially beneficial for little bustards (Estrada *et al.*, 2015), and these practices are significantly more frequent on hunting estates with high-intensity releasing (Arroyo *et al.*, 2012). This is a plausible hypothesis that could explain why little bustards have decreased throughout the province except on hunting estates with high-intensity releases, and further study is required to verify it. The fact that breeding little bustards have not decreased in these areas is perhaps unexpected, given that hunting activity is a source of disturbance and affects the behaviour of this species (Casas *et al.*, 2009; Tarjuelo *et al.*, 2015), and in autumn, hunting activity is very intensive. We note that we have worked with breeding male data, and thus, we have no information on the changes in numbers of females and young, and their response to hunting management. Nevertheless, management practices such as game crops and predator control should also be beneficial for females and their offspring (Morales *et al.*, 2013; Tarjuelo *et al.*, 2013).

Neither our study design nor our results allow us to support or refute the hypothesis of an indirect impact of releases on wild little bustards through the transmission of parasites. This impact could be masked by hunting management factors (e.g. predator control or habitat management), compensating for the detrimental effects of parasite transmission or making the area more attractive for little bustards. If the latter occurs and parasite transmission takes place more strongly on these estates than elsewhere (owing to higher densities), it is possible that they may be functioning to some extent as ecological traps.

In summary, our results confirm a substantial difference in little bustard counts between 2005 and 2016, a negative population change of little bustards within the species' Spanish stronghold that was observed in the last national survey (García de La Morena *et al.*, 2018). They also show the magnitude of red-legged partridge releasing for hunting in the region, but failed to detect any negative relationship between releasing and little bustard population change. This study raises several questions for future studies to address. In particular, it is crucial to little bustard conservation to understand the mechanisms by which management of hunting estates with high-intensity releasing may facilitate the persistence of male little bustards and to assess whether such management also benefits females and young. Also, given the large spatial overlap between areas with little bustards and those with high-intensity releasing, future work should establish whether parasites transfer from released red-legged partridges to little bustards, and with what consequences. Meanwhile, the evidence presented here and from the literature suggests that at least some of the package of management measures carried out on intensive shooting estates could be useful for little bustard conservation. It would be important to identify which ones are beneficial to encourage them elsewhere.

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Supplementary material

Appendix A

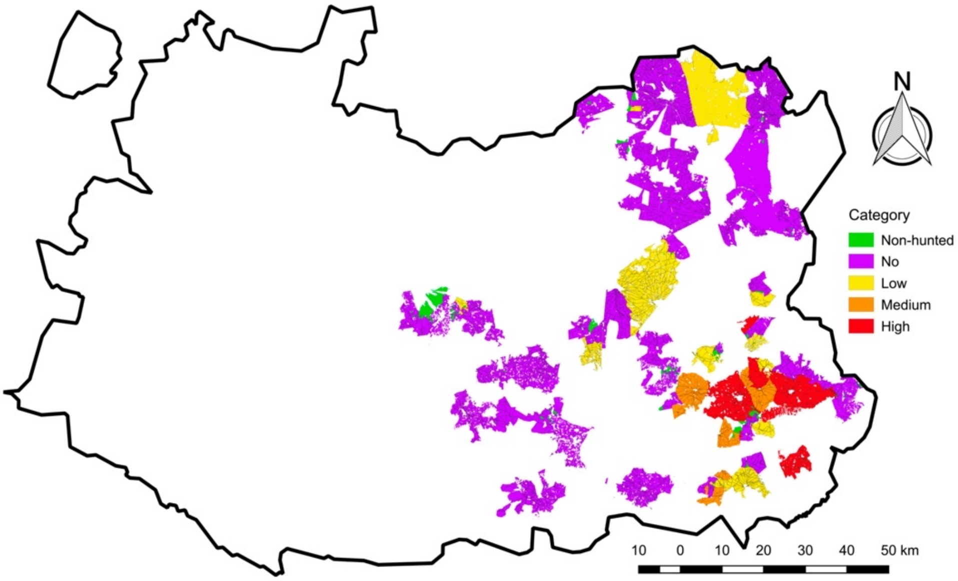

Figure A1. Spatial distribution of studied hunting estates by category of releasing intensity. “No” indicates hunting estates without releases, “Low” releases occurred in less than five of the study years with fewer than four partridges per ha, “Medium” releases occurred in more than five study years, with 4 to 15 partridges per ha, “High” releases were made in more than five study years with more than 15 partridges per ha and “Non-hunted” indicates non-hunting areas. “Non-hunted” areas are numerous (N=234), but most of them are very small (interspersed small green dots). “Non-hunted” areas are numerous (N=234), but most of them are very small (interspersed small green dots)

4

BESTELAKO FAKTORE ANTROPIKOAK

(ehizaren intentsifikazioa)

IV. ARTIKULUA

Ba al dago loturarik mehatxatutako basoilo txikiaren populazio-aldaketen eta ehizarako egindako eper gorrien askatzeen artean? Espainia erdialdean egindako eskala handiko ikerketa

- **Laburpena**
- **Sarrera**
- **Eztabaida**

Xabier Cabodevilla, Nicholas J. Aebischer, François Mougeot,
Manuel B. Morales, Beatriz Arroyo

European Journal of Wildlife Research, 66(2), 1-10.

Ba al dago loturarik mehatxatutako basoilo txikiaren populazio-aldaketen eta ehizarako egindako eper gorrien askatzeen artean? Espainia erdialdean egindako eskala handiko ikerketa

Laburpena

Ehiza helburu gisa duten haztegietan hazitako animalien askatzeak gero eta kudeaketa-praktika arruntagoa bilakatzen ari dira. Hala ere, askatze horiek ondorio negatiboak izan ditzakete bestelako basa-espezie sinpatrikoetan, adibidez parasitoen transmisioaren bitartez. Hemen, alde batetik, 15 urtez (2002-2016) Espainiako Ciudad Real probintzian egin diren eper gorrien askatzeen intentsitatea eta espazio-denbora patroiak dokumentatu ditugu. Eta, beste aldetik, 2005etik 2016ra izandako basoilo txikiaren ugaritasun-aldaketak zenbatetsi ditugu (2005ean eta 2016an eginiko erroldetan oinarrituta), eta ugaritasun-aldaketa horiek eper gorrien askatzeekin erlazionatu ditugu. Gure estimazioen arabera, probintzia-mailan urtero > 600.000 eper gorri askatu ziren, gutxienez lurraldearen % 20an zehar. Askatzeen intentsitatea aldakorra izan zen ehiza-barrutien artean, eta gorabeherak izan zituen ikertutako 15 urteetan zehar, ziurrenik, 2008-2014 bitartean gertatutako krisi ekonomikoa dela eta. Oro har, basoilo txikien kopurua % 46 murriztu zen bi zenbaketen artean, eta murrizketa handiagoa izan zen probintziaren mendebaldean. Espero ez bezala, eper gorri gehien askatu zituzten ehiza-barrutietan bakarrik murriztu ziren basoilo txikiaren kopuruak. Hori barruti horietan egiten den kudeaketaren ondorioa izan liteke, edota basoilo txikiari mesede egin dioten beste faktore batzuen ondorio, hala nola ehizarako ereintzak, harraparien kontrola edo habitataren kalitatea.

Gako-hitzak

Alectoris rufa, ehizarako ereintzak, haztegietan hazitako hegaztiak, kudeaketa, populazio-murrizketa, *Tetrax tetrax*.

1. Sarrera

Gizadiaren historian zehar, ehiza eta haren kudeaketa nabarmen aldatu dira (Washburn eta Lancaster, 2017), eta, gaur egun, Europako zenbait lekutan, ehizaren intentsifikazio-prozesu bat izaten ari da. Adibidez, ehiza-hegaztien populazioak handitzeko, haztegietan hazitako hegaztien askatzeak gero eta ohikoagoak dira (Arroyo eta Beja, 2002; Aebischer, 2019). Ehiza xeheko espezieen kasuan, horrek, batzuetan, milioika hegazti askatzea dakar (Mondain-Monval eta Girard, 2000; Noer *et al.*, 2008; Champagnon *et al.*, 2009; Caro *et al.*, 2014; VKM, 2017; Aebischer, 2019). Hala ere, praktika horrek arazo potentzial asko dakarzkie askatutako espezieen basa-populazioei (Champagnon *et al.*, 2012): espeziearen portaeran, demografian eta morfologian gertatzen diren aldaketak, patogenoak sakabanatzea, edota basa-populazioen genetikaren kalitatea okertzea (Tompkins *et al.*, 2000; Villanúa *et al.*, 2006; Arroyo eta Beja, 2002; Champagnon *et al.*, 2010; Champagnon *et al.*, 2012; Díaz-Fernández *et al.*, 2013). Gainera, espezie sinpatrikoei ere eragin diezaieke, baliabideak lortzeko lehiagatik edo patogenoen sakabanatzea dela-eta (Prenter *et al.*, 2004; Bicknell *et al.*, 2010); hala ere, habitataren kudeaketari lotutako ondorio positiboak ere deskribatu dira (Mustin *et al.*, 2018).

Eper gorria (*Alectoris rufa*) Europan askatutako ehiza-espezieetako bat da (Arroyo eta Beja, 2002). Erresuma Batuan, Frantzian eta Espainian espezie horretako milioika ale askatzen dira urtero (Tupigny, 1996; Caro *et al.*, 2014; Aebischer, 2019). Espainian, eper gorria oso ehiza-espezie garrantzitsua da kopuruz eta sozialki (Andueza *et al.*, 2018), eta Espainia erdialdeko ehiza xeheko barrutien % 94an ehizatzen da (Ríos-Saldaña, 2010). Datu ofizialen arabera, herrialde horretan, urtero 2,5-4 milioi eper gorri ehizatzen dira (MAAMA, 2014). Kopuru hori eper gorri basatien populazio ugaltzailea baino altuagoa izan daiteke, populazio ugaltzailea 1,7-3,7 bikotez osatua baita (Blanco-Aguiar *et al.*, 2004). Hainbeste eper gorri ehizatu ahal izateak erlazionaturik dago haztegietan hazitako eper gorrien askatzearekin; izan ere, azken hamarkadetan, askatutako eper gorri kopurua asko handitu da, urtero milioika hegazti askatzen baitira (Caro *et al.*, 2014).

Kudeaketa-praktika gisa, ehizarako egindako animalien askatzeak polemikoak dira (Sokos *et al.*, 2008; Gamborg eta Jensen, 2017; Mustin *et al.*, 2018; Avery, 2019), baita ehiza-sektorearen baitan ere (Delibes-Mateos *et al.*, 2014; Delibes-Mateos *et al.*, 2015; Gamborg *et al.*, 2016). Espainian, askatzeen onura nagusia ekonomikoa dela dirudi;

izan ere, badirudi ehiza komertzialari eusteko modu bakarra dela (Delibes-Mateos *et al.*, 2015). Hala ere, askatzeak (gaur egun egiten diren bezala) ez dute nahitaez laguntzen basa-populazioak berreskuratzen; adibidez, basa-populazioen genetikaren kalitatea okertzea eragin dezakete (Blanco-Aguiar *et al.*, 2008; Casas *et al.*, 2012). Gainera, haztegietan hazitako eper gorriek eper gorri basatiek baino patogeno gehiago dituzte (Millán *et al.*, 2004; Pagès-Manté *et al.*, 2007), eta eper gorri horien askatzeek eper gorri basatiak patogeno horien eraginpean jar ditzakete (Millán *et al.*, 2004; Villanúa *et al.*, 2008; Díaz-Sánchez *et al.*, 2012a, b). Bestalde, eper horiek askatzeak, eper gorri basatiei ez ezik, beste espezie sinpatriko batzuei ere eragiteko ahalmena dute. Adibidez, basoilo txikia (*Tetrax tetrax*), Espainiako nekazal inguruneko estepako hegazti babestu bat, zeinaren populazioak % 48ko jaitsiera jasan duen 2005etik 2016ra (García de la Morena *et al.*, 2018). Villanúa *et al.*-ek (2007) deskribatu zuten basoilo txiki batean itxuraz askatutako eper gorrietatik zetorren parasito berri baten agerpena, eta hori arazo bat izan daiteke. Bestalde, basoilo txikiak ugariagoak dira azerien kontrol-maila altua duten ehiza-barrutietan (Estrada *et al.*, 2015), eta eper gorri gehien askatzen dituzten barrutietan harraparien kontrola intentsiboagoa da (Arroyo *et al.*, 2012).

Ikerketa honek bi helburu nagusi zituen: lehenik eta behin, Ciudad Realen (Espainiako erdialdean) egiten diren eper gorrien askatzeen espazio-denbora patroiak dokumentatzea, eta, bigarrenik, askatze horiek probintzia horretako basoilo txikiaren populazioaren aldaketekin loturarik daukaten ebaluatzea. Ciudad Real eper gorri gehien askatzen diren Espainiako probintzietariko bat denez (Ríos-Saldaña, 2010) eta Iberiar penintsulako basoilo txikiaren gotorleku garrantzitsua ere badenez (García de la Morena *et al.*, 2018; Casas *et al.*, 2019), basoilo txikiaren populazio-aldaketak ehiza-barrutietan egiten diren askatze-intentsitate desberdinekin alderatu genituen.

4. Eztabaida

4.1. Hazitegietan hazitako eper gorrien askatzeen espazio-denbora patroiak

Ciudad Realeko datu ofizialen arabera, ehizarako kudeaketa-praktika honen erabilpena asko ugaritu zen 2002-2008 bitartean. Joera hori aldatu egin zen 2008aren ondoren, seguruenik krisi ekonomikoaren ondorioz (Fernández-Albertos *et al.* 2013; Caro *et al.* 2014; Rehman *et al.* 2017), zeinak, dirudienez, ezberdin eragin zien ehiza-barruti ez-intentsibo eta intentsiboei. Ehiza-barruti ez-intentsibo batzuek eper gorriak kopuru berean askatzen jarraitu zuten, baina askok hegazti horiek askatzeari utzi zioten. Ehiza-barruti intentsibo guztiek, aldiz, askatzeekin jarraitu zuten, baina askatutako eper gorrien kopurua murriztu zuten. Barruti intentsiboak errentagarriagoak dira (Arroyo *et al.* 2017); potentzialki, erresilientzia handiagoa izan dezakete finantza-arazoen aurrean, eta horietako askok, askatzeari utzi beharrean, nahiago izan zuten askatutako eper gorrien kopurua murriztu. Barruti ez-intentsiboek ez dute haien errentagarritasuna handitzen eper gorrien askatzeekin (Arroyo *et al.* 2017), eta, hortaz, posible da arazo ekonomikoengatik erabaki izana askatzeak erabat uztea. Kudeaketa-jarduera horren erabilerak behera egin zuen 2013ra arte ehiza intentsiboko barrutietan. Azken urteetan, Espainian, krisi ekonomikoaren urterik txarrenen amaierarekin batera, 2008-2013 bitartean (Rahman *et al.* 2017), askaturiko eper gorrien kopurua handitzen hasi zen berriro. Horrek erakusten du ehiza-barruti intentsiboek zer garrantzi ematen dioten eper gorrien askatzeei, askaturiko eper gorrien kopurua handitzen hasi baitzen egoera ekonomikoa hobetu bezain laster.

Datu ofizialen arabera, Ciudad Realen 2008 eta 2012 bitartean urtero 600.000 eper gorri askatu ziren, nahiz eta, ziurrenik, askatu ziren eperren benetako kopurua handiagoa izan. Probintzia horretan, 1.370 ehiza barrutietatik 575ek (% 42) barruti horien ehizako plan teknikoetan adierazi zuten eper gorriak askatuko zituztela (Ríos-Saldaña 2010), baina, 2016ra arte, horietatik % 29k soilik eskatu dute ofizialki eper gorriak askatzeko baimena (n = 165, barruti guztien % 12a). Ehizako plan teknikoetan eper gorriak askatzeko asmoa adierazi zuten ehiza-barrutiek ez dute zertan hegaztiak askatu. Hala ere, baimena eskatu ahal duten eta baimena eskatu duten barruti-kopuruen diferentziak adieraz dezake barruti batzuek ez dutela urtero baimena eskatzen. Hori bereziki garrantzitsua izan liteke hegazti asko askatzen ez dituzten barruti ez-intentsiboetan (ikus Caro *et al.* 2014). Beste ikerketa batean, eskualde bereko 51 barruti ez-intentsiboetako kudeatzaileak (ausaz aukeratuak) elkarrizketatu ziren, eta % 35ek adierazi zuten aurreko urtean eper gorriak askatu zituela (Arroyo *et al.* 2012). Portzentaje hori, askatzeak egiteko baimena eskatu zuten barrutien ehunekoari baino, ehizako plan teknikoetan agertzen denari gerturatzen zaio.

Eper gorrien askatzeak eremu jakin batzuetan kontzentratzen ziren, batez ere probintziaren hego-ekialdean. Aurkitutako espazio-patroi horrek iradokitzen du ehiza-barruti jakin batek egindako askatzeak, nolabait, inguruko ehiza-barrutiek egindako askatzeek baldintzatuta egon daitezkeela. Ehiza-kudeatzaileei egindako elkarrizketetan ere iradokitzen da hori (BA, argitaratu gabeko datuak). Nolanahi ere, gure emaitzek adierazten dute eper gorrien askatzeak handiak direla, ez bakarrik kopuruari dagokionez, baita azalera espazialari dagokionez ere, Ciudad Realeko probintziaren azaleraren % 20an, gutxienez, gertatzen baitira.

4.2. Basoilo txikiaren ugaritasunaren aldaketak eta horrek eper gorrien askatze-intentsitatearekin duen erlazioa

Basoilo txikiaren Espainiako populazioak —mendebaldeko Europako handiena (García de la Morena *et al.* 2006)— % 48ko murrizketa jasan du 11 urtez (García de la Morena *et al.* 2018). Espainiako erkidegoen arabera, populazio-jaitsiera hori aldakorra izan da, eta Gaztela-Mantxak, Ciudad Real probintzia barne hartzen duenak, basoilo txikiaren Espainiako populazioaren % 60ari eusten dio oraindik (García de la Morena *et al.* 2018). Hala ere, gure emaitzek erakutsi zuten Ciudad Realen basoilo txikiaren populazioak ere beherakada handia izan zuela, eta 2005eko eta 2016ko zenbaketen artean % 46ko aldea dagoela. Estimazio horren sinesgarritasun-tartea (% 31-61) kontuan hartuta, García de la Morena *et al.*-ek (2018) zenbatetsitako % 37ko aldaketaren antzekoa da. Probintziaren mendebaldean, populazioaren beherakada ekialdean baino handiagoa izan zen, eta, bestalde, probintziaren iparraldean eta hegoaldean, populazioaren beherakada txikiagoa izan zen probintziaren erdialdean baino. Banaketa espazial hori, neurri batean behintzat, espeziearen gizarte-dinamikaren bidez azal liteke. Erakarpen koespezifiko handia eta lekak eratzen dituen espeziea denez (Jiguet *et al.* 2000, Morales *et al.* 2014), bere populazioen murrizketa orokorraren ondorioz, dentsitate txikiko eremuetako gizabanakoak gainerako dentsitate handiko eremuetara joan daitezke, hala nola probintziaren hego-ekialdera, Inchaustik eta Bretagnollek (2005) proposatutako mekanismoari jarraikiz. Mekanismoa edozein dela ere, nabarmena da basoilo txikiaren populazioak probintziaren hego-ekialdean gutxiago jaitsi zirela (edo kontzentratu egin zirela), eper gorrien askatze intentsiboenak egin ziren lekuan.

Emaitza hori askatze-intentsitatearen arabera sailkatutako ehiza-barrutien eskala-mailan ere aurkitu zen: basoilo txikiaren dentsitateak nabarmen aldatu ez ziren eremu bakarrak askatze-intentsitate handieneko ehiza-barrutietan zeuden. Horrek iradokitzen du, horrelako ehiza-barrutietan, basoilo txikiari positiboki eragiten dioten faktoreak egon daitezkeela. Faktore horiek habitataren kudeaketarekin edo beste kudeaketa-modu batzuekin lotuta egon daitezke, hala nola elikagaien eta uraren hornidurarekin edo harraparien kontrolarekin (Draycott *et al.* 2008; Fletcher *et al.* 2010; Smith *et al.* 2010). Habitaten kalitateari edo kantitateari buruzko daturik ez genuen jaso, baina beste ikerketa batzuek frogatu dute eper gorriak maiz askatzen dituzten barrutietan egindako kudeaketa-praktika batzuk onuragarriak izan daitezkeela beste espezie batzuentzat (Draycott *et al.* 2008; Estrada *et al.* 2015). Praktika horien artean, harrapakarien kontrola eta ehizarako laboreen hornidura (ehizarako bereziki landatutako laboreak, biltzen ez direnak) dira, seguruenik, basoilo txikientzako onuragarrienak (Estrada *et al.* 2015), eta praktika horiek askoz ere ohikoagoak dira askatze-intentsitate handiko ehiza-barrutietan (Arroyo *et al.* 2012). Hipotesi hori onargarria da, eta probintzia osoan, askatze-intentsitate handiko ehiza-barrutietan izan ezik, gertatutako basoilo txikien murrizketa azal lezake. Hala ere, ikerketa gehiago egin behar dira hori egiaztatzeko. Eremu horietan ugalketa-garaiko basoilo txikien populazioa murriztu ez izana ustekabekoa izan daiteke; izan ere, ehiza-jarduera espezie horren jokaerari eragiten dion asaldura bat da (Casas *et al.* 2009; Tarjuelo *et al.* 2015), eta udazkenean ehiza-jarduera oso handia da. Nabarmendu behar dugu arren datuekin lan egin dugula, eta, beraz, ez dugula informaziorik emeen eta kumeen kopuruan izandako aldaketei buruz, ezta horiek ehizaren kudeaketari emandako erantzunari buruz ere. Hala ere, kudeaketa-praktikak, hala nola ehizarako laboreak eta harraparien kontrola, onuragarriak izan beharko lirateke emeentzat eta haien kumeentzat (Morales *et al.* 2013; Tarjuelo *et al.* 2013).

Ez gure ikerketaren diseinuak ez gure emaitzek ez digute aukerarik ematen askatzeak, parasitoen transmisioaren bidez, basoilo txikietan duten zeharkako inpaktuaren hipotesia babesteko edo ezeztatzeko. Ehizaren kudeaketak (adibidez, harraparien kontrolak edo habitataren kudeaketak) inpaktu hori estal dezakete, parasitoen transmisioaren ondorio kaltegarriak konpentsatuz, edota basoilo txikientzako eremua erakargarriago eginez. Azken hori gertatuz gero, eta parasitoen transmisioa barruti horietan beste barrutietan baino intentsitate handiagoz gertatzen bada (dentsitate handiagoak direla eta), baliteke barruti horiek tranpa ekologiko gisa jokatzea neurri batean.

Laburbilduz, gure emaitzek berresten dute 2005 eta 2016ko basoilo txikiaren zenbaketen artean alde nabarmena dagoela, Espainiako basoilo txikiaren gotorlekuan emandako populazio-aldaketa negatiboa (García de La Morena *et al.* 2018). Era berean, eskualdean ehizarako egiten diren eper gorrien askatzeen tamaina erakusten dute, baina ez dute erlazio negatiborik hauteman askatzeen eta basoilo txikiaren populazio-aldaketaren artean. Ikerlan honek hainbat gai planteatzen ditu, etorkizuneko ikerketetan jorratu beharrekoak. Bereziki, basoilo txikia kontserbatzeko, ezinbestekoa da, batetik, ulertzea askatze-intentsitate handiko barrutietan egiten diren kudeaketa-praktiken artean zeintzuk diren onuragarriak arrentzat, eta, bestetik, ebaluatzea kudeaketa horiek emeei eta kumeei ere mesede egiten ote dieten. Gainera, askatze-intentsitate handiko barrutien eta basoilo txikiak dituzten zonen arteko gainjartze espazial handia dela eta, etorkizuneko ikerketek zehaztu beharko lukete askatutako eperretatik basoilo txikietara parasitoak transferitzen diren eta horren ondorioak zeintzuk diren. Bitartean, hemen aurkeztutako datuek eta datu bibliografikoek iradokitzen dute ehiza intentsiboko barrutietan aplikaturiko kudeaketa-neurri batzuk, gutxienez, basoilo txikia kontserbatzeko baliagarriak izan daitezkeela. Onuragarriak diren kudeaketa-neurriak identifikatzea garrantzitsua litzateke, horiek beste leku batzuetan sustatzeko.

Esker onak

Eskerrak Luis Basurtori (Ciudad Real Probintziako Kontseilua; JCCM) hazitegietan hazitako eper gorrien askatzeen datu ofizialak atzitzea baimendu izanagatik. Basoilo txikiaren errolden datuak SEO-BirdLife-k partekatu zituen adeitasunez. Xabier Cabodevillak Eusko Jaurlaritzaren Hezkuntza, Unibertsitate eta Ikerketa Sailaren doktoretza-bekaren eta mugimendu-bekaren babesarekin burutu zuen lana (Erref.: PRE_2018_2_0273 eta EP_2019_1_0070). Ikerketa hau Eskualde Garapenerako Europako Funtsak (Feder) eta Gaztela-Mantxako Komunitateen Batzordeak finantziatutako AGROPERDIZ (SBPLY/17/180501/000245) proiektuaren barne dago.

5

FARMLAND BIRDS' DIET AND HEALTH STATUS

PAPER V

Simultaneous analysis of the intestinal parasites and diet through eDNA metabarcoding

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Integrative Zoology (Under review)

Simultaneous analysis of the intestinal parasites and diet through eDNA metabarcoding

Abstract

Agricultural expansion and intensification are having a huge impact on plant and arthropod diversity and abundance, affecting food availability for farmland birds. Difficult food access, in turn, can lead to immunosuppression and a higher incidence of parasites. In the studies designed to examine changes in the diet of birds and their parasites, metabarcoding is proving particularly useful. This technique requires mini-barcodes capable of amplifying the DNA of target organisms from faecal environmental DNA. To help to understand the impact of agricultural expansion on biodiversity, this study sought to design and identify mini-barcodes that might simultaneously assess diet and intestinal parasites from the faeces of farmland birds. The capacity to identify diet and parasites of two existing and three developed mini-barcodes was tested "in silico" in relation to the behaviour of a reference eukaryotic barcode. Among the newly designed mini-barcodes, MiniB18S_81 showed the higher taxonomic coverage of eukaryotic taxa and a greater amplification and identification capacity for diet and parasite taxa sequences. Moreover, when it was tested on faecal samples from five different steppe bird species, MiniB18S_81 showed high taxonomic resolution of the most relevant diet and parasite phyla, Arthropoda, Nematoda, Platyhelminthes and Apicomplexa, at the order level. Thus, the mini-barcode developed emerges as an excellent tool to simultaneously provide detailed information regarding the diet and parasites of birds, essential for conservation and management.

Keywords

Biomonitoring; broad-spectrum primers; eukaryotic mini-barcodes; faecal eDNA; farmland birds; parasitology

1. Introduction

Owing to ever-changing climate and land-use patterns, biodiversity is increasingly under threat (Pimm *et al.*, 1995; Jetz *et al.*, 2007). In the last 50 years, especially, agricultural expansion has brought about changes in land-use that have severely compromised biodiversity (Meyer and Turner, 1992; Matson *et al.*, 1997; Pain and Pienkowski, 1997; Newton, 1998; Chamberlain *et al.*, 2000; Benton *et al.*, 2003). Among other consequences, the expansion of croplands is diminishing plant species abundance and richness (Wilson *et al.*, 1999; Storkey *et al.*, 2012; Fonderflick *et al.*, 2020), which ultimately affect both the seed banks (Wilson *et al.*, 1999; Andreasen *et al.*, 2018) and the seasonality of seed availability (Newton, 2004). Furthermore, as the seeds used in intensive agriculture are mostly treated with pesticides, this has devastating repercussions on the birds that ingest these seeds (Prosser and Hart, 2005; Lopez-Antia *et al.*, 2015; Millot *et al.*, 2017; Eng *et al.*, 2019). The intensification of agriculture has been also identified as the main driver of the decline of insect populations (Benton *et al.*, 2002), which are another important dietary component of many bird species, and their declines are already having a notable impact on insectivorous bird communities (Bowler *et al.*, 2019). Land-use changes and reduced food availability are a stress factor for birds, affecting their health and immune response (Kitaysky *et al.*, 1999; Hoi-Leitner *et al.*, 2001; Kitaysky *et al.*, 2007; Pigeon *et al.*, 2013; Almasi *et al.*, 2015) by lowering their defences against parasites (Korschgen *et al.*, 1978; Wakelin, 1996; Nordling *et al.*, 1998). Synergistic effects of food availability, parasitism and stress have been observed on bird population densities (Chapman *et al.*, 2006). Disease/parasitism and nutrition often interact to determine the abundance of wildlife populations. Helminthic and protozoan parasites can directly impact host survival and reproduction through pathological effects and indirectly by compromising the host's health state (Coop and Holmes, 1996; Murray *et al.*, 1998). Hence, parasitism is also an important factor to consider as a potential risk for both wild and farm-reared bird species (Lafferty, 1997; Dunn *et al.*, 2014; Stockdale *et al.*, 2015; Cabodevilla *et al.*, 2020).

To date, the studies designed to address the diet of farmland birds have paid most attention to available trophic resources (plants and arthropods), without confirming whether these were consumed or not (Salamolard and Moreau, 1999; Holland *et al.*, 2006; Faria *et al.*, 2012; Holland *et al.*, 2012), whereas the studies focusing on the ingested prey have examined the diet through visual identification of the remains of prey exoskeletons present in the faeces of the birds (Jiguet, 2002; Browne *et al.*, 2006; Holland *et al.*, 2006; Bravo *et al.*, 2017). However, this identification method, despite it is widely used for diet analysis, does not generally go beyond the ordinal taxonomic level and few individuals are identified at deeper levels (Jiguet, 2002; Browne *et al.*, 2006; Bravo *et al.*, 2017). In addition, some of the ingested preys could remain undetected or unidentified because of the difficulties in identifying them after undergoing digestion (Moreby, 1988; Pompanon *et al.*, 2012). The case of parasitological studies conducted in farmland birds is similar as most have involved the visual identification of parasites (Browne *et al.*, 2006; Okulewicz and Sitko, 2012; Rengifo-Herrera *et al.*, 2014; Presswell and Lagrue, 2016), often via the dissection of dead animals (Villanúa *et al.*, 2008; Santoro *et al.*, 2010; Okulewicz and Sitko, 2012). However, in non-invasive studies of faecal samples, the most abundant parasites can be visually identified although low intensity parasitism is hardly discernible (Rengifo-Herrera *et al.*, 2014; Presswell and Lagrue, 2016). Moreover, taxonomic identification based on the morphological appearance of the eggs of many species is also limited or even impossible (Browne *et al.*, 2006; Presswell and Lagrue, 2016).

The introduction twenty years ago of environmental DNA (hereafter eDNA) analysis opened a new avenue for ecology (Taberlet *et al.*, 2012a). This method has quickly gained importance in parallel with new - generation sequencing technology (Shokralla *et al.*, 2012; Taberlet *et al.*, 2012a, b) and is becoming increasingly popular for the study of diet, microbes and parasites (Pompanon *et al.*, 2012; Shokralla *et al.*, 2012; Taberlet *et al.*, 2012b; Bass *et al.*, 2015; Kerley *et al.*, 2018). Through eDNA analysis, relevant information can be obtained from non-invasive samples such as faeces (Srivathsan *et al.*, 2016), thus avoiding the need to examine dead animals. The use of faecal samples allows to investigate the host genetics, parasites, gut microbiota and diet (Srivathsan *et al.*, 2016). However, faeces usually contain various substances that act as PCR inhibitors (Lantz, 1997; Wilson, 1997; Rådström *et al.*, 2004), and thus, the design of proper mini-barcodes for eDNA analysis in faeces is needed.

Many different barcodes (usually longer than 300 bp) have been designed for DNA metabarcoding, both specific and broad-spectrum (Prosser *et al.*, 2013; Hadziavdic *et al.*, 2014; Van Steenkiste *et al.*, 2015; Cheng *et al.*, 2016; Krehenwinkel *et al.*, 2018). Nevertheless, the length of the barcode used in environmental metabarcoding studies should be carefully chosen (Hajibabaei *et al.*, 2006; Deagle *et al.*, 2006; Deagle *et al.*, 2007; Taberlet *et al.*, 2012a) because the DNA from water, soil, air or faeces is often degraded (Deagle *et al.*, 2006; Yu *et al.*, 2012; Taberlet *et al.*, 2012b). Studies that have focused on the metabarcoding of eukaryotic eDNA have already described specific mini-barcodes (shorter than 300 bp) for different taxa (Epp *et al.*, 2012; Pompanon *et al.*, 2012), although they provide a narrow view of the sample. On the contrary, broad-spectrum mini-barcodes can provide information on both target taxa and on their overall contribution to a higher category taxon, allowing to get a better understanding on the sample. Thus, the use of proper broad-spectrum mini-barcodes may allow to get relevant information on diet and intestinal parasites in the same analysis. However, as far as we know, the eukaryotic mini-barcodes available have not been tested with this purpose in mind. In addition, although metabarcoding cannot be considered a quantitative tool, several studies have shown some quantitative capacity of this method (Evans *et al.*, 2016; Lamb *et al.*, 2019; Piñol *et al.*, 2019). Accordingly, it should be possible to compare the proportions of each taxon among similar samples, e.g., water samples from different ponds of faeces collected in different seasons (Pompanon *et al.*, 2012). The use of candidate mini-barcodes to identify taxa, nevertheless, depends on the existence of a robust reference sequence data (Clarke *et al.*, 2014). Moreover, for an accurate estimate of biodiversity, a sufficiently variable DNA region needs to be amplified. The most used DNA genes for barcode design are mitochondrial cytochrome c oxidase subunit 1 (COI) and cytochrome b (cytb), ribosomal RNA genes 12S rRNA, 16S rRNA and 18S rRNA gene sequences, and nucleic internal transcribed spacer regions 1 and 2 (i.e. ITS1 and ITS2) and Ribulose bisphosphate carboxylase large chain (rbcL) (Hajibabaei *et al.*, 2007; Pompanon *et al.*, 2012; Andújar *et al.*, 2018; Djurhuus *et al.*, 2020). Among these, the 18S rRNA gene spans an especially variable region for which there exists a robust and constantly expanding reference dataset (Hadziavdic *et al.*, 2014).

The main goal of this study was to identify an appropriate mini-barcode for eDNA analysis on faecal samples to retrieve information on both the diet and the intestinal parasites of birds. We assessed already existing mini-barcodes as well as self-designed mini-barcodes targeting the 18S or 28S rRNA genes in terms of their suitability for their use on bird faecal samples. Once we had identified a candidate mini-barcode, it was tested empirically using a high-throughput sequencing approach on faecal samples of different steppe bird species.

2. Materials and methods

2.1. Mini-barcode design

We used a dataset of 5000 sequences selected randomly from SILVA SSU v132 reference and non-redundant database (Quast *et al.*, 2013), downloaded via the function *obisilva* of OBITools software (Boyer *et al.*, 2016). The selection process was based on sequence annotation of the SILVA database including 1000 sequences for each phylum Phragmoplastophyta, Apicomplexa, Arthropoda, Nematoda and Platyhelminthes. The sequences were then aligned in Geneious software v11.1.4 (Biomatters Ltd, New Zealand; Kearse *et al.*, 2012) and the resulting alignment formed the baseline for the design of the primers. Primer design was performed using Geneious software v11.1.4. with the following parameters: primer length 18–27 nucleotides, melting temperature (T_m) 57–63°C and GC content 30–80%. Next, we assessed mini-barcodes for their potential to form dimers or alternative amplicons. Two approaches were implemented: a) 100 mini-barcodes (amplicon size < 200 bp) were designed to cover the sequences of Nematoda and Platyhelminthes phyla (2000 sequences; **Table A1**, Appendix A); and b) 20 mini-barcodes (amplicon size 100-200 bp) were designed using the full dataset of 5000 sequences (**Table A2**, Appendix A). Mini-barcodes were then selected according to the following criteria:

i) Mini-barcodes appearing in both approaches.
ii) Mini-barcodes covering a variable region (V1 to V9) of the 18S rRNA gene.
iii) Mini-barcodes covering the edge of the alignment area were discarded as in this region there was no information for many sequences.
iv) In the case of mini-barcodes generated with similar primers, we selected the longer mini-barcode.

After this filtering, three mini-barcodes were selected, two for the V3 region (MiniB18S_41 and MiniB18S_43) and one for the V7-V8 region (MiniB18S_81) (**Table 1**).

Table 1. List of barcodes tested in this study. More information on amplicon lengths is provided as **Figure A2**, Appendix A.

Barcode	Primer	Mean length	Reference
MiniB18S_41	**F**: CAGGGTTCGATTCCGGAGAG **R**: CACCAGACTTGCCCTCCAAT	156 bp (mini-barcode)	This study
MiniB18S_43	**F**: AACGGCTACCACATCCAAGG **R**: CACCAGACTTGCCCTCCAAT	125 bp (mini-barcode)	This study
MiniB18S_81	**F**: GGCCGTTCTTAGTTGGTGGA **R**: CCCGGACATCTAAGGGCATC	150 bp (mini-barcode)	This study
566F/1200R	**566F**: CAGCAGCCGCGGTAATTCC **1200R**: CCCGTGTTGAGTCAAATTAAGC	610 bp (large-barcode)	Hadziavdic *et al.* 2014
nucLSUDf1/nucLSUDr1	**nucLSUDf1**: CGTCTTGAAACACGGACCAAG **nucLSUDr1**: GCATAGTTCACCATCTTTCGGG	156 bp (mini-barcode)	Sonnenberg *et al.* 2007
Short28SF/ Short28SR	**Short28SF**: GTGTAACAACTCACCTGCCG **Short28SR**: GCTACTACCACCAAGATCTG	154 bp (mini-barcode)	Vestheim & Jarman 2008

2.2. Mini-barcode test on real tissue

We first tested whether the designed mini-barcodes (**Table 1**) could amplify DNA in simple PCRs conducted on 7 tissue samples of 6 different phyla: a nematode (*Ascaridia galli* Schrank, 1788), a cestode (*Raillietina cesticillus* Molin, 1858), an insect (*Apis mellifera* Linnæus, 1758), an arachnid (*Ischyropsalis pyrenaea* Simon, 1872), a leech (*Helobdella stagnalis* Linnæus, 1758), a gastropod (*Cepaea hortensis* O.F.Müller, 1774) and a bird (*Scolopax rusticola* Linnæus, 1758). DNA extraction from tissues was performed using the kit DNeasy® Blood & Tissue Kit (Ref. 69506) (QIAGEN). All PCR reactions (20 µl) were carried out using 1 µl of DNA in reactions with 1.25 µl of MgCl (25 mM), 2 µl of dNTPs (20 mM), 0.25 of 10X Buffer B, 0.3 µl of each forward (20 pM) and reverse (20 pM) primers, 1 µl of BSA (10mg/ml) and 0.25 µl of BIOTAQ™ DNA Polymerase of Bioline (ref. BIO-21060; 5 U/µl). The thermocycle conditions were: 96°C for 1 minute and 35 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 1 minute. PCR amplification capacity was confirmed by electrophoresis in 1.5% TBE agarose gel. All tested barcodes showed capacity to amplify the DNA of these 7 different tissue samples belonging to 6 different phyla (Nematoda, Cestoda, Annelida, Mollusca, Arthropoda, and Vertebrata).

2.3. "In silico" PCR

We also checked by "in silico" PCR the new mini-barcodes (**Table 1**) together with the two eukaryotic mini-barcodes of the 28S rRNA gene most widely used in next generation sequencing (NGS) diet studies (Pompanon *et al.*, 2012): nucLSUDf1/nucLSUDr1 (Sonnenberg *et al.*, 2007) and Short28SF/Short28SR (Vestheim and Jarman, 2008). In addition, we compared the taxonomic coverage and resolution of these mini-barcodes to those of the reference eukaryotic barcode F566/R1200 (Hadziavdic *et al.*, 2014) (**Table 1**). This barcode has been identified as the best eukaryotic barcode among "de novo" designed and previously existing universal eukaryotic 18S rRNA barcodes (Hadziavdic *et al.*, 2014).

All mini-barcodes were “in silico” tested using the program ecoPCR (Ficetola *et al.*, 2010) and OBITools (Boyer *et al.*, 2016). First, we used the *obisilva* function to download the ecoPCR format SILVA 132 SSU reference (cleaned to keep only high quality sequences) and non-redundant dataset. Likewise, we downloaded the ecoPCR format SILVA 132 LSU reference dataset. For large subunits (LSU), SILVA has no non-redundant version. Second, we used the function *ecotaxstat* of ecoPCR (Ficetola *et al.*, 2010) to test the taxonomic coverage of the 3 designed mini-barcodes, F566/R1200 eukaryotic long barcode (650 bp), and nucLSUDf1/nucLSUDr1 and Short28SF/Short28SR 28S mini-barcodes on Bacteria, Archaea and Eukarya. In the same way, we used the functions *ecotaxstat* and *ecotaxspecificity* to test their taxonomic coverage and resolution capacity on 10 eukaryotic phyla (**Table A3**, Appendix A): Apicomplexa, Nematoda and Platyhelminthes including main parasites of birds; Phragmoplastophyta, Annelida, Arthropoda and Mollusca which may appear in birds' diets; Ascomycota and Basidiomycota to check for fungal sample contamination; and Vertebrata to check if the barcodes could identify the host. We especially focused on the phyla Phragmoplastophyta, Apicomplexa, Arthropoda, Nematoda and Platyhelminthes, as we anticipated these would be the taxa mostly represented in the diet and parasites of birds. The *ecotaxspecificity* option -*e* 0 was used, which does not allow any mismatches. Taxonomic classification was assigned according to SILVA 's reference database (www.arb-silva.de).

2.4. Metabarcoding proof

Finally, based on both the taxonomic coverage and the resolution capacities revealed in the “in silico” PCRs, we selected the MiniB18S_81 mini-barcode for the assessment in a real metabarcoding analysis. The samples tested were faecal samples from 12 bird flocks of 5 different steppe bird species; 1 flock of farmed red-legged partridges (*Alectoris rufa*), 2 flocks of wild red-legged partridges (*Alectoris rufa*), 4 flocks of pin-tailed sandgrouse (*Pterocles alchata*), 1 flock of black-bellied sandgrouse (*Pterocles orientalis*), 2 flocks of great bustards (*Otis tarda*) and 2 flocks of little bustards (*Tetrax tetrax*). All the samples were collected between 2016 and 2017 in the province of Ciudad Real (Spain) with the exception of the samples of a pin-tailed sandgrouse flock collected in 2017 in the province of Lleida (Spain).

Faeces were collected in the roosts without trapping any birds and, whenever possible, fresh faeces from 20 individuals of each flock were collected. DNA extractions were performed using the QIAamp® DNA Stool Mini Kit from QIAGEN (Ref. 51504). From 15 to 20 DNA samples were obtained per flock (DNA could not be extracted from every sample). The concentration (μg/ml) and quality (A260/280 and A260/230 ratios) of each DNA sample were measured using the NanoVue Plus spectrophotometer (GE Healthcare). Samples from each flock were mixed according to DNA quantity and quality in 3 pools of 5-7 samples, one of high, one of medium and one of low quantity (**Table A1**, Appendix A). Subsequently, DNA was amplified using PCR from extracts using each of the designed primers (miniB18S_81F and miniB18S_81R) (**Table 1**) at the Analytical Services (SGIker) of the University of the Basque Country (UPV/EHU). To retrieve enough amplified DNA for the sequencing reaction, three PCR reactions were run per sample pool. Samples were purified and a second reaction was performed to index each amplified product and attach Illumina index using the Illumina Nextera v2 kit. We used the same Illumina index for the three pools prepared for each flock. Amplification (20 μl) was carried out using 1 μl of DNA in a reaction mix containing 1.25 μl of MgCl (25 mM), 2 μl of dNTPs (20 mM), 0.25 of 10X Buffer B, 0.3 μl of each forward (20 pM) and reverse (20 pM) primer, 1 μl of BSA (10mg/ml) and 0.25 μl of GoTaq® Flexi DNA Polymerase from Promega (ref. M829; 5 U/μl). The thermocycle conditions were: 96°C for 1 minute and 35 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 1 minute. Once amplified, PCR outputs were sequenced in an Illumina MiSeq NGS platform (sequencing of 2x150 bp paired-end reads) with the MiSeq Reagent Kit v2, following the manufacturer's instructions.

Bioinformatic sequencing output tests were conducted using the Cutadapt (Martin, 2011) and Usearch (Edgar, 2010) software packages. First, the primers were cut and the forward and reverse sequences combined. We used the *join.fastq* function of Usearch instead of merging forward and reverse sequences to avoid losing sequences of amplicons longer than 300 bp. The sequences were then cleaned (*fastq_filter* fuction of Usearch), the unique sequences and OTUs were identify using *fastx_uniques* and *cluster_otus* functions of Usearch respectively, and OTU table was built based on 97% OTUs using the *usearch_global* function. Subsequently, the *sintax* function of Usearch was used to predict the taxonomy of the OTUs obtained with a *cutoff* of 0.8 and a combined 18S and 16S dataset. The reference dataset was built combining the SILVA 18S v123 dataset (Quast *et al.*, 2013) with the RDP 16S v16 training set (Cole *et al.*, 2014), both provided by the Usearch platform. We opted for the RDP 16S v16 training dataset instead of a complete SILVA, Greengenes or RDP 16S dataset because of the high level of mistaken sequences reported for these datasets (Edgar, 2018).

All the graphs were constructed using the *ggplot2* package (Wickham, 2016) in R v3.6.1 (R Core Team, 2019).

3. Results

3.1. “In silico” testing

All the tested mini-barcodes showed short mean amplicon lengths (**Table 1**), as well as a quite constant amplicon length (**Figure A1**, Appendix A). Although the five mini-barcodes generated amplicons between 30 and 900 bp, percentages of sequences longer than 300 bp were negligible (0.06% MiniB18S_41; 0.05% MiniB18S_43; 1.16% MiniB18S_81, 0.34% nucLSUDf1/nucLSUDr1 and 0.32% Short28SF/Short28SR). In the case of MiniB18S_81, cestodes showed larger amplicons with a mean length of 299 bp.

Both the five mini-barcodes and the reference eukaryotic barcode showed high taxonomic coverage for the eukaryotic organisms and null capacity to amplify organisms of the taxa Bacteria and Archaea (**Figure 1**). Of the three mini-barcodes designed in this study, MiniB18S_81 showed the highest taxonomic coverage for eukaryotic organisms. This mini-barcode amplified 91.1% of the eukaryotic sequences present in the SILVA reference database, which is the highest score obtained among the mini-barcodes tested in this study. Only the large barcode 566F/1200R showed a slightly better taxonomic coverage for eukaryotic organisms (92.6%; **Figure 1**). In addition, all barcodes showed a good capacity to amplify eukaryotic phyla sequences: MiniB18S_41 amplified sequences of 131 out of the 138 phyla in the SSU reference database (i.e. the 94.9% of the phyla), MiniB18S_43 amplified 129 out of the 138 phyla (93.5%), MiniB18S_81 amplified 132 out of the 138 phyla (95.7%), 566F/1200R amplified 136 out of the 138 phyla (98.6%), nucLSUDf1/nucLSUDr1 amplified sequences of 85 of the 88 phyla in the LSU reference database (96.6%), and Short28SF/Short28SR amplified 85 of the 88 phyla (96.6%).

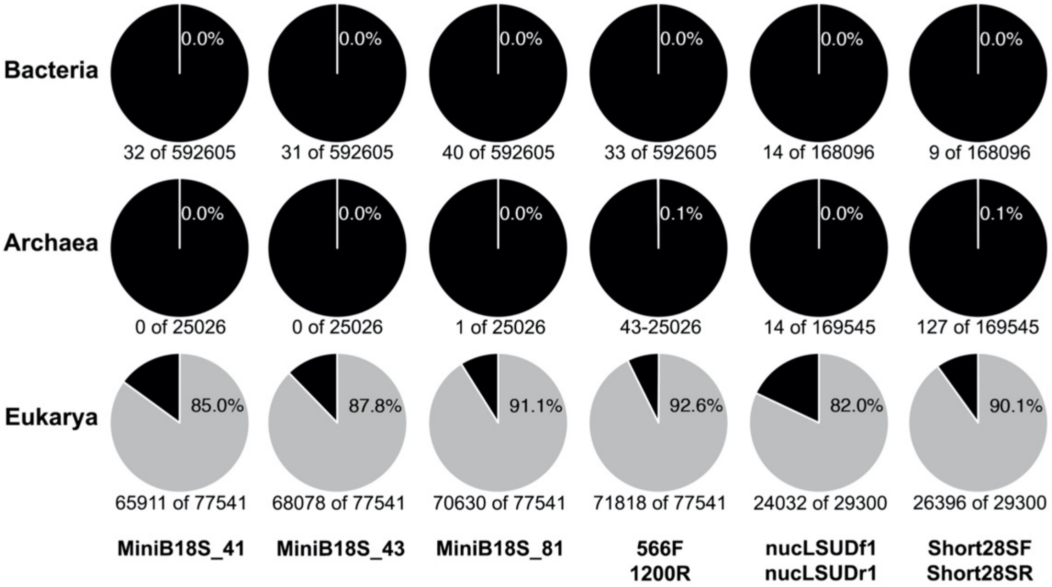

Figure 1. "In silico" taxonomic coverage capacity of barcodes of Bacteria, Archaea and Eukarya taxa. Grey colour represents the proportion of amplified sequences and black colour the proportion of non-amplified sequences. Inside of each graph is the percentage of amplified sequences and below the graph is the number of amplify sequences upon total sequences of this taxa in reference data set.

All barcodes were able to amplify large numbers of organisms of the 10 target phyla (**Table A3**, Appendix A). However, MiniB18S_81 amplified more than 90% of the sequences of 7 of these 10 phyla and showed the best taxonomic coverage among the mini-barcodes tested, as good as the taxonomic coverage of the large barcode 566F/1200R (**Table A2**, Appendix A). Within the existing 28S mini-barcodes, nucLSUDf1/nucLSUDr1 showed the poorest taxonomic coverage of several phyla, amplifying less than 80% of the sequences of 5 out of the 10 phyla examined (**Table A3**, Appendix A). MiniB18S_43 and nucLSUDf1/nucLSUDr1 mini-barcodes showed the worst capacity to detect sequences of the phyla Phragmoplastophyta, Arthropoda, Nematoda, Platyhelminthes and Apicomplexa (**Figure 2**). The former did not provide a high taxonomic coverage for Nematodes and the latter behaved poorly on all the taxa, especially for Nematoda and Apicomplexa (**Figure 2**). The MiniB18S_81 mini-barcode provided the best results, showing an excellent taxonomic coverage on Arthropoda, Nematoda and Platyhelminthes, despite a slightly lower taxonomic coverage for the Apicomplexa phylum than MiniB18S_41, MiniB18S_43 and Short28SF/Short28SR (**Figure 2**). Finally, all the five mini-barcodes and reference 566F/1200R barcode displayed a high taxonomic coverage for the Fungi kingdom and its phyla Ascomycota and Basidiomycota (**Table A3**, Appendix A).

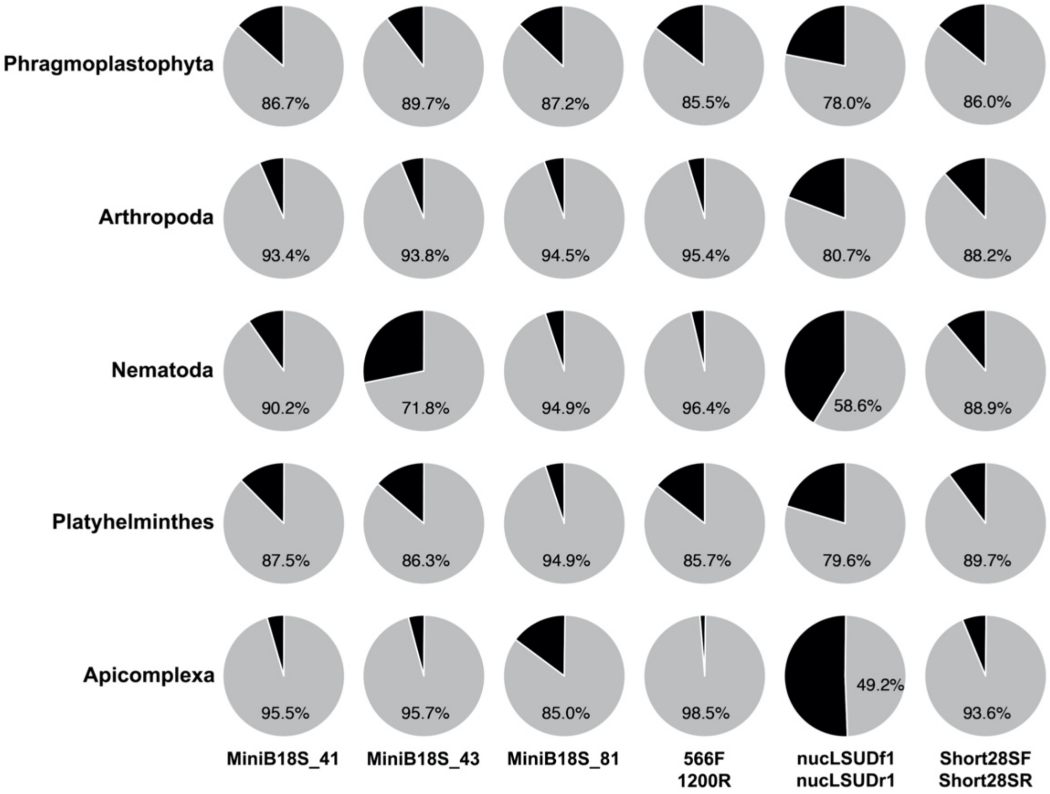

Figure 2. "In silico" taxonomic coverage of barcodes of the phyla Phragmoplastophyta, Arthropoda, Nematoda, Platyhelminthes and Apicomplexa. Grey colour represents the proportion of amplified sequences and black colour the proportion of non-amplified sequences. Inside of each graph is the percentage of amplified sequences of the respective taxon. For all results of taxonomic coverage, go to **Table A3**, Appendix A.

Table 2. "In silico" resolution capacity (in percentage) of barcodes for the phyla Phragmoplastophyta, Arthropoda, Nematoda, Platyhelminthes and Apicomplexa. Resolution percentages shown only for the taxonomy categories for which there were sufficient data available. The number of unambiguously identified taxa among the total number of taxa available for each taxonomic category in the reference dataset is shown in brackets. To see all the taxonomic resolution results, go to **Table A3**, Appendix A.

Arthropoda	**MiniB18S_41**	**MiniB18S_43**	**MiniB18S_81**	**566F/1200R**	**nucLSUDf1/nucLSUDr1**	**Short28SF/Short28SR**
Class	**71.4** (10 of 14)	**69.2** (9 of 13)	**76.9** (10 of 13)	**85.7** (12 of 14)	**100** (11 of 11)	**90.9** (10 of 11)
Order	**52.6** (40 of 76)	**50** (38 of 76)	**73** (54 of 74)	**89.5** (68 of 76)	**100** (4 of 4)	**100** (4 of 4)
Species	**21.1** (2623 of 12445)	**19.5** (2434 of 12489)	**59.2** (7451 of 12591)	**76.8** (9752 of 12700)	**62.4** (2835 of 4542)	**52.9** (2626 of 4960)

Phragmoplastophyta	**MiniB18S_41**	**MiniB18S_43**	**MiniB18S_81**	**566F/1200R**	**nucLSUDf1/nucLSUDr1**	**Short28SF/Short28SR**
Class	**33.3** (1 of 3)	**33.3** (1 of 3)	**100** (3 of 3)	**100** (3 of 3)		
Order	**8.6** (10 of 117)	**8.6** (10 of 117)	**31.2** (37 of 119)	**80.3** (94 of 117)		
Genus	**18.6** (336 of 1804)	**17.0** (307 of 1808)	**39.4** (706 of 1791)	**87.3** (1531 of 1753)		
Species	**29.4** (2265 of 7709)	**28.3** (2254 of 7975)	**34.5** (2676 of 7756)	**65.9** (5013 of 7609)	**29.7** (2391 of 8061)	**31.7** (2820 of 8884)

Nematoda	**MiniB18S_41**	**MiniB18S_43**	**MiniB18S_81**	**566F/1200R**	**nucLSUDf1/nucLSUDr1**	**Short28SF/Short28SR**
Class	**100** (2 of 2)	**100** (2 of 2)	**100** (2 of 2)	**0** (0 of 2)	**100** (2 of 2)	**100** (2 of 2)
Order	**26.67** (4 of 15)	**20** (3 of 15)	**53.3** (8 of 15)	**80** (12 of 15)	**60** (3 of 5)	**60** (3 of 5)
Species	**47.5** (932 of 1961)	**46.6** (727 of 1560)	**61.6** (1270 of 2062)	**86.4** (1811 of 2095)	**58.7** (229 of 390)	**33.3** (197 of 591)

Platyhelminthes	**MiniB18S_41**	**MiniB18S_43**	**MiniB18S_81**	**566F/1200R**	**nucLSUDf1/nucLSUDr1**	**Short28SF/Short28SR**
Class	**100** (6 of 6)	**100** (6 of 6)	**100** (6 of 6)	**100** (6 of 6)	**100** (5 of 5)	**100** (5 of 5)
Order	**79.5** (31 of 39)	**71** (27 of 38)	**89.7** (35 of 39)	**94.9** (37 of 39)	**100** (8 of 8)	**100** (8 of 8)
Family	**86.11** (31 of 36)	**83.8** (31 of 37)	**100** (37 of 37)	**100** (37 of 37)		
Species	**52.2** (900 of 1723)	**49.3** (837 of 1699)	**81.5** (1523 of 1869)	**92.9** (1567 of 1687)	**33.1** (174 of 526)	**30.7** (182 of 593)

Apicomplexa	**MiniB18S_41**	**MiniB18S_43**	**MiniB18S_81**	**566F/1200R**	**nucLSUDf1/nucLSUDr1**	**Short28SF/Short28SR**
Class	**100** (4 of 4)	**100** (4 of 4)	**100** (4 of 4)	**100** (4 of 4)	**100** (2 of 2)	**100** (2 of 2)
Order	**100** (5 of 5)	**100** (5 of 5)	**100** (5 of 5)	**100** (5 of 5)	**100** (4 of 4)	**100** (5 of 5)
Family	**100** (5 of 5)	**100** (5 of 5)	**100** (5 of 5)	**100** (5 of 5)		
Genus	**76.2** (48 of 63)	**73.8** (45 of 61)	**70.97** (44 of 62)	**96.9** (63 of 65)	**81.8** (9 of 11)	**83.3** (10 of 12)
Species	**34.03** (487 of 1431)	**31.6** (453 of 1435)	**41.3** (526 of 1274)	**76** (1122 of 1477)	**34.7** (43 of 124)	**36** (85 of 236)

The taxonomic resolution of the large barcode 566F/1200R was higher than any of the mini-barcodes (**Table 2**; **Table A3**, Appendix A). Nevertheless, the resolution capacity of the five mini-barcodes was good, and MiniB18S_81 showed the best resolution for the phyla Phragmoplastophyta, Arthropoda, Nematoda, Platyhelminthes and Apicomplexa (**Table 2**). In fact, this mini-barcode offered high taxonomic resolution at the order level within the phyla Arthropoda, Nematoda, Platyhelminthes and Apicomplexa, at the family level in Platyhelminthes and Apicomplexa, at the genus level in Apicomplexa, and at the species level in Arthropoda, Nematoda and Platyhelminthes. However, no results were obtained at the family and genus levels for various phyla as this information was lacking in the reference dataset (**Table 2**).

3.2. Metabarcoding proof

The MiniB18S_81 mini-barcode provided a coverage of more than 100,000 reads per sample and high-quality sequences, with an average of 66% of forward/reverse combined sequences showing less than one expected error (others were discarded). Moreover, on average, 80.4% of reads were assigned to an OTU with 97% identity. Taking as a reference both the SILVA 18S v123 dataset and RDP 16S v16 training dataset, the MiniB18S_81 mini-barcode identified 1367 of 1384 OTUs recovered as eukaryotic organisms with certainty, whereas no sequences were assigned to Bacteria or Archaea.

Our metabarcoding analysis results were also consistent with the data obtained through "in silico" PCR. The MiniB18S_81 mini-barcode showed an excellent taxonomic resolution capacity at the order level for Apicomplexa, Arthropoda, Nematoda and Platyhelminthes, while for plants it only showed good resolution at the class level (**Table 3**). In the Apicomplexa phylum, this mini-barcode also offered good resolution capacity at the family and genus levels (**Table 3**). However, no results were obtained at the family and genus levels for various phyla, probably due to the lack of information in the reference dataset regarding these taxonomic levels and phyla (**Table 3**). We did not included the Annelida and Mollusca phyla in Table 3 as only two OTUs of each were detected in this analysis. MiniB18S_81 also showed good taxonomic resolution at the class level in Ascomycota and Basidiomycota (**Table 3**). In contrast, the taxonomic resolution for the Vertebrata phylum was poor, which is in line with the results of the "in silico" approach. In addition, this mini-barcode was capable of unambiguously identifying some genera. Among these, we should highlight *Cryptosporidium* spp. and *Blastocystis* spp., two groups of microorganisms of increasing interest due to their impact on human health.

Table 3. Resolution capacity (in percentage) of the mini-barcode MiniB18S for different taxa according to the metabarcoding test. Phragmoplastophyta, Arthropoda, Nematoda, Platyhelminthes and Apicomplexa are presented in bold. Resolution percentages shown only for the taxonomy categories for which there was sufficient taxonomic information in the reference dataset. Annelida and Mollusca excluded due to lack of information.

MiniB18S_81		**Taxonomic resolution**					
Kingdom	**Phylum**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Alveolata		82.9	75	71.1	55.3	39.5	5.3
	Apicomplexa		**81.3**	**81.3**	**62.5**	**62.5**	**6.3**
Chloroplastida		89.4	84.8	7.1		2.4	4.1
	Phragmoplastophyta		**93.2**	**7.7**		**2.7**	**4.6**
Fungi		84	59.5	40.5	10.5	10	3
	Ascomycota		67.1	50.6	10.6	9.4	2.4
	Basidiomycota		84.6	48.1	15.4	19.2	5.8
Metazoa		89.0	72.0	63.3	0.5		16.5
	Arthropoda		**95.9**	**84.7**			**17.3**
	Nematoda		**100**	**94.4**			**44.4**
	Platyhelminthes		**91.7**	**83.3**	**8.3**		
	Vertebrata		17.6				8.8

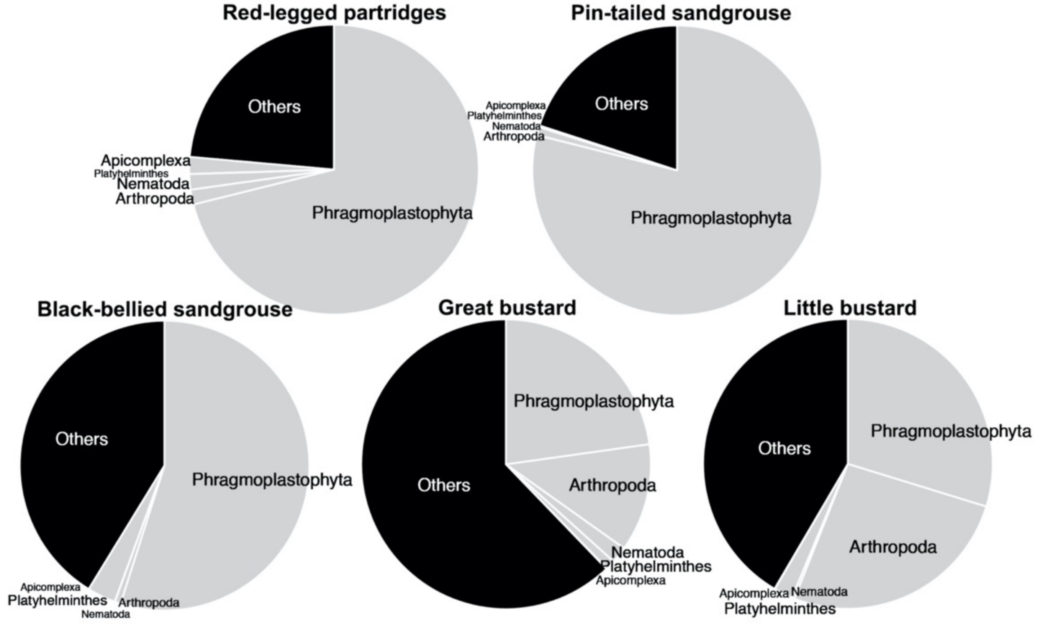

Figure 3. Mean percentages of reads of the phyla Phragmoplastophyta, Arthropoda, Nematoda, Platyhelminthes and Apicomplexa by bird species.

The use of MiniB18S_81 mini-barcode allowed to estimate the percentage of reads for each taxon in relation to the total reads obtained for each sample (**Figure 3**), which allowed to estimate the contribution of each taxon to the birds diet, gut microbiota or parasite community.

4. Discussion

Faecal samples are of great value as a non-invasive means to gather information on the feeding ecology and parasite infestation of species, supporting the management and conservation of their populations. This study aimed to identify the best mini-barcode of eukaryote 18S rRNA to be used in studies based on faeces metagenomics that could simultaneously analyse the diet and parasites in birds, with special emphasis on threatened farmland birds. Among the mini-barcodes developed in this study, MiniB18S_81 (of gene 18S rRNA) in particular showed a great taxonomic coverage of almost all eukaryotic taxa, as well as a good taxonomic resolution of the most interesting phyla associated with the diet and parasitology of birds (Jiguet, 2002; Villanúa *et al.*, 2008; Santoro *et al.*, 2010; Bravo *et al.*, 2017) through both "in silico" and real metabarcoding testing. Thus, the MiniB18S_81 mini-barcode is able to provide a highly valuable information for farmland bird conservation.

All the tested mini-barcodes displayed short and homogeneous amplicon lengths, obtaining in general sequences shorter than 300 bp. In the case of MiniB18S_81, the amplicons longer than 300 bp corresponded mainly to the taxonomic group Cestoda, probably related to the insertion of more than 100 bp in the V7-V8 region of the 18S gene. Although the species belonging to this phylum are parasites, DNA of cestodes in faeces samples is usually not as degraded as other faecal DNA, since it is extracted directly from tissues (adults, larvae or eggs). Thus, we strongly believe that this methodological drawback should not be a problem when studying the parasitology on faecal samples.

The variability of the V7-V8 region of the 18S gene was not totally unexpected, since this region is much more variable than the V3 region (Hadziavdic *et al.*, 2014). In fact, this seems to be the reason why the MiniB18S_81 mini-barcode has a higher taxonomic coverage and resolution capacity than the MiniB18S_41 and the MiniB18S_43 mini-barcodes. When we compared the features of the newly-designed

MiniB18S_81 with two of the eukaryotic mini-barcodes most widely used in NGS diet studies, it proved to be slightly superior to the Short28SF/Short28SR 28S mini-barcode (Vestheim and Jarman, 2008) in terms of the taxonomic coverage for four out of the five target phyla.

Overall, both the MiniB18S_81 and the Short28SF/Short28SR mini-barcodes showed wide taxonomic coverage and resolution of all the phyla examined along. However, the available reference datasets for LSU (the DNA region amplified by the Short28SF/Short28SR mini-barcode) are not as long and purified as those for SSU (the DNA region amplified by the MiniB18S_81 mini-barcode), and without a robust reference database, information is lost (Clarke *et al.*, 2014). Likewise, the MiniB18S_81 mini-barcode showed a much wider taxonomic coverage than the 28S mini-barcode nucLSUDf1/nucLSUDr1 (Sonnenberg *et al.*, 2007), which indeed, among the tested mini-barcodes, proved to be the most inefficient tool for eDNA analysis of diet and parasites on bird faecal samples.

In the metabarcoding test conducted on faeces of different steppe bird species, MiniB18S_81 proved to have a great taxonomic resolution capacity at the order level of the targeted phyla. Especially, in the case of Apicomplexa, Arthropoda, Nematoda, and Platyhelminthes, where more than 80% of the obtained OTUs were unambiguously identified at order level. With regards to the Phragmoplastophyta, the taxonomic resolution at class level provided an overall picture of the importance of plants in the birds' diet. Hence, the MiniB18S_81 mini-barcode provides information on the contribution to the bird's diet of different arthropod orders, other invertebrates, and plants, as well as information about the bird's parasites, at least at the order level. The lower taxonomic resolution observed at species level in the metabarcoding test is likely attributable to the fact that many of the OTUs recovered corresponded to organisms that were not included in the reference dataset, and thus it does not allow to assess the real resolution capacity of the mini-barcode at this level. Although the percentage of reads obtained for each taxa cannot be considered quantitative of the real contribution of these taxa to the gut microbiota (Evans *et al.*, 2016; Lamb *et al.*, 2019; Piñol *et al.*, 2019), this percentage could be useful for comparative studies.

The capacity of MiniB18S_81 to unambiguously identify some pathogens such as the genera *Cryptosporidium* spp. and *Blastocystis* spp. suggests its possible use for estimating parasitic loads. In effect, there is much interest in *Blastocystis* spp. because of their impacts on human health (Tan, 2008; Scanlan, 2012) and zoonotic capacity (Greige *et al.*, 2018). This parasite has proved to be far more common in wildlife than previously considered, due to the limitations of previous diagnostic techniques, and the lack of surveillance in wildlife (Parkar *et al.*, 2007). It was long considered to be of no clinical significance, but there is growing consensus that these parasites can have major impact on host health and thus influence population survival and reproduction (Thompson *et al.*, 2010). Studies of *Blastocystis* spp. have often been based on the presence/absence of OTUs and this new mini-barcode could provide information on the proportions of this organism in the host's gut microbiota especially useful for comparative studies. This will help to determine the prevalence of these gut parasites in the population over time, as well as to assess the potential impact of *Blastocystis* spp. on wildlife health.

Both "in silico" and real metabarcoding testing also showed that MiniB18S_81 mini-barcode displays good taxonomic coverage for the phyla Ascomycota and Basidiomycota. This is particularly crucial when working with eDNA, as environmental contamination with fungi could be a problem (Bohmann *et al.*, 2014). By using this broad-spectrum mini-barcode proposed here, fungal contamination can be detected and contaminated samples discarded.

In conclusion, the MiniB18S_81 mini-barcode is a promising new tool for simultaneous biomonitoring of diet and intestinal parasites through eDNA metabarcoding of faecal samples. It provides useful information about the arthropod orders and plants in the diet, as well as about the contributions of Nematoda, Platyhelminthes and Apicomplexa parasites to the gut microbiota. Understanding the ecological determinants of animal density is a central question in the field of species conservation, especially if we consider the current rate of human interference. In this context the MiniB18S_81 mini-barcode could provide a better insight on the ecology of farmland birds and help to elucidate the reasons for their populations decline.

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Supplementary material

Appendix A

Table A1. List of mini-barcodes designed based on 2000 Nematoda and Platyhelminthes DNA sequences

Barcode	Forward	Reverse	Product Size	Tm Fw	Tm Rv
MiniB18S_1	AACCTGGTTGATCCTGCCAG	GGTCAGGGCTTTGTTGCATG	181	60	60
MiniB18S_2	AACCTGGTTGATCCTGCCAG	AGGTCAGGGCTTTGTTGCAT	182	60	60.2
MiniB18S_3	ACAACCTGGTTGATCCTGCC	CAAGGTCAGGGCTTTGTTGC	186	60.3	60
MiniB18S_4	CAACCTGGTTGATCCTGCCA	CAAGGTCAGGGCTTTGTTGC	185	60.3	60
MiniB18S_5	AACCTGGTTGATCCTGCCAG	CAAGGTCAGGGCTTTGTTGC	184	60	60
MiniB18S_6	CATGCAACAAAGCCCTGACC	CAAGCCCGGTTGGTTTTGTT	62	60	59.8
MiniB18S_7	GCAACAAAGCCCTGACCTTG	CAAGCCCGGTTGGTTTTGTT	59	60	59.8
MiniB18S_8	CATGCAACAAAGCCCTGACC	GTCACCAAAAACGGGCCAAG	78	60	60.2
MiniB18S_9	GCAACAAAGCCCTGACCTTG	GTCACCAAAAACGGGCCAAG	75	60	60.2
MiniB18S_10	AACAAAACCAACCGGGCTTG	CCCTGATTCTCCGTTACCCG	160	59.8	59.9
MiniB18S_11	GCAACAAAGCCCTGACCTTG	CCCTGATTCTCCGTTACCCG	199	60	59.9
MiniB18S_12	AACAAAACCAACCGGGCTTG	CTCTCCGGAATCGAACCCTG	175	59.8	59.9
MiniB18S_13	CGGGTAACGGAGAATCAGGG	CTCTCCGGAATCGAACCCTG	35	59.9	59.9
MiniB18S_14	CGGGTAACGGAGAATCAGGG	CGTTTCTCAGGCTCCCTCTC	50	59.9	59.8
MiniB18S_15	CAGGGTTCGATTCCGGAGAG	CGTTTCTCAGGCTCCCTCTC	35	59.9	59.8
MiniB18S_16	CTTGGCCCGTTTTTGGTGAC	CCTTGGATGTGGTAGCCGTT	190	60.2	60
MiniB18S_17	GAGAGGGAGCCTGAGAAACG	CCTTGGATGTGGTAGCCGTT	36	59.8	60
MiniB18S_18	CGGGTAACGGAGAATCAGGG	CCTTGGATGTGGTAGCCGTT	66	59.9	60
MiniB18S_19	CAGGGTTCGATTCCGGAGAG	CCTTGGATGTGGTAGCCGTT	51	59.9	60
MiniB18S_20	GAGAGGGAGCCTGAGAAACG	CTGCCTTCCTTGGATGTGGT	43	59.8	60
MiniB18S_21	CGGGTAACGGAGAATCAGGG	CTGCCTTCCTTGGATGTGGT	73	59.9	60
MiniB18S_22	CAGGGTTCGATTCCGGAGAG	CTGCCTTCCTTGGATGTGGT	58	59.9	60
MiniB18S_23	AACGGCTACCACATCCAAGG	CTGCCTTCCTTGGATGTGGT	27	60	60
MiniB18S_24	GAGAGGGAGCCTGAGAAACG	CGTGCTGGGAGTGGGTAATT	73	59.8	60
MiniB18S_25	CGGGTAACGGAGAATCAGGG	CGTGCTGGGAGTGGGTAATT	103	59.9	60
MiniB18S_26	CAGGGTTCGATTCCGGAGAG	CGTGCTGGGAGTGGGTAATT	88	59.9	60
MiniB18S_27	ACCACATCCAAGGAAGGCAG	CGTGCTGGGAGTGGGTAATT	50	60	60
MiniB18S_28	AACGGCTACCACATCCAAGG	CGTGCTGGGAGTGGGTAATT	57	60	60
MiniB18S_29	CGGGTAACGGAGAATCAGGG	GCCCTCCAATTGATCCTCGT	200	59.9	59.8
MiniB18S_30	CAGGGTTCGATTCCGGAGAG	GCCCTCCAATTGATCCTCGT	185	59.9	59.8
MiniB18S_31	ACCACATCCAAGGAAGGCAG	GCCCTCCAATTGATCCTCGT	147	60	59.8
MiniB18S_32	AACGGCTACCACATCCAAGG	GCCCTCCAATTGATCCTCGT	154	60	59.8
MiniB18S_33	AATTACCCACTCCCAGCACG	GCCCTCCAATTGATCCTCGT	117	60	59.8
MiniB18S_34	CAGGGTTCGATTCCGGAGAG	TGCCCTCCAATTGATCCTCG	186	59.9	59.8
MiniB18S_35	ACCACATCCAAGGAAGGCAG	TGCCCTCCAATTGATCCTCG	148	60	59.8
MiniB18S_36	AACGGCTACCACATCCAAGG	TGCCCTCCAATTGATCCTCG	155	60	59.8
MiniB18S_37	AATTACCCACTCCCAGCACG	TGCCCTCCAATTGATCCTCG	118	60	59.8
MiniB18S_38	ACGAGGATCAATTGGAGGGC	CACCAGACTTGCCCTCCAAT	30	59.8	60
MiniB18S_39	CGAGGATCAATTGGAGGGCA	CACCAGACTTGCCCTCCAAT	29	59.8	60
MiniB18S_40	GAGAGGGAGCCTGAGAAACG	CACCAGACTTGCCCTCCAAT	180	59.8	60
MiniB18S_41	CAGGGTTCGATTCCGGAGAG	CACCAGACTTGCCCTCCAAT	195	59.9	60
MiniB18S_42	ACCACATCCAAGGAAGGCAG	CACCAGACTTGCCCTCCAAT	157	60	60
MiniB18S_43	AACGGCTACCACATCCAAGG	CACCAGACTTGCCCTCCAAT	164	60	60
MiniB18S_44	AATTACCCACTCCCAGCACG	CACCAGACTTGCCCTCCAAT	127	60	60
MiniB18S_45	ATTGGAGGGCAAGTCTGGTG	CGAGCTTTTTAACCGCAGCA	84	60	59.8

MiniB18S_46	AATTACCCACTCCCAGCACG	CGAGCTTTTTAACCGCAGCA	191	60	59.8
MiniB18S_47	ATTGGAGGGCAAGTCTGGTG	ACGAGCTTTTTAACCGCAGC	85	60	59.8
MiniB18S_48	AATTACCCACTCCCAGCACG	ACGAGCTTTTTAACCGCAGC	192	60	59.8
MiniB18S_49	ACGAGGATCAATTGGAGGGC	CGGACCCAGATCCAACTACG	112	59.8	59.9
MiniB18S_50	CGAGGATCAATTGGAGGGCA	CGGACCCAGATCCAACTACG	111	59.8	59.9
MiniB18S_51	ATTGGAGGGCAAGTCTGGTG	CGGACCCAGATCCAACTACG	102	60	59.9
MiniB18S_52	GCTGCGGTTAAAAAGCTCGT	AGCGAAGAGGCAGTACAACC	64	59.8	60
MiniB18S_53	TGCTGCGGTTAAAAAGCTCG	AGCGAAGAGGCAGTACAACC	65	59.8	60
MiniB18S_54	ACGAGGATCAATTGGAGGGC	AGCGAAGAGGCAGTACAACC	139	59.8	60
MiniB18S_55	CGAGGATCAATTGGAGGGCA	AGCGAAGAGGCAGTACAACC	138	59.8	60
MiniB18S_56	CGTAGTTGGATCTGGGTCCG	AGCGAAGAGGCAGTACAACC	47	59.9	60
MiniB18S_57	ATTGGAGGGCAAGTCTGGTG	AGCGAAGAGGCAGTACAACC	129	60	60
MiniB18S_58	ATTGGAGGGCAAGTCTGGTG	GACAGCGAAGAGGCAGTACA	132	60	59.8
MiniB18S_59	TGTACTGCCTCTTCGCTGTC	AGGAAACCGACAGCGAAGAG	28	59.8	60
MiniB18S_60	GCTGCGGTTAAAAAGCTCGT	AGGAAACCGACAGCGAAGAG	75	59.8	60
MiniB18S_61	TGCTGCGGTTAAAAAGCTCG	AGGAAACCGACAGCGAAGAG	76	59.8	60
MiniB18S_62	ACGAGGATCAATTGGAGGGC	AGGAAACCGACAGCGAAGAG	150	59.8	60
MiniB18S_63	CGAGGATCAATTGGAGGGCA	AGGAAACCGACAGCGAAGAG	149	59.8	60
MiniB18S_64	CGTAGTTGGATCTGGGTCCG	AGGAAACCGACAGCGAAGAG	58	59.9	60
MiniB18S_65	ATTGGAGGGCAAGTCTGGTG	AGGAAACCGACAGCGAAGAG	140	60	60
MiniB18S_66	GGTTGTACTGCCTCTTCGCT	AGGAAACCGACAGCGAAGAG	31	60	60
MiniB18S_67	TTCGTATGGCGGCGTTAGAG	CGCCTTCGAACCTCTGACTT	116	60.2	60
MiniB18S_68	AAGTCAGAGGTTCGAAGGCG	ACTTTGGTTTCCCGGGACTG	115	60	60.2
MiniB18S_69	GCGATCAGATACCGCCCTAG	GACTTTGGTTTCCCGGGACT	99	60	59.9
MiniB18S_70	AAGTCAGAGGTTCGAAGGCG	GACTTTGGTTTCCCGGGACT	116	60	59.9
MiniB18S_71	GCGATCAGATACCGCCCTAG	AGACTTTGGTTTCCCGGGAC	100	59.8	59.9
MiniB18S_72	AAGTCAGAGGTTCGAAGGCG	AGACTTTGGTTTCCCGGGAC	117	60	59.9
MiniB18S_73	AAGTCAGAGGTTCGAAGGCG	CCCCCGGAACCAAAGACTTT	130	60	60.2
MiniB18S_74	TTGATTCGGTGGTTGGTGGT	TCCACCAACTAAGAACGGCC	44	59.8	60
MiniB18S_75	GGCCGTTCTTAGTTGGTGGA	GCAGGCTAGAGTCTCGTTCG	66	60	60.2
MiniB18S_76	GGCCGTTCTTAGTTGGTGGA	GCCGCTTGTCCCTCTAAGAA	112	60	59.8
MiniB18S_77	TGGCCGTTCTTAGTTGGTGG	CCCGGACATCTAAGGGCATC	169	60.3	60
MiniB18S_78	TTCTTAGAGGGACAAGCGGC	CCCGGACATCTAAGGGCATC	76	59.8	60
MiniB18S_79	CGAACGAGACTCTAGCCTGC	CCCGGACATCTAAGGGCATC	122	60.2	60
MiniB18S_80	TTGATTCGGTGGTTGGTGGT	CCCGGACATCTAAGGGCATC	192	59.8	60
MiniB18S_81	GGCCGTTCTTAGTTGGTGGA	CCCGGACATCTAAGGGCATC	168	60	60
MiniB18S_82	GATGCCCTTAGATGTCCGGG	CAATCCCCGATCCCAGTCAC	118	60	60.2
MiniB18S_83	GATGCCCTTAGATGTCCGGG	ATTGCAATCCCCGATCCCAG	122	60	60.2
MiniB18S_84	GATGCCCTTAGATGTCCGGG	TGTGTACAAAGGGCAGGGAC	200	60	59.9
MiniB18S_85	GTGACTGGGATCGGGGATTG	TTCAATCGGAGTAGCGACGG	126	60.2	59.9
MiniB18S_86	CTGGGATCGGGGATTGCAAT	TTCAATCGGAGTAGCGACGG	122	60.2	59.9
MiniB18S_87	GTCCCTGCCCTTTGTACACA	TTCAATCGGAGTAGCGACGG	44	59.9	59.9
MiniB18S_88	TCCCTGCCCTTTGTACACAC	TTCAATCGGAGTAGCGACGG	43	59.9	59.9
MiniB18S_89	GTGACTGGGATCGGGGATTG	GAGCCACCACTACAACACCA	166	60.2	59.9
MiniB18S_90	CTGGGATCGGGGATTGCAAT	GAGCCACCACTACAACACCA	162	60.2	59.9
MiniB18S_91	GTCCCTGCCCTTTGTACACA	GAGCCACCACTACAACACCA	84	59.9	59.9
MiniB18S_92	TCCCTGCCCTTTGTACACAC	GAGCCACCACTACAACACCA	83	59.9	59.9
MiniB18S_93	CCGTCGCTACTCCGATTGAA	GAGCCACCACTACAACACCA	60	59.9	59.9
MiniB18S_94	GTCCCTGCCCTTTGTACACA	TTCTCACCAAGAGCCACCAC	94	59.9	59.9
MiniB18S_95	TCCCTGCCCTTTGTACACAC	TTCTCACCAAGAGCCACCAC	93	59.9	59.9
MiniB18S_96	TGGTGTTGTAGTGGTGGCTC	TTCTCACCAAGAGCCACCAC	30	59.9	59.9
MiniB18S_97	CCGTCGCTACTCCGATTGAA	TTCTCACCAAGAGCCACCAC	70	59.9	59.9
MiniB18S_98	TGGTGTTGTAGTGGTGGCTC	TTTGGCGCTTCTCACCAAGA	38	59.9	60.2
MiniB18S_99	CCGTCGCTACTCCGATTGAA	TTTGGCGCTTCTCACCAAGA	78	59.9	60.2
MiniB18S_100	GAGCAGGATGAAGCCAGAGG	CGGACCTCCACCAGAGTTTC	39	60.2	60

Table A2. List of mini-barcodes designed based on the full dataset of 5000 DNA sequences: 1000 sequences per phylum (Phragmoplastophyta, Apicomplexa, Arthropoda, Nematoda and Platyhelminthes). Shown in bold are the primers selected for this study.

ID	Forward	Reverse	In Parasite DB	Product Size
MiniB18S_101	TTGCCCGTTTTGGTGACTCT	CCTTGGATGTGGTAGCCGTT	No	188
MiniB18S_102	CTTAGCTGATCGCACGGTCT	CCTTGGATGTGGTAGCCGTT	No	162
MiniB18S_103	CTTAGCTGATCGCACGGTCT	CTGCCTTCCTTGGATGTGGT	No	169
MiniB18S_104	CGGGTAACGGAGAATCAGGG	ACAGACCTCAGAGCCCGTAT	No	147
MiniB18S_105	CAGGGTTCGATTCCGGAGAG	ACAGACCTCAGAGCCCGTAT	No	132
MiniB18S_106	AACGGCTACCACATCCAAGG	ACAGACCTCAGAGCCCGTAT	No	101
MiniB18S_41	**CAGGGTTCGATTCCGGAGAG**	**CACCAGACTTGCCCTCCAAT**	**Yes**	**192**
MiniB18S_42	ACCACATCCAAGGAAGGCAG	CACCAGACTTGCCCTCCAAT	Yes	154
MiniB18S_43	**AACGGCTACCACATCCAAGG**	**CACCAGACTTGCCCTCCAAT**	**Yes**	**161**
MiniB18S_107	TCTTGATTCGGTGGGTGGTG	CCCGGACATCTAAGGGCATC	No	192
MiniB18S_81	**GGCCGTTCTTAGTTGGTGGA**	**CCCGGACATCTAAGGGCATC**	**Yes**	**166**
MiniB18S_108	GATGCCCTTAGATGTCCGGG	ACCCCTATCCCCATCACGAA	No	114
MiniB18S_109	GATGCCCTTAGATGTCCGGG	AACCCCTATCCCCATCACGA	No	115
MiniB18S_110	GATGCCCTTAGATGTCCGGG	CAACCCCTATCCCCATCACG	No	116
MiniB18S_84	GATGCCCTTAGATGTCCGGG	TGTGTACAAAGGGCAGGGAC	Yes	197
MiniB18S_111	TTCGTGATGGGGATAGGGGT	TGTGTACAAAGGGCAGGGAC	No	103
MiniB18S_112	TCGTGATGGGGATAGGGGTT	TGTGTACAAAGGGCAGGGAC	No	102
MiniB18S_113	GATGCCCTTAGATGTCCGGG	GTGTGTACAAAGGGCAGGGA	No	198
MiniB18S_114	TTCGTGATGGGGATAGGGGT	GTGTGTACAAAGGGCAGGGA	No	104
MiniB18S_115	TCGTGATGGGGATAGGGGTT	GTGTGTACAAAGGGCAGGGA	No	103

Table A3. Taxonomic coverage and resolution (in percentage) of each primer pair across the 10 target phyla and their respective kingdoms by taxonomic level. Taxonomical classification according to SILVA's reference database. N seq. indicates the number of sequences of these taxa present in the reference dataset.

MiniB18S_41			Taxonomic coverage						Taxonomic resolution					
Kingdom	**Phylum**	**N seq.**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Alveolata		11651	100.0	100.0	100.0	97.6	94.6	87.4	28.6	46.7	60.0	46.3	60.2	36.7
	Apicomplexa	1499		100.0	100.0	100.0	96.9	95.5		100.0	100.0	100.0	76.2	34.0
Chloroplastida		12166	100.0	93.3	98.1		98.8	89.3	75.0	28.6	12.8		20.6	26.9
	Phragmoplastophyta	8895		100.0	98.3		99.1	86.7		33.3	8.5		18.6	29.4
Fungi		15748	100.0	100.0	88.5	88.1	85.4	81.5	33.3	43.9	34.9	31.3	33.1	17.3
	Ascomycota	7992		100.0	76.3	76.3	77.0	73.0		27.3	23.9	27.0	30.8	16.1
	Basidiomycota	5824		100.0	100.0	100.0	97.7	95.1		47.4	36.4	25.7	26.4	12.4
Metazoa		25557	100.0	96.7	97.4	97.5		91.6	62.9	57.5	52.0	77.9		29.4
	Annelida	1669		100.0	100.0	100.0		97.4		50.0	26.1	80.0		27.4
	Arthropoda	13319		100.0	98.7			93.4		71.4	52.6			21.1
	Mollusca	1586		100	100			97.5		86	62			17.80
	Nematoda	2173		100.0	100.0			90.2		100.0	26.7			47.5
	Platyhelminthes	1969		100.0	100.0	97.3		87.5		100.0	79.5	86.1		52.2
	Vertebrata	1137		84.6	91.7			79.2		9.1	18.2			41.8

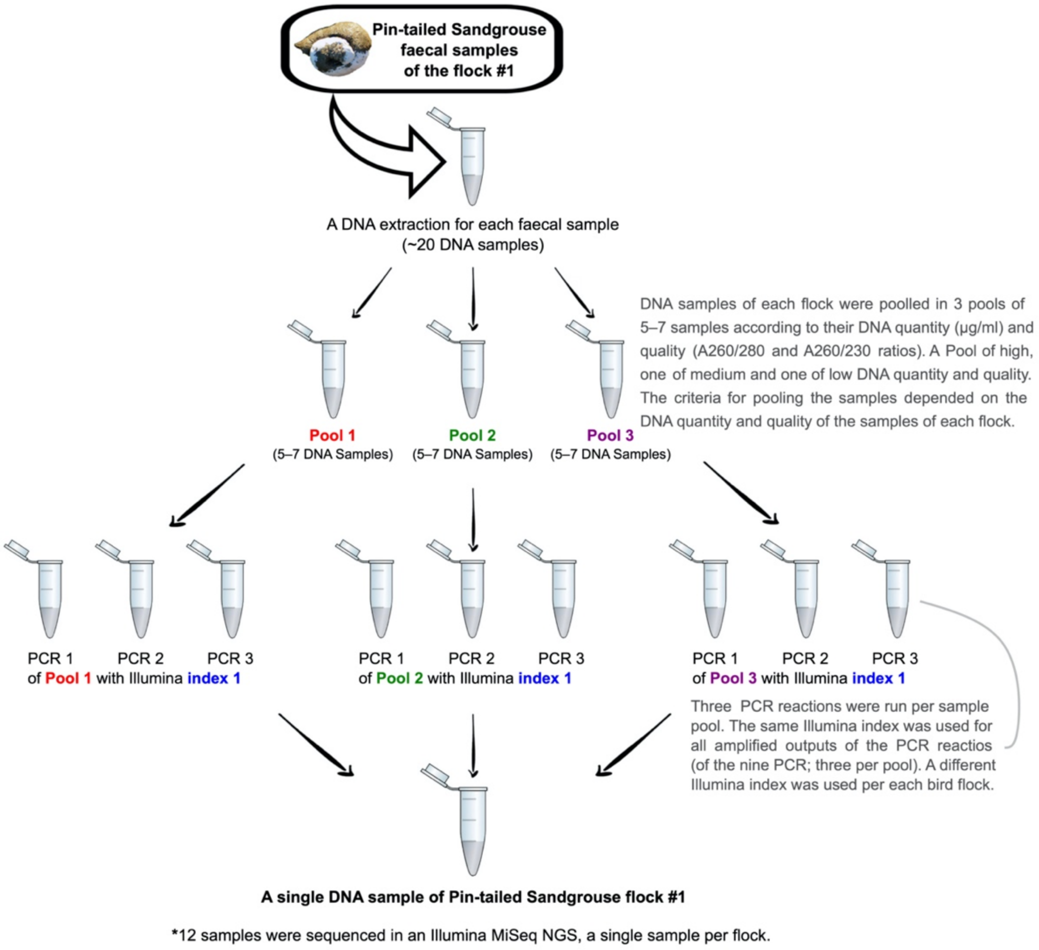
MiniB18S_43			Taxonomic coverage						Taxonomic resolution					
Kingdom	**Phylum**	**N seq.**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Alveolata		11651	100.0	100.0	100.0	100.0	91.8	89.2	28.6	46.7	60.0	45.2	57.6	33.6
	Apicomplexa	1499		100.0	100.0	100.0	93.8	95.7		100.0	100.0	100.0	73.8	31.6
Chloroplastida		12166	100.0	100.0	98.7		99.1	91.6	75.0	33.3	13.4		19.1	25.5
	Phragmoplastophyta	8895		100.0	98.3		99.3	89.7		33.3	8.5		17.0	28.3
Fungi		15748	100.0	86.0	88.5	90.1	90.7	90.0	33.3	36.7	31.9	28.2	27.2	15.6
	Ascomycota	7992		100.0	98.9	98.6	97.6	94.5		27.3	26.1	24.4	25.2	14.4
	Basidiomycota	5824		57.9	68.2	78.6	79.4	84.2		27.3	33.3	24.2	20.7	9.9
Metazoa		25557	91.4	96.7	96.4	97.5		90.4	59.4	51.7	46.8	75.3		27.0
	Annelida	1669		100.0	100.0	100.0		96.6		50.0	26.1	80.0		24.4
	Arthropoda	13319		92.9	98.7			93.8		69.2	50.0			19.5
	Mollusca	1586		100.0	100.0			97.5		85.7	61.5			16.4
	Nematoda	2173		100.0	100.0			71.8		100.0	20.0			46.6
	Platyhelminthes	1969		100.0	97.4	100.0		86.3		100.0	71.0	83.8		49.3
	Vertebrata	1137		92.3	91.7			80.5		16.7	18.2			39.3

MiniB18S_81			Taxonomic coverage						Taxonomic resolution					
Kingdom	**Phylum**	**N seq.**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Alveolata		11651	100.0	100.0	100.0	100.0	95.2	93.9	28.6	40.0	40.0	42.9	61.6	38.3
	Apicomplexa	1499		100.0	100.0	100.0	95.4	85.0		100.0	100.0	100.0	71.0	41.3
Chloroplastida		12166	100.0	100.0	99.4		98.3	89.4	75.0	53.3	31.6		40.2	32.8
	Phragmoplastophyta	8895		100.0	100.0		98.4	87.2		100.0	31.1		39.4	34.5
Fungi		15748	93.3	100.0	100.0	96.6	94.2	92.4	50.0	45.6	43.5	40.2	38.1	20.0
	Ascomycota	7992		100.0	100.0	98.3	95.3	90.8		27.3	35.5	33.9	32.6	18.0
	Basidiomycota	5824		100.0	100.0	99.1	96.7	96.3		57.9	45.4	40.4	38.8	14.5
Metazoa		25557	97.1	98.9	98.7	97.5		93.7	58.8	61.8	69.2	89.6		58.6
	Annelida	1669		100.0	100.0	100.0		97.5		100.0	87.0	100.0		56.9
	Arthropoda	13319		92.9	96.1			94.5		76.9	73.0			59.2
	Mollusca	1586		100.0	100.0			96.5		100.0	84.6			42.5
	Nematoda	2173		100.0	100.0			94.9		100.0	53.3			61.6
	Platyhelminthes	1969		100.0	100.0	100.0		94.9		100.0	89.7	100.0		81.5
	Vertebrata	1137		92.3	91.7			71.7		50.0	63.6			40.5

566F/1200R			Taxonomic coverage						Taxonomic resolution					
Kingdom	**Phylum**	**N seq.**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Alveolata		11651	100.0	100.0	100.0	100.0	99.7	94.1	57.1	80.0	65.0	61.9	87.9	79.8
	Apicomplexa	1499		100.0	100.0	100.0	100.0	98.5		100.0	100.0	100.0	96.9	76.0
Chloroplastida		12166	100.0	100.0	100.0		98.8	89.8	75.0	60.0	72.3		84.1	64.1
	Phragmoplastophyta	8895		100.0	98.3		96.3	85.5		100.0	80.3		87.3	65.8
Fungi		15748	93.3	98.3	99.5	99.0	97.7	95.6	100.0	96.4	86.3	81.4	81.4	60.5
	Ascomycota	7992		100.0	100.0	97.9	97.1	94.1		90.9	84.9	74.7	75.1	49.8
	Basidiomycota	5824		100.0	100.0	100.0	98.7	97.2		100.0	84.8	85.7	88.3	67.3
Metazoa		25557	100.0	97.8	98.0	97.5		93.9	85.7	86.4	90.7	98.7		79.1
	Annelida	1669		100.0	100.0	100.0		97.0		100.0	91.3	100.0		78.0
	Arthropoda	13319		100.0	98.7			95.4		85.7	89.5			76.8
	Mollusca	1586		100.0	100.0			97.4		71.4	92.3			66.0
	Nematoda	2173		100.0	100.0			96.4		0.0	80.0			86.4
	Platyhelminthes	1969		100.0	100.0	100.0		85.7		100.0	94.9	100.0		92.9
	Vertebrata	1137		92.3	91.7			76.5		75.0	100.0			71.8

nucLSUDf1/nucLSUDr1			Taxonomic coverage						Taxonomic resolution					
Kingdom	Phylum	N seq.	Phylum	Class	Order	Family	Genus	Species	Phylum	Class	Order	Family	Genus	Species
Alveolata		510	100.0	100.0	90.9	92.9	91.9	68.0	100.0	100.0	100.0	100.0	91.2	38.3
	Apicomplexa	252		100.0	80.0		91.7	49.2		100.0	100.0		81.8	34.7
Chloroplastida		10340		100.0				78.0		100.0				29.7
	Phragmoplastophyta	10332						78.0						29.7
Fungi		5129	90.9	100.0	100.0			89.0	70.0	69.0	73.3			20.0
	Ascomycota	3527		100.0	100.0			88.8		66.7	68.0			16.6
	Basidiomycota	1104		100.0	100.0			88.8		87.5	77.8			24.8
Metazoa		11007	100.0	100.0	100.0			80.9	100.0	94.7	92.0			57.2
	Annelida	369		100.0				96.7		100.0				71.7
	Arthropoda	5625		100.0	100.0			80.7		100.0	100.0			62.4
	Mollusca	874		100.0	100.0			91.8		83.3	100.0			46.8
	Nematoda	665		100.0	100.0			58.6		100.0	60.0			58.7
	Platyhelminthes	661		100.0	100.0			79.6		100.0	100.0			33.1
	Vertebrata	508		50.0				59.1						35.3

Short28SF/Short28SR			Taxonomic coverage						Taxonomic resolution					
Kingdom	Phylum	N seq.	Phylum	Class	Order	Family	Genus	Species	Phylum	Class	Order	Family	Genus	Species
Alveolata		510	100.0	100.0	100.0	100.0	100.0	95.3	80.0	85.7	90.9	100.0	83.8	36.2
	Apicomplexa	252		100.0	100.0		100.0	93.6		100.0	100.0		83.3	36.0
Chloroplastida		10340		100.0				86.0		100.0				31.7
	Phragmoplastophyta	10332						86.0						31.7
Fungi		5129	90.9	100.0	100.0			92.6	80.0	62.1	66.7			16.2
	Ascomycota	3527		100.0	100.0			91.5		41.7	60.0			14.0
	Basidiomycota	1104		100.0	100.0			94.6		75.0	55.6			17.3
Metazoa		11007	100.0	100.0	100.0			91.2	77.8	82.5	92.0			46.0
	Annelida	369		100.0				98.1		100.0				53.3
	Arthropoda	5625		100.0	100.0			88.2		90.9	100.0			52.9
	Mollusca	874		100.0	100.0			97.9		83.3	100.0			37.0
	Nematoda	665		100.0	100.0			88.9		100.0	60.0			33.3
	Platyhelminthes	661		100.0	100.0			89.7		100.0	100.0			30.7
	Vertebrata	508		50.0				81.9						35.1



DNA samples of each flock were poolled in 3 pools of 5–7 samples according to their DNA quantity (μg/ml) and quality (A260/280 and A260/230 ratios). A Pool of high, one of medium and one of low DNA quantity and quality. The criteria for pooling the samples depended on the DNA quantity and quality of the samples of each flock.

Three PCR reactions were run per sample pool. The same Illumina index was used for all amplified outputs of the PCR reactios (of the nine PCR; three per pool). A different Illumina index was used per each bird flock.

*12 samples were sequenced in an Illumina MiSeq NGS, a single sample per flock.

Figure A1. Diagram of the sample processing, from faecal samples to sequencing in an Illumina MiSeq NGS platform

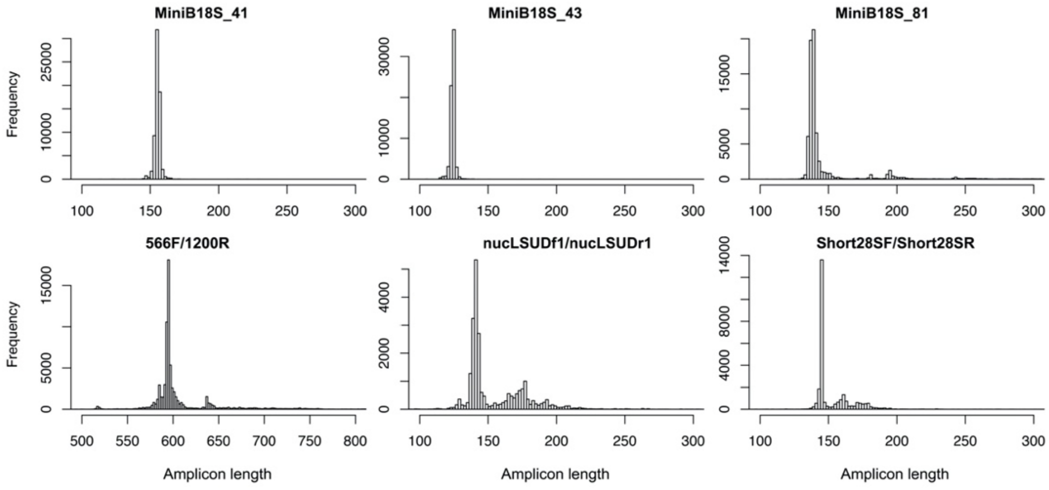


Figure S2. Amplicon lengths in each barcode

PAPER VI

Metabarcoding insights onto the diet and trophic diversity of six declining farmland birds

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Metabarcoding insights onto the diet and trophic diversity of six declining farmland birds

Abstract

Knowledge of feeding ecology of declining species, such as farmland birds, is essential to address their conservation requirements, especially when their habitats are suffering important reductions of trophic resources.

In this study, we apply a metabarcoding approach to describe the diet composition of six of the most significant farmland birds inhabiting European cereal pseudo-steppes: little bustard, great bustard, pin-tailed sandgrouse, black-bellied sandgrouse, red-legged partridge, and common quail. We further studied seasonal diet variations (autumn to spring) in all species but the common quail, whose diet was studied during spring and summer.

We show that their diets mostly consisted of plants, although in the case of little bustard (34%) and great bustard (12%) arthropods are also highly relevant. Among arthropods, we found high proportions of thrips, spiders, and springtails, which were previously unreported in their diet, and some taxa that could be used as antiparasitic food. Moreover, we report that little bustard's diet is the least rich of that of all studied species, and that diet of all these species is less diverse in winter than in autumn and spring. Diet composition of these declining species supports the importance of natural and semi-natural vegetation and landscape mosaics that can provide a wide variety of arthropods, plants, and seeds all year-round.

Keywords

Biomonitoring; faecal eDNA; little bustard; pin-tailed sandgrouse; red-legged partridge; steppe birds

1. Introduction

In recent decades, agricultural landscapes have changed dramatically worldwide (Tscharntke *et al.*, 2005). Since the 1960s, farmland intensification supported by subsidies of the European Common Agricultural Policy (CAP), which involves plot enlargement, mechanization, and wide use of synthetic fertilizers and phytosanitary products, has changed agricultural landscapes in Europe. After the 1990s, agricultural intensification was further increased by the globalization of commodity markets and CAP reforms, which also led to land abandonment in less favored areas (Van Zanten *et al.*, 2014).

All these processes led, under most circumstances, to a homogenization of agricultural landscapes by creating larger plots with sparse natural or semi-natural elements(Jongman, 2002). Unfortunately, agricultural intensification has had severe consequences for biodiversity (Stoate *et al.*, 2001; Donald *et al.*, 2006; Storkey *et al.*, 2012), and it is considered the main driver of the current biodiversity loss in Europe (Stoate *et al.*, 2001). Farmland birds are an excellent example of this, as their populations have strongly declined across Europe over the last decades concomitantly with agriculture intensification (Benton *et al.*, 2003), despite successive reforms of EU's CAP to try and reduce the environmental impacts of modern farming (Traba and Morales, 2019). Within an increasingly intensive agricultural landscape, interspersed patches of natural vegetation and fallow land (agricultural plots that are not cultivated during one or more growing seasons) are extremely important for farmland birds, because they provide a large number of resources, such as food, breeding areas and cover for the protection of chicks (Mcmahon *et al.*, 2010; Traba and Morales, 2019; Tarjuelo *et al.*, 2020a). In the Iberian Peninsula, where the soil is poor and artificial fertilizers were not common until recently, fallow land was a widespread and common practice in the traditional agrarian landscapes, as part of a rotation system for the land (Tarjuelo *et al.*, 2020b). Thus, fallow land in Spain reached more than 50% of Europe's fallow land surface (Tarjuelo *et al.*, 2020b), thanks to the extensive nature of agriculture practiced in this region for centuries (Donázar *et al.*, 1997). Biogeographical, environmental, and agricultural model reasons have been decisive for the Iberian Peninsula to become one of the most important areas in Europe for the conservation of steppe birds (Santos and Suárez, 2005; Mcmahon *et al.*, 2010; Traba and Morales, 2019). However, due to the more recent intensification of agriculture, fallow land in Spain has decreased by 1.1 million hectares in the last 15 years (Traba and Morales, 2019; Tarjuelo *et al.*, 2020a), leading to more homogeneous landscapes, with strong negative consequences for steppe birds and farmland biodiversity, and probably huge impacts on the trophic resources available for steppe bird species. This involves the reduction of plant and insect species abundance and richness (Wilson *et al.*, 1999; Benton *et al.*, 2002; Storkey *et al.*, 2012; Raven and Wagner, 2021), and consequently seed banks (Wilson *et al.*, 1999; Andreasen *et al.*, 2018) and the seasonality of seed availability (Newton, 2004).

Considering that more than 80% of steppe bird species have an unfavorable conservation status at the European level (Burfield, 2005) and that agricultural intensification has a direct impact on the ecosystem's trophic resources and their availability (Wilson *et al.*, 1999; Benton *et al.*, 2002; Benton *et al.*, 2003; Storkey *et al.*, 2012; Andreasen *et al.*, 2018), knowledge of the trophic ecology of these species takes on special relevance. The study of feeding ecology is essential to understand the biological and ecological requirements of species and, therefore, for proper management and conservation of their populations and habitats. Unfortunately, the information available on the trophic ecology of many declining steppe birds is limited (Del Hoyo *et al.*, 1992; Madroño *et al.*, 2004). The few studies that exist describe the diet of some of these species based on visual (micro- and/or macroscopic) studies of crops, stomachs, or feces contents (Suárez *et al.*, 1999; Jiguet, 2002; Bravo *et al.*, 2012). These techniques may have a strong bias since the detection probability is strongly influenced by digestion, even with the impossibility of detecting some taxa in feces due to their complete digestion (Pompanon *et al.*, 2012). Unlike the traditional techniques for diet study, the modern genetic methods through fecal eDNA metabarcoding can easily go beyond the detection capabilities of visual identification (Pompanon *et al.*, 2012; Shokralla *et al.*, 2012). These methods are becoming increasingly popular for the study of diet, and although they also have some important biases regarding amplification process (Pompanon *et al.*, 2012; Shokralla *et al.*, 2012), they allow a highly accurate description of species trophic ecology (Pompanon *et al.*, 2012).

This study aims to describe the diet of some of the most significant steppe bird and farmland game bird species of the European cereal pseudo-steppes through DNA-metabarcoding of species′ fecal samples. All these farmland birds inhabit increasingly intensified agricultural landscapes and are currently declining (Traba and Morales, 2019; Mougeot *et al.*, 2021) so there is an urgent need to improve knowledge about their diet to correctly understand their trophic requirements and conservation needs. We assessed the diet of four steppe birds, the little bustard (*Tetrax tetrax*), the great bustard (*Otis tarda*), the pin-tailed sandgrouse (*Pterocles alchata*), and the black-bellied sandgrouse (*Pterocles orientalis*) and two farmland game birds, the red-legged partridge (*Alectoris rufa*) and the common quail (*Coturnix coturnix* Linnæus, 1758). The diet of all these species is not yet well known, and here we focused on the relative importance of plants and invertebrates (Annelida, Arthropoda, and Mollusca) in their diet. With this level of taxonomic resolution, we compared diet composition and richness between species. For four species that could be sampled from autumn to spring, we also describe seasonal variations in diet. We provide the most up-to-date comprehensive understanding of their food requirements in a rapidly changing environment.

2. Materials and methods

2.1. Ethics Statement

All actions were performed according to Spanish law. Fecal samples of all species, except for common quail fecal samples, were collected in a manner that did not disrupt the birds' routine (see below). Common quail fecal samples were collected during ringing seasons. Ringed common quails were caught by qualified persons (expert ringers endorsed by the Aranzadi Ringing Scheme) and with the relevant administrative permission, issued by the Provincial Council of Alava (permission number: 17/009), and all of them were released in perfect condition after ringing them. None were retained for the purpose of collecting their feces; we only collected fecal samples from those common quails that defecated during the ringing process.

2.2. Sampled species

The study was carried out in Spain, a region where agricultural pseudo-steppes are home to the most important European populations of some threatened steppe-bird species, such as little bustard, great bustard and pin-tailed sandgrouse, and which also hold a big population of black-bellied sandgrouse (Madroño *et al.*, 2004; García de la Morena *et al.*, 2018). Most of these species are classified as vulnerable in Spain (Real Decreto 139/2011). Moreover, all of them have suffered a population decline to some extent throughout the last decade (García de la Morena *et al.*, 2018; Palacín, 2019; Cabodevilla *et al.*, 2020a; Mougeot *et al.*, 2021), with strong declines of -48% (2005-2016) and -43% (2005 and 2019) in the case of little bustard and black-bellied sandgrouse (García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020a; Mougeot *et al.*, 2021). The Iberian Peninsula also holds most of the wild (native) red-legged partridge population (Blanco-Aguiar *et al.*, 2003) and an important common quail population (Rodríguez-Teijeiro *et al.*, 2003). Red-legged partridge and common quail are game species of high socio-economic importance, especially the former, which is the main small game-bird species in Spain (Andueza *et al.*, 2018). All four steppe-bird and the two game-bird species rely on the pseudo-steppes and extensive farmland ecosystem and its resources (Madroño *et al.*, 2004) but despite their ecological and socio-economic importance, their trophic ecology is poorly studied and it has never been assessed using a molecular approach (Palacios *et al.*, 1975; Del Hoyo *et al.*, 1992; Lane *et al.*, 1999; Suárez *et al.*, 1999; Jiguet, 2002; Bravo *et al.*, 2012; Bravo *et al.*, 2017).

2.3. Sample collection

All samples were collected in Spain, most of them in the province of Ciudad Real (39° 0′ N, 4° 0′ W), but also in Navarra (42° 49′ N, 1° 39′ W), Lleida (41° 48′ N, 1° 28′ W), and Araba (42° 50′ N, 2° 45′ W) (the latter only for common quail; **Fig. 1**). Most samples were collected during the non-breeding season (autumn, winter, and early spring) of 2017 and 2018, except for the little bustard samples from Lleida (collected in winter and early spring of 2011, 2012, and 2013) and common quail samples (collected throughout the breeding season -late spring and summer- of 2017). The breeding season of these species extends from April to August (Del Hoyo *et al.*, 1992), so here we use the term breeding season to cover this period and the term non-breeding season to cover the period from September to early April. We collected feces samples in roosting places used to spend the night, so as not to alter the birds' routine, except in the case of quails, whose feces were collected during the scientific ringing process of these birds (work done by expert ringers from the Aranzadi Science Society, with the corresponding administrative authorization). During the non-breeding period, studied species (except for quails) usually gather in flocks. We sampled in roosting places early morning where we had located flocks at dusk. Within roosts, we sampled feces that were at least 2m apart to avoid sampling the same individuals twice. For quail, to be consistent, we group all individual birds ringed within a single breeding season within a single "group", although this species does not form flocks during the breeding season and the samples are not even from the same date. This should be considered when our results will be interpreted. We collected fecal samples of a total of 40 flocks, distributed as follows: six flocks of great bustard, 14 flocks of little bustard, 10 flocks of pin-tailed sandgrouse, two flocks of black-bellied sandgrouse, seven flocks of red-legged partridge, and a group of common quail. In the case of the two little bustard's flocks from Lleida, they were not flocks perse, as they include samples from different years. When possible, feces of 20 individuals per flock were collected. In the field, samples were collected in individual zipper bags, avoiding cross-contamination, and stored in cold conditions until they reached the laboratory, where they were frozen at -80 °C for a week and then kept at -20 °C until they were processed.

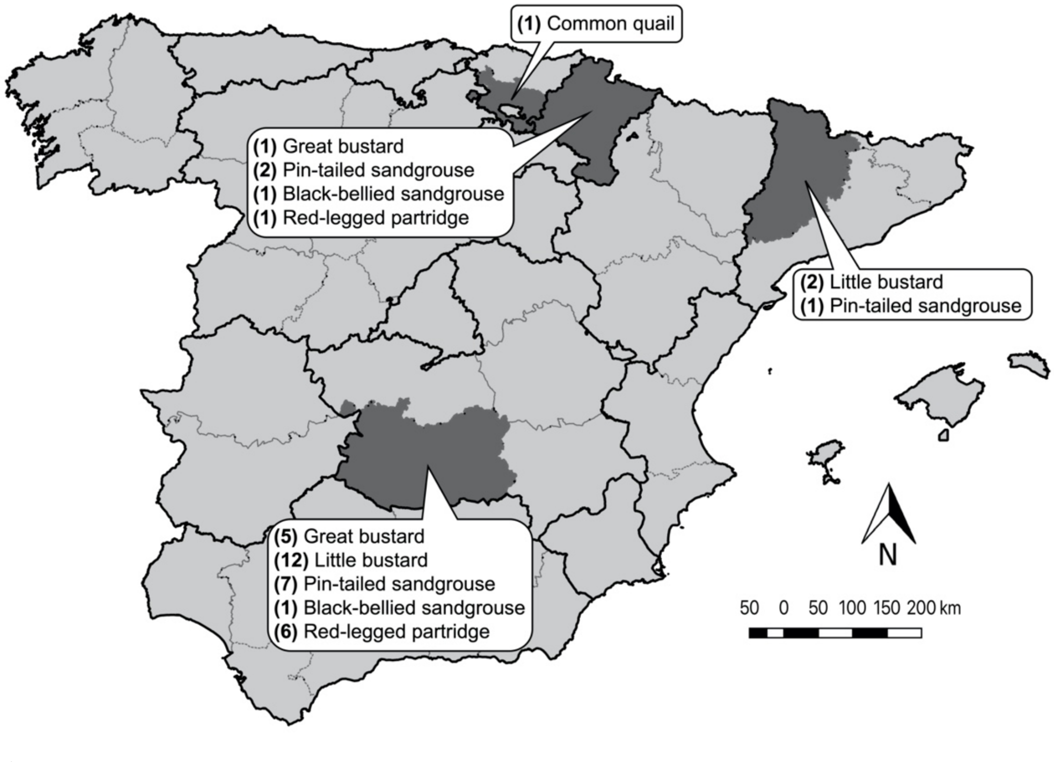

Figure 1. Map of Spanish regions with the spatial distribution of each species samples. The number of flocks per species sampled in each region is shown in brackets.

2.4. Sample processing

DNA extractions were performed using the QIAamp® DNA Stool Mini Kit from QIAGEN (Ref. 51504). From 1 to 20 (14 on average) DNA samples were obtained per flock (**Table A1**, Appendix A). We used a NanoVue Plus spectrophotometer (GE Healthcare) to measure the concentration (µg/ml) and quality (A260/280 and A260/230 ratios) of each DNA sample. Although we extracted DNA from each fecal sample, we considered each flock as a unique sample for analyses, and during the preparation of the libraries, samples of the same flock were mixed by adding them the same index. First, samples from each flock were mixed according to DNA quantity and quality in 3 pools of 1-7 samples, one of high, one of medium, and one of low quantity and quality (**Fig. A1**, Appendix A). Subsequently, PCR amplification of each sample pool of each bird flock was performed using the Eukaryotic mini-barcode miniB18S_81 designed by Cabodevilla *et al.* (2020b) at the Analytical Services (SGIker) of the University of the Basque Country (UPV/EHU). Samples were purified and a second reaction was performed to index each amplified product and attach Illumina index using the Illumina Nextera v2 kit. We added the same index to the 3 pools of each bird flock, using a different index per flock (**Fig. A1**, Appendix A). The amplification was carried out according to Cabodevilla *et al.* (2020b), using the same DNA quantity, reaction mix, and thermocycler conditions. PCR outputs were sequenced in an Illumina MiSeq NGS platform (sequencing of 2x150 bp paired-end reads) with the MiSeq Reagent Kit v2, following the manufacturer's instructions.

2.5. Bioinformatics and statistical analyses

The sequencing output was processed using the Cutadapt (Martin, 2011) and Usearch (Edgar, 2010) software packages. First, the primers were cut and forward and reverse sequences were merged (77.34% of sequences merged correctly). The sequences were then cleaned, filtering them by expected errors (*fastq_filter* function of Usearch, allowing a single expected error; 97% passed), the unique sequences and the OTUs were identify using *fastx_uniques* and *cluster_otus* functions of Usearch respectively, and the OTU table was built based on 97% OTUs using the *usearch_global* function. Subsequently, we used the *sintax* function of Usearch and the SILVA 18S v123 reference dataset (Quast *et al.*, 2013) to predict the taxonomy of the obtained OTUs. We used a *cutoff* of 0.8 to identify taxonomy at the kingdom, phylum, and class levels and a *cutoff* of 0.7 to identify taxonomy at the order level. We did not include sequences of the 16S gene on our reference data set, since used primers do not amplify prokaryotic organisms (Cabodevilla *et al.*, 2020b). Then we filtered the OTU table, selecting only those OTUs that could belong to the studied birds' diet, OTUs belonged to Chloroplastida, Arthropoda, Mollusca (only those of Gastropoda class), and Annelida (only those of Clitellata class) taxa. Among OTUs belonging to the phylum Arthropoda, those OTUs belonging to the classes Maxillopoda and Ostracoda (organisms inhabiting the water) and to the order Phthiraptera (ectoparasites) were also omitted, as these were probably not part of the steppe birds' diet but they are environmental contamination. Once filtered, we normalized the OTU table transforming the data to proportions, using the Total Sum Normalization method (TSS), by dividing reads of each OTU of each group by the total read of this group (McKnight *et al.*, 2019).

We used the OTU table to describe the diets of the species analyzed based on the mean values and SD of the percentage of reads (estimate as: number of reads of OTUs of specific taxa / total number of reads * 100) and the percentage of OTUs (estimate as: number of OTUs of specific taxa / total number of OTUs * 100). The percentage of reads represents a measure of the abundance of the OTUs of specific taxa while the percentage of OTUs represents a measure of richness within a specific taxon. For common quail it was not possible to obtain mean values and SD since, as we group all individual samples in a single group, we only obtained one data. Thus, we used these value to describe the composition of plants (sequences belonging to the Chloroplast kingdom) and invertebrates (Annelida, Arthropoda, and Mollusca phyla, from the Metazoo kingdom) of the diet of the studied bird species, describing in detail the composition of the animal part of the diet (Arthropoda phylum classes belonging to the diet and Insecta class orders). In the case of the plants, the used mini-barcode shows very low resolution capacities at the order level, so we decided to include such results at the kingdom level since most OTUs belonged to the same class (Embryophyta) and same phylum (Phragmoplastophyta; Cabodevilla *et al.*, 2020b). As common quail samples were collected at a different time of year than those for other species, the results for this species were considered separately. We carried out an ANOVA in R v3.6.161 to check if there were differences in OTUs richness between bird species. Our response variable was the number of OTUs per bird flock and the explanatory variable was the species. We break down the results of the ANOVA by species applying a Tukey post-hoc test, using the *lsmeans* package in R (Russell, 2016; R Core Team, 2019. In addition, in the case of great bustard, little bustard, pin-tailed sandgrouse, and red-legged partridges we also made a comparison of diet by season, comparing their diet in autumn, winter, and spring. We classified samples in relation to season within the non-breeding season as follows: from September to November were considered as autumn, from December to February as winter, and from March to April as spring. In addition to the direct comparisons, differences in diet between species and between seasons within species were tested by Non-Metric Multidimensional Scaling (NMDS) and Permutational Multivariate Analysis of Variance (PERMANOVA) models (*metaMDS* and *adonis* functions of vegan v2.4-2 package in R; Oksanen *et al.*, 2013; R Core Team, 2019). For this purpose, the database was transformed by applying a fourth root. NMDS is an ordination technique that uses a Bray-Curtis matrix of ranked similarities and displays samples in low-dimensional space while retaining as nearly as possible the similarity rankings between samples.

3. Results

We achieved a mean coverage of 130006 reads (min: 41632, max: 227268) belonging to diet operational taxonomic units (OTUs) per sample and high-quality sequences. On average, diet reads accounted for 69.58% of the total reads. These reads were classified into 879 OTUs, 636 OTUs of plants, 235 OTUs of arthropods, 3 OTUs of annelids, and 5 OTUs of mollusks.

We found clear differences in diet composition between studied species based on the results of the NMDS analysis (**Fig. 2** NMDS axis 1) and PERMANOVA test ($F_{5,39}$ = 2.64, $P < 0.001$; **Fig. 2**). The diet of both bustard species was more similar to each other than to those of sandgrouse and partridges. In the case of common quail, although we only had one sample, its breeding season diet appeared to be quite different from the non-breeding diet of the other species according to both NMDS axis (**Fig. 2**).

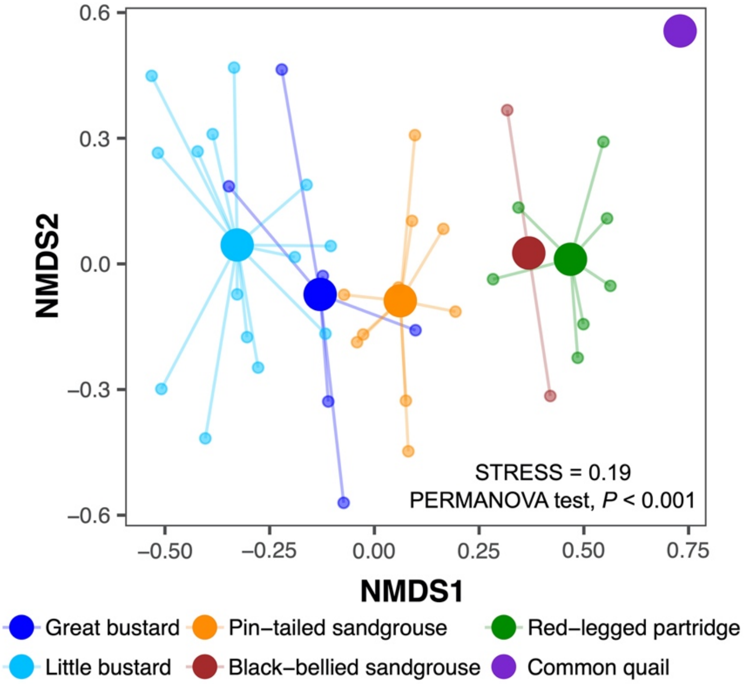

Figure 2. Graphical illustration of the results of the NMDS analysis of the studied birds' diet (OTUs). Larger dots represent the centroid of the flocks of each species, while smaller dots represent the individual flock data of each species.

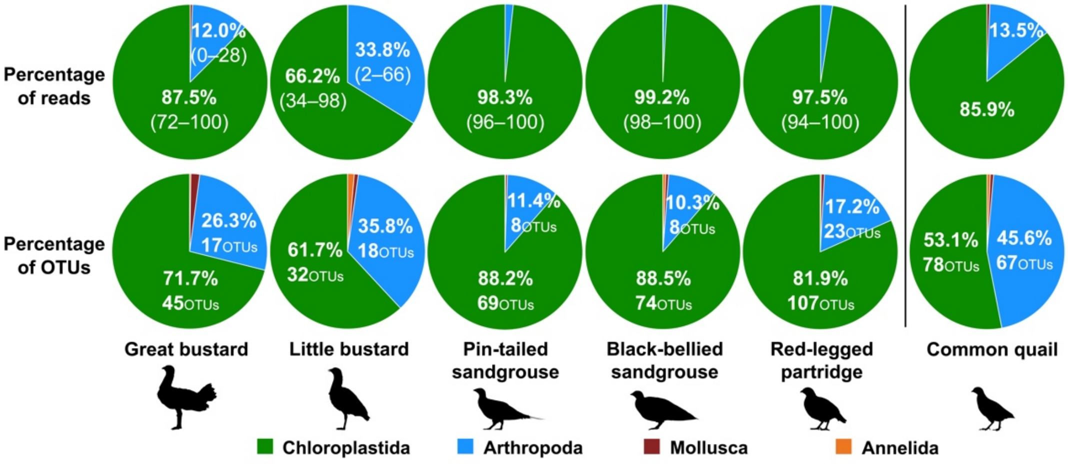

Figure 3. Mean percentage of reads (a measure of abundance) and OTUs (a measure of richness) of the Chloroplastida, Arthropoda, Mollusca, and Annelida taxa present on the diet of studied farmland bird species. Percentages >10% are specified numerically and in the case of reads, SD is indicated in brackets. In the OTUs graphs, OTUs richness is also indicated. Note that samples of great bustard, little bustard, pin-tailed sandgrouse, black-bellied sandgrouse, and red-legged partridges were collected during the non-breeding season, whereas samples of common quail were collected during the breeding season.

Differences between species were also clear when we checked the proportion of reads and OTUs of each taxon obtained from sequencing (**Fig. 3** and **Fig. 4**). The results showed that the plant material was the most important component of the diet of all six species, but the importance of invertebrates in the diet strongly differed among them (**Fig. 3**). Invertebrates contributed to the diet of all species, but they were especially important for both bustard species in autumn, winter, and spring and common quail during the breeding season. In all species, the contribution of invertebrates to diet was mostly made up by arthropods, while mollusks and annelids seemed to be rarely taken.

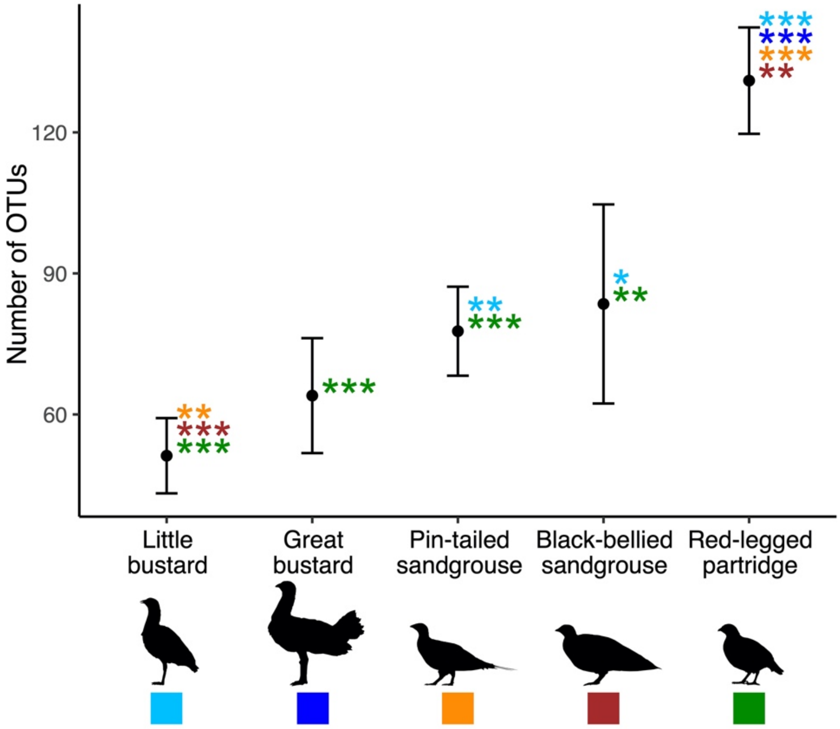

Figure 4. OTUs richness in the diet of studied species. The whiskers represent the 95% confidence intervals. The statistical significance of the Tukey post-hoc test is provided by * symbols. *** $P < 0.001$. ** $P < 0.01$. * $P < 0.05$. The color of the * corresponds to the color of the species with which the comparison is made. For those comparisons that were not statistically significant, no significance data are provided. For detailed statistical results of Tukey post-hoc tests, see **Table A2** , Appendix A.

The ANOVA test showed that there were significant differences in OTU richness among species ($F_{4,34} = 35.45$, $P < 0.001$; **Fig. 4**). During the non-breeding season, the diet of red-legged partridges was the richest, with an average of 131 OTUs (95% Confidence Interval (CI) = 120–142 CI), followed by the diets of black-bellied sandgrouse (84 OTUs, 62–1 05 CI), pin-tailed sandgrouse (78 OTUs, 68–87 CI) and great bustard (64 OTUs, 52–76 CI). The diet of little bustard (51 OTUs, 43–59 CI) was the least rich. In the case of the two bustard species, around 30% of the OTUs belonged to Arthropoda taxa (**Fig. 3**). In contrast to the large differences found among species, we found low variability in OTU richness within species, as shown by the 95% Confidence Intervals (**Fig. 4**), even if samples were collected in different seasons or different geographical regions. During the breeding season, common quail showed a high OTUs richness (147 OTUs), although it has to be taken into account that sampled birds were not a flock per se (i.e., they include birds captured in different dates and probably that foraged in the different places), so these data are not comparable with those of other species.

Regarding the arthropods that compose the diet of these species, there were large differences between species (**Fig. 5**). The order Thysanoptera (Insecta) was represented in the diet of all species sampled in the non-breeding season (**Fig. 5**), although considering its contribution to the total diet, it represented only 5.6% of total reads in little bustard, 3.1% of total reads in great bustard, and less than 0.85% of total reads in the other species. In the case of the bustard species, insects were the most common prey, especially those of the orders Coleoptera, Diptera, and Thysanoptera, and for Great bustard also Hymenoptera and Orthoptera (**Fig. 5**). Sandgrouse rarely fed on arthropods and the small number of arthropods in their diet was mainly composed of thrips (Thysanoptera) in the case of the pin-tailed sandgrouse, and of springtails (Ellipura) in the case of the black-bellied sandgrouse. Red-legged partridges also mainly fed on plants, although when they fed on arthropods, they mostly took arachnids, hymenopters, and thrips. During the breeding season, common quail showed a very different diet (**Fig. 5**), with a large proportion of its diet composed of organisms of taxa Arachnida and Ellipura, while insects represented less than 25% of the reads. Besides, we found some taxa in the studied species diet whose species usually sow high toxicity. Thus, we found Hymenoptera (mostly ants) in the diet of all species, *Scolopendra* genera in the diet of great bustard (in 2 of 6 flocks) and little bustard (in 3 of 14 flocks), another Chilopoda taxa in the common quail's diet and an organism of Meloidae family in little bustard's diet (in 1 of 14 flocks).

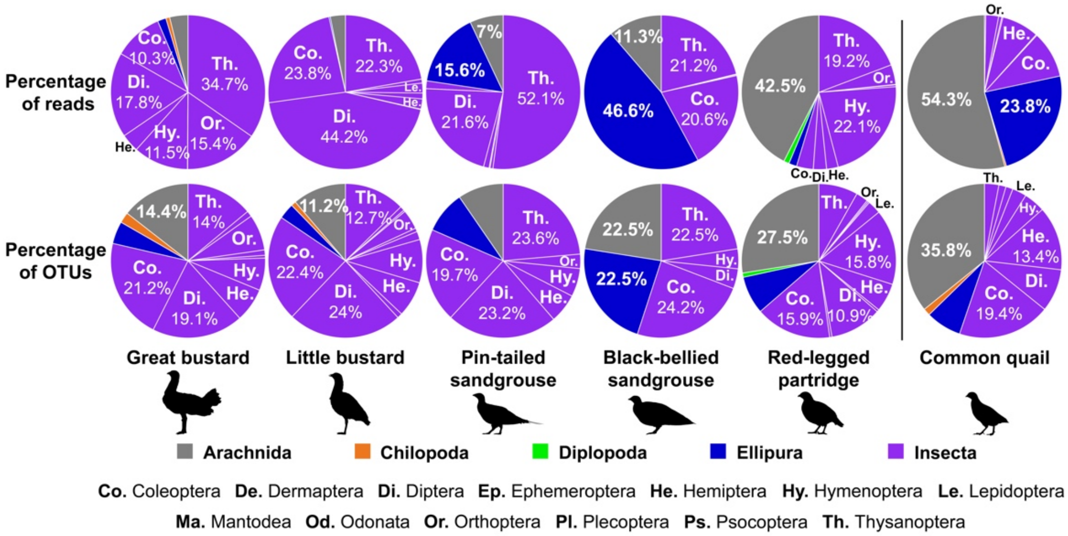

Figure 5. Mean percentage of reads (a measure of abundance) and OTUs (a measure of richness) of each phylum of Arthropoda present on the diet of studied farmland bird species, with phylum Insecta data broken down into orders. Percentages >10% are detailed numerically. Among Insect's orders, those with a percentage >2% are identified. Note that samples of great bustard, little bustard, pin-tailed sandgrouse, black-bellied sandgrouse, and red-legged partridges were collected during the non-breeding season, whereas samples of common quail were collected during the breeding season.

The NMDS and PERMANOVA analyses of seasonal diet differences for great bustard, little bustard, pin-tailed sandgrouse, and red-legged partridge showed significant differences between autumn, winter, and spring ($F_{11,36} = 2.08$, $P < 0.001$; **Fig. 6**). Those differences were also found for little bustard ($F_{2,13} = 2.08$, $P < 0.01$) and pin-tailed sandgrouse ($F_{2,9} = 1.61$, $P < 0.01$) when species were analysed separately by PERMANOVA testing. In the case of great bustard ($F_{2,5} = 0.45$, $P = 0.13$) and red-legged partridge ($F_{2,6} = 1.27$, $P = 0.13$) we did not find significant seasonal differences, although this could be due to the small sample size (6 flock of great bustard and 7 flock of red-legged partridge, with a single flock of each species sampled in winter). In all species, the autumn diet was the most different (**Fig. 6**).

Although the OTUs composition on little bustard and pin-tailed sandgrouse varied significantly between seasons (**Fig. 6**), the proportions of plants and invertebrates in their diet did not change as much. The proportion of plants and arthropods in the little bustard´s diet was similar in autumn and spring, with the proportion of arthropods (percentage of reads) being lower in winter. Pin-tailed sandgrouse fed slightly more on arthropods in winter than in the other two seasons (percentage of reads; **Fig. 7**) although, in general, arthropods did not fare much in their diet. Red-legged partridges also rarely fed on arthropods during the non-breeding season, although these were more common in their autumn diet. There were clear differences in the proportion of plants and invertebrates (percentage of reads) between seasons in great bustard diet, with higher consumption of arthropods in autumn, while in winter they barely fed on them (according to the percentage of reads; **Fig. 7**). In all species, the total OTU richness was very similar in the three seasons (although it was a bit lower in winter; **Fig. 7**). In this respect, the great bustard was also the species that showed the largest differences.

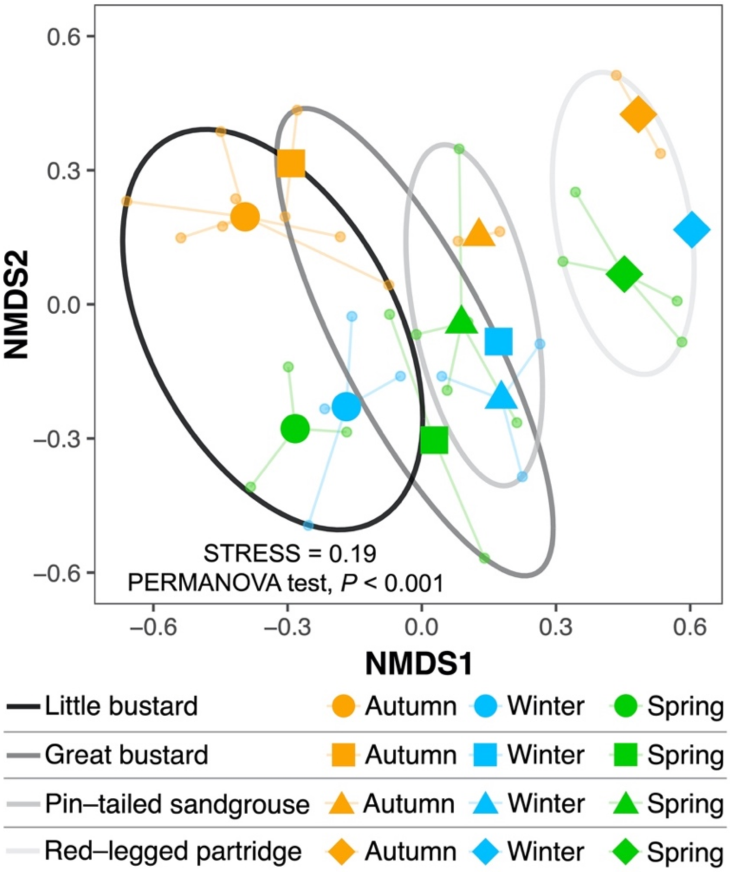

Figure 6. Graphical illustration of the NMDS base on diet OTUs of little bustard, great bustard, pin-tailed sandgrouse, and red-legged partridge represented by season. Large symbols represent the centroid of the flocks of each species in each season, while small dots represent data of each different flock per species and season. The circles group data of each species.

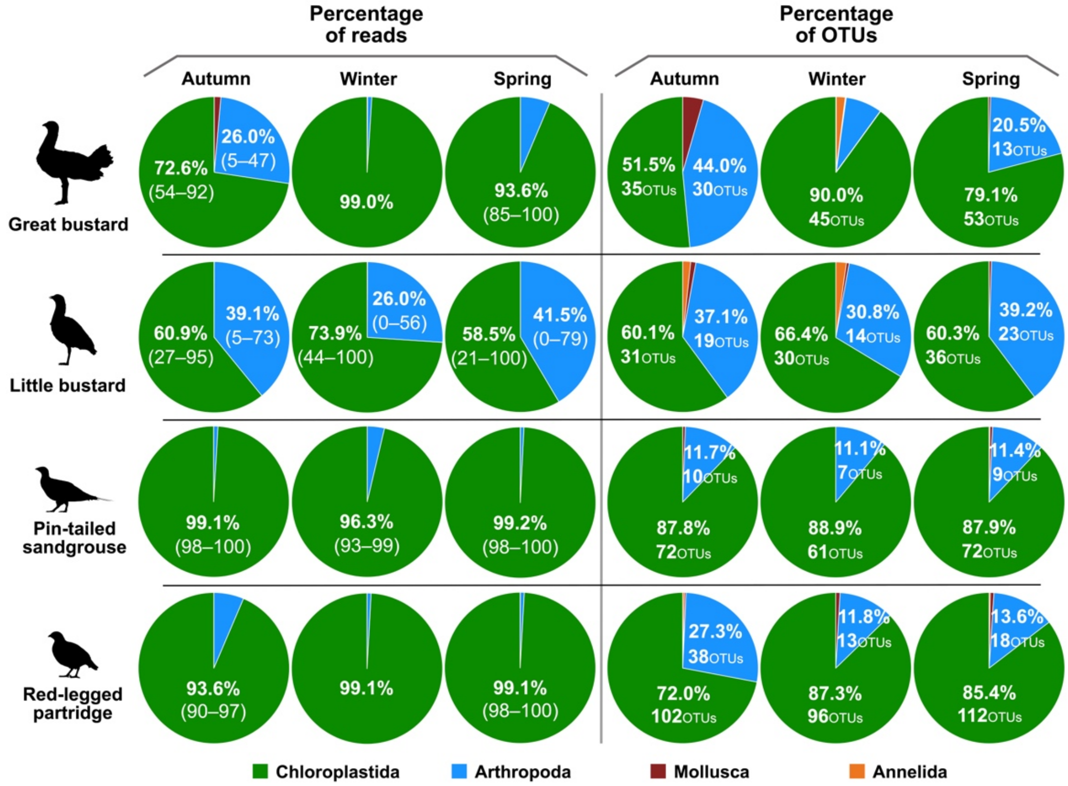

Figure 7. Mean percentage of reads (a measure of abundance) and OTUs (a measure of richness) of the Chloroplastida, Arthropoda, Mollusca, and Annelida taxa present on the diet of great bustard, little bustard, pin-tailed sandgrouse, and red-legged partridge in autumn, winter and spring. Percentages >10% are detailed numerically and in the case of reads, SD is indicated in brackets. In the OTUs graphs, OTUs richness is also indicated. For Great Bustard and Red-legged Partridge, only one flock was sampled in winter so no SDs are provided.

Unlike the plant/invertebrate proportions in the diet, there were strong differences in the proportion of consumed arthropod taxa between seasons (percentage of reads) in all species, although the consumed arthropod taxa richness (percentage of OTUs) did not change as much (**Fig. 8**). These differences were largely due to the fact that in all species the importance of thrips (Thysanoptera) was quite low in autumn while they contributed greatly to arthropod diets of pin-tailed sandgrouse and little bustard during winter and spring and of great bustard and red-legged partridge during spring (percentage of reads; **Fig. 8**). Great bustard showed the largest seasonal differences in arthropod consumption, with high importance of Diptera and Hymenoptera in autumn and very high importance of Orthoptera in winter (although all reads belong to a single OTU). Regarding the little bustard´s diet, Coleoptera and Diptera were important in all seasons. In pin-tailed sandgrouse diet, springtails (Ellipura) were important in autumn and winter, Diptera was important in autumn and spring diet, and arachnids appeared also with 12% of arthropod reads in the spring diet (**Fig. 8**). Red-legged partridge's arthropod diet was largely composed of arachnids in autumn and winter. In autumn, Hymenoptera also made up a large part of its arthropod diet.

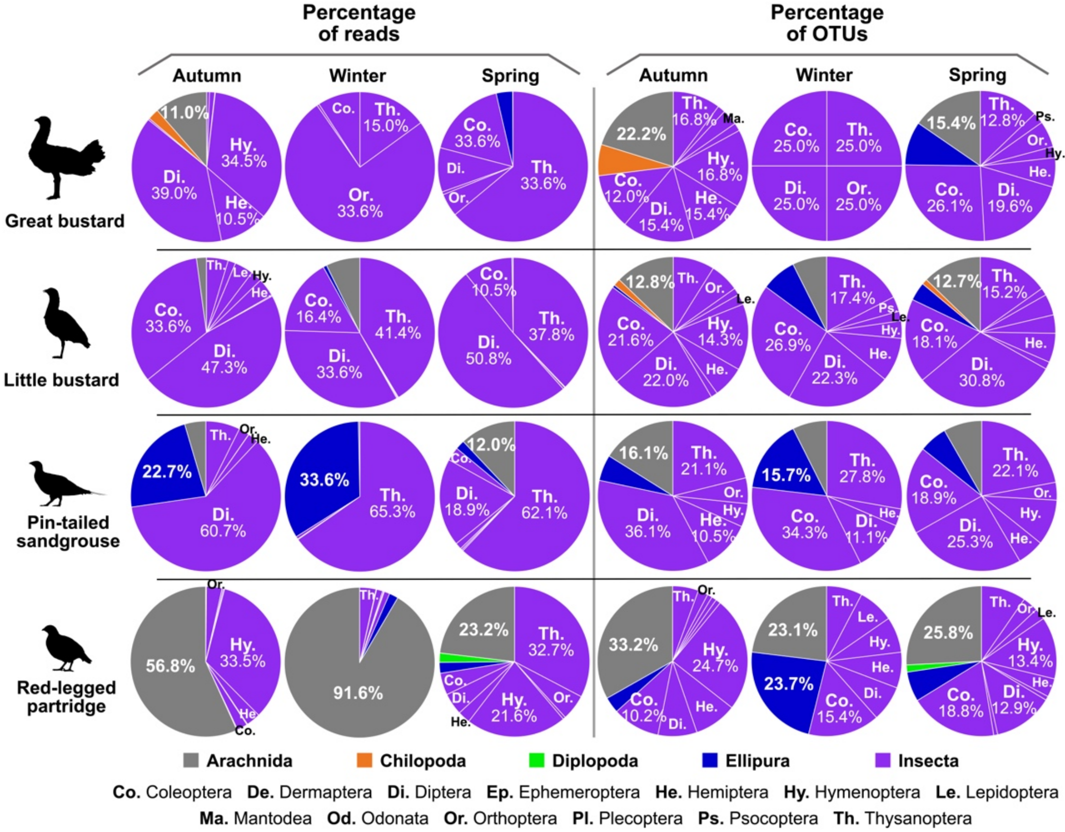

Figure 8. Mean percentage of reads (a measure of abundance) and OTUs (a measure of richness) of each phylum of Arthropoda present on the diet of great bustard, little bustard, pin-tailed sandgrouse, and red-legged partridge in autumn, winter, and spring, with phylum Insecta data broken down into orders. Percentages >10% are detailed numerically. Among Insect's orders, those with a percentage >2% are identified. For Great Bustard and Red-legged Partridge, only one flock was sampled in winter so no SDs are provided.

4. Discussion

Foraging strategies and diet selection play an essential role in individual survival and reproduction (Martin, 1987), and their study becomes crucial when it comes to endangered species such as steppe birds or declining gamebirds of high socioeconomic importance. In this work, we used, for the first time, a metabarcoding approach to study the diet of these species, which has provided new and relevant data on their diet composition, including the first detailed description of the arthropod diet of pin-tailed sandgrouse, black-bellied sandgrouse, red-legged partridge, and common quail. Our results showed clear differences between studied species' diets, with the highest diet similarities among the species most closely related phylogenetically. Little bustards´ diet was more similar to that of great bustards (both belong to the Otidae family), with important proportions of arthropods (Coleoptera, Diptera, and Thysanoptera and for Great bustard also Hymenoptera and Orthoptera) in both species. Pin-tailed sandgrouse diet was more similar to that of black-bellied sandgrouse (both belong to the Pteroclidae family), being composed mostly by plants, with small proportions of arthropods (Thysanoptera, Ellipura, Diptera, Coleoptera, and Arachnida) in both species. Red-legged partridge diet was also mostly composed of plants and a small proportion of arthropods (Arachnida, Thysanoptera, and Hymenoptera), although results showed high arthropod OTU richness. In the case of common quail, arthropods were an important part of its diet during the breeding season, especially arachnids and springtails (Ellipura). Our results also provided reliable data on differences in diet richness between species, which was very constant among flocks within species, suggesting that it may be a characteristic of each species. The little bustard was the species with the lowest non-breeding diet richness, showing half of pin-tailed sandgrouse plant OTUs, even if usually both species constitute mixed flocks during the non-breeding period (Martín *et al.*, 2010). This result probably indicates a high specificity regarding each species diet, and thus relatively low trophic plasticity. On the other hand, red-legged partridges showed by far the highest diet richness, even when data was broken down into seasons, which probably indicates much greater flexibility in its food selection. This species can exploit many more habitats than the other species, such as vineyards, scrublands, and olive groves (Del Hoyo *et al.*, 1992).

Regarding the arthropods that compose the diet of these species, we found some taxa that had not been described (or only occasionally) previously in these species' diets (Jiguet, 2002; Bravo *et al.*, 2012; Bravo *et al.*, 2017), such as thrips (Thysanoptera), arachnids (Arachnida) or springtails (Ellipura), and for which our results indicate that they are important arthropod prey. These organisms are often completely digested and therefore underestimated when the diet is analyzed through visual identification in fecal samples (Pompanon *et al.*, 2012). We found large proportions of thrips within the arthropods that compose the diet of little bustard, great bustard, pin-tailed sandgrouse, black-bellied sandgrouse, and red-legged partridge. Thrips were present in all the samples analyzed in this study and were particularly frequent during winter and early spring. Thysanoptera is an order of very small insects, many of them phytophagous, which develop their life cycle in plants (Morse and Hoddle, 2006; Goldarazena, 2015). Considering their small size (0.3 – 14 mm) and cosmopolitan distribution (Goldarazena, 2015), birds may consume them indirectly through the accidental intake of plants colonized by thrips. In the case of bustards, the species with the highest intake of thrips, this could be due to the high intake of legumes that these species have in winter (Bravo *et al.*, 2012; Bravo *et al.*, 2017). However, the selection of plants colonized by thrips may also be intentional because they contain a higher level of nutrients than plants without thrips. In the case of sandgrouse diet, thrips represented a lower proportion of their arthropod diet, possibly because sandgrouse feed mainly on seeds rather than on leaves (Del Hoyo *et al.*, 1992; Suárez *et al.*, 1999). This could also explain why thrips were less frequent in all species in their autumn diet, when crop fields are mostly bare or newly sown. Future studies should specifically assess whether these bird species actively select plants colonized by thrips. If this was the case, steppe birds could be providing an important ecosystem service in terms of pest control (Ndang'ang'a *et al.*, 2013), since many thrips species present invasive characteristics and could be vectors of different viruses that are detrimental to crops (Morse and Hoddle, 2006; Goldarazena, 2015).

Our study also revealed the high importance of arachnids in the common quail's diet, which was as yet unknown; our results clearly showed a large intake of this invertebrate phylum by this species. Birds have been described as important predators of spiders in forest habitats, but not in open ecosystems (Gunnarsson, 2007). Common quails may feed on spiders because they have high nutritious value and lack defensive chemicals (unlike ants) that might be toxic or repelling (Gunnarsson, 2007).

In that context, we found that the arthropod diet of some species contained, in low proportions, some taxa such as ants, *Scolopendra* spp. and Meloidae (that include toxic species) that might be consumed for auto-medication, and used as antiparasitic food (Lee *et al.*, 2015; Lima *et al.*, 2016; Whitman *et al.*, 2019). Previous studies have demonstrated the use of some plant species and insects of the Meloidae family as antiparasitic agents by great bustards (Bravo *et al.*, 2014; Bolívar *et al.*, 2020).

However, as far as we know, this is the first time that Meloidae is described in the diet of the little bustard and that *Scolopendra* spp is reported in the diet of both great bustard and little bustard. In the case of ants, they were mainly consumed in autumn. During this season, due to the emergence of winged ants, ant availability may be particularly high and these may be taken opportunistically as a readily available food resource, but ants could also be consumed for antiparasitic use (Lima *et al.*, 2016).

Our results also showed that diet composition varied across seasons, with important changes in the proportion of consumed arthropod taxa, being less diverse in winter than in autumn and spring. These results probably reflected differences in arthropod availability in the different seasons (Boyer *et al.*, 2003). However, except for the great bustard diet, the ratio between plants and invertebrates in the diet did not change as much, and plant and arthropod richness was quite constant throughout the non-breeding season. The results obtained for the great bustard (a strong reduction in the proportion of arthropods in its winter diet) are in line with previous studies (Palacios *et al.*, 1975), showing that the winter diet of great bustards was based almost exclusively on vegetable matter. On the other hand, the low variation in arthropod taxa richness in the little bustard diet between the seasons may reflect this bird's high affinity for certain prey and low trophic plasticity. The red-legged partridge, by contrast, showed both high plant taxa richness and high arthropod taxa richness, although the proportion of arthropod reads in its diet was low. This showed the low trophic specificity and high trophic plasticity of this species, at least in comparison with the other studied species. There were probably also important changes in the proportions of consumed plant taxa, but unfortunately, our methodology did not allow us to break down plant data (Cabodevilla *et al.*, 2020b), and this should be assessed in future studies.

Our results have relevant implications for the little bustard, a threatened species in sharp decline (García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020a) and the species with the least diverse diet (especially regarding plants) and with the highest proportion of arthropods on his diet during the non-breeding season. According to our results, the little bustard diet during the non-breeding season may include a higher proportion of arthropods than estimated by previous studies based on visual analysis of feces (Bravo *et al.*, 2017). It should be noted that most of the arthropods described in their winter diet (thrips, flies, and spiders) belong to taxa with few chitinous structures, potentially less detectable by a visual study of remains in the feces. By contrast, the proportion of Coleoptera in their winter diet was small. With all this in mind, the little bustard is possibly the most vulnerable species to changes in trophic resources, such as the loss of weed and arthropod biodiversity and abundance resulting from agriculture intensification (Benton *et al.*, 2002). During winter, this species has been seen using legume fields, stubbles and fallow lands (Silva *et al.*, 2004; Cuscó *et al.*, 2018; González del Portillo *et al.*, 2021). Alfalfa fields provide an important plant trophic resource that the species uses throughout the year (Bravo *et al.*, 2017; Cuscó *et al.*, 2018; González del Portillo *et al.*, 2021) and also provide a high abundance of arthropods (González del Portillo *et al.*, 2021). Stubbles, although they maintain a lower abundance of arthropods, are positively selected because their vegetation structure facilitates the location and capture of prey (González del Portillo *et al.*, 2021). Moreover, natural and semi-natural environments (such as vegetation growing in fallow land) play an essential role for arthropod communities, providing over-wintering areas for that group and maintaining an overall greater abundance and richness of arthropods than crop lands (Pfiffner and Luka, 2000; Hendrickx *et al.*, 2007). A strong association between the decline in little bustard abundance and the decline in the fallow surface has been shown (Traba and Morales, 2019), suggesting a potential link between these two factors. This relationship between arthropods and fallow land reinforces the need for a new CAP that guarantees the maintenance of fallow land in the European agroecosystems if little bustard populations, as well as those of other farmland birds, are to be conserved (Traba and Morales, 2019; Tarjuelo *et al.*, 2020b).

The proportion of arthropods in the non-breeding diet of sandgrouse species was very small, consistent with a high dependence on vegetable trophic resources. Pin-tailed sandgrouse and black-bellied sandgrouse are mainly granivorous species (Del Hoyo *et al.*, 1992), so they rely on the availability of seeds and seed banks. Seed availability is highly variable between habitats and among seasons, being also strongly influenced by agricultural intensification (Newton, 2004; Tarjuelo *et al.*, 2019). Within arable land, natural and semi-natural vegetation growing in grassy field boundaries and fallow land provides an important stock of seeds, being thus important habitat elements for these species (Tarjuelo *et al.*, 2019).

Within these landscapes in continuous intensification, updated knowledge on species diet becomes essential to address their conservation. In this line, our results provided relevant data on the diet of these declining species throughout the non-breeding season (the least studied season so far), especially with regard to invertebrates. Moreover, they showed the importance of arthropods in these species' diets, proving the need to adequately maintain the arthropod populations within agricultural landscapes. This could be crucial to address the conservation of endangered species. In the future, this study should be complemented by a detailed assessment of vegetable diet, which would help to clarify the extent to which these birds feed on cultivated crops, including pesticide coated seeds (Tarjuelo *et al.*, 2020a), and the role that fallows and semi-natural habitats play as food sources. Besides, the study of diet throughout the breeding season should be also assessed, as it is known that in the breeding season diet could change significantly (Palacios *et al.*, 1975; Jiguet, 2002). Moreover, chicks might need a diet richer in protein for growth, as highlighted for great bustard and red-legged partridge, whose chicks rely on arthropod diets (Green, 1984; Jiguet, 2002; Bravo *et al.*, 2012), but the trophic requirements of sandgrouse or little bustard´s chicks have not yet been described in depth (Jiguet, 2002). To properly address the conservation needs of these species, especially of threatened steppe bird species, agriculture should be managed in a way that ensures fulfilling trophic needs. This could be achieved in a sustainable way by promoting agri-environmental schemes attractive and profitable for farmers. These measures should be focused on increasing the complexity of the ecosystems, to shift these ecosystems towards a mosaic landscape, which can provide a wide variety of arthropods, plants, and seeds throughout the year.

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Supplementary material

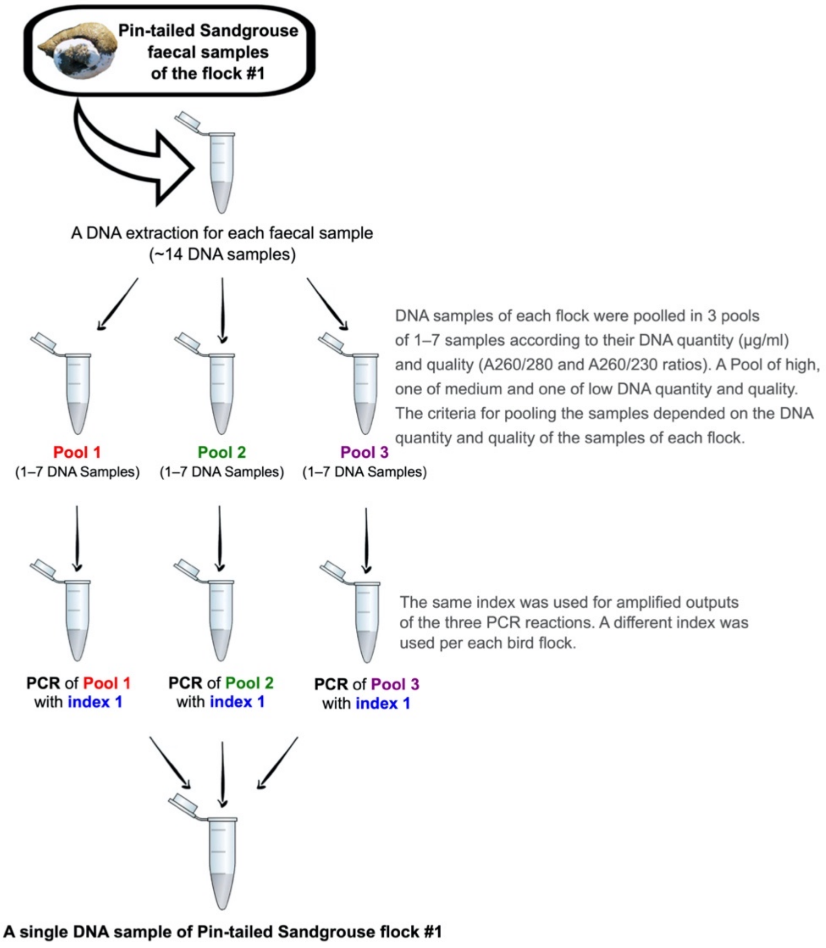
Appendix A

Table A1. DNA samples collected by species flock, with information on flock's origin and sampling season.

Species	Flock	DNA samples	Province	Season
Little bustard	1	10	Ciudad Real	Early spring
Little bustard	2	17	Lleida	Winter
Little bustard	3	16	Lleida	Early spring
Little bustard	4	16	Ciudad Real	Winter
Little bustard	5	15	Ciudad Real	Winter
Little bustard	6	19	Ciudad Real	Winter
Little bustard	7	11	Ciudad Real	Autumn
Little bustard	8	20	Ciudad Real	Autumn
Little bustard	9	14	Ciudad Real	Autumn
Little bustard	10	13	Ciudad Real	Autumn
Little bustard	11	19	Ciudad Real	Autumn
Little bustard	12	18	Ciudad Real	Early spring
Little bustard	13	20	Ciudad Real	Autumn
Little bustard	14	18	Ciudad Real	Autumn
Great bustard	1	6	Ciudad Real	Early spring
Great bustard	2	7	Navarra	Early spring
Great bustard	3	17	Ciudad Real	Winter
Great bustard	4	17	Ciudad Real	Autumn
Great bustard	5	11	Ciudad Real	Autumn
Great bustard	6	1	Ciudad Real	Early spring
Pin-tailed sandgrouse	1	11	Navarra	Early spring
Pin-tailed sandgrouse	2	16	Navarra	Early spring
Pin-tailed sandgrouse	3	15	Ciudad Real	Early spring
Pin-tailed sandgrouse	4	13	Lleida	Winter
Pin-tailed sandgrouse	5	16	Ciudad Real	Winter
Pin-tailed sandgrouse	6	17	Ciudad Real	Autumn
Pin-tailed sandgrouse	7	16	Ciudad Real	Winter
Pin-tailed sandgrouse	8	14	Ciudad Real	Autumn
Pin-tailed sandgrouse	9	15	Ciudad Real	Early spring
Pin-tailed sandgrouse	10	15	Ciudad Real	Early spring
Black-bellied sandgrouse	1	20	Navarra	Autumn
Black-bellied sandgrouse	2	18	Ciudad Real	Winter
Red-legged partridge	1	11	Ciudad Real	Autumn
Red-legged partridge	2	15	Ciudad Real	Autumn
Red-legged partridge	3	14	Ciudad Real	Winter
Red-legged partridge	4	11	Ciudad Real	Early spring
Red-legged partridge	5	9	Ciudad Real	Early spring
Red-legged partridge	6	6	Navarra	Early spring
Red-legged partridge	7	9	Ciudad Real	Early spring
Common quail	1	16	Alava	Spring-summer

Table A2. Statistical results of Tukey post-hoc analysis for the ANOVA model for differences on OTUs richness among species.

Species	*Degrees of freedom*	*t.ratio*	*P*
LB * GB	34	-1.78	0.40
LB * PTS	34	-4.34	< **0.01**
LB * BBS	34	-2.90	< **0.05**
LB * RLP	34	-11.70	< **0.001**
GB * PTS	34	-1.80	0.39
GB * BBS	34	-1.62	0.49
GB * RLP	34	-8.17	< **0.001**
PTS * BBS	34	-0.51	0.99
PTS * RLP	34	-7.34	< **0.001**
BBS * RLP	34	-4.02	< **0.01**



DNA samples of each flock were poolled in 3 pools of 1–7 samples according to their DNA quantity (μg/ml) and quality (A260/280 and A260/230 ratios). A Pool of high, one of medium and one of low DNA quantity and quality. The criteria for pooling the samples depended on the DNA quantity and quality of the samples of each flock.

The same index was used for amplified outputs of the three PCR reactions. A different index was used per each bird flock.

*40 samples were sequenced in an Illumina MiSeq NGS, a single sample per flock.

PAPER VII

A semi-quantitative approximation to *Blastocystis* spp. infections in farmland birds: very high parasitic load in protected Bustards

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In preparation

A semi-quantitative approximation to *Blastocystis* spp. infections in farmland birds: very high parasitic load in protected Bustards

Abstract

Many farmland birds are in a sharp decline due to agricultural intensification. Drivers of decline may also be individual stressors, which may increase their susceptibility to parasitic infections. Among protist parasites, *Blastocystis* spp. is one of the most widely distributed and least studied in wildlife, although it is zoonotic and it is known that its pathogenic capacity increases in immunosuppressed hosts.

In this study we tested a metabarcoding approach to semi-quantitatively estimate *Blastocystis* spp. parasitic load, combining it with phylogenetic inference to identify *Blastocystis* subtypes. We used this approach to evaluate the importance of *Blastocystis* spp. and its subtypes on 6 farmland bird species (great bustard, little bustard, pin-tailed sandgrouse, black-bellied sandgrouse, red-legged partridge, and common quail), including also farm-reared red-legged partridges.

Our results showed that the proposed approach is able to identify *Blastocystis* subtypes and provides a semi-quantitative parasitic load of *Blastocystis* spp. and its subtypes. On the other hand, we found that all studied bird species were infected by *Blastocystis* spp., although the most abundant subtypes differ between species. Farm-reared birds were found infected mostly by *Blastocystis* subtype 6, a zoonotic subtype very rare in wild birds. In general, wild birds did not show high parasitic loads, with the exception of bustards. In the case of the little bustard, the parasitic load was extremely high. Furthermore, this species was found to be coinfected by many *Blastocystis* OTUs (12 OTUs were found per flock), and a high proportion of *Blastocystis* reads belonged to the zoonotic subtype 7. Our results highlight that *Blastocystis* spp. and their potential effects should be considered in the context of population health and conservation management of this highly threatened species. In general, this study provides a tool for the study of *Blastocystis* spp. and the first assessment of the importance of this parasite and its subtypes in farmland birds, showing the great importance that this organism could have for the conservation of some species highly susceptible to stress.

Keywords

Blastocystis subtypes; *Eimeria*; farmland birds’ health status; farm-reared red-legged partridges; little bustard; protist parasites

1. Introduction

Blastocystis spp. is a strict anaerobic protist parasite that can be found in the intestinal tract of many different animals including humans (Ramírez *et al.*, 2014; Betts *et al.*, 2018; Greige *et al.*, 2018; Katsumata *et al.*, 2018; Wang *et al.*, 2018; Asghari *et al.*, 2019; Piubelli *et al.*, 2019). After several decades studying it (Tan, 2008) 22 subtypes (hereafter ST) of *Blastocystis* have been described (Stensvold and Clark, 2020), nine of them in humans, including some zoonotic ones (Iguchi *et al.*, 2007; Tan 2008; Lewicki *et al.*, 2016; Cian *et al.*, 2017; Greige *et al.*, 2018). Although the pathogenic potential of *Blastocystis* spp. seems clear (Hussein *et al.*, 2008; Puthia *et al.*, 2008; Tan, 2008; Elwakil and Hewedi, 2010; Ajjampur and Tan, 2016), it has been equally found in sick and healthy individuals (Scanlan and Marchesi, 2008; Scanlan, 2012; Adamu *et al.*, 2013), and thus it is not yet understood why this organism is sometimes pathogenic and sometimes not (Scanlan, 2012). However, it is known that its effect is over the gut bacterial microbiota (Scanlan, 2012; Defaye *et al.*, 2018; Nieves-Ramírez *et al.*, 2018; Yason *et al.*, 2019) and its pathogenic capacity increases in immunosuppressed hosts (Llibre *et al.*, 1989; Adamu *et al.*, 2013; Chandramathi *et al.*, 2014; Ahmed and Karanis 2018).

The mechanisms by which *Blastocystis* spp. produces changes on the gut microbiota are still unclear. Probably it is a consequence of the *Blastocystis* spp. grazing activity over gut bacteria (Dunn *et al.*, 1989; Tan, 2008; Nieves-Ramírez *et al.*, 2018). If this were so, the effect over gut microbiota would depend on both the feeding preferences of each *Blastocystis* ST (Yason *et al.*, 2019) and the parasitic load (Scanlan, 2012), and this could explain the differences in pathology between cases. On the other hand, the changes produced by *Blastocystis* spp. on the gut microbiota could also be a secondary consequence of other *Blastocystis* spp. activities (Yason *et al.*, 2019). Either way, a thorough understanding of the *Blastocystis* spp. pathogenic capacity is essential to properly assess the impact of this organism in human and wildlife health. In fact, it could be a great conservation concern for highly stressed wild animal populations, as its pathogenic capacity increases in immunosuppressed and stressed hosts (Chandramathi *et al.*, 2014). Unfortunately, *Blastocystis* spp. has hardly been studied in wildlife, even though it is a very common and widely distributed organism (Clark *et al.*, 2013). This could be especially relevant for declining species, as may be the case for many farmland birds, which are under continuous environmental stress, mainly due to changes in agriculture and other human activities (Donald *et al.*, 2001).

A good example would be the little bustard (*Tetrax tetrax*), which, in Spain, has suffered a population decrease of ca. 50% in 11 years (García de la Morena *et al*., 2018; Cabodevilla *et al*., 2020a) and is easily stressed (Marco *et al*., 2006; Ponjoan *et al*., 2008; Tarjuelo *et al*., 2015). In this context, the estimation of *Blastocystis* spp. parasitic load in these species could be the key. Unfortunately, the studies so far both in humans and in animals have focused only on the presence and the prevalence of *Blastocystis* spp. and its STs (Nagel *et al*., 2016; Beghini *et al*., 2017; Betts *et al*., 2018; Greige *et al*., 2018). The estimation of *Blastocystis* STs parasitic load and its use in comparative studies would provide relevant information on the *Blastocystis* spp. pathogenic capacity, bringing some light to this unclear topic.

The *Blastocystis* STs could be assessed through a metabarcoding approach (Maloney *et al*., 2020). This technique does not give a real proportion of the organisms present in the samples, but could quickly and efficiently identify all *Blastocystis* STs present in the sample and could provide a semi-quantitative representation of the importance of each taxa in the sample (Evans *et al*., 2016; Lamb *et al*., 2019; Piñol *et al*., 2019). In this context, valuable information can be obtained by combining non-invasive samples, such as faeces, and the application of high-throughput sequencing (HTS) techniques. The advent of HTS has simplified the characterization of complex faecal DNA and now allows for characterization of the different aspects of species ecology. Theoretically, the use of Eukariotic broad-spectrum barcodes could allow the estimation of a semi-quantitative value of *Blastocystis* spp. over the other organisms present in the gut (Evans *et al*., 2016; Lamb *et al*., 2019; Piñol *et al*., 2019). Thus, it might be an appropriate tool to perform comparative studies of *Blastocystis* spp. representation (a semi-quantitative parasitic load) on gut microbiota, which could easily be estimated from faecal samples. However, faecal DNA tends to be quite degraded (Deagle *et al*., 2006; Yu *et al*., 2012; Taberlet *et al*., 2012a), so the length of the barcode used is critical (Hajibabaei *et al*., 2006; Deagle *et al*., 2006; Deagle *et al*., 2007; Taberlet *et al*., 2012b). Mini-barcodes of less than 200pb should be the ideal barcodes; however, these short amplicons usually have lower taxonomic resolution capacity. Recently, Cabodevilla *et al*. (2020b) described a Eukariotic broad-spectrum mini-barcode (150bp) able to amplify Bastocystis spp. and with enough resolution to identify unambiguously the *Blastocystis* spp. amplicons at genus level. However, they did not assess the capacity of this mini-barcode to identify the STs of *Blastocystis* spp., an essential information in the study of this organism.

Here we used a two-step approach to assess the importance of *Blastocystis* spp. in declining farmland birds. First, we evaluated the "in silico" and "in vivo" capacity of MiniB18S_81 mini-barcode (Cabodevilla *et al.*, 2020b) to amplify and identify *Blastocystis* spp. and its STs, as well as its capacity to estimate a semi-quantitative parasitic load of *Blastocystis* spp., which could be used in comparative studies. Second, we applied this mini-barcode to describe and evaluate the presence and the parasitic load of *Blastocystis* spp. in four endangered steppe bird species, two declining wild farmland game bird species and farm-reared individuals of one of those game bird species. We also determined which STs appear in each of these birds and their importance. We expected to find higher abundances of *Blastocystis* spp. sequences in declining steppe birds under high stress, and differences in *Blastocystis* STs infecting wild and farm-reared birds.

2. Materials and methods

2.1. Fine-tuning the methodology: “In silico” testing

To evaluate the in silico capacity of the primers, we downloaded the SILVA SSU v132 reference (cleaned to keep only high quality sequences) and non-redundant dataset (Quast *et al.*, 2013) in ecoPCR format using the *obisilva* function of Obitools (Boyer *et al.*, 2016) software. Subsequently, the *obigrep* function of Obitools was used to select the sequences of *Blastocystis* spp. present in SILVA SSU v132 dataset. Then, we used this *Blastocystis* spp. dataset to perform an "in silico" PCR using the software ecoPCR (Ficetola *et al.*, 2010) with the primers miniB18S_81F and miniB18S_81R designed by Cabodevilla *et al.* (2020b). The taxonomic coverage of the primers on *Blastocystis* spp. sequences was estimated using the *ecotaxstat* function of Obitools software. To assess the taxonomic resolution of the primers at ST level, we performed a phylogenetic reconstruction on a dataset combining the amplicons obtained after the “in silico” PCR with a reference dataset including the reference sequences proposed by Yoshikawa *et al.* (2016). This reference dataset included three sequences per ST when possible (**Table A1**, Appendix A) from ST1 to ST17. Sequences were aligned with Mafft v.7 online version (Katoh *et al.*, 2002) using Q-INS-i algorithm and default values for the rest of the parameters for the alignment (Katoh and Toh, 2008). The phylogenetic inference was carried out using Bayesian Inference (BI) with MrBayes 3.2.2 at CIPRES Science Gateway (Miller *et al.*, 2010), running it for $20x10^6$

generations with a 25% burn-in value. The best evolutionary model was selected applying the Akaike Information Criterion (AIC) (Akaike, 1974) as implemented in jModelTest v.2.1.6 (Darriba *et al.*, 2012). The genus *Proteromonas* (GenBank accession number: U37108) was used as outgroup.

2.2. Assessing *Blastocystis* spp. and its STs in farmland birds

2.2.1. Studied species, sample collection and DNA extraction

Here we have used the same samples that were collected to study the diet of these birds (see methods and **Fig. 1** of the **paper VI** in chapter 5), and we have also included samples from 5 red-legged partridge farms. Thus, we analysed samples of flocks of six different farmland bird species: six flocks of great bustard (*Otis tarda*), 14 flocks of little bustard (*Tetrax tetrax*), 10 flocks of pin-tailed sandgrouse (*Pterocles alchata*), two flocks of black-bellied sandgrouse (*Pterocles orientalis*), seven groups of wild red-legged partridge (*Alectoris rufa*), five groups (farms) of farm-reared red-legged partridge (*Alectoris rufa*) and a group of common quail (*Coturnix coturnix*). Common quail faeces were collected during the scientific ringing process of these birds (work done by expert ringers from Aranzadi Science Society, with the corresponding administrative authorization). Farm-reared red-legged partridge samples were collected in Spain during autumn of 2016. Red-legged partridge is a game species of high socio-economic importance, being the most important small game-bird species in Spain (Andueza *et al.*, 2018). However, as is shown in paper IV in chapter 4 that the wild red-legged partridge populations in central Spain (one of the most important areas for breeding) are in sharp decline, with a loss of 50% of their population between 2010 and 2017, and among the management practices carried out for the species are included massive releases of farm-reared red-legged partridge, with several million of these birds being released annually in Spain (Cabodevilla *et al.*, 2020a; Caro *et al.*, 2014). These farm-reared birds are potentially stressed and in contact with humans, so we also included them in the study to check if they carry any zoonotic *Blastocystis* ST and if they have higher parasitic loads than wild birds. The four studied steppe bird species (great bustard, little bustard, pin-tailed sandgrouse and black-bellied sandgrouse) are classified as vulnerable in Spain (Madroño *et al.*, 2004) and some of them are suffering strong population declines. The little bustard has suffered a decline of 48% during 2005-2016 in Spain (García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020a). The black-bellied sandgrouse population of peninsular Spain has also declined by 31% between 2005 and 2019 (Mougeot *et al.*, 2021). The pin-tailed sandgrouse population is overall more stable but has been declining in the northern part of its distribution range (-37% in the Ebro valley) during the same period (Mougeot *et al.*, 2021). As mentioned in the methods section of the second article of chapter 4, when possible, fresh faeces of around 20 individuals per flock, group or farm were collected. The samples were frozen at -80 ºC for a week and then stored in a freezer at -20ºC until processing.

The DNA extraction of samples was performed using the QIAamp® DNA Stool Mini Kit of Quiagen (Ref. 51504), and the DNA concentration (µg/ml) and quality was assessed through NanoVue Plus spectrophotometer (GE Healthcare). After DNA extractions, on average we had 14 DNA samples per flock (**Table A2**, Appendix A).

2.2.2. Metabarcoding analysis

Although we extracted DNA from each individual faecal sample, we considered each flock as a unique sample for analyses, and before the preparation of the libraries, samples of the same flock were mixed. For common quail, to be consistent with the approach used in other species, we grouped samples from all individual birds ringed within a single breeding season within a single "group", although this species does not form flocks during the breeding season and the samples are not even from the same date. First, the DNA samples of each flock were mixed into 3 pools of 1–7 samples according to the DNA quantity and quality, a pool of high quantity, a pool of medium quantity and a pool of poor quantity for each flock (see; **Fig. A1**, Appendix A of the **paper VI** in chapter 5). DNA of each pool was PCR amplified from extracts using miniB18S_81 mini-barcode designed by Cabodevilla *et al.* (2020b), at the Analytical Services (SGIker) of the University of the Basque Country, UPV/EHU. Samples were purified and a second reaction was performed to index each amplified product and attach Illumina adaptors using the Illumina Nextera v2 kit. The same Illumina adaptor was added to the three pools of each bird flock. Amplification (20 µl) was carried out using 1 µl of DNA in reactions with 1.25 µl of MgCl (25 mM), 2 µl of dNTPs (20 mM), 0.25 of 10X Buffer B, 0.3 µl of each forward (20 pM) and reverse (20 pM) primers, 1µl of BSA (10mg/ml) and 0.25 µl of GoTaq® Flexi DNA Polymerase of Promega (ref. M829; 5 U/µl). The amplification was performed under the following thermocycle conditions: 96°C for 1 minute and 35 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 1 minute. Once amplified, we used an Illumina MiSeq NGS platform (sequencing of 2x150 bp paired-end reads) with the MiSeq Reagent Kit v2 to sequence the samples.

2.2.3. Bioinformatics analysis

Illumina's output was cleaned up and analysed trough Cutadapt (Martin, 2011) and Usearch (Edgar, 2010) softwares. First, we removed the primers with Cutadapt software. Subsequently, using Usearch, forward and reverse sequences were merged (76.2% of sequences merged correctly) and then they were filtered by expected errors (allowing a single expected error; 96.6% passed). Retained sequences were clustered into Operational Taxonomic Units (OTUs) with Usearch using a 97% similarity threshold and, then, an OTU table was built. We used the SILVA 18S v123 dataset (Quast *et al.*, 2013) to assign taxonomy to the obtained OTUs through the function *sintax* of Usearch software with a *cutoff* of 0.75 (with a *cutoff* of 0.8 some Stramenopiles sequences were misidentified). We did not include sequences of the 16S gene in this reference dataset because our primers do not amplify prokaryotic organisms (Cabodevilla *et al.*, 2020b). After taxonomic assignment, the OTUs identified as hosts (bird DNA) and humans were removed from the OTU table. Finally, this cleaned OTU table was normalized transforming the data to proportions. We used Total Sum Normalization method (TSS) to normalize the OTU table, dividing the reads of each OTU of each sample by the total read of that group (McKnight *et al.*, 2019). Since the proportions of *Blastocystis* spp. reads were likely to be very low, those proportions were multiplied by 100 and transformed into percentages.

Using the SILVA 18S v123 dataset, 22 OTUs were identified as *Blastocystis* spp. However, in order not to overlook any *Blastocystis* spp. sequences, all the OTUs assigned to Stramenopiles but without genus identification were blasted against NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Through this procedure, eight more OTUs were assigned to *Blastocystis* spp. To determine the ST of the OTUs assigned to this protist, we performed a phylogenetic reconstruction on a sequence matrix combining our OTUs' sequences with the reference dataset (see above for analysis settings).

Lastly, we estimated the percentage and standard error (SE) of *Blastocystis* spp. and each ST reads among total reads of each flock and among total protist's reads (reads of OTUs identified as kingdom Alveolata, Amoebozoa, Centrohelida, Discicristoidea, Discoba, Rhizaria, and Stramenopiles, as well as OTUs identified as philum Rigifilida, Apusomonadidae, Choanoflagellida, and Ichthyosporea) of each flock. We also estimated the percentage and SE of protist's reads among total reads of each flock. To better understand the health status of these species, we also estimated the percentage of *Eimeria* spp. and *Cryptosporidium* spp. among total reads, as these two organisms are described also as important protist parasites of birds (Millán, 2009) and used mini-barcodes allow to unambiguously identify them (Cabodevilla *et al.*, 2020b). The graphical representations were carried out using the package *ggplot2* of R (Wickham, 2016), on R v3.6.1 (R Core Team, 2019).

3. Results

3.1. Fine-tuning the methodology: "In silico" testing

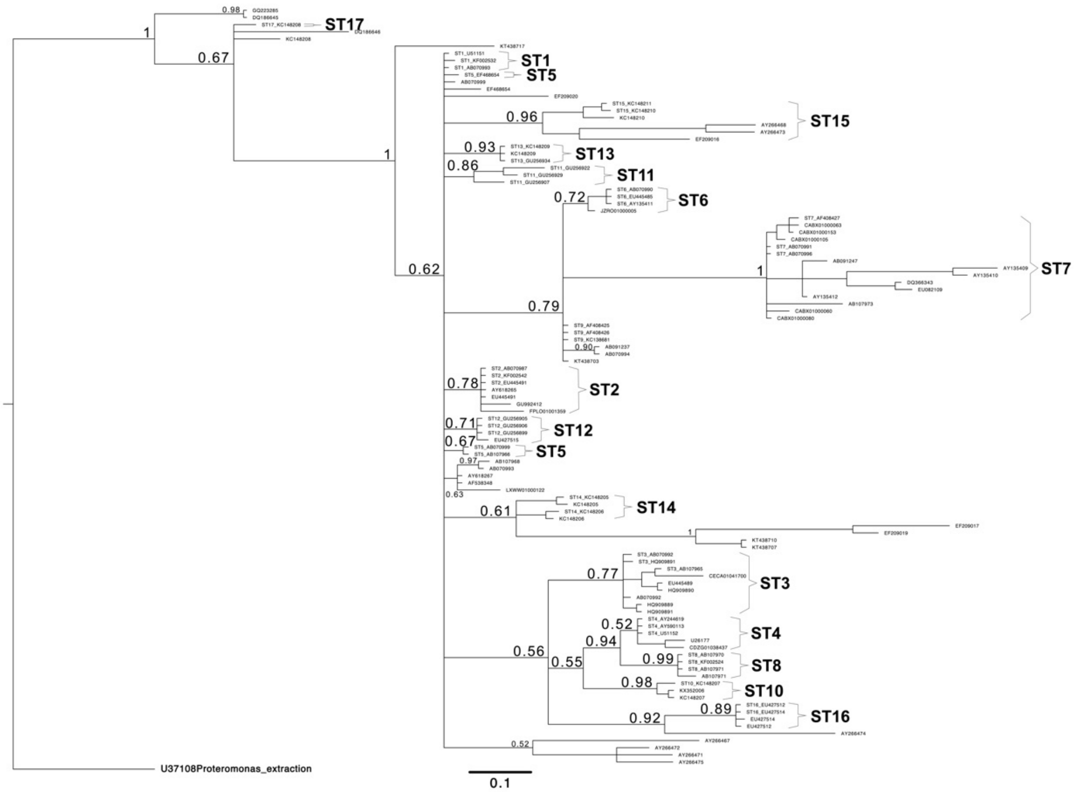

Figure 1. Phylogenetic inference of reference data set and amplicons obtained from in silico PCR.

Using the miniB18S_81 mini-barcode, we obtained a taxonomic coverage of 98.4% on SILVA dataset's *Blastocystis* spp. sequences (63 of 64 were amplified). In addition, the phylogenetic tree yielded by the analysis allowed us to identify the *Blastocystis* ST of the obtained amplicons (**Fig.1**). Although amplicon length is quite short (130 bp), it showed enough variability to discriminate among *Blastocystis* ST, except for the ST1, ST5 and ST9 that did not form monophyletic groups (**Fig.1**). Thus, our results show that this mini-barcode has indeed a high capacity and resolution to amplify and identify *Blastocysti*s spp. from faecal samples, and that phylogenetic inference is a good tool that allows in most cases to identify *Blastocystis* STs. However, in most cases, we had insufficient support to establish the phylogenetic relationships between them.

3.2. Assessing *Blastocystis* spp. and its STs in farmland birds

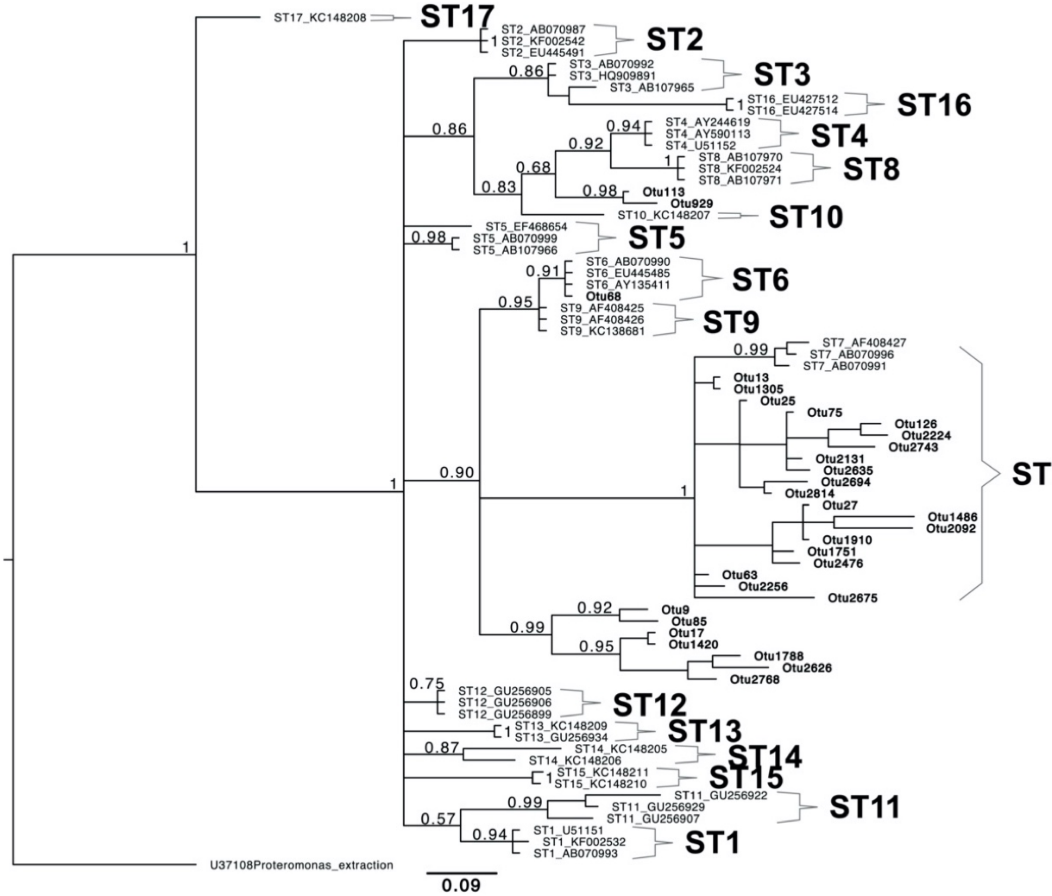

Figure 2. OTUs identification at *Blastocystis* ST level base on phylogenetic inference.

Table 1. Mean percentage of each of identified *Blastocystis* STs reads among total reads by species. Percentages above 0.5% are in bold.

Species	ST6	ST7	Related to **ST4** and **ST8**	Related to **ST6**, **ST7**, and **ST9**
GB	0.0005%	0.02%	0%	**9.43%**
LB	0.0002%	**10.00%**	0.0002%	**10.40%**
PTS	0.0002%	0.006%	0.12%	0.008%
BBS	0%	0.01%	0%	0.01%
w-RLP	0.0001%	0.006%	0.0003%	0.005%
f-RLP	**0.51%**	0.006%	0%	0.005%
CQ	0%	0.003%	0%	0.02%

Through metabarcoding analysis of farmland birds' faecal samples we obtained 2767 OTUs (excluding OTUs identified as bird´s and human's), among which 30 OTUs were identified as *Blastocystis* spp. The ST assessment by phylogenetic inference, allowed us to classify obtained *Blastocystis* spp. OTUs in STs (**Fig. 2**). 20 OTUs were identified as ST7, one OTU was identified as ST6, and nine OTUs were not unambiguously identified. Among those nine OTUs, two were strongly related to ST4 and ST8 and seven were strongly related to ST6, ST7, and ST9 (**Fig. 2**). The OTU unambiguously identified as ST6 (OTU68) was very rare in wild farmland birds (representing a less than the 0.2% of *Blastocystis* spp. reads in all species except for wild red-legged partridge, for which it represented 1.1%) but extremely common on farm-reared red-legged partridge, as it represented the 97.8% of *Blastocystis* spp. sequences on farm-reared red-legged partridge samples. Moreover, this OTU was found in 4 out of the 5 checked red-legged partridge farms. Without the OTU68, reads of *Blastocystis* spp. represented the same percentage of total reads on wild red-legged partridge as on farm-reared red-legged partridge (0.01% and 0.01% respectively; **Table 1**). On the other hand, the two OTUs related to ST4 and ST8 were only found on wild red-legged partridge, little bustard, and pin-tailed sandgrouse samples. This two OTUs were extremely rare on wild red-legged partridge and little bustard samples (around the 0.0002% of total reads in both species, which represented 2% and 0.0009% of *Blastocystis* spp. reads in each species respectively), but they were common on pin-tailed sandgrouse, representing 89% of *Blastocystis* spp. reads found in this species (**Table 1**). Overall, the most abundant *Blastocystis* spp. OTUs were found in little bustard and great bustard (**Table 1**). In the case of little bustard, OTU9 (classified close to ST6, ST7, and ST9) was the most common, representing 10% of total reads. OTU13, OTU25, and OTU27 (classified as ST7) were also very abundant, representing 6.8%, 1.1% and 1.5% of little bustard total reads respectively. In the case of great bustard, OTU17, OTU85, and OTU2768 (classified close to ST6, ST7, and ST9) were the most common, representing each of them more than 1% of total reads.

Regarding the co-occurrence of OTUs, co-infection by different STs seems to be very common, as more than 4 different OTUs were found per flock in all species (**Fig. 3a**). This seemed to be especially relevant in little bustard, as 12 OTUs were found per flock. Moreover, 16 of the 30 *Blastocystis* spp. OTUs were found to be infecting more than one species, and 3 of them were found in all species.

Our results showed that *Blastocystis* spp. was extremely common in bustards, especially in little bustard, in relation to both richness and abundance (**Fig. 3a** and **Fig. 3b**), showing much higher values than any of the other species, including farm reared birds. In fact, *Blastocystis* spp. reads represented 9.5% of total reads in great bustard and 20.4% of total reads in little bustard, while they represented less than 0.2% of total reads in the other species, with the exception of farm-reared red-legged partridge, for which *Blastocystis* spp. reads represented the 0.5% of total reads (**Fig. 3b**). In relation to the protist reads obtained, we found that, in great bustard and little bustard they were highly related to *Blastocystis* spp. reads (**Fig. 3c** and **Fig. 3d**). *Blastocystis* spp. reads represented more than 80% of protist reads in great bustard and little bustard, while they represented less than 20% of protist reads in other the species (**Fig. 3d**). Excluding *Blastocystis* spp. reads from protist reads, the percentage of protist reads among total reads was lower than 2.5%, except for farm-reared red-legged partridge, in which case they represented 6.8% of total reads. This high percentage of non-*Blastocystis* protist reads found in farm-reared red-legged partridge was mostly due to the high percentage of *Eimeria* spp. reads (6.3% of total reads) found. These organisms were rare in wild farmland birds (**Fig. A1**, Appendix A). We also found much higher abundance of *Cryptosporidium* spp. (0.2% of total reads) in farm-reared red-legged partridge than in wild farmland birds (**Fig. A1**, Appendix A).

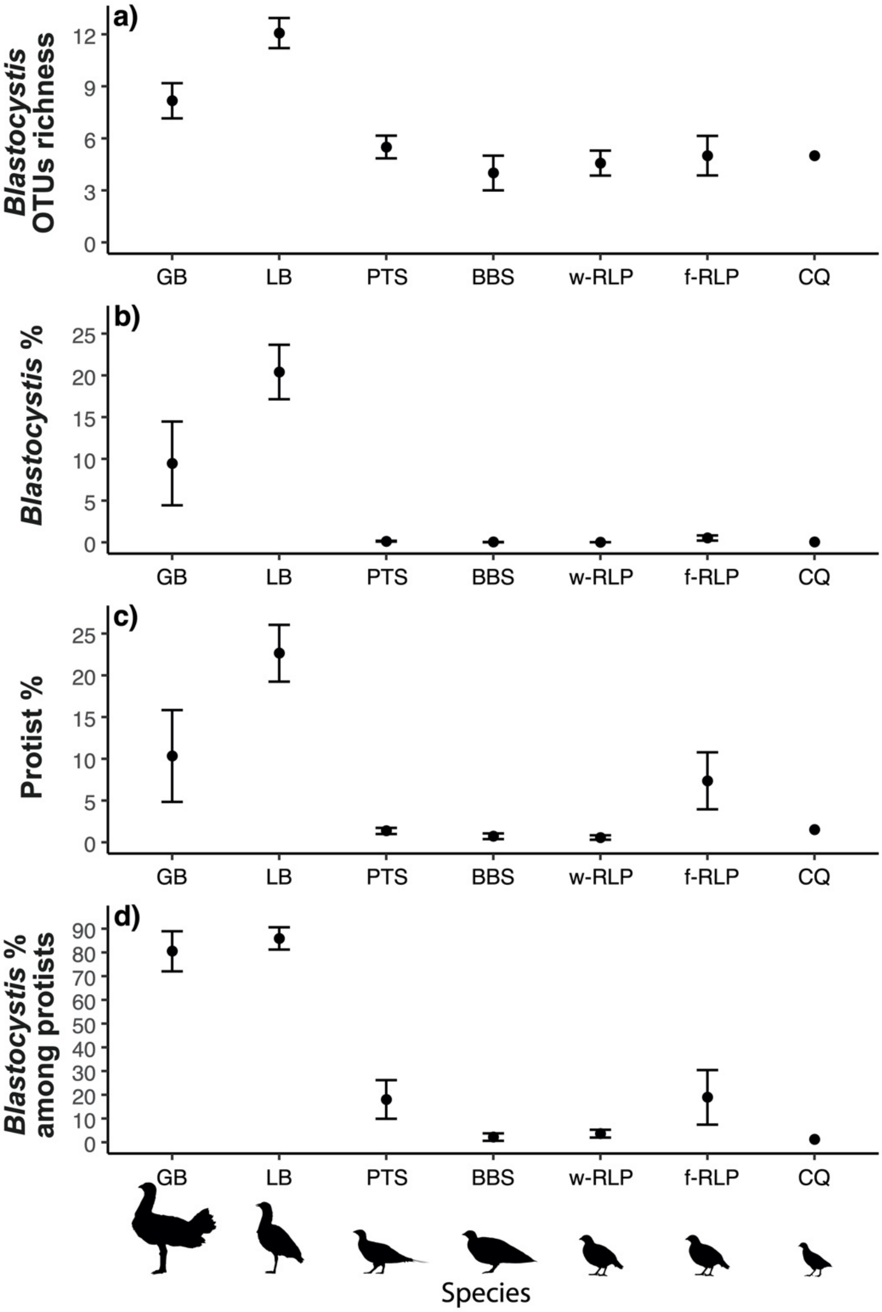

Figure 3. (a) *Blastocystis* OTUs richness (±SE) found in each of studied species. (b) Percentage of *Blastocystis* spp. sequences (±SE) over all amplified sequences and (d) over all amplified protozoan sequences found in each of studied species. (c) Percentage of protist's sequences (±SE) over all amplified sequences found in each of studied species. Note that only one group of common quail was sampled and therefore no SE are reported.

4. Discussion

So far, little attention has been paid to the parasitic load of *Blastocystis* spp. and its STs, while this could be the key to understanding when and how this organism is pathogenic. Our results showed that metabarcoding could be a useful tool to semi-quantitatively estimate *Blastocystis* spp. parasitic load, as well as to identify all STs present in faecal samples and their importance at once. The MiniB18S_81 mini-barcode described by Cabodevilla *et al.* (2020b) showed high capacity and resolution to amplify and identify *Blastocystis* spp. from faecal samples. Moreover, the use of phylogenetic inference showed to be a good tool that allows in most cases the identification of *Blastocystis* STs. Thus, this approach provides a semi-quantitative value of *Blastocystis* STs abundance, a percentage that will allow to assess the importance of *Blastocystis* STs in different organisms and habitats and to perform comparative studies, bringing a new perspective on *Blastocystis* ecology and its pathogenic capacity. However, in future studies it would be advisable to enlarge the reference database, which will probably improve the resolution of phylogenetic inference.

Using the metabarcoding analysis of farmland birds' faecal samples, we found *Blastocystis* spp. in all the studied bird species. However, the abundance and richness of *Blastocystis* OTUs in species' guts was clearly different among species. On average pin-tailed sandgrouse, black-bellied sandgrouse, wild red-legged partridge, farm-reared red-legged partridge, and common quail showed around five *Blastocystis* OTUs per flock and *Blastocystis* spp. reads represented less than 1% of total reads. Great bustard and little bustard, instead, showed much higher richness and abundance of *Blastocystis* spp. than the rest of the species. Although the role of *Blastocystis* spp. in the gut microbiota of these hosts is not known, proliferation of this parasite in humans and other species has been associated to immunocompromised or stressed individuals (Adamu *et al.*, 2013; Chandramathi *et al.*, 2014; Ahmed and Karanis, 2018). This could indicate that sampled great bustard and little bustard individuals were under severe stress or hint to pathogenic potential of *Blastocystis* spp. in these species.

In addition, phylogenetic inference allowed us to identify the ST of *Blastocystis* OTUs found: one ST6 OTU, 20 ST7 OTUs, two OTUs related to ST4 and ST8, and seven OTUs related to ST6, ST7, and ST9. Most of the 30 *Blastocystis* OTUs found on the studied species faeces were close to ST6 and ST7, as is common in birds (Stensvold *et al.*, 2009; Cian *et al.*, 2017; Greige *et al.*, 2018; Maloney *et al.*, 2020), although some of them were also related to ST4 and ST8. All *Blastocystis* STs found have also been described in humans and are therefore very likely to have zoonotic capacity (Parkar *et al.*, 2007; Cian *et al.*, 2017; Greige *et al.*, 2018), although the OTUs unequivocally identified as specific ST are the most likely to be zoonotic. ST6 is a common zoonotic ST that has been described in farm reared birds (Lewicki *et al.*, 2016; Greige *et al.*, 2018). In this study, it has been found almost exclusively in farm reared birds which have close contact with humans, so this gives rise to uncertainty about the directionality of ST6 transmission, which could reach to farm-reared red-legged partridge from its human keepers rather than the other way around. In addition, these results show that captive and wild animals of the same species may carry different *Blascocystis* STs. ST7 has been shown to have a major impact on the human gut microbiota through its diet (Yason *et al.*, 2019) and seems to have significant impact over the beneficial bacteria *Bifidobacterium* and *Lactobacillus* (Yason *et al.*, 2019). In our study this ST was highly important in little bustard (more than 10% of total reads), while in other species it represented less than 0.05% of total reads. Given that little bustard is not a bird with intensive contact with humans, the zoonotic risk seems low. However, from a conservation point of view, this high abundance of ST7 in little bustard might have relevant consequences on little bustard gut microbiota and thus, on the species' population dynamics.

Regarding the percentage of protist reads, in general it was very low, except for great bustard, little bustard, and farm-reared red-legged partridge. The percentage of protist reads in great bustard and little bustard was strongly related to the percentage of *Blastocystis* spp. but in the case of farm-reared red-legged partridge, this was fully correlated with the percentage of the coccidian *Eimeria* spp. In addition, farm-reared red-legged partridge also showed infection by *Cryptosporidium* spp. These two protists are considered important parasites of birds (Millán, 2009) and may have a relevant impact on birds’ health (Millán, 2009; Ryan, 2010). Both have been previously described as important parasites on red-legged partridge farms (Pagès-Manté *et al.*, 2007; Millán, 2009; Naciri *et al.*, 2011). As these organisms were rarely found in wild animals, as suggested by other studies (Villanúa *et al.*, 2007; Villanúa *et al.*, 2008), our results showed that farm-reared birds are infected by different parasites than wild birds and their release into the wild may be a risk to the health of wild populations.

Among all species, our results showed that *Blastocystis* spp. is extremely abundant in little bustard gut (*Blastocystis* spp. reads represent the 20% of total reads), and thus it may be of serious concern for its conservation. Little bustard is a heavily threatened species, whose populations have declined in more than 50% over the past 11 years (García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020a). The negative population dynamics of this species is strongly linked to habitat loss due to agricultural intensification (Traba and Morales, 2019), a strong change in the agroecosystem that pushes it into a highly stressful situation (Tarjuelo *et al.*, 2015). Moreover, little bustard is *per se* a species prone to show physiological stress (Marco *et al.*, 2006; Ponjoan *et al.*, 2008; Tarjuelo *et al.*, 2015), which probably makes it very susceptible to infection by *Blastocystis* spp. Future studies should assess the impact of *Blastocystis* spp. and its ST on little bustard as well as the relation between the *Blastocystis* spp. parasitic load, physiological stress and demographic consequences of little bustard populations.

In conclusion, this study shows the benefit of metabarcoding for comparative studies of *Blastocystis* spp. parasitic load. This methodology may provide relevant and interesting results that could explain the impact of *Blastocystis* spp. and its ST on the gut microbiota on wildlife and the human health. On the other hand, our results highlight the importance of further research on the incidence and impact of *Blastocystis* spp. on wildlife, as it could differ widely from captive animals. In addition, our results provide new and relevant information on the incidence and parasitic load of *Blastocystis* spp. and its STs in some threatened farmland birds, showing that this organism has a high incidence in some of the most threatened Spanish steppe birds. These findings highlight the imperative need of future research to evaluate to what extent *Blastocystis* spp. can be a conservation issue for these declining species.

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Supplementary material

Appendix A

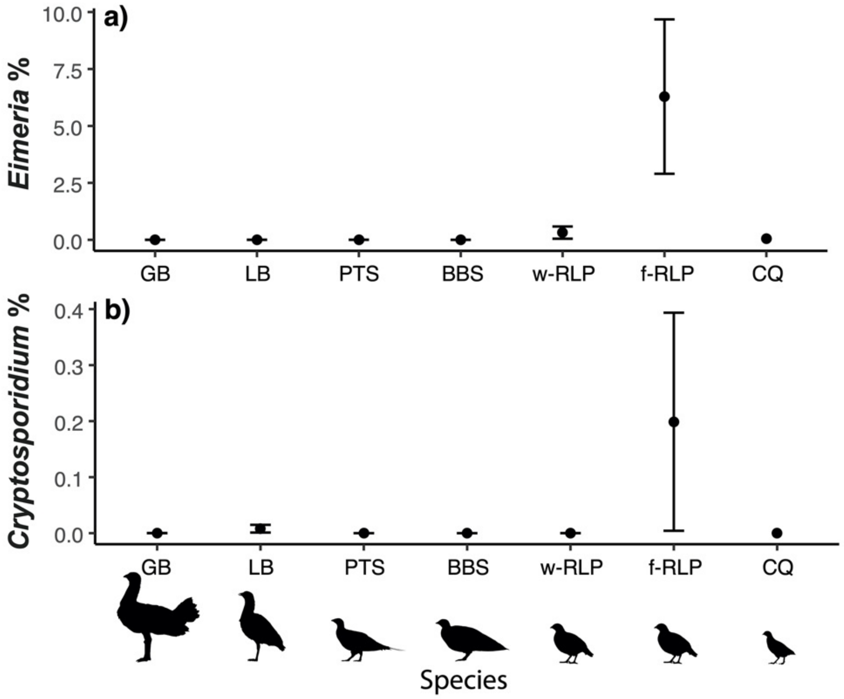

Figure A1. (a) Percentage of *Eimeria* spp. sequences (±SE) over all amplified sequences found in each of studied species. (B) Percentage of *Cryptosporidium* spp. sequences (±SE) over all amplified sequences found in each of studied species. Note that only one group of common quail was sampled and therefore no SE are reported.

Table A1. Used reference sequences of *Blastocystis* ST proposed by Yoshikawa *et al.* (2016) for Phylogenetic inferences.

ST	**Host**	**Accession no. in GenBank**
1	Human	U51151
1	Human	KF002532
1	Chicken	AB070993
2	Human	AB070987
2	Human	KF002542
2	Monkey	EU445491
3	Human	AB070992
3	Colobus	HQ909891
3	Cattle	AB107965
4	Human	AY244619
4	Rat	AY590113
4	Guinea pig	U51152
5	Human	EF468654
5	Pig	AB070999
5	Cattle	AB107966
6	Human	AB070990
6	Chicken	EU445485
6	Turkey	AY135411
7	Human	AF408427
7	Human	AB070991
7	Quail	AB070996
8	Lemur	AB107970
8	Opossum	KF002524
8	Pheasant	AB107971
9	Human	AF408425
9	Human	AF408426
9	Human	KC138681
10	Camel	KC148207
11	Elephant	GU256922
11	Elephant	GU256929
11	Elephant	GU256907
12	Giraffe	GU256905
12	Giraffe	GU256906
12	Giraffe	GU256899
13	Mouse deer	KC148209
13	Quokka	GU256934
14	Cattle	KC148205
14	Mouflon	KC148206
15	Gibbon	KC148211
15	Camel	KC148210
16	Kangaroo	EU427512
16	Kangaroo	EU427514
17	Gundi	KC148208

Table A2. DNA samples collected by species flock, with information on flock's origin.

Species	Flock	DNA	Province
Little bustard	1	10	Ciudad Real
Little bustard	2	17	Lleida
Little bustard	3	16	Lleida
Little bustard	4	16	Ciudad Real
Little bustard	5	15	Ciudad Real
Little bustard	6	19	Ciudad Real
Little bustard	7	11	Ciudad Real
Little bustard	8	20	Ciudad Real
Little bustard	9	14	Ciudad Real
Little bustard	10	13	Ciudad Real
Little bustard	11	19	Ciudad Real
Little bustard	12	18	Ciudad Real
Little bustard	13	20	Ciudad Real
Little bustard	14	18	Ciudad Real
Great bustard	1	6	Ciudad Real
Great bustard	2	7	Navarra
Great bustard	3	17	Ciudad Real
Great bustard	4	17	Ciudad Real
Great bustard	5	11	Ciudad Real
Great bustard	6	1	Ciudad Real
Pin-tailed sandgrouse	1	11	Navarra
Pin-tailed sandgrouse	2	16	Navarra
Pin-tailed sandgrouse	3	15	Ciudad Real
Pin-tailed sandgrouse	4	13	Lleida
Pin-tailed sandgrouse	5	16	Ciudad Real
Pin-tailed sandgrouse	6	17	Ciudad Real
Pin-tailed sandgrouse	7	16	Ciudad Real
Pin-tailed sandgrouse	8	14	Ciudad Real
Pin-tailed sandgrouse	9	15	Ciudad Real
Pin-tailed sandgrouse	10	15	Ciudad Real
Black-bellied sandgrouse	1	20	Navarra
Black-bellied sandgrouse	2	18	Ciudad Real
Common quail	1	16	Alava
Red-legged partridge	1	11	Ciudad Real
Red-legged partridge	2	15	Ciudad Real
Red-legged partridge	3	14	Ciudad Real
Red-legged partridge	4	11	Ciudad Real
Red-legged partridge	5	9	Ciudad Real
Red-legged partridge	6	6	Navarra
Red-legged partridge	7	9	Ciudad Real
Farm red-legged partridge	1	10	Spain
Farm red-legged partridge	2	10	Spain
Farm red-legged partridge	3	20	Spain
Farm red-legged partridge	4	19	Spain
Farm red-legged partridge	5	21	Spain

5

NEKAZAL INGURUNEKO HEGAZTIEN DIETA ETA OSASUN-EGOERA

V. ARTIKULUA

Hesteetako parasitoen eta dietaren aldibereko azterketa eDNA metabarcoding-aren bitartez

- **Laburpena**
- **Sarrera**
- **Eztabaida**

Xabier Cabodevilla, Benjamín J. Gómez-Moliner,
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Integrative Zoology (Errebizio prozesuan)

Hesteetako parasitoen eta dietaren aldibereko azterketa eDNA metabarcoding-aren bitartez

Laburpena

Nekazaritzaren hedapena eta intentsifikazioa eragin handia ari dira izaten landareen eta artropodoen aniztasunean eta ugaritasunean, eta horrek eragina du nekazal inguruneko hegaztientzako elikagaien eskuragarritasunean. Elikagaiak eskuratzeko zailtasunak, era berean, immunogutxitzea eta parasito-intzidentzia handitzea eragin ditzake. Hegaztien dieta-aldaketak eta horien parasitoak aztertzeko diseinatutako ikerlanetan, metabarcoding-a oso baliagarria izaten ari da. Teknika horrek helburu diren organismoen DNA gorotzetatik anplifikatzeko gai diren mini-barcode-ak behar ditu. Nekazaritzaren hedapenak biodibertsitatean duen eragina ulertzen laguntzeko, azterlan honen helburua zen nekazal inguruneko hegaztien gorotzetatik dieta eta hesteetako parasitoak aldi berean ebaluatu ahal izateko mini-barcode-ak diseinatu eta identifikatzea. Erreferentziazko barcode eukariotiko batekin konparatuz, ezagunak ziren bi eta guk diseinatutako hiru mini-barcode-en dieta eta parasitoak identifikatzeko gaitasuna frogatu zen "in silico". Mini-barcode berrien artean, MiniB18S_81ek erakutsi zuen taxon eukariotoen estaldura taxonomiko handiena, baita dieta-taxonen eta parasito-taxonen sekuentziak anplifikatzeko eta identifikatzeko gaitasun handiena ere. Gainera, estepako bost hegazti espezieren lagin fekaletan probatu zenean, MiniB18S_81ek dieta-taxon eta parasito-taxon garrantzitsuenak (Arthropoda, Nematoda, Platyhelminthes eta Apicomplexa) ordena-mailan identifikatzeko gaitasuna erakutsi zuen. Horrela, garatutako mini-barcode berria tresna bikaina da aldi berean hegaztien dieta eta parasitoei buruzko informazio zehatza lortzeko, zeina funtsezkoa baita hegazti hauek kontserbatzeko eta kudeatzeko.

Gako-hitzak

Biomonitorizazioa, eDNA fekala, espektro zabaleko primerrak, mini-barcode eukariotikoak, nekazal inguruneko hegaztiak, parasitologia

1. Sarrera

Klima eta lurzoruaren erabilera-ereduak etengabe aldatzen ari direnez, biodibertsitatea gero eta mehatxatuago dago (Pimm *et al.*, 1995; Jetz *et al.*, 2007). Azken 50 urteotan bereziki, nekazaritzaren hedapenak aldaketak eragin ditu lurzoruaren erabileran, eta horrek biodibertsitatea arrisku larrian jarri du (Meyer eta Turner, 1992; Matson *et al.*, 1997; Pain eta Pienkowski, 1997; Newton, 1998; Chamberlain *et al.*, 2000; Benton *et al.*, 2003). Beste ondorio batzuen artean, laborantza-lurren hedapena landare-espezieen ugaritasunean eta aberastasunean murrizketak ari da eragiten (Wilson *et al.*, 1999; Storkey *et al.*, 2012; Fonderflick *et al.*, 2020), zeinak hazi-bankuetan (Wilson *et al.*, 1999; Andreasen *et al.*, 2018) eta hazien eskuragarritasunaren urtarokotasunean eragina daukan (Newton, 2004). Gainera, nekazaritza intentsiboan ereiten diren hazi gehienak pestizidekin tratatzen direnez, horietaz elikatzen diren hegaztietan ondorio larriak dituzte (Prosser eta Hart, 2005; López-Antia *et al.*, 2015; Millot *et al.*, 2017; Eng *et al.*, 2019). Nekazaritzaren intentsifikazioa intsektuen populazioen gainbeheraren arrazoi nagusi gisa ere identifikatu izan da (Benton *et al.*, 2002), zeinak hegazti askoren dietaren osagai garrantzitsu bat diren, eta horiek gutxitzeak eragin nabarmena du dagoeneko hegazti intsektujaleen komunitateetan (Bowler *et al.*, 2019). Hegaztientzat, lurraren erabileran gertatzen diren aldaketak eta elikagaiaren eskuragarritasuna murriztea estres-faktoreak dira, eta hegaztien osasunari eta erantzun immunitarioari eragiten diete (Kitaysky *et al.*, 1999; Hoi-Leitner *et al.*, 2001; Kitaysky *et al.*, 2007; Pigeon *et al.*, 2013; Almasi *et al.*, 2015), eta parasitoen aurkako defentsak gutxitzen (Korschgen *et al.*, 1978; Wakelin, 1996; Nordling *et al.*, 1998). Elikagaien eskuragarritasunak, parasitismoak eta estresak hegazti-populazioen dentsitateetan dituzten eragin sinergikoak ezagunak dira (Chapman *et al.*, 2006). Gaixotasunek/parasitismoak eta elikadurak elkarrekin eragiten dute basa-animalien populazioen ugaritasuna modulatzeko. Parasito helmintikoek eta protozoarioek ostalariaren biziraupenean eta ugalketan eragin zuzena izan dezakete efektu patologikoen bidez, baita zeharkako eragina ere, ostalariaren osasun-egoera arriskuan jartzean (Coop eta Holmes, 1996; Murray *et al.*, 1998). Beraz, parasitismoa ere kontuan hartu beharreko faktore garrantzitsua da hegazti basati zein haztegietan hazitako hegazti espezieentzat (Lafferty, 1997; Dunn *et al.*, 2014; Stockdale *et al.*, 2015; Cabodevilla *et al.*, 2020).

Orain arte, nekazal inguruneko hegaztien dieta aztertzeko diseinatutako ikerketek arreta handiagoa jarri diete eskura dauden baliabide trofikoei (landareak eta artropodoak), horien kontsumoari baino (Salamolard eta Moreau, 1999; Holland *et al.*, 2006; Faria *et al.*, 2012; Holland *et al.*, 2012). Aldiz, irentsitako harrapakinetan oinarritutako ikerketak hegaztien gorotzetan aurkitutako harrapakinen exoeskeletoen hondarren begi bistazko identifikazioan oinarritu dira (Jiguet, 2002; Browne *et al.*, 2006; Holland *et al.*, 2006; Bravo *et al.*, 2017). Hala ere, identifikazio-metodo hori dieta aztertzeko asko erabiltzen den arren, ez da maila taxonomiko ordinaletik haratago joaten, eta maila sakonagoetan ale gutxi identifikatzen dira (Jiguet, 2002; Browne *et al.*, 2006; Bravo *et al.*, 2017). Gainera, baliteke irentsitako harrapakin batzuk ez detektatzea edo ez identifikatzea digestioa jasan ondoren, hondarrak identifikatzeko zailtasunen ondorioz (Moreby, 1988; Pompanon *et al.*, 2012). Nekazal inguruneko hegaztien parasitoak aztertu dituzten ikerketen kasua antzekoa da, parasitoen identifikazioa gehienetan bisuz egin baita (Browne *et al.*, 2006; Okulewicz eta Sitko, 2012; Rengifo-Herrera *et al.*, 2014; Presswell eta Lagrue, 2016), sarritan hildako hegaztien disekzioaren bidez (Villanúa *et al.*, 2008; Santoro *et al.*, 2010; Okulewicz eta Sitko, 2012). Hala ere, gorotzen ikerketa ez-inbaditzaileetan, parasitorik ugarienak bisuz identifika daitezke, nahiz eta intentsitate txikiko parasitismoa ia ez den bereizgarria (Rengifo-Herrera *et al.*, 2014; Presswell eta Lagrue, 2016). Gainera, morfologikoki, parasito-espezie askoren arrautzen identifikazio taxonomikoa ere mugatua edo ezinezkoa da (Browne *et al.*, 2006; Presswell eta Lagrue, 2016).

Duela hogei urte ingurumen-DNAren (aurrerantzean eDNA) analisia egiten hasi zenean, ekologiarako bide berri bat ireki zen (Taberlet *et al.*, 2012b). Metodo hori berehala zabaldu zen, belaunaldi berriko sekuentziazio-teknologiarekin batera (Shokralla *et al.*, 2012; Taberlet *et al.*, 2012a,b), eta gero eta ohikoagoa da dieta, mikrobioak eta parasitoak aztertzeko (Pompanon *et al.*, 2012; Shokralla *et al.*, 2012; Taberlet *et al.*, 2012a; Bass *et al.*, 2015; Kerley *et al.*, 2018). Lagin ez-inbaditzaileetatik abiatuta, hala nola gorozkietatik, eDNAren analisiaren bidez informazio garrantzitsua lor daiteke (Srivathsan *et al.*, 2016), hildako animaliak aztertu beharra saihestuz. Gorotzen erabilerak ostalariaren genetika, parasitoak, hesteetako mikrobiota eta dieta ikertzea ahalbidetzen du (Srivathsan *et al.*, 2016). Hala ere, gorotzek PCRaren inhibitzaile gisa jarduten duten hainbat substantzia izaten dituzte (Lantz, 1997; Wilson, 1997; Rådström *et al.*, 2004), eta, horregatik, beharrezkoa da gorotzen eDNA aztertzeko mini-barcode aproposak diseinatzea.

Barcode ezberdin asko diseinatu dira (300 pb baino luzeagoak normalean) DNAren metabarcoding-analisiak burutzeko, espezifikoak zein espektro zabalekoak (Prosser *et al.*, 2013; Hadziavdic *et al.*, 2014; Van Steenkiste *et al.*, 2015; Cheng *et al.*, 2016; Krehenwinkel *et al.*, 2018). Hala ere, ingurumeneko laginak aztertzen dituzten metabarcoding-eko ikerlanetan erabilitako barcode-en luzera kontu handiz aukeratu behar da (Hajibabaei *et al.*, 2006; Deagle *et al.*, 2006; Deagle *et al.*, 2007; Taberlet *et al.*, 2012b), normalean, uraren, lurraren, airearen edo gorotzen DNA nahiko degradatua egoten delako (Deagle *et al.*, 2006; Yu *et al.*, 2012; Taberlet *et al.*, 2012a). Eukarioten eDNAn zentratu diren ikerketek hainbat taxonentzako mini-barcode (300 pb baino laburragoak) espezifiko ezberdinak deskribatu dituzte (Epp *et al.*, 2012; Pompanon *et al.*, 2012), baina laginaren ikuspegi mugatua ematen dute. Aldiz, espektro zabaleko mini-barcode-ek helburu diren taxonei buruzko informazioa eman dezakete, baita hauek kategoria altuagoko taxon bati egiten dioten ekarpen globalari buruzkoa ere, eta horrek lagina hobeto ulertzea ahalbidetzen du. Horrela, espektro zabaleko mini-barcode egokiak erabiliz, dietari eta hesteetako parasitoei buruzko informazio garrantzitsua lor daiteke analisi berean. Hala ere, eskuragarri dauden mini-barcode eukariotoak ez dira probatu asmo horrekin. Gainera, nahiz eta metabarcoding-a ezin den tresna kuantitatibotzat hartu, zenbait ikerlanek metodo horren nolabaiteko gaitasun kuantitatiboa erakutsi dute (Evans *et al.*, 2016; Lamb *et al.*, 2019; Piñol *et al.*, 2019). Ondorioz, antzeko laginak erabiliz, lortutako taxonen proportzioak alderatzea posible izan beharko litzateke, adibidez, urtaro eta urmael ezberdinetan jasotako ur-laginak (Pompanon *et al.*, 2012). Baina, mini-barcode-ak aukeratzekoan, argi eduki behar da horien potentziala helburu diren taxonen erreferentziako sekuentzia-datu sendoen existentziaren araberakoa izango dela (Clarke *et al.*, 2014). Gainera, biodibertsitatearen estimazio zehatza egiteko, DNAren eskualde nahikoa aldakor bat anplifikatzea beharrezkoa da. DNA-geneak, non barcode gehienak diseinatzen diren, honako hauek dira: mitokondrietako cytochrome c oxidase subunit 1 (COI) eta cytochrome b (cytb), 12S rRNA, 16S rRNA eta 18S rRNA gene erribosomikoak eta nukleoko ITS1, ITS2 eta rbcL (Hajibabaei *et al.*, 2007; Pompanon *et al.*, 2012; Andújar *et al.*, 2018; Djurhuus *et al.*, 2020). Horien artean, 18S rRNA geneak eskualde bereziki aldakor bat hartzen du; gainera, badago erreferentziazko datu-base sendo bat gene horrentzat, zeina etengabe hedatzen ari den (Hadziavdic *et al.*, 2014).

Ikerketa honen helburu nagusia gorotzen eDNAtik hegaztien dieta eta parasitoei buruzko informazioa lortu dezakeen mini-barcode egokia identifikatzea zen.

Lehenik, 28S rRNA geneko mini-barcode ezagunak eta guk diseinatutako 18S rRNA geneko mini-barcode-ak ebaluatu genituen, hegaztien gorotz-laginetan erabiltzeko egokitasunari dagokionez. Mini-barcode egokiena hautatu ostean, estepako hegazti-espezie desberdinen gorotz-laginetan enpirikoki probatu genuen hori.

4. Eztabaida

Gorotz-laginak oso baliagarriak dira modu ez-inbaditzailean espezieen elikaduraren ekologiari eta espezieen parasito-infestazioei buruzko informazioa biltzeko, populazioen kudeaketa eta kontserbazioa bultzatuz. Ikerketa honen helburua ARNr 18S eukariotikoa anplifikatzeko gai den mini-barcode onena identifikatzea zen, hegaztien gorotzen metagenomikan oinarrituriko hegaztien dieta eta parasitoen ikerketetan erabiltzeko, batez ere mehatxatutako nekazal inguruneko hegaztietan. Ikerlan honetan garatutako mini-barcode-en artean, MiniB18S_81ek (18S rRNA), bereziki, ia taxon eukarioto guztientzat estaldura taxonomiko handia erakutsi zuen, bai "in silico" bai metabarcoding-probetan. Gainera, bereizmen taxonomiko ona azaldu zuen hegaztien dietaren osagai eta hegaztien endoparasito diren taxon garrantzitsuenentzat (Jiguet, 2002; Villanúa *et al.*, 2008; Santoro *et al.*, 2010; Bravo *et al.*, 2017). Horrela, MiniB18S_81 mini-barcode-ak oso informazio baliotsua eman dezake nekazal inguruneko hegaztiak kontserbatzeko.

Probatutako mini-barcode guztiek anplikoi-luzera laburrak eta homogeneoak erakutsi zituzten, eta, oro har, 300 bp baino sekuentzia laburragoak lortu ziren. MiniB18S_81en kasuan, 300 bp-tik gorako anplikoiak, nagusiki, Cestoda talde taxonomikoari zegozkion, ziur aski, talde honetan 18S geneko V7-V8 eskualdean 100 bp-tik gorako txertatze bat dagoelako. Talde horretako espezieak parasitoak diren arren, gorotz-laginetan aurkitutako haien DNA ez da beste DNA fekala bezain degradatua egoten, zestodoen DNA zuzenean organismo horien ehunetatik (helduak, larbak edo arrautzak) ateratzen baita. Beraz, zestodoen anplikoi luzeek ez lukete arazo bat izan behar lagin fekalak erabiliz parasitologia aztertzerakoan.

18S genearen V7-V8 eskualdearen aldakortasuna ez zen guztiz ustekabekoa, eskualde hori V3 eskualdea baino askoz aldakorragoa baita (Hadziavdic *et al.*, 2014). Izan ere, badirudi hori dela MiniB18S_81 mini-barcode-ak MiniB18S_41 eta MiniB18S_43 mini-barcode-ek baino estaldura eta bereizmen taxonomiko ahalmen handiagoa izatearen arrazoia. MiniB18S_81en ezaugarriak dietei buruzko ikerlanetan gehien erabiltzen diren bi mini-barcode eukariotoekin konparatu genituenean, horrek helburu ziren bost filumetatik laurentzat Short28SF/Short28SR 28S mini-barcode-a (Vestheim eta Jarman, 2008) baino estaldura taxonomiko handiagoa aurkeztu zuen.

Oro har, MiniB18S_81 eta Short28SF/Short28SR mini-barcode-ek estaldura eta bereizmen taxonomiko zabala aurkeztu zuten aztertutako filum guztietan. Hala ere, LSUrako (Short28SF/Short28SR mini-barcode-arekin anplifikatutako DNA eskualdea) eskuragarri dauden erreferentziazko datu-baseak ez dira SSUrako (MiniB18S_81 mini-barcode-arekin anplifikatutako DNA eskualdea) eskuragarri dauden erreferentziazko datu-baseak bezain handiak eta araztuak, eta erreferentziazko datu-base sendorik gabe, informazioa galtzen da (Clarke *et al.*, 2014). Halaber, MiniB18S_81 mini-barcode-ak nucLSUDf1/nucLSUDr1 (Sonnenberg *et al.*, 2007) 28S mini-barcode-ak baino estaldura taxonomiko askoz zabalagoa erakutsi zuen. Izan ere, azkenengo hori tresnarik ez-eraginkorrena izan zen, probatutako mini-barcode-en artean, hegaztien lagin fekaletatik abiatuta dieta eta parasitoak aztertzeko.

Estepako hainbat hegazti-espezietako gorotzetan egindako metabarcoding-proban, MiniB18S_81ek frogatu zuen bereizmen taxonomiko handia zuela helburu ziren filumetan, ordena-mailan. Bereziki, Apicomplexa, Arthropoda, Nematoda eta Platyhelminthesen kasuan, non lortutako OTUen % 80 baino gehiago zalantzarik gabe identifikatu ziren ordena-mailan. Phragmoplastophytari dagokionez, klase-mailako bereizmen taxonomikoak landareek hegaztien dietan duten garrantziaren irudi orokorra eman zuen. Beraz, MiniB18S_81 mini-barcode-ak informazioa ematen du artropodoek (ordena-mailan), beste ornogabe batzuek eta landareek hegaztien dietan egiten duten ekarpenari buruz, bai eta hegaztien parasitoei buruzko informazioa ere, ordena-mailan behintzat. Metabarcoding-proban espezie-mailan ikusitako ebazpen taxonomiko txikia lortutako OTU asko erreferentziazko datu-basean sartuta ez egoteari egotz dakioke, eta, beraz, horrek ez luke mini-barcode-ak maila horretan duen benetako bereizmen-gaitasuna ebaluatzeko aukerarik emango. Taxon bakoitzerako lortutako irakurketen ehunekoa ezin da hartu taxon horiek hesteetako mikrobiotari egiten dioten benetako ekarpen kuantitatibotzat (Evans *et al.*, 2016; Lamb *et al.*, 2019; Piñol *et al.*, 2019), baina ehuneko hori erabilgarria izan liteke ikerlan konparatiboak burutzeko.

MiniB18S_81ek *Cryptosporidium* spp. eta *Blastocystis* spp. generoak bezalako patogeno batzuk zalantzarik gabe identifikatzeko gaitasunak iradokitzen du karga parasitarioak zenbatesteko erabil daitezkeela. Hain zuzen ere, giza osasunean dituen eraginak direla eta (Tan, 2008; Scanlan, 2012) eta gaitasun zoonotikoa izateagatik (Greige *et al*., 2018), *Blastocystis* spp. interes handiko organismoa da. Parasito hori animalia basatietan lehen uste baino askoz arruntagoa dela frogatu da, aurreko diagnostiko-tekniken mugak eta fauna basatiaren jarraipen eza direla eta (Parkar *et al*., 2007). Denbora luzez garrantzi klinikorik ez zuela pentsatu zen, baina gero eta adostasun handiagoa dago kontrakoaren alde, hau da, parasito horrek ostalariaren osasunean eragin handia izan dezakeela eta, beraz, biztanleriaren biziraupenean eta ugalketan eragin dezakeela (Thompson *et al*., 2010). *Blastocystis* spp.-ri buruzko ikerketak sarritan OTUen presentzian/absentzian oinarritu dira, eta mini-barcode berri honek organismo honek ostalariaren hesteetako mikrobiotan dituen proportzioei buruzko informazioa eman lezake, azterketa konparatiboak egiteko bereziki erabilgarria dena. Horrek lagunduko du zehazten hesteetako parasito horiek denboran zehar biztanlerian duten prebalentzia, bai eta *Blastocystis* spp.-k faunaren osasunean izan dezakeen eragina ebaluatzen ere.

Bai "in silico", bai metabarcoding-probek erakutsi zuten MiniB18S_81 mini-barcode-ak Ascomycota eta Basidiomycota filoentzako estaldura taxonomiko ona duela. Hori oso garrantzitsua da eDNArekin lan egiten denean, onddoek eragindako kutsadura-arazo bat izan baitaitezke (Bohmann *et al*., 2014). Hemen proposatutako espektro zabaleko mini-barcode-a erabiliz, onddoek eragindako kutsadura hauteman daiteke, eta kutsatutako laginak baztertu.

Laburbilduz, MiniB18S_81 mini-barcode-a dietaren eta hesteetako parasitoen aldi bereko biomonitorizazioa egiteko tresna berri eta esperantzagarria da, lagin fekalen eDNAren metabarcoding-aren bidez. Hegaztien dietan agertzen diren artropodo ordenei eta landareei buruzko informazio erabilgarria ematen du, baita Nematoda, Platyhelminthes eta Apicomplexa parasitoei buruzkoa ere. Animalien dentsitatea baldintzatzen duten faktore ekologikoak ulertzea funtsezkoa da espezieen kontserbazioaren alde lan egin ahal izateko, bereziki gaur egungo giza interferentziaren tasa kontuan hartuta. Testuinguru horretan, MiniB18S_81 mini-barcode-ak nekazal inguruneko hegaztien ekologiari buruzko informazio garrantzitsua eman dezake, eta haien populazioen gainbeheraren arrazoiak argitzen lagundu.

Esker onak

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VI. ARTIKULUA

Metabarcoding-ak gainbeheran dauden nekazal inguruneko sei hegaztiren dieta eta dibertsitate trofikoa ulertzea ahalbidetzen du

- **Laburpena**
- **Sarrera**
- **Eztabaida**

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Scientific Reports (Errebizio prozesuan)

Metabarcoding-ak gainbeheran dauden nekazal inguruneko sei hegaztiren dieta eta dibertsitate trofikoa ulertzea ahalbidetzen du

Laburpena

Gainbeheran dauden espezieen elikaduraren ekologia ezagutzea haien kontserbazio-premiei aurre egiteko funtsezkoa da, batez ere haien habitatetan baliabide trofikoen murrizketa handiak gertatzen direnean.

Ikerlan honetan, metabarcoding-a erabili dugu Europako estepetako zereal-laboreen lurretan bizi diren nekazal inguruneko sei hegazti esanguratsuenen dietaren konposizioa deskribatzeko: basoilo txikia, basoilo handia, ganga azpizuria, ganga azpibeltza, eper gorria eta galeperra. Gainera, espezie horien dietaren urtaroko aldakuntzak (udazkenetik udaberrira) aztertu ditugu, galeperrarena izan ezik, haren dieta udaberrian eta udan aztertu baitzen.

Hegazti horien dieta, nagusiki, landareez osatuta dagoela frogatu da, basoilo txikiaren kasuan (% 34) eta basoilo handiaren kasuan (% 12), artropodoak ere oso garrantzitsuak diren arren. Artropodoen artean, tripsak, armiarmak eta kolenboloak (orain arte horien dietan deskribatu ez direnak) eta parasitoen aurkako elikagai gisa erabil daitezkeen taxon batzuk daude. Gainera, aurkitu dugu basoilo txikiaren dieta zela pobreena aztertutako espezie guztien dieten artean eta, bestalde, espezie horien guztien neguko dieta udazkenekoa edo udaberrikoa baino pobreagoa zela. Gainbeheran dauden espezie hauen dietaren konposizioak landaredi natural eta erdi-naturalaren eta mosaiko-egitura duen nekazal ingurunearen garrantzia erakusten du, zeina, urte osoan zehar, artropodo, landare eta hazi ugari emateko gai izan behar den.

Gako-hitzak

Basoilo txikia; biomonitorizazioa; eper gorria; estepetako hegaztiak; ganga azpizuria; gorotzen ingurumen DNA

1. Sarrera

Azken hamarkadetan, mundu osoan zehar izugarri aldatu dira nekazaritza-paisaiak (Tscharntke *et al.*, 2005). Hirurogeiko hamarkadaz geroztik, Europako Nekazaritza Politika Bateratuaren (NPB) dirulaguntzek babestutako nekazaritza-lurren intentsifikazioak aldaketa handiak ekarri zituen Europako nekazaritza-paisaietan, hala nola lursailak handitzea, mekanizazioa eta ongarri sintetikoen eta produktu fitosanitarioen erabilera. 1990eko hamarkadatik aurrera, oinarrizko produktuen merkatuen globalizazioaren eta NPBren erreformen ondorioz, nekazaritzaren intentsifikazioa are gehiago handitu zen, eta horrek nekazaritza-eremu pobreenetan lurrak lantzeari uztea eragin zuen (Van Zanten *et al.*, 2014).

Intentsifikazio-prozesu horiek nekazaritza-paisaiak homogeneizatzea ekarri zuten, elementu natural edo erdi-natural gutxiago dituzten partzela handiagoak sortuz (Jongman, 2002). Zoritxarrez, nekazaritzaren intentsifikazioak ondorio larriak izan ditu biodibertsitatean ere (Stoate *et al.*, 2001; Donald *et al.*, 2006; Storkey *et al.*, 2012), eta Europako biodibertsitatearen egungo galeraren eragile nagusitzat jotzen da (Stoate *et al.*, 2001). Nekazal inguruneko hegaztiak horren adibide bikaina dira; izan ere, Europa osoan asko murriztu dira haien populazioak azken hamarkadetan, nekazaritzaren intentsifikazioaren hedapenarekin batera (Benton *et al.*, 2003), nahiz eta Europar Batasunak, nekazaritzaren intentsifikazioaren inpaktuak murrizten saiatzeko, NPBri hainbat erreforma egin (Traba eta Morales, 2019). Nekazaritza-paisaia gero eta intentsiboago baten barruan, landaredi naturala eta lugorriak oso garrantzitsuak dira nekazal inguruneko hegaztientzat, baliabide ugari ematen baitituzte, hala nola elikagaiak, habia egiteko lekuak eta txitak babesteko estaldura (McMahon *et al.*, 2010; Traba eta Morales, 2019; Tarjuelo *et al.*, 2020a). Iberiar penintsulan, non lurra nahiko pobrea den eta duela gutxi arte ongarri artifizialak ez ziren asko erabiltzen, lugorria oso ohiko nekazaritza-praktika zen, lurraren laboreen errotazio-sistema tradizionalaren barne (Tarjuelo *et al.*, 2020b). Hala, Europako lugorri-azaleraren % 50 baino gehiago Espainian zegoen (Tarjuelo *et al.*, 2020b), eskualde horretan mendeetan zehar egindako nekazaritza estentsiboaren ondorioz (Donázar *et al.*, 1997). Iberiar penintsula Europa mailan estepetako hegaztien kontserbaziorako gune garrantzitsuenetako bat izatearen arrazoi nagusienak eskualdearen biogeografia, ingurumena eta nekazaritza-eredu tradizionalak izan dira. (Santos eta Suárez, 2005; McMahon *et al.*, 2010; Traba eta Morales, 2019). Hala ere, nekazaritzaren intentsifikazioaren ondorioz, Espainian lugorriak 1,1 milioi hektarea egin du behera azken 15 urteetan (Traba eta Morales, 2019; Tarjuelo *et al.*, 2020a), eta paisaia homogeneoagoak sortu dira, zeinek ondorio negatibo handiak izan dituzten estepetako hegazti eta nekazal inguruneko bestelako biodibertsitatearentzat. Horrek, ziurrenik, espezie horientzat garrantzitsuak diren baliabide trofikoetan ere inpaktu izugarriak izan ditu, hala nola landare- eta intsektu-espezieen ugaritasunean eta aberastasunean (Wilson *et al.*, 1999; Benton *et al.*, 2002; Storkey *et al.*, 2012; Raven eta Wagner, 2021), hazi-bankuen hazien ugaritasunean (Wilson *et al.*, 1999; Andreasen *et al.*, 2018) eta hazien eskuragarritasunaren urtarokotasunean (Newton, 2004).

Kontuan hartuta Europako estepako hegazti-espezieen % 80k baino gehiagok kontserbazio-egoera txarra dutela (Burfield, 2005) eta nekazaritzaren intentsifikazioak espezie horien ekosistemen baliabide trofikoetan eta haien erabilgarritasunean eragin zuzena duela (Wilson *et al.*, 1999; Benton *et al.*, 2002; Benton *et al.*, 2003; Storkey *et al.*, 2012; Andreasen *et al.*, 2018), espezie horien ekologia trofikoari buruzko ezagutzak garrantzi handia du. Ekologia trofikoaren ikerketa funtsezkoa da espezieen eskakizun biologikoak eta ekologikoak ulertzeko, eta, beraz, haien populazioak eta habitatak behar bezala kudeatzeko eta kontserbatzeko beharrezkoa. Zoritxarrez, gainbeheran dauden estepako hegazti askoren ekologia trofikoari buruzko informazioa oso mugatua da (Del Hoyo et al., 1992; Madroño *et al.*, 2004). Horri buruz egindako ikerketa urrietan, espezie horietako batzuen dieta ikertzeko, papoen, urdailen edo gorotzen edukien ikusizko azterketetan (mikro edo makroskopikoak) oinarritu dira (Suárez *et al.*, 1999; Jiguet, 2002; Bravo *et al.*, 2012). Teknika horiek alborapen handia izan dezakete; izan ere, digestioak eragin handia du detekzio-probabilitatean, eta, taxon batzuen kasuan, detektatzeko ezintasuna ere eragin dezake (Pompanon *et al.*, 2012). Dieta ikertzeko teknika tradizionalak ez bezala, metodo genetiko modernoak gai dira alborapen horiek gainditzeko (Pompanon *et al.*, 2012; Shokralla *et al.*, 2012). Metodo horiek, gainera, alborapen garrantzitsuak dauzkate anplifikazio-prozesuari dagokionez ere (Pompanon *et al.*, 2012; Shokralla *et al.*, 2012); hala ere, gero eta ezagunagoak dira dieta ikertzeko, espezieen ekologia trofikoaren deskribapen oso zehatza ahalbidetzen baitute (Pompanon *et al.*, 2012).

Ikerketa honek, espezieen gorotz-laginen DNAren metabarcoding-aren bidez, Europako estepetako hegaztien eta hegazti zinegetikoen espezie esanguratsuenetako batzuen dieta deskribatzea du helburu. Nekazal inguruneko hegazti horiek guztiak gero eta nekazaritza-paisaia intentsifikatuagoetan bizi dira, eta, gaur egun, gainbeheran daude (Traba eta Morales, 2019; Mougeot *et al.*, 2021). Horregatik, haien dietari buruzko ezagutza hobetzea premiazkoa da, haien eskakizun trofikoak eta kontserbazio-beharrak behar bezala ulertzeko. Estepetako lau hegaztiren dietak –basoilo txikia (*Tetrax tetrax*), basoilo handia (*Otis tarda*), ganga azpizuria (*Pterocles alchata*) eta ganga azpibeltza (*Pterocles orientalis*)– eta nekazal inguruneko bi hegazti zinegetikoren dietak –eper gorria (*Alectoris rufa*) eta galeperra (*Coturnix coturnix*)– ebaluatu ditugu. Espezie horien dieta oraindik ez da ondo ezagutzen. Ikerketa honetan, landareek eta ornogabeek (Annelida, Arthropoda eta Mollusca) espezie hauen dietan duten garrantzi erlatiboan zentratu gara, dietaren osaera eta espezie bakoitzaren dietaren aberastasuna deskribatuz. Udazkenetik udaberrira lagindu ahal izan ziren lau espezieen kasuan, dietan izandako urtaroko aldakuntzak ere deskribatu genituen. Hortaz, hemen espezie hauen elikadura-premiei buruzko informazio eguneratua ematen da, azkar aldatzen den ingurune batean.

4. Eztabaida

Banakoen biziraupenean eta ugalketan funtsezko eginkizuna dute elikagaia bilatzeko eta hautatzeko estrategiek (Martin, 1987), eta, hortaz, funtsezkoa da galtzeko arriskuan dauden espezieetan hori ikertzea, hala nola estepetako hegaztietan edo gainbeheran dauden eta garrantzi sozioekonomiko handia duten hegazti zinegetikoetan. Lan honetan, lehen aldiz, metabarcoding-a erabili da espezie horien dieta aztertzeko, eta espezie horien dietaren osaerari buruzko informazio berri eta garrantzitsua eman du, ganga azpizuriaren, ganga azpibeltzaren, eper gorriaren eta galeperraren artropodoen dietaren lehen deskribapen zehatza barne. Gure emaitzek argi eta garbi erakutsi zuten aztertutako espezieen dieten artean desberdintasun handiak zeudela, filogenetikoki lotura estuena zuten espezieen artean antzekotasun gehiago egonda. Basoilo txikiaren dieta basoilo handiaren dietarekiko antzekoagoa zen (biak Otidae familiakoak dira), eta artropodo proportzio handiak aurkezten zituzten (Coleoptera, Diptera, eta Thysanoptera, eta basoilo handiaren kasuan, Hymenoptera eta Orthoptera ere bai). Ganga azpizuriaren dieta ganga azpibeltzaren dietaren antzekoagoa izan zen (biak Pteroclidae familiakoak dira); nagusiki landarez osatua zegoen, eta artropodo proportzioa txikia zen bi espezieetan (Thysanoptera, Ellipura, Gotera, Coleoptera eta Arachnida). Eper gorriaren dieta ere landarez eta artropodo-proportzio txiki batez osatua zegoen (Arachnida, Thysanoptera eta

Hymenoptera), emaitzek, artropodoen OTU-aberastasun handia erakutsi zuten arren. Galeperrari dagokionez, ugalketa-garaian, artropodoak bere dietaren zati garrantzitsu bat izan ziren, bereziki araknidoak eta kolenboloak (Ellipura). Gure emaitzek espezieen arteko dietaren aberastasunaren desberdintasunei buruzko datu fidagarriak ere eman zituzten, zeina oso konstantea izan zen espezie bakoitzeko saldoen artean. Hortaz, horrek iradokitzen du dietaren aberastasuna espezie bakoitzaren ezaugarri bat izan daitekeela. Ugalketa-garaitik kanpo, basoilo txikia izan zen dietaren aberastasun baxuena aurkeztu zuen espeziea, ganga azpizuriak aurkeztutako landare-OTUen erdia erakutsiz, nahiz eta normalean bi espezieek saldo mistoak osatzen dituzten ugalketa-garaitik kanpo (Martín *et al.*, 2010). Emaitza horrek adierazten du, ziurrenik, espezie bakoitzak espezifikotasun handia duela dietari dagokionez, eta, beraz, plastikotasun trofiko erlatiboki baxua. Bestalde, eper gorriak erakutsi zuen, alde handiarekin, dieta aberatsena, baita datuak urtaroka banatu zirenean ere. Horrek adierazten du, ziur aski, eper gorriak malgutasun handiagoa duela elikagaien hautaketan; izan ere, espezie horrek beste espezieek baino askoz habitat gehiago ustiatu ditzake, hala nola mahastiak, sastrakadiak eta olibondoak (Del Hoyo *et al.*, 1992).

Espezieen dieta osatzen duten artropodoei dagokienez, aurrez deskribatu ez ziren (edo noizean behin bakarrik deskribatu ziren) taxon batzuk aurkitu genituen espezie hauen dietetan (Jiguet, 2002; Bravo *et al.*, 2012; Bravo *et al.*, 2017), hala nola tripsak (Thysanoptera), araknidoak (Arachnida) edo kolenboloak (Ellipura); gure emaitzen arabera, garrantzitsuak dira hegazti hauen dietetan. Organismo horiek erabat digerituta egon ohi dira gorotzetan, eta, beraz, gutxietsi egiten dira gorotz-laginak identifikazio bisualaren bidez aztertzen direnean (Pompanon *et al.*, 2012). Basoilo txikiaren dieta osatzen duten artropodoen artean, trips-proportzio handiak aurkitu genituen, bai eta basoilo handiaren, ganga azpizuriaren, ganga azpibeltzaren eta eper gorriaren dietan ere. Tripsak ikerketa honetan aztertutako lagin guztietan agertu ziren, eta ugariak izan ziren, bereziki, neguan eta udaberri hasieran. Thysanoptera landareetan bizi-zikloa garatzen duten intsektu oso txikien ordena bat da, horietako asko fitofagoak direlarik (Morse eta Hoddle, 2006; Goldarazena, 2015). Tamaina txikia (0,3-14 mm) eta banaketa kosmopolita (Goldarazena, 2015) kontuan hartuta, hegaztiek zeharka kontsumi ditzakete, tripsek kolonizatutako landareak ustekabean irentsiz. Basoiloen kasuan, hau da, tripsak gehien jaten dituzten espezieen kasuan, espezie hauek neguan lekadun asko jatearen ondorioa izango litzateke (Bravo *et al.*, 2012; Bravo *et al.*, 2017). Hala ere, tripsez kolonizatutako landareen hautaketa ere nahita egin daiteke, tripsik gabeko landareek baino elikagai gehiago dituztelako. Gangen dietaren kasuan, tripsak euren artropodo-dietaren proportzio txikiagoa izan ziren, beharbada, gangak, nagusiki, haziez elikatzen direlako, eta ez hostoez (Del Hoyo *et al.*, 1992; Suárez *et al.*, 1999). Horrek, halaber, azal lezake espezie guztietan tripsak udazkeneko dietan hain ohikoak ez izatearen arrazoia, labore-lur gehienak biluzik edo erein berriak baitaude. Etorkizunean, espezifikoki ebaluatu beharko litzateke tripsek kolonizatutako landareak modu aktiboan hautatzen ote dituzten hegazti-espezie hauek. Horrela izanda, baliteke estepetako hegaztiek, izurrien kontrolari dagokionez, zerbitzu ekosistemiko garrantzitsua ematea (Ndang'ang'a *et al.*, 2013); izan ere, trips-espezie askok ezaugarri inbaditzaileak dituzte eta laboreetarako kaltegarriak diren birus desberdinen bektoreak izan daitezke (Morse eta Hoddle, 2006; Goldarazena, 2015).

Gure ikerketak, halaber, agerian utzi zuen araknidoek garrantzi handia zutela galeperraren dietan, orain arte ezezaguna baitzen; gure emaitzek argi eta garbi erakutsi zuten espezie horrek ornogabe horietako asko irensten zituela. Bestalde, deskribatu da armiarmak hegaztien harrapakin garrantzitsuak direla baso-habitatetan, baina ez ekosistema irekietan (Gunnarsson, 2007). Galeperrak armiarmez elikatzearen arrazoia izan daiteke horiek elikadura-balio handia izatea eta toxikoak edo uxagarriak izan daitezkeen substantzia kimiko defentsiborik ez edukitzea (inurriek ez bezala) (Gunnarsson, 2007).

Testuinguru horretan, espezie batzuen dietan, proportzio txikian, inurriak, *Scolopendra* spp. eta Meloidae (espezie toxikoak barneren dituena) taxonak agertu ziren, besteak beste, zeinak automedikaziorako kontsumitu ahal dituzten eta elikagai antiparasitario gisa erabil daitezkeen (Lee *et al.*, 2015; Lima *et al.*, 2016; Whitman *et al.*, 2019). Beste ikerketa batzuek erakutsi dute basoilo handiek Meloidae familiako intsektu-espezie batzuk eta landare batzuk parasitoen aurkako agente gisa erabiltzen dituztela (Bravo *et al.*, 2014; Bolívar *et al.*, 2020). Hala ere, Meloidae familia basoilo txikien dietan agertzen den lehen aldia da, eta *Scolopendra* spp. basoilo txikien eta basoilo handien dietan aurkitzen den lehen aldia ere bai. Inurrien kasuan, batez ere udazkenean kontsumitu ziren. Urtaro horretan hegodun inurriak agertzen direnez, inurrien eskuragarritasuna bereziki handia izan daiteke, eta hegaztiek modu oportunistan har ditzakete. Hala ere, inurriak parasitoen aurkako erabilerarako ere kontsumi daitezke (Lima *et al.*, 2016).

Gure emaitzek erakutsi zuten, halaber, urtaroetan zehar, dietaren osaera aldatu egiten zela, eta artropodoen taxonen proportzioan aldaketa handiak gertatu zirela; hala, neguko dietak udazken eta udaberriko dieta baino dibertsitate baxuagoa erakutsi zuen. Emaitza horiek, ziurrenik, laginketa-tokietan artropodoen eskuragarritasunean dauden aldeak islatzen dituzte (Boyer *et al.*, 2003). Hala ere, basoilo txikiaren dietan izan ezik, urtaroen artean ez zen hainbeste aldatu dietan aurkitutako landareen eta ornogabeen arteko proportzioa, eta landare eta artropodoen aberastasuna nahiko konstantea izan zen ugalketa-garaitik kanpo. Basoilo handiarentzat lortutako emaitzak (artropodoen proportzioaren murrizketa handia neguko dietan) bat datoz aurreko ikerlanekin (Palacios *et al.*, 1975), eta horien neguko dieta ia erabat landare-gaietan oinarritzen zela erakusten zuten. Bestalde, urtaroen artean basoilo txikiaren dietan artropodo-taxonen aberastasunean gertatzen diren aldaketa txikiak isla dezake hegazti honek zenbait harrapakinekiko duen afinitate handia eta plastikotasun trofiko txikia. Eper gorriak, aldiz, landare-taxonen eta artropodo-taxonen aberastasun handia erakutsi zuen, bere dietan artropodoen irakurketen proportzioa txikia izan zen arren. Horrek frogatzen du espezie honen espezifikotasun trofiko baxua eta plastikotasun trofiko handia, gutxienez aztertutako gainerako espezieekin alderatuta. Ziurrenik, kontsumitutako landare-taxonen proportzioetan ere aldaketa handiak gertatu ziren, baina, tamalez, gure metodologiak ez zigun ahalbidetu landareen datuak xehatzea (Cabodevilla *et al.*, 2020b), eta hori etorkizuneko ikerketetan ebaluatu beharko litzateke.

Gure emaitzek ondorio garrantzitsuak dituzte basoilo txikiarentzat, mehatxatuko eta gainbeheran dagoen espeziea (García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020a), dietarik pobreena duen espeziea (bereziki landareei dagokienez) eta ugalketa-garaitik kanpo artropodoen proportziorik handiena duen espeziea denez gero. Gure emaitzen arabera, ugalketa-garaitik kanpoko basoilo txikiaren dietak gorotzen analisi bisualean oinarritutako aurreko azterketek zenbatetsitakoak baino artropodoen proportzio handiagoa barne har dezake (Bravo *et al.*, 2017). Aipatzekoa da neguko dietan deskribatutako artropodo gehienak (tripsak, dipteroak eta armiarmak) egitura kitinoso gutxi dituzten taxonetakoak direla, eta, beharbada, ez direla hain hautemangarriak gorotzen azterketa bisual baten bidez. Aitzitik, koleopteroen proportzioa txikia zen neguko dietan. Hori guztia kontuan hartuta, litekeena da basoilo txikia izatea baliabide trofikoen aldaketekiko kalteberena den espeziea, hala nola belarren eta artropodoen biodibertsitatearen eta ugaritasunaren galerarekiko (Benton *et al.*, 2002). Neguan, basoilo txikiak lekadunak, uztondoak eta lugorriak erabiltzen ditu (Silva *et al.*, 2004; Cuscó *et al.*, 2018; González del Portillo *et al.*, 2021). Alpapa-sailak espezie horrek urtean zehar erabiltzen dituen baliabide trofiko begetal garrantzitsuak dira (Bravo *et al.*, 2017; Cuscó *et al.*, 2018; González del Portillo *et al.*, 2021), eta horiek artropodoen ugaritasun handia ere badute (González del Portillo *et al.*, 2021). Uztondoak, artropodo gutxiago mantentzen dituzten arren, positiboki hautatuak dira, euren landare-egiturak harrapakinak aurkitu eta harrapatzea errazten duelako (González del Portillo *et al.*, 2021). Gainera, ingurune naturalek eta erdi-naturalek (lugorrietan hazten den landaredia, adibidez) funtsezko eginkizuna betetzen dute artropodoen komunitateentzat, hibernazio-eremu gisa; gainera, halakoetan, artropodoen ugaritasun eta aberastasun globala laborantza-lurretan baino handiagoa da (Pfiffner eta Luka, 2000; Hendrickx *et al.*, 2007). Basoilo txikiaren ugaritasunaren murrizketaren eta lugorrien azaleraren murrizketaren artean erlazio handia dagoela dirudi (Traba eta Morales, 2019), zeinak bi faktore horien arteko lotura posible bat iradokitzen duen. Artropodoen eta lugorriaren arteko erlazio horrek indartu egiten du NPB berri baten beharra; basoilo txikiaren populazioak kontserbatu nahi badira, Europako nekazaritza-ekosistemetan lugorriari eustea bermatu behar du, eta nekazal inguruneko beste hegazti batzuentzat ere onuragarria litzateke (Traba eta Morales, 2019; Tarjuelo *et al.*, 2020b).

Ugalketa-garaitik kanpo ganga-espezieen dietan agertzen diren artropodoen proportzioa oso txikia izan zen; landare-baliabide trofikoekiko mendekotasun handiaren isla da hori. Ganga azpizuria eta ganga azpibeltza, nagusiki, espezie hazi-jaleak dira (Del Hoyo *et al.*, 1992), eta, beraz, hazien eskuragarritasunaren eta hazi-bankuen mende daude. Hazien eskuragarritasuna oso aldakorra da habitaten eta urtaroen arabera, eta nekazaritzaren intentsifikazioak nabarmenki eragiten dio (Newton, 2004; Tarjuelo *et al.*, 2019). Nekazaritza-lurretan, belardien mugetan eta lugorrietan hazten den landaredi naturalak eta erdi-naturalak hazi-erreserba handia ematen dute, eta, beraz, espezie hauentzat habitat-elementu garrantzitsuak dira (Tarjuelo *et al.*, 2019).

Etengabe intentsifikatzen ari diren paisaia hauetan, espezieen dietaren ezagutza eguneratua ezinbestekoa da espezieen kontserbazioari ekiteko. Ildo horretan, gure emaitzek datu garrantzitsuak eman zituzten gainbeheran dauden espezie horien ugalketa-garaitik kanpoko dietari buruz (orain arte gutxien aztertu den garaia), bereziki ornogabeei dagokienez. Gainera, artropodoek espezie hauen dietan duten garrantzia erakutsi zuten, eta artropodoen populazioak nekazaritza-inguruneetan behar bezala mantentzeko beharra agerian jarri. Hori funtsezkoa izan liteke galtzeko arriskuan dauden espezieen kontserbazioari heltzeko. Etorkizunean, landare-dietaren ebaluazio zehatz batekin osatu beharko litzateke hemen egindako lana, zeinak argitzen lagunduko lukeen, batetik, hegazti hauek laboreez zenbateraino elikatzen diren, pestizidaz blindatutako haziak barne (Tarjuelo *et al.*, 2020a), eta, bestetik, lugorriek eta habitat erdi-naturalek elikagai-iturri gisa duten eginkizuna. Gainera, ugalketa-garaiko dietaren azterketa ere ebaluatu behar da, jakina baita hazkuntza-garaian dieta nabarmen alda daitekeela (Palacios *et al.*, 1975; Jiguet, 2002). Gainera, baliteke txitek proteinetan aberatsagoa den dieta bat behar izatea hazteko, basoilo handiaren eta eper gorriaren kasuan agerian geratu den bezala, zeinen txitak artropodoz elikatu behar diren (Green, 1984; Jiguet, 2002; Bravo *et al.*, 2012). Oraindik ez dira sakon deskribatu gangen edo basoilo txikiaren txiten eskakizun trofikoak (Jiguet, 2002). Espezie horiek, batez ere arriskuan dauden estepetako hegazti-espezieak, kontserbatzeko premiei behar bezala aurre egiteko, nekazaritza-lurrak espezieen behar trofikoak asetzen direla bermatzeko moduan kudeatu beharko lirateke. Nekazarientzat erakargarriak eta errentagarriak diren nekazaritza-ingurumeneko laguntzak sustatuz lor liteke hori. Neurri horiek ekosistemen konplexutasuna handitzean zentratu beharko lirateke, nekazaritza-lurrak mosaiko moduko paisaia bihur daitezen, urtean zehar, artropodo-, landare- eta hazi-ugaritasun altua mantentzeko gai baitira.

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VII. ARTIKULUA

Nekazal inguruneko hegaztietan ematen den *Blastocystis* spp.-ren infekzioetara hurbilketa erdi-kuantitatiboa: karga parasitario oso handia basoilo babestuetan

- **Laburpena**
- **Sarrera**
- **Eztabaida**

Xabier Cabodevilla, Beatriz Arroyo, Amaia Caro,
Ursula Höfle, María J. Madeira

Prestatzen

Nekazal inguruneko hegaztietan ematen den *Blastocystis* spp.-ren infekzioetara hurbilketa erdi-kuantitatiboa: karga parasitario oso handia basoilo babestuetan

Laburpena

Nekazaritzaren intentsifikazioaren ondorioz, nekazal inguruneko hegazti asko gainbeheran daude. Gainbeheraren eragileen artean, estres-faktore indibidualak ere izan daitezke, zeinek infekzio parasitarioekiko sentikortasuna areagotu dezaketen. Parasito protisten artean, *Blastocystis* spp. da fauna basatian gehien banatua eta gutxien ikertua dagoenetako bat, zoonotikoa izan arren eta bere ahalmen patogenikoa banako immunodeprimituetan handitzen den arren.

Ikerlan honetan, *Blastocystis* spp. delakoaren karga parasitarioa era erdi-kuantitatiboan balioesteko metabarcoding-ikuspegi bat probatu genuen, eta saiatu ginen, teknika hori inferentzia filogenetikoarekin konbinatuz, *Blastocystis* subtipoak identifikatzen. Metodologia hori erabili genuen nekazal inguruneko sei hegazti-espezietan *Blastocystis* spp.-k eta haren subtipoek duten garrantzia ebaluatzeko (basoilo handia, basoilo txikia, ganga azpizuria, ganga azpibeltza, eper gorria eta galeperra), baita haztegietan hazitako eper gorrietan ere.

Gure emaitzek erakutsi zuten proposatutako metodologia gai dela *Blastocystis* subtipoak identifikatzeko, bai eta *Blastocystis* spp. eta haren subtipoen karga parasitario erdi-kuantitatiboa zenbatesteko erabilgarria ere. Bestalde, ikertutako hegazti-espezie guztiak *Blastocystis* spp.-z infektatuak zeudela adierazi zuten datuek, espezieen artean subtipo ugarienak ezberdinak izan ziren arren. Haztegietan hazitako hegaztiak, nagusiki, *Blastocystis* 6. subtipoaz infektatuak aurkitu ziren; hegazti basatietan, ez zen ohikoa subtipo zoonotiko hori. Oro har, hegazti basatiek ez zuten karga parasitario handirik izan, basoiloek izan ezik. Basoilo txikiaren kasuan, karga parasitarioa oso handia zen. Gainera, ikusi zen espezie hori *Blastocystis*eko OTU askorekin koinfektatuta zegoela (12 OTU aurkitu ziren saldo bakoitzeko), eta *Blastocystis*eko DNA-irakurketen proportzio handi bat 7. subtipoari zegozkien. Gure emaitzek agerian uzten dute *Blastocystis* spp.-k eta horrek izan ditzaketen ondorioak kontuan hartu beharreko gauzak direla mehatxatuta dagoen espezie horren populazioen osasunaren kudeaketa eta kontserbazioari dagokienez. Oro har, ikerlan honetan *Blastocystis* spp.-ri buruzko ikerketak egiteko metodologia berri bat aurkezten da, eta parasito horrek eta horren subtipoek nekazal inguruneko hegaztietan duten garrantziaren lehen ebaluazioa egiten da, zeinak adierazten duen organismo horrek garrantzi handia izan dezakeela estresa jasateko arrisku handia duten espezie batzuen kontserbaziorako.

Gako-hitzak

Basoilo txikia; *Blastocystis* subtipoak; *Eimeria*; haztegietan hazitako eper gorriak; nekazal inguruneko hegaztien osasuna; parasito protistak

1. Sarrera

Blastocystis spp. parasito protista anaerobio zorrotza da, zeina animalia ezberdin askoren hestean aurki daitekeen, besteak beste, gizakietan (Ramírez *et al.*, 2014; Betts *et al.*, 2018; Greige *et al.*, 2018; Katsumata *et al.*, 2018; Wang *et al.*, 2018; Asghari *et al.*, 2019; Piubelli *et al.*, 2019). Hainbat hamarkadatan zehar organismo hori ikertu ondoren (Tan, 2008), *Blastocystis*en 22 subtipo (aurrerantzean, ST) deskribatu dira (Stensvold eta Clark, 2020); horietako bederatzi gizakian aurkitu dira, zoonotikoak diren batzuk barne (Iguchi *et al.*, 2007; Tan, 2008; Lewicki *et al.*, 2016; Cian *et al.*, 2017; Greige *et al.*, 2018). Nahiz eta *Blastocystis* spp.-ren potentzial patogenikoak argia dirudien (Hussein *et al.*, 2008; Puthia *et al.*, 2008; Tan, 2008; Elwakil eta Hewedi, 2010; Ajjampur eta Tan, 2016), berdin aurkitu da gaixo zein osasuntsu dauden pertsonetan (Scanlan eta Marchesi, 2008; Scanlan, 2012; Adamu *et al.*, 2013), eta, hortaz, ez dago oraindik argi zergatik batzuetan patogenikoa den eta beste batzuetan ez (Scanlan, 2012). Hala ere, jakina da hesteetako mikrobiota bakterianoan duela eragina (Scanlan, 2012; Defaye *et al.*, 2018; Nieves-Ramírez *et al.*, 2018; Yason *et al.*, 2019), eta haren gaitasun patogenikoa handitu egiten dela banako immunodeprimituetan (Llibre *et al.*, 1989; Adamu *et al.*, 2013; Chandramathi *et al.*, 2014; Ahmed eta Karanis, 2018).

Blastocystis spp.-k hesteetako mikrobiotan aldaketak eragiteko erabiltzen dituen mekanismoak oraindik ez daude argi. Litekeena da *Blastocystis* spp. hesteetako bakterioez elikatzearen ondorioa izatea (Dunn *et al.*, 1989; Tan, 2008; Nieves-Ramírez *et al.*, 2018). Hori horrela balitz, hesteetako mikrobiotaren gaineko eragina *Blastocystis*en ST (Yason *et al.*, 2019) bakoitzaren elikadura-lehentasunen eta karga parasitarioaren (Scanlan, 2012) araberakoa izango litzateke, eta horrek azal litzake, ikerketetan, patogenotasunean aurkitutako desberdintasunak. Bestalde, *Blastocystis* spp.-k hesteetako mikrobiotan eragindako aldaketak *Blastocystis* spp.-ren beste jarduera batzuen bigarren mailako ondorio izan litezke (Yason *et al.*, 2019). Edonola ere, *Blastocystis* spp.-ren gaitasun patogenikoa sakonki ezagutzea funtsezkoa da, organismo horrek gizakien osasunean eta fauna basatian duen eragina behar bezala ulertzeko. Izan ere, oso estresatuta dauden animalia basatien populazioak kontserbatzeko kezka handia izan liteke, banako immunodeprimitu eta estresatuetan areagotu egiten baita horren gaitasun patogenikoa (Chandramathi *et al.*, 2014). Zoritxarrez, *Blastocystis* spp.-k fauna basatian duen intzidentzia ia ez da ikertu, oso organismo arrunta eta hedatua izan arren (Clark *et al.*, 2013). Gainbeheran dauden espezieentzat bereziki garrantzitsua izan liteke hori, adibidez, nekazal inguruneko hegazti askorentzat, batez ere nekazaritzan eta beste giza jarduera batzuetan izandako aldaketen ondorioz inguruneko etengabeko estresa jasaten baitute (Donald *et al.*, 2001). Horren adibide ona basoilo txikia izango litzateke (*Tetrax tetrax*): Espainian, 11 urtean populazioaren % 50 inguruko beherakada izan du (García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020a), eta erraz estresatzen da (Marco *et al.*, 2006; Ponjoan *et al.*, 2008; Tarjuelo *et al.*, 2015). Testuinguru honetan, espezie hauetan gakoa izan liteke *Blastocystis* spp.-ren karga parasitarioaren estimazioa. Zoritxarrez, orain arte gizakietan eta animalietan egindako ikerketek *Blastocystis* spp. eta bere STen presentzian eta prebalentzian soilik jarri dute arreta (Nagel *et al.*, 2016; Beghini *et al.*, 2017; Betts *et al.*, 2018; Greige *et al.*, 2018). *Blastocystis* STen karga parasitarioaren estimazioak egiteak eta ikerlan konparatiboetan erabiltzeak informazio garrantzitsua emango luke *Blastocystis* spp.-ren gaitasun patogenikoari buruz.

Blastocystis STak metabarcoding-metodologia erabiliz ebalua litezke (Maloney *et al.*, 2020). Teknika horrek ez du laginetan dauden organismoen benetako proportzioa ematen, baina azkar eta eraginkortasunez identifika ditzake laginean dauden *Blastocystis* ST guztiak, eta taxon bakoitzak laginean duen garrantziaren ugaritasun erdi-kuantitatiboa eman dezake (Evans *et al.*, 2016; Lamb *et al.*, 2019; Piñol *et al.*, 2019). Testuinguru honetan, informazio baliotsua lor daiteke lagin ez-inbaditzaileak (gorotzak, adibidez) eta errendimendu handiko sekuentziazio-teknikak (HTS) konbinatuz. HTSren agerpenak gorotzen DNA konplexuaren karakterizazioa sinplifikatu zuen, eta, orain, aukera ematen du espezieen ekologiaren alderdi berri ezberdinak aztertzeko. Teorian, espektro zabaleko barcode eukariotikoen erabilerak aukera eman lezake, hesteetan dauden organismoen artean, *Blastocystis* spp.-ren balio erdi-kuantitatiboa balioesteko (Evans *et al.*, 2016; Lamb *et al.*, 2019; Piñol *et al.*, 2019). Horrela, metodologia hau egokia izan liteke *Blastocystis* spp.-k hesteetako mikrobiotan adierazten duen proportzioaren (karga parasitario erdi-kuantitatiboa) azterketa konparatiboak egiteko, eta erraz estimatu liteke gorotz-laginetatik abiatuta. Hala ere, gorotzetako DNA nahiko degradatuta egon ohi da (Deagle *et al.*, 2006; Yu *et al.*, 2012; Taberlet *et al.*, 2012a); beraz, erabilitako barcode-en luzera funtsezkoa da (Hajibabaei *et al.*, 2006; Deagle *et al.*, 2006, 2007; Taberlet *et al.*, 2012b). 200 bp baino gutxiagoko mini-barcode-ak idealak izan dira; hala ere, anplikoi motz horiek bereizmen taxonomiko txikiagoa izaten dute. Duela gutxi, Cabodevilla *et al.*-ek (2020b) espektro zabaleko mini-barcode eukariotiko bat deskribatu zuten (150 pb), Bastocystis spp. anplifikatzeko gai zena eta, genero-mailan, *Blastocystis* spp.-ren anplikoiak anbiguotasunik gabe identifikatzeko bereizmen nahikoa zuena. Hala ere, lan horretan ez zuten ebaluatu mini-barcode horrek *Blastocystis* STak identifikatzeko duen gaitasuna, zeina organismo horren ikerketan funtsezko informazioa den.

Ikerketa honetan, *Blastocystis* spp.-k gainbeheran dauden nekazal inguruneko hegaztietan duen garrantzia ebaluatzeko, bi urratseko metodologia erabili genuen. Lehenik, ebaluatu genuen MiniB18S_81 mini-barcode-ak (Cabodevilla *et al.*, 2020b) *Blastocystis* spp. eta bere STak anplifikatzeko eta identifikatzeko duen "in silico" eta "in vivo" gaitasuna, bai eta *Blastocystis* spp.-ren karga parasitario erdi-kuantitatiboa estimatzeko duen gaitasuna ere. Bigarrenik, mini-barcode hori erabili genuen galtzeko arriskuan dauden estepetako lau hegazti-espezietan, gainbeheran dauden hegazti zinegetikoen bi espezietan eta espezie horietako baten haztegietan hazitako banakoetan *Blastocystis* spp.-ren presentzia eta karga parasitarioa deskribatzeko eta aztertzeko. Hegazti horietako bakoitzari eragiten dieten STak zein diren eta zein garrantzi duten ere ikertu genuen. Estres handia jasaten zuten gainbeheran dauden estepetako hegaztietan *Blastocystis* spp.-ren sekuentzia gehiago aurkitzea espero genuen; halaber, espero genuen desberdintasunak aurkitzea hegazti basatien eta haztegietan hazitako hegaztien artean, horiei eragiten dieten *Blastocystis* STei dagokienez.

4. Eztabaida

Orain arte, *Blastocystis* spp.-ren eta horren STen karga parasitarioan arreta gutxi jarri da, eta hori gakoa izan liteke organismo horren patogenotasuna ulertzeko. Gure emaitzek erakutsi zuten metabarcoding-a tresna baliagarria izan daitekeela *Blastocystis* spp.-ren karga parasitarioa modu erdi-kuantitatiboan balioesteko, bai eta gorotz-laginetan dauden ST guztiak identifikatzeko eta horiek duten garrantzia zehazteko ere. Cabodevilla *et al.*-ek (2020b) deskribatutako MiniB18S_81 mini-barcode-ak gaitasun eta bereizmen handia erakutsi zuen gorotz-laginetatik *Blastocystis* spp. anplifikatzeko eta identifikatzeko. Gainera, inferentzia filogenetikoa *Blastocystis*en STak identifikatzeko tresna on bat dela frogatu zuten, zeinak, kasu gehienetan, *Blastocystis* STak identifikatzea ahalbidetu zuen. Horrela, frogatu zen metodologia hori gai zela *Blastocystis* STen ugaritasunaren balio erdi-kuantitatiboa emateko, eta, portzentaje horri esker, *Blastocystis* STek organismo eta habitat ezberdinetan duten garrantzia ebaluatu eta konparaziozko azterketak egin ahal izango dira, *Blastocystis* spp.-ren ekologiari eta gaitasun patogenikoari buruzko ikuspegi berri bat emanez. Hala ere, etorkizunean erreferentziazko datu-basea zabaltzea gomendagarria litzateke, inferentzia filogenetikoaren bereizmen-ahalmena hobetuko bailuke.

Nekazal inguruneko hegaztien gorotz-laginen metabarcoding-analisiaren bidez, *Blastocystis* spp. parasitoa aurkitu dugu aztertutako hegazti-espezie guztietan. Hala ere, espezieen hesteetan aurkitutako *Blastocystis* OTUen ugaritasuna eta aberastasuna oso ezberdinak izan ziren espezieen artean. Batez beste, ganga azpizuriak, ganga azpibeltzak, eper gorri basatiak, haztegietan hazitako eper gorriak eta galeperrak saldo bakoitzeko bost *Blastocystis* OTU inguru erakutsi zituzten, eta *Blastocystis* spp.-ren irakurketak lortutako DNA-irakurketa guztien % 1 baino gutxiago izan ziren espezie horietan. Basoiloetan, aldiz, *Blastocystis* spp.-ren aberastasuna eta ugaritasuna gainerako espezieena baino askoz handiagoa izan zen. Ostalari horien hesteetako mikrobiotan *Blastocystis* spp.-k duen zeregina ezezaguna den arren, gizakietan eta beste espezie batzuetan, parasito horren ugaritasuna banako immunodeprimituekin edo estresatuekin erlazionatu da (Adamu *et al.*, 2013; Chandramathi *et al.*, 2014; Ahmed eta Karanis, 2018). Lagindutako basoilo handiak eta basoilo txikiak estres larrian zeudela adieraz lezake horrek, edo *Blastocystis* spp.-k espezie horietan duen potentzial patogenikoa iradoki.

Gainera, inferentzia filogenetikoari esker, aurkitutako *Blastocystis* OTUen STak identifikatu ahal izan genituen: ST6 OTU bat, 20 ST7 OTU, ST4 eta ST8rekin erlazionatutako bi OTU, eta ST6, ST7 eta ST9rekin erlazionatutako zazpi OTU. Aztertutako espezieen gorotzetan aurkitutako *Blastocystis*en 30 OTUetatik gehienak ST6 eta ST7rekin erlazionatuta zeuden, hegaztietan ohikoa den bezala (Stensvold *et al.*, 2009; Cian *et al.*, 2017; Greige *et al.*, 2018; Maloney *et al.*, 2020), horietako batzuk ST4 eta ST8rekin ere erlazionatuta zeuden arren. Aurkitutako *Blastocystis* ST guztiak gizakietan ere deskribatu dira, eta, beraz, oso litekeena da zoonotikoak izatea (Parkar *et al.* 2007; Cian *et al.* 2017; Greige *et al.* 2018); hala ere, zalantzarik gabe ST espezifiko gisa identifikatutako OTUak dira zoonotikoak izateko probabilitate handiena dutenak. ST6a ST zoonotiko ezagun bat da, haztegietan hazitako hegaztietan deskribatu dena (Lewicki *et al.*, 2016; Greige *et al.*, 2018). Ikerlan honetan, ST hau ia bakarrik haztegietan hazitako hegaztietan aurkitu zen, hau da, gizakiekin kontaktu estua duten hegaztietan, eta, beraz, horrek zalantzak sortzen ditu ST6aren transmisioaren norabideari buruz; izan ere, haztegietan hazitako eper gorrietara horien giza zaintzaileengandik irits daiteke, eta ez alderantziz. Gainera, emaitzek erakutsi zuten, espezie bereko hegaztien artean, haztegietan hazitako banakoek eta banako basatiek *Blastocystis* ST desberdinak izan ditzaketela. ST7ari dagokionez, frogatu da giza hesteetako mikrobiotaz elikatzearen ondorioz Bifidobacterium eta Lactobacillus bakterio onuragarrietan eragin negatibo nabarmena duela (Yason *et al.*, 2019). Ikerketa honetan, ST hori oso ugaria zen basoilo txikietan (irakurketa guztien % 10 baino gehiago); beste espezie batzuetan, berriz, irakurketa guztien % 0,05 baino gutxiago izan zen. Basoilo txikia ez denez gizakiekin kontaktu zuzena duen hegaztia, arrisku zoonotikoa txikia dela dirudi. Hala ere, kontserbazioaren ikuspegitik, basoilo txikien hestean ST7 ugari egoteak ondorio garrantzitsuak izan ditzake espezie horren hesteetako mikrobiotan, eta, beraz, espeziearen populazioaren dinamikan ere bai.

Protisten DNAren irakurketen ehunekoari dagokionez, oro har oso txikia izan zen, basoilo handiaren, basoilo txikiaren eta haztegietan hazitako eper gorriaren kasuan izan ezik. Basoiloetan aurkitutako protisten irakurketen proportzio altua *Blastocystis* spp.-ren proportzioarekin oso lotuta zegoen, baina haztegietan hazitako eper gorrien kasuan, erabat lotuta zegoen *Eimeria* spp. kokzidianoaren proportzioarekin. Gainera, haztegietan hazitako eper gorriek *Cryptosporidium* spp. parasitoaren infekzioa ere aurkeztu zuten. Bi protista horiek hegaztien parasito garrantzitsutzat jotzen dira (Millan, 2009), eta eragin nabarmena izan dezakete hegaztien osasunean (Millan, 2009; Ryan, 2010). Biak deskribatu dira haztegietan hazitako eper gorrien parasito garrantzitsu gisa (Pagès-Manté *et al.*, 2007; Millán, 2009; Naciri *et al.*, 2011). Organismo horiek oso gutxitan aurkitu zirenez animali basatietan, eta beste ikerketa batzuek iradokitzen duten bezala (Villanúa *et al.*, 2007; Villanúa *et al.*, 2008), gure emaitzek erakutsi zuten hegazti basatiek eta haztegietan hazitako hegaztiek parasito ezberdinak dituztela, eta, hortaz, haztegietan hazitako hegaztiak naturan askatzea arriskutsua izan daitekeela hegazti basatien populazioen osasunerako.

Ikertutako espezieen artean, gure emaitzek erakutsi zuten *Blastocystis* spp. basoilo txikiaren hesteetan oso ugaria dela (*Blastocystis* spp.-ren irakurketak irakurketa guztien % 20a izanik), eta, beraz, parasito hori oso kezkagarria izan daiteke espezie horren kontserbaziorako. Basoilo txikia oso mehatxatuta dagoen espeziea da: azken 11 urteetan, Espainiako populazioaren % 50 galdu da (García de la Morena *et al.*, 2018; Cabodevilla *et al.*, 2020a). Espezie horren populazio-dinamika negatiboa oso lotuta dago habitataren galerarekin, nekazaritzaren intentsifikazioaren ondorioz

(Traba eta Morales, 2019); intentsifikazio horrek aldaketa handiak ekarri ditu agroekosisteman, eta horrek basoilo txikiaren populazioak estres handiko egoera batera bultzatzen ditu (Tarjuelo *et al.*, 2015). Gainera, basoilo txikia berez estres fisiologikoa agertzeko erraztasuna duen espeziea da (Marco *et al.*, 2006; Ponjoan *et al.*, 2008; Tarjuelo *et al.*, 2015), eta horrek *Blastocystis* spp.-ren infekzioa izateko aukera handitzen ditu. Etorkizunean, *Blastocystis* spp. eta horren STek basoilo txikian duten eragina ebaluatu beharko litzateke, baita *Blastocystis* spp.-ren karga parasitarioaren, estres fisiologikoaren eta basoilo txikiaren populazioen demografiaren arteko erlazioa ere.

Ondorioz, azterlan honek erakusten du metabarcoding-ak onura dakarrela, *Blastocystis* spp.-ren karga parasitarioaren azterketa konparatiboak ahalbidetzen baititu. Metodologia honek emaitza garrantzitsuak eta interesgarriak eman ditzake, *Blastocystis* spp.-k eta bere STek hesteetako mikrobiotan eta faunan eta giza osasunean duten eragina azal dezaketenak. Bestalde, gure emaitzek agerian uzten dute *Blastocystis* spp.-k fauna basatian duen eraginari eta inpaktuari buruz ikertzen jarraitzearen garrantzia, haztegietan hazitako animaliekin alderatuta ezberdintasun handia egon baitaiteke. Gainera, gure emaitzek informazio berria eta garrantzitsua ematen dute *Blastocystis* spp.-k eta bere STek mehatxatutako nekazal inguruneko hegazti batzuetan duten eraginari eta karga parasitarioari buruz, eta erakusten dute organismo horrek eragin handia duela mehatxaturik dauden Espainiako estepetako hegazti batzuetan. Emaitzek agerian uzten dute zer garrantzi duen etorkizunean *Blastocystis* spp. gainbeheran dauden espezie horientzat zenbaterainoko kontserbazio-arazoa izan daitekeen ebaluatzeak.

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6

SYNTHESIS

Synthesis

In this thesis a multidisciplinary approach was used to bring some light to important aspects involved in the decline of farmland birds. The new knowledge presented in this thesis is significant in order to adequately address the conservation of farmland birds. It provides relevant information on the implications of changes in land-use and agricultural practices and of other anthropogenic factors for farmland birds, as well as on the diet and parasites affecting these birds, which may be in turn related to habitat and anthropogenic practices.

This section aims to synthesise and bring together the contributions of this thesis. It is not a discussion as each article included in the thesis has its own detailed discussion.

Effects of land-uses and farming practices

Agricultural intensification is the main factor driving declines of farmland bird species (Donald *et al.*, 2006). Agricultural intensification continues to expand and this is unlikely to slow down in next decades (Kehoe *et al.*, 2017; Zabel *et al.*, 2019). Thus, it is important to have information on the effect of agricultural intensification on these birds' populations, broken down by intensification measures, land-uses, and agricultural practices. This will allow to develop more environmentally responsible productive farming systems and appropriate compensatory measures to mitigate the adverse effects of each intensification measure.

Regarding this, the results in this thesis highlight the importance of a mosaic farmland landscape structure, with parches of natural and semi-natural (fallow land) vegetation and traditional rain-fed crop systems.

Thus, we found that both the implementation of irrigation in arable land (involving important changes in land-use and the loss of natural vegetation) and the modernization of vineyards (involving important changes in vine's structure and management) drive significant changes on local bird communities. The irrigation of rain-fed cereal systems is extremely detrimental for farmland bird populations, leading to strong and rapid decline of farmland species occurrence probabilities and thus species richness. Our results demonstrate that irrigation, which is associated with changes in land-use and vegetation structure, has a strong negative impact on some threatened farmland bird species, such as the little bustard or greater short-toed lark, and on some important farmland game species, such as red-legged partridge. The very few number of species benefited by the irrigation suggests that, in the long-term, irrigation of arable land may lead to substantial negative changes in local bird communities, with less diversity and a lack of ecologically-important farmland species.

The modernisation of vineyards, on the other hand, did not result in a general negative effect on bird occurrence probabilities. This modernization involves higher vines and the systematic use of irrigation (by dripping systems) throughout the summer, with fertigation being applied in a third of the vineyards. Its effect depends on the species, with some important farmland birds, such as the red-legged partridge, using trellised vineyards more than traditional vineyards when available, although occurrence in vineyard plots was overall higher when they were surrounded by arable land. Thus, our results indicate that some agricultural species could be attracted to the trellised vineyards during the summer in search of shelter or water, after the cereal crops have been harvested.

However, the preference of trellised vs traditional vineyard plots by red-legged partridges in summer contrasts with the negative relationship found between red-legged partridge abundance and availability of trellised vineyards at the landscape scale. This relationship shows that the higher the proportion of trellised vineyards, the lower the abundance of red-legged partridge. This pattern is consistent with a negative effect of nitrate exposure in trellis vineyards on red-legged partridge populations, and with experimental studies that showed adverse effects on nitrate water consumption on bird physiology and health (Rodríguez-Estival *et al.*, 2010). In fact, the negative relationship between trellis vineyards and red-legged partridge abundance was less strong in those areas sensitive to nitrate pollution, where certain farming practices as well as the amount of fertilisers that can be applied to irrigation water is more strongly regulated (Order of 07/02/2011, JCCM). Thus, when fertigation is applied, the dripping irrigation in rain-fed vineyards could act as an ecological trap for farmland birds, attracting them for shade and water but negatively affecting their health. This should be studied in depth in the future.

Moreover, our results showed that red-legged partridge abundance was positively related to the proportion of natural vegetation and the availability of rain-fed vineyards in the landscape, which highlights the importance of traditional agroecosystems for farmland birds, which usually involve smaller plot sizes, higher proportion of fallow land, higher crop heterogeneity, and higher surface of natural vegetation (Matson *et al.*, 1997).

Other anthropic factors (hunting intensification)

As mentioned above, agriculture intensification drives the decline of several farmland bird populations (Donald *et al.*, 2006), including traditionally important game species, such as the red-legged partridge. In fact, we have also assessed that the Castilla La-Mancha population of red-legged partridge, a region holding one of the main wild red-legged partridge populations, has decreased more than 50% in the last decade. This is a strong population decline of an ecologically and socio-economically highly relevant species.

This has led to an intensification of game management, aiming to sustain the hunting activity. Hunting management intensification implies that measures such as the provision of food and water, the provision of game crops (crops planted specifically for the game that are not harvested, providing food and shelter), or predator control have become quite common (Mustin *et al.*, 2018). These measures aim to reduce the negative impact of agriculture intensification and so could be also positive for non-game farmland birds (Mustin *et al.*, 2018). However, hunting intensification also involves the increasing use of releases of farm-reared animals, which could be detrimental for both wild game birds and other sympatric bird species (Villanúa *et al.*, 2007; Díaz-Sánchez *et al.*, 2012; Mustin *et al.*, 2018; Aebischer, 2019). Thus, it is important to better understand the implications of hunting management intensification to farmland birds.

To elucidate this, we checked if there was any relationship between the release intensity of farm-reared red-legged partridges and the population trend of little bustards, a highly threatened farmland species, in central Spain. First we described the release intensity, finding that annually between 300,000 and 700,000 farm-reared red-legged partridges were officially released in at least 20% of a province of 19,813 km^2 between 2002 and 2016. This amount fluctuated between years and was strongly affected by the economic crisis of 2008-2014. Second, we assessed the population decline affecting little bustard populations in same area, which was estimated as a population loss of 46% between 2005 and 2016, which was more pronounced in the west of the province. Finally, we also showed that the weakest decline of little bustard breeding population was observed in hunting grounds with higher intensity of farm-reared red-legged partridge releases. From a biological point of view, a positive effect of partridge release intensity on little bustard breeding populations do not seem to have a clear justification. So, it is not appropriate to say that releases are the causal reason for this positive effect. This relationship is probably a reflection of the positive effect of other management measures that are more frequent on estates of high release intensity, such as the provision of game crops or predator control, (Estrada *et al.*, 2015; Mustin *et al.*, 2018) or else may be driven by habitat quality, which may be better in those hunting estates. Thus, it appears that some measures associated with hunting intensification or habitat quality may minimise the effect of agricultural intensification. This should be studied in more detail in the future.

Farmland birds' diet and health status

In order to accurately understand how these intensification measures affect wildlife populations, it is imperative to have an accurate knowledge on some important biological aspects of these species, such as their diet and health status, two scarcely studied aspects of most farmland birds. In particular, the health status of their populations is largely unknown, and most diet studies are based on traditional methods that may have identification biases.

In this sense, within this thesis we describe a new mini-barcode, which is designed to provide simultaneously information on diet and parasites of birds by the metabarcoding of their faeces. This is a new tool able to provide information at order level of the arthropods and at class level of the plants that compose birds´ diet, as well as to provide information at order level of parasites (flatworms, nematodes and protozoa) affecting birds, even at genera level in some protozoa groups. Moreover, we showed that the metabarcoding of faecal samples with this mini-barcode, combining with phylogenetic inference, is a good approach to amplify and identify the parasite *Blastocystis* spp. and its subtypes. This is a parasite to consider, given that in recent years there is growing consensus that these parasites can have major impact on host health and thus influence population survival and reproduction. We used this mini-barcode to bring new knowledge, from a molecular point of view, of the autumn-winter period diet and *Blastocystis* spp. infection of six threatened farmland bird species: little bustard, great bustard, pin-tailed sandgrouse, black-bellied sandgrouse, red-legged partridge, and common quail (the latter, for spring-summer period) .

Regarding their diet, our results showed that in all cases it was mostly composed by plants, although arthropods were also very important in diet of some species, such as little bustard (34%), great bustard (12%), and common quail (14%). The molecular approach allowed to describe high proportions of thrips, spiders and springtails in their diet. The high importance of these arthropods in the diet of some of these birds was unknown and some of them have been described for first time in this work. Moreover, we also found that diet richness is very constant among flocks within species, suggesting that it may be a characteristic of each species. Among species, little bustard showed the lowest non-breeding diet richness, amounting to half of pin-tailed sandgrouse plant richness, even if usually both species constitute mixed flocks during the non-breeding period. This result probably indicates a high specificity regarding each species diet, and highlights the vulnerability of little bustards to a decrease in abundance of their used food items.

Overall, the diet composition of the studied farmland birds highlights again the need for an heterogenous environment capable of providing stable trophic resources throughout the year, plants, seeds, and arthropods, and, therefore, able to support farmland bird populations. A farmland landscape with a mosaic structure that holds important proportions of natural and semi-natural vegetation (weeds), that also maintains the stubbles, and, therefore, provides trophic resources in different seasons (Mcmahon *et al.*, 2010; Traba and Morales, 2019; Tarjuelo *et al.*, 2020a). This is fully in line with our results on the effect of land-use, where the importance of natural vegetation was already shown, surely in part due to its contribution of trophic resources.

On the other hand, our results showed that *Blastocystis* spp. is affecting all studied farmland bird species. Interestingly, we only found the zoonotic subtype 6 in farm-reared birds, giving rise to uncertainty about the directionality of ST6 transmission, which could reach to farm-reared red-legged partridge from its human keepers rather than the other way around. According to parasitic load, both bustard species, but especially the little bustard, showed very high abundances of *Blastocystis* spp. in their gut microbiota. Moreover, little bustard flocks were coinfected by many different *Blastocystis* spp., and among these the parasitic load of zoonotic subtype 7 was very high. This result is probably indicative of the delicate health status of little bustard populations, which are likely to be subject to severe stress and therefore immunosuppressed, as it is a species that is easily stressed (Marco *et al.*, 2006; Ponjoan *et al.*, 2008), which is under a huge pressure by farmland intensification and human activity (Tarjuelo *et al.*, 2015). In future studies, the implications on birds´ health status of a high *Blastocystis* spp. parasitic load should be assessed, as well as infections by other parasites, such as coccidia, nematodes and flatworms.

The future of agriculture and farmland bird conservation

The fast intensification of the agricultural environment in the last decades has involved major changes in the structure and management of agroecosystems, with serious consequences for biodiversity. This is especially relevant in developing countries, where the expansion of agriculture is proving to be devastating for wildlife (Kehoe *et al.*, 2017; Zabel *et al.*, 2019). However, as proven in this thesis and other studies (Donald *et al.*, 2002), at the European level the agricultural intensification resulting from the Common Agricultural Policy (CAP) has also had a negative impact and it is clear that so far the measures taken within the successive renewals of the CAP have not been able to completely mitigate this negative effect. In fact, as demonstrated in this thesis and other contemporary works, at present the most important European populations of some highly relevant farmland bird species, such as little bustard, pin-tailed sandgrouse, black-bellied sandgrouse, Dupont's lark, or red-legged partridge, continue to decline sharply (García de la Morena *et al.*, 2018; Gómez-Catasús *et al.*, 2018; Cabodevilla *et al.*, 2020; Mougeot *et al.*, 2021). Thus, although every renewal of the CAP is greener, there is still a large amount of work to be done to achieve sustainable agriculture.

In recent years, different research has highlighted that, to address the conservation of these species, it is important to maintain fallow land and to manage it appropriately to maintain semi-natural vegetation (Sanz-Pérez *et al.*, 2019; Traba and Morales, 2019; Tarjuelo *et al.*, 2020b). However, this measure alone will hardly be sufficient if monoculture, the use of agrochemicals and the expansion of irrigation, among others, are not limited. In this sense, maintaining traditional rain-fed farmlands and limiting the use of agrochemicals, fertilisers and coated seeds, at least in the Special Protection Areas (SPA), are crucial to preserve bird populations.

Traditional rain-fed farmlands are heterogeneous agricultural landscapes, with a diversity of rain-fed crops, with small plots, stubbles in winter, where some plots are left fallow each year, the boundaries between plots are preserved, and even in some regions where plots of natural vegetation are maintained. An agricultural environment which is currently becoming less and less profitable and which the agri-environmental measures proposed in the CAP are not being able to promote at the desired level. Thus, the delicate situation of many farmland birds in continuous decline requires to rethink agricultural policies, which should contribute to the sustainability and competitiveness of European agriculture, but also to better and more integrated conservation of these species' habitats.

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SINTESIA

Sintesia

Tesi honetan, nekazal inguruneko hegaztien gainbeherarekin zerikusia duten alderdiak argitzeko, diziplina anitzeko ikuspegia erabili da. Tesi honetan aurkezten den ezagutza berria esanguratsua da nekazal inguruneko hegaztien kontserbazioa behar bezala lantzeko. Hala nola, nekazaritza-lurzoruaren erabileran eta nekazaritza-jardueretan izandako aldaketek eta beste faktore antropogeniko batzuek nekazal inguruneko hegaztientzat dituzten ondorioei buruzko informazio garrantzitsua, bai eta hegazti horien dietari eta horiei eragiten dieten parasitoei buruz ere, zeinak, aldi berean, habitatarekin eta praktika antropogenikoekin lotuta egon daitezkeen.

Atal honek tesi honen ekarpenak bateratzea eta laburbiltzea du helburu. Ez da eztabaida bat, tesian sartutako artikulu bakoitzak bere eztabaida zehatza baitu.

Lurzoruaren erabileren eta nekazaritza-jardueren ondorioak

Nekazaritzaren intentsifikazioa da nekazal inguruneko hegaztien gutxitzea bultzatzen duen faktore nagusia (Donald *et al.*, 2006). Gaur egun, nekazaritzaren intentsifikazioak areagotzen jarraitzen du, eta ez dirudi hurrengo hamarkadetan areagotze hori geldituko denik (Kehoe *et al.*, 2017; Zabel *et al.*, 2019). Horregatik, garrantzitsua da nekazaritzaren intentsifikazioak hegazti horien populazioetan duen eraginari buruzko informazioa zehatza izatea, intentsifikazio-neurri bakoitzaren eragin zehatza aztertuz. Horri esker, ingurumenarekiko errespetagarriagoak diren nekazaritza-sistema produktiboak garatu ahal izango dira, bai eta intentsifikazio-neurri bakoitzaren ondorio kaltegarriak arintzeko konpentsazio-neurri egokiak proposatu ere.

Ildo horretan, tesi honen emaitzek mosaiko-egitura duen nekazaritza-paisaiaren garrantzia nabarmentzen dute, landaredi naturaleko eta erdi-naturaleko (lugorria) lursailak eta lehorreko nekazaritza-sistema tradizionala dituen nekazaritza-ingurunea.

Horrela, gure emaitzek adierazten dute laborantza-lurretan ureztapena ezartzeak (horrek lurzoruaren erabileran eragindako aldaketa handiak eta landaredi naturalaren galera barne) eta mahastien modernizazioak (horrek mahastien egituran eta erabileran eragindako aldaketa handiak barne) aldaketa esanguratsuak eragiten dituztela tokiko hegaztien komunitateetan. Nekazal inguruneko hegaztientzat, lehorreko zerealen nekazaritza-sistemak ureztatzea oso kaltegarria da; izan ere, espezie horien agertzeko probabilitatean murriztapen nabarmen eta azkarra eragiten du eta, beraz, espezie horien aberastasunean ere bai. Gure emaitzek erakusten dutenez, ureztatzeak, lurzoruaren erabileran eta landarediaren egituran izandako aldaketei lotuta, eragin negatibo handia du arriskuan dauden nekazal inguruneko hegazti batzuetan, hala nola basoilo txikiarengan edo txoriandre arruntarengan, baita nekazal inguruneko espezie zinegetiko garrantzitsu batzuetan ere, esate baterako, eper gorriarengan. Ureztatzea oso espezie gutxirentzako onuragarria izateak iradokitzen du nekazaritza-lurrak ureztatzeak, epe luzera, tokiko hegaztien komunitateetan aldaketa negatibo nabarmenak eragin ditzakeela, eta, hala, komunitateen aniztasuna murriztu eta garrantzi ekologikoko espezieak galdu daitezkeela.

Mahastien modernizazioak, ordea, ez zuen hegaztiak agertzeko probabilitatean eragin negatibo orokorrik izan. Modernizazio horrek dakar mahatsondo altuagoak, ureztatze sistemikoa (tanta-jarioko sistemen bidez), eta fertirrigazioa mahastien herenean aplikatzea. Bestalde, horren eragina espeziearen araberakoa da; izan ere, nekazal inguruneko hegazti garrantzitsu batzuek, eper gorriak adibidez, aukera izanez gero, beso-sistemako mahastiak gehiago erabiltzen dituzte mahasti tradizionalak baino, nahiz eta haien presentzia arruntagoa izan mahastiak laborantza-lurrez inguratuta daudenean. Horrela, gure emaitzen arabera, udan zehar, zerealen laboreak uztatu ondoren, beso-sistemako mahastiak nekazal inguruneko zenbait espezie erakar ditzake, babes edo ur bila gerturatu baitaitezke.

Hala ere, udan zehar eper gorriek beso-sistemako mahastiak nahiago izateak, paisaia-eskalan eper gorriaren ugaritasunaren eta beso-sistemako mahastien arteko erlazio negatiboarekin kontraste egiten du. Erlazio horrek erakusten du beso-sistemako mahastien proportzioa zenbat eta handiagoa izan, eper gorriaren ugaritasuna orduan eta txikiagoa dela. Patroi hori koherentea da beso-sistemako mahastietako nitratoekiko esposizioak eper gorriaren populazioetan izan dezakeen eragin negatibo hipotetiko batekin, baita nitratodun uraren kontsumoak hegaztien fisiologian eta osasunean dituen ondorio kaltegarriak erakutsi dituzten ikerketa esperimentalekin ere (Rodriguez-Estival *et al.*, 2010). Izan ere, beso-sistemako mahastien eta eper gorriaren ugaritasunaren arteko erlazio negatiboa ez zen hain handia izan nitratoen kutsadurarekiko sentikorrak diren zonaldeetan, non nekazaritza-jarduera jakin batzuk, ureztatzeko urari aplika dakizkiokeen ongarrien kopurua barne, gehiago araututa dauden (2011/02/07ko Agindua, JCCM). Hortaz, lehorreko mahastietan tantakako ureztatzearen bidezko fertirrigazioa aplikatzeak tranpa ekologiko gisa joka dezake nekazal inguruneko hegaztientzat, itzal eta ur bila hurbildu baitaitezke, baina ur horrek haien osasunari kalte egin diezaioke. Hori sakon aztertu beharko litzateke etorkizunean.

Gainera, gure emaitzek erakutsi zuten eper gorriaren ugaritasuna positiboki lotuta zegoela landaredi naturalaren proportzioarekin eta lehorreko mahastien eskuragarritasunarekin. Horrek agerian uzten du nekazaritza-ekosistema tradizionalek garrantzi handia dutela nekazal inguruneko hegaztientzat; horiek tamaina txikiagoko lursailak, lugorri-proportzio handiagoa, laboreen heterogeneotasun handiagoa eta landaredi naturalaren azalera handiagoa izan ohi dute (Matson *et al.*, 1997).

Bestelako faktore antropikoak (ehizaren intentsifikazioa)

Lehen aipatu bezala, nekazaritzaren intentsifikazioak nekazal inguruneko zenbait hegazti-populazioren gainbehera bultzatzen du (Donald *et al.*, 2006), tradizioz garrantzitsuak diren espezie zinegetikoak barne, eper gorria kasu. Izan ere, Gaztela-Mantxako eper gorrien populazioa, eper gorri basatien populazio nagusietako bat izanik, % 50 baino gehiago murriztu da azken hamarkadan. Garrantzi ekologiko eta sozioekonomiko handiko espezie baten populazioaren beherakada larria da hori.

Horren ondorioz, ehiza-jarduerari eusteko ehizaren kudeaketa intentsifikatu egin da. Ehizaren kudeaketaren intentsifikatzeak neurri ezberdinak barne hartzen ditu, hala nola elikagaien eta uraren hornidura, ehiza-laboreen hornidura (espezie zinegetikoei laguntzeko uztatzen ez diren laboreak, elikagaia eta babesa emateko), edo harrapariak kontrolatzea (Mustin *et al.*, 2018). Neurri horien helburua nekazaritzaren intentsifikazioaren eragin negatiboa murriztea da, eta, beraz, nekazal inguruneko hegazti ez zinegetikoentzat ere positiboak izan daitezke (Mustin *et al.*, 2018). Hala ere, ehizaren intentsifikazioak haztegietan hazitako animaliak askatzea ere dakar, eta hori kaltegarria izan liteke bai hegazti zinegetiko basatientzat, bai beste hegazti sinpatriko batzuentzat (Villanúa *et al.*, 2007; Díaz-Sánchez *et al.*, 2012; Mustin *et al.*, 2018; Aebischer, 2019). Horregatik, garrantzitsua da ehizaren kudeaketaren intentsifikazioak nekazal inguruneko hegaztietan dituen ondorioak hobeto ulertzea.

Hori argitzeko, Espainiako erdialdean haztegietan hazitako eper gorrien askatze-intentsitatearen eta oso mehatxatuta dagoen basoilo txikiaren populazioaren joeraren artean loturarik ba ote zegoen aztertu genuen. Lehenik eta behin, askatzeen intentsitatea deskribatu genuen, non ikusi genuen 19.813 km2-ko probintzia baten azaleraren % 20an, 2002 eta 2016 bitartean, urtero ofizialki haztegietan hazitako 300.000-700.000 eper gorri askatu zirela. Kopuru hori aldakorra izan zen urteen artean, eta 2008-2014ko krisi ekonomikoak eragin handia izan zuen askatze-kopuruan. Bigarrenik, eremu bereko basoilo txikiaren populazioari eragiten dien beherakada ebaluatu genuen, eta balioztatu zen 2005 eta 2016 bitartean populazioaren % 46 galdu zela; beherakada nabarmenagoa izan zen probintziaren mendebaldean. Azkenik, ikusi genuen basoilo txikiaren populazio ugaltzailearen beherakada txikiena haztegietan hazitako eper gorria gehien askatzen zuten ehiza-barrutietan gertatu zela. Ikuspuntu biologiko batetik, ez dirudi justifikazio argirik duenik eper gorrien askatzearen intentsitateak basoilo txikiaren populazio ugaltzailean eragin positiboa izateak. Beraz, ez litzateke egokia izango efektu positibo horren kausa askatzeak direla esatea. Litekeena da erlazio hori askatze-intentsitate handiko barrutietan ohikoak diren beste kudeaketa-neurri batzuen eragin positiboaren isla izatea, hala nola ehiza-laboreen hornidura edo harraparien kontrola (Estrada *et al.*, 2015; Mustin *et al.*, 2018), edo habitataren kalitateak eragindakoa, zeina ehiza-barruti horietan hobeagoa izan daitekeen. Hala, badirudi ehizaren intentsifikazioari edo habitataren kalitateari lotutako zenbait neurrik nekazaritzaren intentsifikazioaren eragina minimiza dezaketela. Hori zehaztasunez aztertu beharko litzateke etorkizunean.

Nekazal inguruneko hegaztien dieta eta osasun-egoera

Intentsifikazio-neurri horiek fauna basatiaren populazioei nola eragiten dieten zehatz-mehatz ulertzeko, ezinbestekoa da espezie horien alderdi biologiko garrantzitsu batzuk ondo ezagutzea, hala nola dieta eta osasun-egoera. Bi alderdi horiek gutxi aztertu dira nekazal inguruneko hegazti gehienetan. Zehazki, populazioen osasun-egoera ez da ezagutzen neurri handi batean, eta dietari buruzko azterlan gehienak identifikazio-arazoak izan ditzaketen metodo tradizionaletan oinarritzen dira.

Ildo horretan, tesi honen barruan, mini-barcode berri bat deskribatzen dugu; hegaztien gorotzen metabarcoding-analisiaren bitartez, dieta eta parasitoei buruzko informazioa aldi berean lortzeko diseinatuta dago. Tresna berri bat da, hegaztien dieta osatzen duten artropodoen informazioa ordena-mailan eta landareen informazioa klase-mailan emateko gai dena, bai eta hegaztiei eragiten dieten parasitoen (platelmintoak, nematodoak eta protozooak) ordena-mailako informazioa emateko gai dena ere, baita protozoo-talde batzuentzat genero-mailara ailegatu ere. Gainera, frogatu dugu gorotz-laginen DNAren metabarcoding bidezko analisia, mini-barcode berria erabiliz, inferentzia filogenetikoarekin konbinatuta, tresna egokia dela *Blastocystis* spp. parasitoa eta haren subtipoak anplifikatzeko eta identifikatzeko. *Blastocystis* spp. kontuan hartu beharreko parasitoa da; izan ere, gero eta argiago dago parasito honek ostalariaren osasunean eragin handia izan dezakeela eta, beraz, populazioaren biziraupenean eta ugalketan eragin dezakeela. Ikuspegi molekularretik, mini-barcode berri hori erabiliz, arriskuan dauden nekazal inguruneko sei hegazti hauen udazken-negu garaiko dieta eta *Blastocystis* spp. parasitoaren infekzioa aztertu ditugu: basoilo txikia, basoilo handia, ganga azpizuria, ganga azpibeltza, eper gorria eta galeperra (azken hori, udaberri-udan).

Dietari dagokionez, gure emaitzek erakutsi zuten kasu guztietan landarez osatuta zegoela, baina artropodoak ere oso garrantzitsuak ziren zenbait espezierentzat, hala nola basoilo txikia (% 34), basoilo handia (% 12) eta galeperra (% 14). Ikuspegi molekularrari esker, hegazti horien dietan tripsen, armiarmen eta kolenboloen proportzio handiak deskribatu ahal izan ziren. Hegazti horietako batzuen dietan artropodo horiek zuten garrantzi handia ezezaguna zen, eta horietako batzuk lehen aldiz deskribatu dira lan honetan. Gainera, aurkitu dugu dietaren aberastasuna oso konstantea dela espezie bakoitzeko saldoen artean, eta horrek iradokitzen du espezie bakoitzaren ezaugarri bat izan daitekeela. Espezieen artean, basoilo txikiak erakusten du ugalketa-garaitik kanpo aberastasun dietetiko txikiena, hau da, ganga azpizuriaren landare-aberastasunaren erdia, nahiz eta normalean bi espezieek saldo mistoak izan ugalketa garaitik kanpo.

Emaitza horrek, ziurrenik, espezie bakoitzaren dietan espezifikotasun handia dagoela adierazten du, eta agerian uzten du basoilo txikiaren kalteberatasuna elikagaien ugaritasuna gutxitzearen aurrean.

Oro har, aztertutako nekazal inguruneko hegaztien dietaren osaerak agerian uzten du berriz ere ingurune heterogeneo baten beharra, alegia, urtean zehar baliabide trofiko egonkorrak dituena, landareak, haziak eta artropodoak, eta, beraz, nekazal inguruneko hegaztien populazioak mantentzeko gai izango dena. Beste hitz batzuetan esanda, landaredi natural eta erdi-naturaleko (belar txarrak) proportzio garrantzitsuak mantentzen dituen mosaiko-egitura duen laborantza-lurren paisaia, uztondoei eusten diena eta, beraz, hainbat urtarotan zehar baliabide trofikoak mantentzen dituena (McMahon *et al.*, 2010; Traba eta Morales, 2019; Tarjuelo *et al.*, 2020a). Hori bat dator guztiz lurzoruaren erabileraren gaineko lortutako emaitzekin; izan ere, landaredi naturalaren garrantzia argi geratzen da lan honetan, eta, ziurrenik, baliabide trofikoei egiten dieten ekarpenarekin loturik dago.

Bestalde, gure emaitzek erakusten dute *Blastocystis* spp.-k aztertu diren nekazal inguruneko hegazti espezie guztiei eragiten diela. Bitxia bada ere, 6. subtipo zoonotikoak haztegietan hazitako hegaztietan baino ez ditugu aurkitzen, eta horrek zalantzan jartzen du ST6-aren transmisioaren norabidea, zeina haztegietan hazitako eper gorrira bere giza zaintzaileengandik abiatuta irits daitekeen, eta ez alderantziz. Karga parasitarioari dagokionez, bi basoilo espezieek, basoilo txikiak bereziki, *Blastocystis* spp.-ren ugaritasun oso handiak erakutsi zituzten euren heste-mikrobiotan. Gainera, basoilo txikiaren saldoak, *Blastocystis* spp. ezberdinez koinfektatuak zeuden, eta, horien artean, 7. subtipo zoonotikoaren karga parasitarioa oso altua zen. Emaitza horrek, ziurrenik, basoilo txikiaren populazioen osasun-egoera delikatua dela adierazten digu; ziur aski, laborantza-lurren eta giza jardueraren intentsifikazioak eraginda (Tarjuelo *et al.*, 2015), estres larria jasaten dute eta, beraz, immunodeprimituak daude, erraz estresatzen den espeziea baita (Marco *et al.*, 2006; Ponjoan *et al.*, 2008). Etorkizunean, *Blastocystis* spp.-ren karga parasitario altuak hegaztien osasun-egoeran dituen ondorioak ebaluatu beharko lirateke, baita beste parasito batzuek eragindako infekzioak ere, hala nola kokzidioak, nematodoak eta platelmintoak.

Nekazaritzaren etorkizuna eta nekazal inguruneko hegaztien kontserbazioa

Azken hamarkadetan gertatutako nekazaritzaren intentsifikazio azkarrak aldaketa handiak eragin ditu nekazaritza-ekosistemen egituran eta kudeaketan, eta ondorio larriak izan ditu biodibertsitatean. Hori bereziki garrantzitsua da garapen-bidean dauden herrialdeetan, nekazaritzaren hedapena oso kaltegarria izaten ari baita basabizitzarako (Kehoe *et al.*, 2017; Zabel *et al.*, 2019). Hala ere, tesi honetan eta beste azterlan batzuetan frogatu den bezala (Donald *et al.*, 2002), Europan, Nekazaritza Politika Bateratuaren (NPB) ondoriozko nekazaritzaren intentsifikazioak ere eragin negatiboak izan ditu, eta argi dago orain arte NPBren ondoz ondoko berrikuntzetan hartutako neurriek ezin izan dutela eragin negatibo hori erabat arindu. Izan ere, tesi honetan eta beste lan garaikide batzuetan erakusten den bezala, gaur egun nekazal inguruneko garrantzi handiko hegazti espezie batzuen Europako populazio garrantzitsuenek, hala nola basoilo txikia, ganga azpizuria, ganga azpibeltza, Portugaleko pirripioa edo eper gorriak, nabarmen jaisten jarraitzen dute (García de la Morena *et al.*, 2018; Gómez-Catasús *et al.*, 2018; Cabodevilla *et al.*, 2020; Mougeot *et al.*, 2021). Hala, NPBren berrikuntza bakoitza ekologikoagoa bada ere, oraindik lan handia dago egiteko nekazaritza jasangarria lortu arte.

Azken urteotan, hainbat ikerketak agerian utzi dute espezie horien kontserbazioari heltzeko, lugorria mantentzea eta behar bezala kudeatzea garrantzitsua dela landaredi erdi-naturalari eusteko (Sanz-Pérez *et al.*, 2019; Traba eta Morales, 2019; Tarjuelo *et al.*, 2020b). Hala ere, neurri hori berez ez da nahikoa izango monolaborantza, agrokimikoen erabilera eta ureztatzearen hedapena mugatzen ez badira, besteak beste. Ildo horretan, lehorreko laborantzako lur tradizionalak mantentzea eta agrokimiko, ongarri eta hazi blindatuen erabilera mugatzea, Hegaztientzako Babes Bereziko Eremuetan gutxienez, funtsezkoak dira hegazti-populazioak babesteko. Lehorreko laborantzako lur tradizionalak nekazaritza-paisaia heterogeneoak dira: lehorreko labore ugari dituztenak, lursail txikiak dituztenak, neguan uztondoak dituztenak, urtero lursail batzuk lugorri uzten dituztenak, lursailen arteko mugak kontserbatzen dituztenak eta baita landaretza naturaleko zenbait lursail mantentzen dituztenak ere. Nekazaritza-ingurune horrek gero eta errentagarritasun txikiagoa du, eta NPBn proposatutako neurriak ez dira gai izan ingurumen hori nahi den mailan sustatzeko. Hortaz, nekazal inguruneko hegazti asko gainbehera bizian daudenez, nekazaritza-politikak birplanteatu behar dira, Europako nekazaritzaren iraunkortasunari eta lehiakortasunari laguntzeko, bai eta espezie horien habitatak hobeto eta integratuago kontserbatzeko ere.

7

CONCLUSIONS

Conclusions

1. The transformation of rain-fed cereal systems to irrigated systems drives strong population declines on farmland bird species, as well as on shrubland, forest, and non-specialist bird species that inhabit these rain-fed agricultural environments.

2. Vineyard modernisation involves changes in vine structures and management. In trellis vineyards, vines are taller, connected by wires, systematically irrigated and one in three trellis vineyards are fertigated.

3. These changes in vine structure and management have effects on species occurrence probability within plots, affecting the species composition within vineyards. In addition, the cover and water provided in trellis vineyards (particularly if vineyard plots are surrounded by arable land) seems to attract species like partridges in summer. If so, fertigation could pose a risk to these species.

4. Red-legged partridge abundance at the landscape level increases with availability of natural vegetation and rain-fed vineyards, while it is lower where proportions of tree crops and irrigated vineyards are higher. The latter relationship seems to be affected by vineyards fertigation, as the negative effect of irrigated vineyards is less pronounced in areas sensitive to nitrate contamination in water, where the amount of fertilizers applied in farmland is more strictly regulated.

5. Dripping fertigation of woody crops within dry environments may therefore pose an important ecological trap to farmland biodiversity, as the shade and water provided by those crops may attract species like red-legged partridges in summer, but may negatively affect their health and demographic parameters.

6. Red-legged partridge population has sharply declined in central Spain, by 51% between 2010 and 2017. Population decline has been steeper in areas where more natural vegetation has been lost and where tree crops have increased.

7. Officially, between 300,000 and 700,000 farm-reared red-legged partridges were released each year between 2002 and 2016 in at least 20% of the province of Ciudad Real. The intensity of releases varied between hunting estates, fluctuated between years and was strongly affected by the economic crisis of 2008-2014.

8. The population of little bustards in Ciudad Real decreased by 46% between 2005 and 2016. However, the only areas where little bustards did not decrease were hunting estates with higher red-legged partridge release intensity. This may be a consequence of management measures that benefit little bustards and are more prevalent on those estates than elsewhere, such as game crop provision or predator control, or a consequence of habitat quality being higher in those estates.

9. The newly designed MiniB18S_81 mini-barcode is a promising new tool for simultaneous biomonitoring birds´ diet and intestinal parasites through eDNA metabarcoding of faecal samples. It showed high taxonomic coverage of eukaryotic taxa and a great amplification, discrimination and identification capacity of diet type and parasite taxa sequences.

10. Little bustard, great bustard, pin-tailed sandgrouse, black-bellied sandgrouse, red-legged partridge, and common quail's diets mostly consisted of plants, although in the case of little bustard (34%) and great bustard (12%) arthropods were also highly relevant even in the non-breeding period. Diet composition of these declining species supports the importance of natural and semi-natural vegetation and landscape mosaics that can provide a wide variety of arthropods, plants, and seeds all year-round.

11. Metabarcoding of faecal samples combining with phylogenetic inference is an innovative approach able to identify *Blastocystis* subtypes and provide a semi-quantitative parasitic load of *Blastocystis* spp. and its subtypes.

12. The *Blastocystis* zoonotic subtype 6 was common in farm-reared red-legged partridges, while it was very rare on wild birds. Moreover, these birds showed higher parasitic loads of *Eimeria* spp. and *Cryptosporidium* spp. than wild ones.

13. Both bustard species, and especially little bustards, showed high *Blastocystis* spp. parasitic loads. Furthermore, little bustard flocks were coinfected by many *Blastocystis* OTUs (12 OTUs were found per flock), and a high portion of *Blastocystis* reads belonged to the zoonotic subtype 7. So, *Blastocystis* spp. should be a factor to be considered for the conservation of this highly threatened species, which could be an indicator that populations of this species are immunosuppressed.

7

ONDORIOAK

Ondorioak

1. Lehorreko zereal-sistemetan ureztatze-sistemak ezartzearen ondorioz, nekazal inguruneko hegazti-espezieen populazioak asko jaitsi dira, eta, era berean, asko jaitsi dira lehorreko nekazaritza-ingurune horietan bizi diren zuhaixka-inguruneko, baso-inguruneko eta espezializatu gabeko hegaztienak ere.

2. Mahastien modernizazioak mahatsondoaren egituretan eta kudeaketan aldaketak ekarri ditu. Beso-sistemako mahastietan, mahatsondoak altuagoak dira, kableez lotuta daude, sistematikoki ureztatzen dira, eta hiru mahastitik bat fertirrigatzen da.

3. Mahatsondoaren egituran eta kudeaketan gertatzen diren aldaketa horiek eragina dute espezieak agertzeko probabilitatean, baita mahastien barruko espezieen osaeran ere. Gainera, udan, beso-sistemako mahastien estaldurak eta ureztatze-urak (bereziki mahastiak laborantza-lurrez inguratuta daudenean) eperrak bezalako espezieak erakartzen dituela dirudi. Hala balitz, fertirrigazioa arriskutsua izan liteke espezie horientzat.

4. Paisaiaren mailan, eper gorriaren ugaritasuna handitu egiten da landaretza naturalarekin eta lehorreko mahastien eskuragarritasunarekin batera; aldiz, zuhaitz-laboreen eta mahasti ureztatuen proportzioa handiagoa denean, txikiagoa da. Azken erlazio hori mahastien fertirrigazioarekin lotuta dagoela dirudi; izan ere, ureztatutako mahastien eragin negatiboa ez da hain nabarmena uretako nitratoen kutsadurarekiko sentikorrak diren eremuetan, non laborantza-lurretan aplikatzen diren ongarrien kopurua zorrotzago araututa dagoen.

5. Beraz, ingurune lehorreko zurezko laboreak tantaka fertirrigatzea tranpa ekologiko garrantzitsua izan daiteke laborantza-lurren biodibertsitaterako; izan ere, udan labore horiek ematen duten itzalak

eta ureztatze-urak eper gorria bezalako espezieak erakar ditzakete, baina haien osasunari eta parametro demografikoei kalte egin diezaiekete.

6. Espainiako erdialdean, eper gorriaren populazioak % 50eko beherakada jasan zuen 2010 eta 2017 bitartean. Beherakada hori nabarmenagoa izan zen landaredi natural gehien galdu zen eta zuhaitz-laboreak ugaritu ziren eremuetan.

7. Ofizialki, 2002 eta 2016 bitartean, gutxienez Ciudad Realgo azaleraren % 20an, urtero haztegietan hazitako 300.000-700.000 eper gorri askatu ziren. Askatzeen intentsitatea aldakorra izan zen ehiza-barrutien artean eta urteen arabera, eta, horretan, 2008 eta 2014 bitarteko krisialdi ekonomikoak eragin handia izan zuen.

8. Ciudad Realen, basoilo txikiaren populazioa % 46 txikitu zen 2005 eta 2016 bitartean. Hala ere, basoilo txikiaren populazioa gutxitu ez zen eremu bakarrak eper gorri gehien askatzen zituzten ehiza-barrutiak izan ziren. Basoilo txikiari mesede egiten dioten eta barruti horietan beste batzuetan baino maizago gertatzen diren kudeaketa-neurrien ondorio izan daiteke hori, hala nola ehiza-laboreak hornitzearen edo harraparien kontrolaren ondorio, edota barruti horietan habitataren kalitatea hobeagoa izatearen ondorio.

9. Guk diseinatutako MiniB18S_81 mini-barcode-a etorkizun handiko tresna berri bat da, gorotz-laginen eDNAren metabarcoding-aren bidez, hegaztien dieta eta hesteetako parasitoen aldi bereko biomonitorizazioa egiteko. Horrek taxon eukariotoen estaldura taxonomiko handia erakutsi zuen, baita hegaztien dietaren parte diren taxonak eta parasitoen taxonak anplifikatzeko, bereizteko eta identifikatzeko gaitasun handia ere.

10. Basoilo txikia, basoilo handia, ganga azpizuria, ganga azpibeltza, eper gorria eta galeperraren dieta batez ere landarez osatuta dago, baina basoilo txikiaren (% 34) eta basoilo handiaren (% 12) kasuan, artropodoak ere oso garrantzitsuak dira ugalketa-garaitik kanpo. Gainbeheran dauden espezie horien dietak landaredi natural eta erdi-naturalaren eta paisaia-mosaikoen garrantzia nabarmentzen du, halakoek urte osoan zehar artropodo, landare eta hazi ugari eman baititzakete.

11. Gorotz-laginen metabarcoding-a gai da, inferentzia filogenetikoarekin konbinatuta, *Blastocystis* subtipoak identifikatzeko eta *Blastocystis* spp.-ren eta haren subtipoen karga parasitarioa era erdi-kuantitatiboan balioztatzeko.

12. *Blastocystis*en 6. subtipo zoonotikoa ohikoa zen haztegietan hazitako eper gorrietan, baina oso arraroa hegazti basatietan. Gainera, hegazti horiek hegazti basatiek baino *Eimeria* spp.-ren eta *Cryptosporidium* spp.-ren karga parasitario handiagoak erakusten zituzten.

13. Bi basoilo espezieek, eta bereziki basoilo txikiak, *Blastocystis* spp.-ren karga parasitario handiak erakutsi zituzten. Gainera, basoilo txikiaren saldoak *Blastocystis* OTU askorekin koinfektatuta zeuden (12 OTU aurkitu ziren saldo bakoitzeko), eta *Blastocystis*eko irakurketen zati handi bat 7. subtipo zoonotikoari zegozkion. Beraz, horrek adieraz dezake espezie horren populazioak immunodeprimituta daudela, eta, beraz, espezie horren kontserbaziorako, *Blastocystis* spp. kontuan hartu beharreko faktorea dela.

OTHER SCIENTIFIC CONTRIBUTIONS

Cabodevilla, X., Moreno-Zarate, L., Arroyo, B., 2018. Differences in wing morphology between juvenile and adult European Turtle Doves *Streptopelia turtur*: implications for migration and predator escape. ***Ibis*** 160(2), 458–463.

Cabodevilla, X., Aguirre, J.I., 2019. The Nesting Synchronisation Process of New Breeders in a White Stork *Ciconia ciconia* Colony. ***Ardeola*** 66(2), 279–289.

Cabodevilla, X. *et al.*, 2020. Age-Related Variation in Wing Shape Differs between Bird Orders: Implications for Interpretation of the Pointedness Index (C2 Axis) in a Size-Constrained Principal Component Analysis (SCCA). ***Ardeola*** 67(2), 341–354.

Campión, D., Elosegi, M.M., **Cabodevilla, X.**, Villanúa, D., 2020. A method for capturing and GPS tracking breeding White-backed Woodpeckers *Dendrocopos leucotos*. ***Bird Study*** 67(2), 239–244.

BESTE EKARPEN ZIENTIFIKO BATZUK

Cabodevilla, X., Moreno-Zarate, L., Arroyo, B., 2018. Differences in wing morphology between juvenile and adult European Turtle Doves *Streptopelia turtur*: implications for migration and predator escape. ***Ibis*** 160(2), 458–463.

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Research contents:

Paper I: Irrigation drives declines in farmland bird communities

Paper II: Vineyard modernization drives changes in bird and mammal occurrence in vineyard plots in dry farmland

Paper III: Farmland composition and farming practices explain spatio-temporal variations in red-legged partridge density in central Spain

Paper IV: Are population changes of endangered little bustards associated with releases of red-legged partridges for hunting? A large-scale study from central Spain

Paper V: Simultaneous analysis of the intestinal parasites and diet through eDNA metabarcoding

Paper VI: Metabarcoding insights onto the diet and trophic diversity of six declining farmland birds

Paper VII: A semi-quantitative approximation to *Blastocystis* spp. infections in farmland birds: very high parasitic load in protected Bustards.

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