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AVIAN SCAVENGERS IN A CHANGING WORLD:
a multidisciplinary conservation approach in the Pyrenees

Pilar Oliva Vidal

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AUS CARRONYAIRES EN UN MÓN CANVIANT:
una aproximació multidisciplinària per a la seva conservació als Pirineus

Pilar Oliva Vidal

Dissertation presented in fulfilment of the requirements for the PhD degree
in Forest and Natural Environment Management.

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Mª Àngels Colomer

Tutor:
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"Una manera de hacer Europa"

All good things are wild and free

Totes les coses bones són salvatges i lliures

Henry David Thoreau

Bearded vulture *Gypaetus barbatus* flying over the Aragonese Pyrenees (Huesca, Spain).
Photo: Pilar Oliva-Vidal

A la meva família

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ABBREVIATIONS AND ACRONYMS

ABPs	Animal by-products not intended for human consumption
AICc	Akaike Information Criterion corrected for small sample size
ΔAICc	Delta Akaike Information Criterion corrected for small sample size
AR	Anticoagulant rodenticide
ASCII	American Standard Code for Information Interchange
BSE	Bovine spongiform encephalopathy
CBD	Convention on Biological Diversity
CI	Confiance Interval
CMS	Convention on the Conservation of Migratory Species of Wild Animals
DARPA	*Departament d'Agricultura, Ramaderia, Pesca i Alimentació*
dMRM	Dynamic multiple reaction monitoring
EC	European Community
ECHA	European Chemical Agency
EDTA	Ethylenediamine tetraacetic acid
EEA	European Environment Acengy
EU	European Union
FGAR	First generation anticoagulant rodenticide
GLM	Generalized Linear Model
GPS	Geographic Position System
HNV	High Nature Value
HWC	Human wildlife conflict
IPBES	Intergovernmental science-policy Platform on Biodiversity and Ecosystem Services
IPM	Integrated Population Models
IUCN	International Union for Conservation of Nature

LC-MS/MS	Liquid chromatography tandem-mass spectrometry
LOQ	Limit of quantification
m a.s.l.	Meters above see level
NSAIDs	Non-steroidal anti-inflammatory drugs
NvCJD	New variant Creutzfeldt-Jakob disease
PA	Protected Area
PAFs	Protection areas for the feeding on necrophagous species of European interest
PDP	Population Dynamic P System
RLI	Red List Index
RSD	Relative standard deviation
RSM	Response Surface Methodology
SE	Standard Error
SFS	Supplementary Feeding Station
SGAR	Second generation anticoagulant rodenticide
$t_{1/2}$	Half-life
UTM	Universal Transverse Mercator
vCJD	Variant Creutzfeldt-Jakob disease
VPC	Veterinary Pharmaceutical Committee
WWF	World Wildlife Fund
ZPAEN	*Zonas de protección para la alimentación de especies necrófagas de interés comunitario*

ABSTRACT

Avian scavengers provide essential ecological, economic and cultural services; yet vultures are among the most threatened groups of birds worldwide and their populations have declined significantly in recent decades. An exception are Iberian vultures whose numbers are stable or even increasing and which play a key role in the future viability of European vulture populations (griffon *Gyps fulvus*, cinereous *Aegypius monachus*, bearded *Gypaetus barbatus* and Egyptian *Neophron percnopterus*). Behind this global collapse lie a wide array of anthropogenic factors, which are accentuating this decline in our human-dominated world. Thus, integrative research linking different disciplines is essential for providing useful insights into the management and conservation of vulture populations in order to design and propose effective management actions.

This thesis combines aspects of different disciplines including behavioural ecology, conservation biology, ecotoxicology and the social sciences as a means of fomenting the scientific understanding of significant emerging threats affecting European avian scavengers and providing a set of evidence-based conservation tools. Within this multidisciplinary framework, this thesis first explores how widespread rural abandonment and subsequent passive rewilding due to woody encroachment affect the functioning of scavenger assemblages in mountain agroecosystems (Chapter 1). This chapter provides evidence that landscape type is the main factor governing scavenging dynamics in rewilding situations through its influence on the composition of scavenger assemblages and their scavenging efficiency. While open landscapes favour faster carrion discovery and exploitation by obligate scavengers and attract more scavengers, in shrublands and, particularly, in forests mammals are the dominant scavengers. Thus, woody encroachment after farmland abandonment could reduce the scavenging efficiency of the assemblage and jeopardize the most abundant and efficient scavenger – that provides crucial scavenging services and facilitatory processes (i.e., the griffon vulture) – while benefitting facultative species, above all mammals. This thesis also demonstrates that, despite the vital regulating service played by griffon vultures in reducing carcass persistence and maintaining healthy ecosystems due to their high carcass consumption rates (Chapter 1), the growing conflict between vultures and livestock could lead to a breakdown in the age-old mutualistic relationship between vultures and humans (Chapter 2). Our findings show that the vulture-livestock conflict is a complex issue accentuated by the typically wide-ranging foraging movements of griffon vultures and the growing amount

of 'fake news' and media misrepresentation that lays the blame for livestock killing on vultures. Mitigation in areas with high extensive livestock numbers – especially close to landfill sites and, above all, during the birthing season – and interdisciplinary awareness campaigns on the coexistence of vultures and livestock are necessary to guarantee the harmony between biodiversity conservation and agropastoral practices (Chapter 2). From an ecotoxicological perspective, this thesis investigates through active monitoring the risk of exposure to second-generation anticoagulant rodenticides in free-living scavenger birds (Chapter 3). Medium-sized avian scavengers such as kites and Egyptian vultures are the species that are most exposed to these pesticides, although of the large obligate avian scavengers bearded vultures showed the highest prevalence. These findings are the first step towards the recognition of the impact of these compounds on scavenging birds and their potential population effects need further investigation. From an ecological modelling angle, our results demonstrate the importance of traditional farming practices of transhumance given its role in increasing carrion availability in mountain landscapes (Chapter 4). However, the seasonal nature of this practice means that the quantitative assessments of food availability must also estimate trophic resources over the whole annual cycle since we found significant seasonal asymmetries. Animal biomass is substantially higher in summer due to transhumance but drops in winter to levels that are unable to satisfy the energetic requirements for the most abundant species (i.e., griffon vultures). The last chapter of this thesis aims to encourage policy managers to adopt strict and effective translocation protocols for restoring European bearded vulture populations without affecting the source Pyrenean population (Chapter 5). Our results show that the extraction of whole clutches and non-territorial adults (≥ 10 years) is preferable to the removal of juveniles since the removal of this age-class from the wild has significant demographic consequences on the source population. Additionally, we highlight how stochastic variations in demographic parameters (e.g., productivity and survival) can substantially change forecasted results and have significant repercussions on conservation outcomes. Thus, it is vital to update the theoretical ecological models used to implement management actions to increase their credibility, efficiency and objectivity. This thesis underlines, above all, the usefulness of developing multidisciplinary approaches aimed at improving and optimizing conservation management actions from an evidence-based perspective. This will help establish priority conservation lines and ensure the coexistence of avian scavengers and humans in an ever-changing world.

RESUM

Les aus carronyaires proporcionen serveis ecològics, econòmics i culturals essencials. No obstant, dins d'aquest gremi, els voltors es troben entre els grups d'ocells més amenaçats a nivell mundial i les seves poblacions han disminuït notablement durant les darreres dècades. Una excepció a aquesta tendència són les poblacions ibèriques, les quals esdevenen estables o fins hi tot han augmentat i, per tant, juguen un paper clau per la futura viabilitat de les poblacions de voltors europeus (voltor comú *Gyps fulvus*, voltor negre *Aegypius monachus*, trencalòs *Gypaetus barbatus* i aufrany *Neophron percnopterus*). Diversos factors antropogènics són la causa d'aquest col·lapse global i podrien accentuar el declivi d'aquestes poblacions en un món dominat pels humans. En aquest context, una investigació integradora que vinculi diferents disciplines pot proporcionar informació valuosa per tal de dissenyar i proposar accions de maneig efectives.

Aquesta tesi combina aspectes de diferents disciplines com l'ecologia del comportament, la biologia de la conservació, l'ecotoxicologia i les ciències socials, per tal de fomentar el coneixement científic envers importants amenaces emergents que afecten a les aus carronyaires europees i proporcionar un conjunt d'eines de conservació basades en l'evidència. En el context d'aquest marc multidisciplinari, aquesta tesi explora en primer terme com l'abandonament rural i el conseqüent canvi en la configuració del paisatge a través de l'expansió natural d'arbustos i boscos ('passive rewilding') afecta el funcionament de les comunitats de carronyaires en agroecosistemes de muntanya (Capítol 1). Aquest capítol evidencia que el tipus de paisatge és el principal factor que governa la dinàmica de consum de carronya a l'influir en la composició i eficiència del gremi de carronyaires. Si bé els paisatges oberts afavoreixen una detecció i explotació més ràpida de les carronyes degut a l'eficient consum per espècies obligades (i.e., voltors) i atrauen un major nombre de carronyaires, en paisatges arbustius i especialment en boscos, els mamífers són els carronyaires dominants. Per tant, els processos de revegetació natural – primer per arbustos i després per boscos – posterior a l'abandonament de terres de cultiu i de pràctiques tradicionals de pasturatge, pot reduir l'eficiència d'aquest gremi i perjudicar l'espècie més eficient en termes de consum i en proporcionar processos tròfics facilitadors (i.e., voltor comú) i al mateix temps, beneficiar espècies facultatives, particularment mamífers. Aquesta tesi també demostra que, malgrat el servei regulador que proporciona el voltor comú en reduir la persistència de les carronyes i, per tant, en mantenir ecosistemes saludables degut a les seves elevades taxes de consum (Capítol 1), el creixent conflicte entre voltors i bestiar podria perjudicar la relació mutualista mil·lenària

entre voltors i humans (Capítol 2). Els nostres resultats evidencien que les interaccions entre voltors i bestiar representen un problema complex d'abordar, en gran mesura degut als habituals grans moviments de campeig dels voltors així com a l'elevat nombre de 'fake news' i tergiversació per part dels mitjans de comunicació que atribueixen la mort de bestiar als atacs de voltors. Per harmonitzar la conservació de la biodiversitat i les pràctiques agropastorals són necessàries mesures de mitigació en zones amb una elevada densitat de bestiar extensiu – especialment les properes a abocadors – essent el període més crític l'època de parts. Paral·lelament, és necessari fomentar campanyes interdisciplinàries de sensibilització sobre la coexistència entre voltors i bestiar (Capítol 2). Des d'una perspectiva ecotoxicològica, aquesta tesi investiga l'exposició a rodenticides anticoagulants de segona generació en aus carronyaires a través d'un monitoreig actiu d'individus capturats i mostrejats al niu (Capítol 3). Les espècies de mida mitjana, com els milans i els aufranys, han estat les més exposades a aquests pesticides, tot i que entre les espècies carronyaires obligades de major grandària el trencalòs ha estat l'espècie amb una major prevalença. Aquest estudi representa el primer pas per reconèixer l'impacte d'aquests compostos en aus carronyaires i els potencials efectes poblacionals requereixen més investigació. Des d'una perspectiva de modelatge ecològic, els nostres resultats demostren la importància de les pràctiques tradicionals de transhumància pel seu paper en incrementar la disponibilitat de carronya en paisatges de muntanya (Capítol 4). No obstant això, degut a la naturalesa estacional d'aquesta pràctica, les estimacions quantitatives de disponibilitat de recursos tròfics han de considerar tot el cicle anual ja que els nostres resultats evidencien asimetries estacionals significatives en la disponibilitat d'aliment. La biomassa animal fou substancialment més elevada a l'estiu degut a la presència de ramats transhumants però baixa fins a nivells que no satisfeien els requeriments energètics del voltor comú – l'espècie més abundant – durant l'hivern. El darrer capítol d'aquesta tesi té com a finalitat proporcionar als gestors protocols de translocació estrictes i eficaços per projectes de reintroducció de poblacions de trencalòs, sense afectar demogràficament a la població d'origen pirinenca (Capítol 5). Els nostres resultats mostren que l'extracció de postes i adults no territorials (≥ 10 anys) és preferible a l'extracció d'individus juvenils ja que l'efecte d'extreure aquesta classe d'edat de la població donant és molt significatiu. A més, variacions estocàstiques en paràmetres demogràfics (per exemple, en la productivitat i la supervivència) poden canviar notablement els resultats obtinguts i repercutir negativament en la conservació d'aquesta espècie. Per tant, és fonamental actualitzar els models ecològics utilitzats per tal d'implementar accions de gestió per incrementar la credibilitat, l'eficiència i l'objectivitat. Aquesta tesi emfatitza en la necessitat de les aproximacions multidisciplinàries per millorar i optimitzar les accions de conservació des d'una perspectiva basada en l'evidència, i per establir línies de conservació prioritàries que preservin la coexistència de les aus carronyaires i els humans en un món en constant canvi.

RESUMEN

Las aves carroñeras proporcionan servicios ecológicos, económicos y culturales esenciales. No obstante, dentro de este gremio, los buitres se encuentran entre los grupos de aves más amenazados a nivel mundial, como demuestra el gran declive sufrido por algunas de sus poblaciones durante las últimas décadas. Una excepción a esta tendencia son los buitres ibéricos, cuyas poblaciones permanecen estables e incluso han aumentado, y juegan un papel clave para la futura viabilidad de las poblaciones de buitres europeos (buitre leonado *Gyps fulvus*, buitre negro *Aegypius monachus*, quebrantahuesos *Gypaetus barbatus* y alimoche *Neophron percnopterus*). Diversos factores antropogénicos son la causa de este colapso global y podrían acentuar el declive de estas poblaciones en un mundo dominado por el ser humano. En este contexto, una investigación integradora que vincule distintas disciplinas puede proporcionar la información necesaria para diseñar y proponer acciones de manejo más efectivas.

Esta tesis combina aspectos de distintas disciplinas como la ecología del comportamiento, la biología de la conservación, la ecotoxicología y las ciencias sociales para fomentar el conocimiento científico acerca de importantes amenazas emergentes que afectan a las aves carroñeras europeas, con objeto de proporcionar un conjunto de herramientas de conservación basadas en la evidencia. En el contexto de este marco multidisciplinario, esta tesis en primer lugar explora como el abandono rural y el consecuente cambio en la configuración del paisaje a través de la expansión natural de matorrales y bosques ('passive rewilding') afecta al funcionamiento de las comunidades de carroñeros en agroecosistemas de montaña (Capítulo 1). Este capítulo evidencia que el tipo de paisaje es el principal factor que gobierna la dinámica de consumo de carroña al influir en la composición y eficiencia de la comunidad de carroñeros. Mientas que los paisajes abiertos favorecen una detección y explotación más rápida de las carroñas debido al eficiente consumo por parte de especies obligadas (i.e., buitres) y atraen mayores abundancias de carroñeros, en paisajes de matorral y, especialmente en bosques, los mamíferos son los carroñeros dominantes. Por tanto, los procesos de sucesión natural – primero por matorrales y después por bosques – después del abandono de tierras de cultivo y de prácticas tradicionales de pastoreo, pueden reducir la eficiencia de este gremio y perjudicar a la especie más eficiente en términos de consumo y de proporcionar procesos facilitadores tróficos (i.e., buitre

leonado) y favorecer a especies facultativas, particularmente mamíferos. Esta tesis también demuestra que, a pesar del importante servicio regulador que prestan los buitres leonados en eliminar la carroña y reducir su persistencia contribuyendo de esta manera a mantener ecosistemas saludables (Capítulo 1), el conflicto entre buitres y ganado puede perjudicar a la relación mutualista milenaria entre buitres y humanos (Capítulo 2). Nuestros resultados muestran que las interacciones entre buitres y ganado representan un problema complejo de abordar, en gran medida debido a los frecuentes y grandes movimientos de campeo de los buitres, así como al elevado número de 'fake news' y de tergiversación por parte de los medios de comunicación que atribuyen la muerte de ganado a los ataques de buitres. Para armonizar la conservación de la biodiversidad y las actividades agropastorales son necesarias medidas de mitigación en zonas con una elevada densidad de ganado extensivo – especialmente las cercanas a vertederos – siendo el período más crítico la época de partos. Además, resulta necesario fomentar campañas interdisciplinarias de sensibilización sobre la coexistencia entre buitres y ganado (Capítulo 2). Desde una perspectiva ecotoxicológica, esta tesis investiga la exposición a rodenticidas anticoagulantes de segunda generación en aves carroñeras a través de un monitoreo activo de individuos capturados y muestreados en el nido (Capítulo 3). Las especies de tamaño medio como los milanos y alimoches, han sido las más expuestas a estos pesticidas mientras que entre las especies de mayor tamaño, lo ha sido el quebrantahuesos. Este estudio representa el primer paso para reconocer el impacto de estos compuestos en aves carroñeras y sus efectos potenciales a nivel poblacional requieren una mayor investigación. Desde una perspectiva de modelado ecológico, nuestros resultados demuestran la importancia de las prácticas tradicionales de trashumancia por el papel que juegan en el incremento de la disponibilidad de carroña en paisajes de montaña (Capítulo 4). No obstante, debido a la naturaleza estacional de esta práctica, las estimaciones cuantitativas de disponibilidad de recursos tróficos tienen que considerar todo el ciclo anual completo ya que nuestros resultados evidencian asimetrías estacionales significativas en la disponibilidad de alimento. La biomasa animal disponible fue substancialmente más elevada en verano debido a la presencia de rebaños trashumantes, pero desciende hasta niveles que no llegan a cubrir los requerimientos energéticos del buitre leonado – la especie más abundante – durante el invierno. El último capítulo de esta tesis tiene como finalidad proporcionar a los gestores protocolos de translocación estrictos y eficaces para proyectos de reintroducción de la población de quebrantahuesos, sin afectar demográficamente a la población de origen pirenaica (Capítulo 5). Nuestros resultados muestran que la extracción de puestas y adultos no territoriales (≥ 10 años) es preferible a la de individuos juveniles, ya que el efecto que tiene la extracción de esta clase de edad en la población donante es muy significativo. Sin embargo, remarcamos que variaciones estocásticas en algunos parámetros demográficos (por ejemplo, en la productividad y la supervivencia) pueden cambiar sustancialmente los resultados obtenidos y por tanto repercutir negativamente en la conservación de esta especie. Por ello, resulta fundamental actualizar los modelos ecológicos utilizados para implementar acciones de gestión que incrementen la credibilidad, eficiencia y objetividad. Esta tesis enfatiza la necesidad de desarrollar enfoques multidisciplinarios para mejorar

y optimizar las acciones de conservación desde una perspectiva basada en la evidencia y, con ello, establecer líneas de conservación prioritarias que preserven la coexistencia de las aves carroñeras y los humanos en un mundo que se encuentra en constante cambio.

GENERAL INTRODUCTION

Little owl *Athene noctua* in the Balaguer steppes, Lleida, Catalonia (Spain)
Photo: Pilar Oliva-Vidal

Biodiversity loss in a changing human-dominated world

The loss of biodiversity in the current human-dominated world (i.e., the 'Anthropocene Epoch' see Vitousek, 1997; Corlett, 2015; Lewis and Maslin, 2015) is one of the most critical environmental challenges on the conservation agenda. There is growing evidence to suggest that recent extinction rates are 100–1000 times greater than their pre-human levels (Pimm et al., 1995, 2014; Pereira et al., 2010; Dirzo et al., 2014). Moreover, habitat loss and fragmentation, one of the major causes of species' declines, is taking place over an extraordinarily short timescale (Novacek and Cleland, 2001). This unprecedented global biodiversity crisis during the past few centuries has led scientists to suggest that a sixth 'mass extinction' is currently already underway (Barnosky et al., 2011; Ceballos et al., 2015).

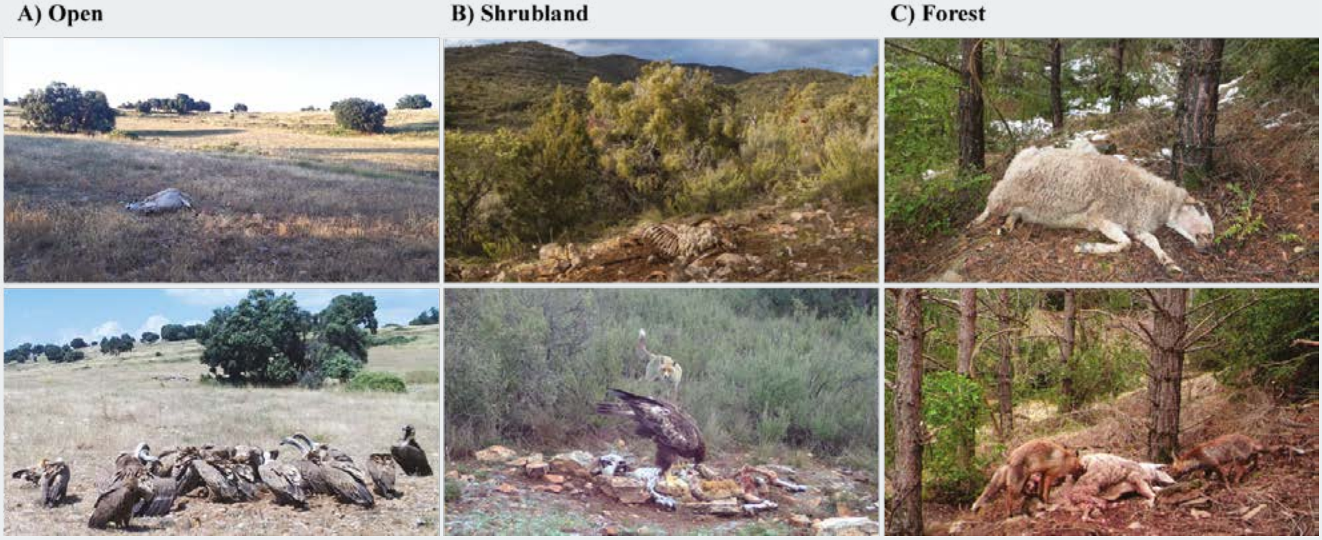
The human population is projected to reach ~10 billion in the twenty-first century (Cohen, 2003), thereby increasing demands for resources and putting ever more pressure on the environment, and the extraction of wild resources, proliferation of infrastructures and conversion of wild habitats into productive land uses will all become forces of global importance (Foley, 2005; Kareiva et al., 2007). At least 75% of the world's terrestrial land surface area has been exposed to land-use/cover change (Venter et al., 2016) and only 23.2% can still be classified as wilderness (Watson et al., 2016) that acts as a buffer against species loss (e.g., areas with no or only minimal levels of human disturbance). Although the extinction risk in wilderness areas is less than half that of non-wilderness regions, such areas are still poorly protected and require further urgent protection (Di Marco et al., 2019). Anthropogenic pressures such as habitat loss/conversion (e.g., intensive agriculture, logging and farmland abandonment; see Box 1), overexploitation, pollution, invasive species and climate change are the main drivers of the current biodiversity crisis (Sala et al., 2000; Godet and Devictor, 2018; IPBES, 2019; Harfoot et al., 2021) and are predicted to accelerate in coming years, thereby perpetuating defaunation processes (i.e., loss or depletion of species from ecological communities, Dirzo et al., 2014) and ecosystem degradation (Jenkins, 2003; Johnson et al., 2017). Furthermore, human activities increase the presence of diverse anthropogenic 'contaminants of emerging concern' including pharmaceutical residues (Ben et al., 2019; Sathishkumar et al., 2020; see Box 2), pesticides/biocides and heavy metals, which are ubiquitous in the environment and are therefore likely to lead to the decline of species affecting ecosystems and human health (Zala and Penn, 2004; Yadav et al., 2021).

BOX 1

Rural landscape transformation: ecological consequences within a scavenging context

Farmland abandonment is one of the most important land-use/cover change of global concern due to the role that agropastoral landscapes play for biodiversity and ecosystem service maintenance (Sala, 2000; Cramer et al., 2008). In Europe, rural population has declined by 17% since 1961 and in the period 2000–2030 at least 20 million ha may be end up being released from agricultural use (Pereira and Navarro, 2015). In rural areas, farmland abandonment entails processes of shrub and forest encroachment with no human control (i.e., 'passive rewilding') involving cascading ecological and landscape effects with some 'loser' and 'winner' species (Pereira and Navarro, 2015). For example, passive rewilding is expected to favour the expansion (and abundance) of wild ungulates and promote the recovery of large predators that historically have been subject to persecution (Falcucci et al., 2006; Acevedo et al., 2011; Martínez-Abraín et al., 2020). Within a scavenging context, although this scenario may result in a greater availability of carrion in such areas, the closure of landscapes may have negative consequences on scavengers adapted to traditional agropastoral landscapes that rely on visual cues when foraging (e.g., avian scavengers, Ruxton and Houston, 2004; Potier et al., 2016). Conversely, vegetation cover may favour scavengers such as mammals that mainly use odour cues to locate food (DeVault and Rhodes, 2002). Moreover, because facultative scavengers do not functionally replace the role of vultures in terms of scavenging, carrion in rewilding landscapes may persist longer and have ecological and sanitary consequences (Markandya et al., 2008; Ogada et al., 2012b; Buechley and Şekercioğlu, 2016).

In the Pyrenees, a region that is home to all four European vultures, as well as important populations of facultative avian and mammal scavengers, 'landscape closure' due to rural and farmland abandonment is common and widespread. The following photographs show the main taxonomic groups exploiting sheep carcasses in three landscapes (open, shrubland and forest) in the Pyrenees where passive rewilding is taking place. Overall, open landscapes promote faster carrion detection and consumption by obligate avian scavengers and attract higher scavenger abundances, possibly due to the trophic facilitatory processes that griffon vultures provide to other scavengers, while in shrublands and forests mammals dominated at carcasses. Picture 'A' shows a group of griffon, a cinereous and an Egyptian vultures feeding on a carcass in an open landscape. Increased vegetation cover (e.g., shrublands) may difficult the rate and speed at which carrion is located by vultures, favouring its exploitation by facultative scavengers (e.g., mammals, raptors and corvids; picture 'B': a golden eagle and a red fox consuming a carcass). Finally, carrion in dense tree cover (e.g., forests), is mainly exploited by mammals (picture 'C': two red foxes consuming a sheep carcass in a Scots pine forest).



In terms of scavenging efficiency (e.g., the ability of scavengers to locate and consume carrion), both carcass detection and consumption times (h) are mediated by landscape characteristics: open < shrulands < forests; birds are faster detectors than mammals in all landscapes. Increased vegetation cover may jeopardize the most abundant and efficient scavengers (i.e., vultures) while favouring less efficient species (i.e., facultative scavengers). Thus, passive rewilding after farmland abandonment is a major factor governing carrion consumption dynamics and shapes the structure and functioning of scavenger assemblages. One of the multiple ecological consequences of such scenario is related to carcass persistence, which increases – on average – up to 4.5 times when carcasses are exploited only by facultative scavengers (80 vs. 362 h with and without griffon vultures, respectively) since consumption rates can increase up to 125 times when vultures are among the consumers. See more details in Chapter 1.

At a global level, at least 28% of the 142,500 species listed on the International Union for Conservation of Nature (IUCN) Red List are threatened with extinction; these include among vertebrates 41% of all amphibians, 26% of all mammals and 13% of all birds (IUCN, 2021). Between 1970 and 2014 anthropogenic pressure caused a 60% decline in the population sizes of vertebrates worldwide (WWF, 2018), and local declines in the abundance of individuals are also frequent and widespread. However, defaunation often remains a largely cryptic phenomenon that can occur rapidly – even in large protected habitats and in species not close to extinction (Craigie et al., 2010; Dirzo et al., 2014) – and be the prelude to species-level extinction (Ceballos and Ehrlich, 2002). A good recent example of a case with an interesting spatial and temporal perspective is that of the population of Iberian ibex *Capra pyrenaica pyrenaica*, endemic to the Iberian Peninsula, that fell drastically to less than 50 individuals in the mid-twentieth century and became extinct in 2000 (Acevedo and Cassinello, 2009). Population-level declines/extirpations and changes in the composition of species in communities can result in the loss of ecological interactions, which may occur before species actually disappear and at higher rates (Valiente-Banuet et al., 2015), and affect the dynamics of populations, communities and ecosystems. For instance, the sudden collapse of vulture populations in South Asia 25 years ago caused by the ingestion of domestic livestock carcasses treated with diclofenac – a non-steroidal anti-inflammatory (NSAID) drug (Green et al., 2004; Oaks et al., 2004; see Box 2) – increased the presence of dogs (*Canis familiaris*) and rats (*Rattus* spp.) at carcasses, resulting in more cases of rabies and leptospirosis in humans and other associated health, economic and ecosystem costs (Markandaya et al., 2008).

Several international initiatives have attempted to coordinate mitigation actions. In 2002, with the signature of the Convention on Biological Diversity (CBD) world leaders committed themselves to achieving by 2010 a significant reduction in the rate of biodiversity loss. However, despite some local successes (e.g., extent of protected areas 'PAs', sustainable forest management and policy responses), the rate of biodiversity loss is not slowing (Butchart et al., 2010; Godet and Devictor, 2018). To objectively quantify progress toward biodiversity conservation, the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) aimed to "provide policy-makers with objective scientific assessments about the state of knowledge regarding the planet's biodiversity, ecosystem and the contributions they make to people" (IPBES, 2019). Therefore, multidisciplinary approaches are urgently needed to better understand and to assess the multifaceted nature of human pressure on biodiversity in order to improve conservation actions from an evidence-based perspective.

Vultures in the Anthropocene: a growing conservation challenge

The status of the world's birds has continued to decline since the first assessment in 1988 (IUCN Red List). While highly threatened species continue to go extinct, many of those that were formerly common and widespread are today in sharp decline. Indeed, although most threatened species have small populations and ranges, certain widespread and numerous species increasingly face extinction. At a global level, ~40% of bird species have declining populations, ~44% remain stable and only ~7% are increasing (Birdlife, 2018). Furthermore, some projections indicate that by 2100, 6–14% of all bird species will be extinct and that 7–25% will be functionally extinct, which will entail a decline in important ecosystem processes such as decomposition, pollination and seed dispersal (Şekercioğlu et al., 2004). This loss of species and greater extinction risk is non-random (Wardle et al., 2011; Dirzo et al., 2014). In birds, species with larger body sizes, low fecundity and high specialization are more likely to be endangered (as occurs in vultures) since their populations are more threatened with extinction if external forces increase their mortality rates (Bennet and Owens, 1997; Sekercioglu et al., 2004). Therefore, threatened species are not evenly distributed among groups and there is a higher proportion of threatened species amongst cranes (73%), Old World vultures (68%), albatrosses (68%) and parrots (29%) (Birdlife, 2018). Of the vultures, Old World species are much more threatened than all birds in general and their populations are declining at a significantly faster rate (McClure et al., 2018; Safford et al., 2019; Fig. 1).

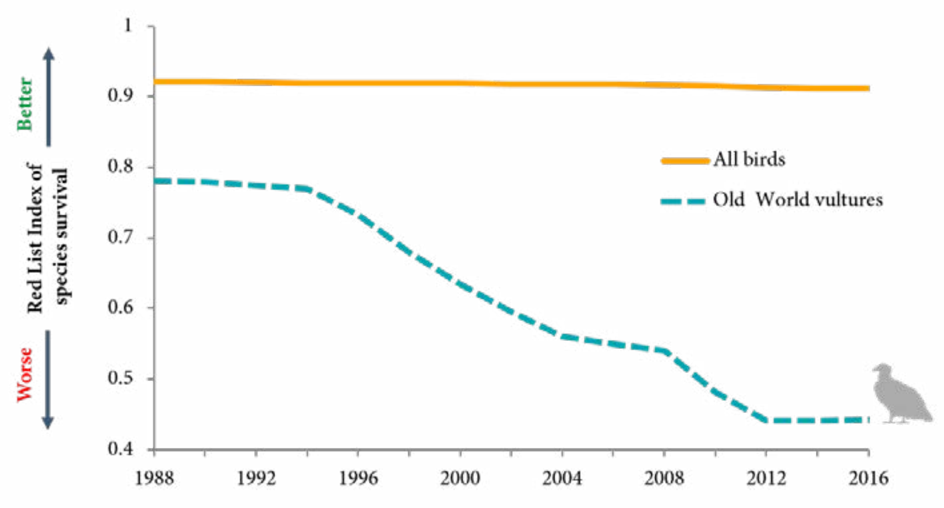


Figure 1. Red List Index (RLI) for Old World vultures and for all birds. A value of 1 equates to all species being categorized as 'Least Concern' and hence that none is expected to go extinct in the near future; a value of 0 would indicate that all species have gone extinct. Modified from BirdLife International (2018).

Of the world's 23 vulture species, the populations of only 13% are on the increase, while 13% remain stable (Table 1). Across Africa, Asia and some parts of Europe, populations are in freefall (e.g., 11 of the 16 are currently listed as globally threatened) and have declined above all in recent years (IUCN Red List; Ogada et al., 2012a). For instance, in 1994, 75% of the 16 Old World vultures were classified as of 'Least Concern' (i.e., not considered to be at risk of extinction) and only the Cape Vulture *Gyps coprotheres* was reported to be globally threatened and classified as 'Vulnerable'. However, today, the picture is completely different and just two species remain of 'Least Concern' while eight (50%) are 'Critically Endangered' and are at risk of imminent extinction due to direct and indirect human pressure (Table 1). For New World vultures, except for the California and Andean condors, information is scarce and the current population trends of some species are unknown.

Table 1. Current continental distribution ranges, conservation status and population trends of the world's 23 vulture species according to the IUCN Red List. The two different orders (Old World vultures in Accipitriformes and New World vultures and condors in Cathartiformes) are shown separately. Modified from Safford et al. (2019).

Species	Range	Global Threat Level[1]	Population trend[1]
Bearded vulture *Gypaetus barbatus*	Europe, Asia, Africa	NT	↘
Cape vulture *Gyps coprotheres*	Africa	EN	↘
Cinereous vulture *Aegypius monachus*	Europe, Asia, (Africa)[2]	NT	↘
Egyptian vulture *Neophron percnopterus*	Europe, Asia, Africa	EN	↘
Griffon vulture *Gyps fulvus*	Europe, Asia, Africa	LC	↗
Himalayan griffon *Gyps himalayensis*	Asia	NT	↘
Hooded vulture *Necrosyrtes monachus*	Africa	CR	↘
Indian vulture *Gyps indicus*	Asia	CR	↘
Lappet-faced vulture *Torgos tracheliotos*	Africa, Asia	EN	↘
Red-headed vulture *Sarcogyps calvus*	Asia	CR	↘
Palm-nut vulture *Gypohierax angolensis*	Africa	LC	↔
Rüppell's vulture *Gyps rueppelli*	Africa, (Europe)[2]	CR	↘
Slender-billed vulture *Gyps tenuirostris*	Asia	CR	↘
White-backed vulture *Gyps africanus*	Africa, (Europe)[2]	CR	↘
White-headed vulture *Trigonoceps occipitalis*	Africa	CR	↘
White-rumped vulture *Gyps bengalensis*	Asia	CR	↘
Andean condor *Vultur gryphus*	South America	VU	↘
Black vulture *Coragyps atratus*	Central and South America	LC	↗
California condor *Gymnogyps californianus*	North America	CR	↗
Greater yellow-headed vulture *Cathartes melambrotus*	South America	LC	↘
King vulture *Sarcoramphus papa*	Central and South America	LC	↘
Lesser yellow-headed vulture *Cathartes burrovianus*	Central and South America	LC	↔
Turkey vulture *Cathartes aura*	North and South America	LC	↔

[1] CR = Critically Endangered; EN = Endangered; NT = Near Threatened; LC = Least Concern; VU = Vulnerable

[2] Cinereous vulture occurs in small numbers in N and W Africa; Rüppell's and White-backed vultures similarly in Europe.

Although some vulture populations in Europe have stabilized or even increased thanks to conservation actions, at global level anthropogenic pressures persist (Safford et al., 2019). Currently, the major threats to vulture populations – the illegal use of poisons, the ingestion of veterinary drugs and other toxic substances (e.g., pesticides) present in human-dominated environments, lead contamination, landscape transformation (e.g., habitat loss and the increase of energy infrastructures such as wind farms) and changes in health policies – are all continuing to grow (Margalida and Ogada, 2018). In addition, in recent times human-vulture cohabitation has been negatively influenced by 'fake news' about the aggressive behaviour of vultures towards livestock that are jeopardizing conservation efforts for Old and New World vultures (Lambertucci et al., 2021).

Poisoning

Avian scavengers in general and vultures in particular are major victims of deliberate poisoning, either intentionally (e.g., belief-based use or to eliminate sentinel species) or unintentionally (e.g., secondary poisoning due to the ingestion of bait targeting livestock predators). Indeed, poisoning is a major threat affecting both Old World (McClure et al., 2018; Margalida and Mateo, 2019; Fig. 2) and New World (Pauli et al., 2018; Plaza et al., 2019; Plaza and Lambertucci, 2021) vultures but remains particularly critical in Asia and Africa (Ogada et al., 2012a; Pfeiffer et al., 2015; Santangeli et al., 2016; Margalida et al., 2019). In Africa, populations of eight species have declined on average by 62% during the past decades mainly due to poisoning and trade in traditional medicines, which together accounted for ~90% of reported deaths (Ogada et al., 2016a).

The behavioural strategies of vultures – notably the accumulation of large numbers of birds at carcasses – often result in catastrophically large numbers of victims in a single poisoning event. Indeed, large-scale mortality events have been frequent in past decades, including several that occurred in Africa. In a shorter timescale (from late 2017 to September 2019), >1,300 vultures were poisoned in southern Africa (Mozambique, South Africa, Botswana, Zambia, Namibia, Tanzania and Kenya) (Margalida et al., 2019; Safford et al., 2019). The victims of this poisoning event included 'Critically Endangered' vulture species as well as mammals such as lions *Panthera leo*. Most events were related to secondary poisoning due to retaliation for carnivore predation on livestock (an example of human-wildlife conflicts 'HWC') or to poachers lacing African elephants *Loxodonta africana* carcasses with large quantities of toxic pesticides to prevent circling vultures from revealing their illicit activities to the authorities (i.e., to foil their function as sentinels). Some body parts were found to be missing from the vultures and lions corpses, suggesting that the trade in body parts for belief-based use (e.g., traditional medicine) is a still major threat to vulture populations (Buij et al., 2016). In fact, > 2000 'Critically Endangered' hooded vultures were killed across eastern Guinea-Bissau (home to ~22% of the world's 197,000 hooded vultures) in September 2019–March 2020 due to intentional poisoning with methiocarb, a carbamate pesticide banned in Europe but still used in this country, to collect vulture heads for belief-based use (Henriques et al., 2020). More recently, in June

2021 nearly 100 birds including griffon and cinereous vultures and black kites were poisoned in Spain after ingesting carbofuran, one of the most toxic of all carbamate pesticides, which was banned in Europe in December 2007. In fact, highly toxic carbamates (e.g., carbofuran and aldicarb) and even strychnine are commonly still used, even though many such products have been banned, particularly in the EU (Berny et al., 2015; Ruiz-Suárez et al., 2015). In Spain alone, in 1992–2017 at least 21,260 animals died after feeding on poisoned bait, the most affected bird being the griffon vulture (1,757 individuals), although 624 cinereous, 325 Egyptian and 48 bearded vultures were also poisoned during this period (de la Bodega et al., 2020). The latest mass poisoning event occurred last March 2022 in Assam (India), when at least 100 Himalayan griffon vultures and a steppe eagle *Aquila nipalensis* were found dead and others sick close to baited goat and dog carcasses, suggesting the occurrence of another HWC-related event. Unfortunately, such events are common in this region and in just two months in January–February 2021 > 56 vultures including the 'Critically Endangered' slender-billed and white-rumped vultures were victims of deliberate poisoning.

Figure 2. Picture showing the propensity of vultures to consume poisoned baits: griffon, cinereous and Egyptian vultures ingesting a non-poisoned bait in the Pre-Pyrenees of Lleida during a field experiment (see Gil-Sánchez et al., 2021).

Veterinary pharmaceuticals: the unprecedented 'Asian Vulture Crisis' was not enough

Populations of three *Gyps* vultures (*G. bengalensis, G. indicus* and *G. tenuirostris*) endemic to South Asia – the former the most abundant large raptor in the world – declined by > 99% during the 1990s (Prakash, 1999; Prakash et al., 2003, 2007) and are still considered to be 'Critically Endangered' (Table 1). The cause of this so-called 'Asian Vulture Crisis' is well understood since in 2004 scientists demonstrated that the ingestion of the NSAID diclofenac was the main cause of this population-level crash (Oaks et al., 2004; see Box 2). The widespread veterinary use of this effective, low-cost product to treat pain, fever and inflammation in livestock meant that animals treated shortly before death became available to vultures. Since low residual levels of this drug remaining in carcasses are sufficient to cause acute renal failure and subsequent death (e.g., within 48h of being ingested; Swan et al., 2006a) the resulting scenario came as no surprise. It has been estimated that < 1% of carcasses containing lethal levels of diclofenac can cause populations to decline at ~50% per year (Green et al., 2004), clear evidence that low environmental levels of pharmaceutical drugs can have a disproportionately large impact on non-target species.

Diclofenac toxicity has been demonstrated in several vulture species (Oaks et al., 2004; Swan et al., 2006a, b; Naidoo et al., 2009; Herrero-Villar et al., 2021) as well as in the steppe eagle (Sharma et al., 2014). Several governments including India, Nepal and Pakistan (2006) and Bangladesh (2010) have banned its production while promoting the use of meloxicam, another NSAID that is non-lethal to *Gyps* vultures (Swan et al., 2006b; Swarup et al., 2007; Naidoo et al., 2008). Since 2010, diclofenac prevalence in cattle carcasses has fallen by 70% (Cuthbert et al., 2014) and population declines have slowed and, encouragingly, even reversed in some areas (Chaudhry et al., 2012; Prakash et al., 2012). However, large 'multi-dose' vials (e.g., 30 ml) authorized for human-use containing diclofenac were administered illegally to livestock (Bowden, 2015) in quantities that are sufficient to eradicate vulture populations (Green et al., 2006). Finally, large human-use 'multi-dose' vials were banned by the Indian government in July 2015 (Bowden, 2015).

Despite this unprecedented catastrophic scenario, in 2013 the Spanish government authorized the veterinary use of diclofenac in cattle, pigs and horses (Margalida et al., 2014a) and renewed its license in 2018 (Moreno-Opo et al., 2021). Taking into account the pivotal role of Spain, home of > 90% of European vulture populations as well as the entire population of the globally threatened Spanish imperial eagle *Aquila adalberti* and important numbers of red kites *Milvus milvus*, this decision seems – to say the least – to be inappropriate (Box 2). In Spain, several supplementary feeding stations (SFSs) – the so-called 'vulture restaurants' – have been set up as a conservation strategy for avian scavengers (Moreno-Opo et al., 2015). In these SFSs, livestock carcasses are often provided from intensive production. Diclofenac should only be used under veterinary supervision and should not to be given to animals that are likely to enter the natural food chain. However, a real risk remains that carcasses containing diclofenac and other veterinary pharmaceuticals be consumed by scavenging birds.

The livestock industry in Spain is an important sector, with at least 25 million pigs and 5.7 million cattle (Margalida et al., 2014b). Indeed, Casas-Díaz et al. (2016) found that griffon vultures were prone to access veterinary pharmaceuticals in livestock carcasses provided at SFSs. For example, in south-east Portugal, 29% of 87 samples of liver, muscle and kidney from seven goats and 25 sheep dumped in SFSs analyzed using a multi-residue method contained antibiotic residues (Gómez-Ramírez et al., 2018). According to these authors, antibiotics were more frequent in goats (42.9%) than in sheep (24.2%); oxytetracycline and trimethroprim were the most common antibiotics, the former in the highest concentrations. As well, of 228 pig and sheep carcasses analyzed in the Iberian Peninsula (Spain and Portugal), at least one (0.44%) was found to contain diclofenac residues and other NSAIDs such as flunixin (1.75%), ketoprofen and meloxicam (0.44%) were also detected (Herrero-Villar et al., 2020). These authors also analyzed 389 carcasses of avian scavengers including 306 griffon, 15 cinereous, 11 Egyptian and 12 bearded vultures, as well as 45 facultative scavenging birds, and detected NSAID residues in 3.6% – specifically, flunixin (1.03%) and meloxicam (2.57%) – of kidney and liver samples. Additionally, they found that flunixin was associated with visceral gout and/or kidney damage in 0.98% of the dead griffon vultures analyzed. In 2012, a griffon vulture corpse was found in an Andalucian (Spain) game hunting reserve with severe visceral gout and the liver and kidneys containing elevated flunixin levels, suggesting that the ingestion of this product was the cause of its death. This was the first reported mortality event from environmental exposure to an NSAID other than diclofenac in vultures (Zorrilla et al., 2015). In addition, ketoprofen and meloxicam were detected in unfertilized eggs from the nests of two bearded vultures at a breeding centre in southern Spain in 2014 and it is thought that the food provided was the most likely source of exposure (Zorrilla et al., 2018). More recently, in September 2020, a cinereous vulture fledgling was found dead in the nest in the Catalan Pre-Pyrenees (NE Spain) due to diclofenac intoxication (Herrero-Villar et al., 2021), the first case of diclofenac poisoning in Europe. Several NSAIDs such as flunixin, ketoprofen, caprofen, aceclofenac, nimesulide and ibuprofen have been reported to be toxic to vultures (Cuthbert et al., 2007, 2011; Taggart et al., 2009; Dama, 2014; Zorrilla et al., 2015; Galligan et al., 2016, 2022; Bowden, 2020), which highlights how insufficiently tested pharmaceutical drugs are a potential minefield for highly susceptible vulture populations. Thus, there is an urgent need to closely monitor free-living avian scavenger populations through multi-species blood sampling, and to study their population dynamics to identify possible regressive trends in demographic parameters and avoid dramatic scenarios like the Asian Vulture Crisis. Green et al. (2016) predicted that the potential rate of decline for the Spanish griffon vulture population provoked by diclofenac could be 0.9–7.7% per year. In light of this finding, in 2021 scientists called for an immediate ban on diclofenac in Europe to avoid its detrimental effects on vulture populations (Margalida et al., 2021).

In this context, we took blood samples from almost 350 Pyrenean obligate and facultative scavenging birds in November 2017–May 2022 to test for the presence of potential toxic compounds such as veterinary drug residues (Oliva-Vidal et al., in prep.). Our multi-species biomonitoring included 76 griffon, 19 cinereous, 95 bearded and 92 Egyptian vultures, 53 red and 8 black kites, and one golden and one Bonelli's eagle. In March 2022, we analyzed 240 samples of plasma in the reference Laboratory of Toxicology at the Institute for Game and Wildlife Research (IREC, Spain). None of the antibiotics tested for (sulfadiazine, trimethoprim, oxytetracycline, ciprofloxacin, sulfadimidine, tetracycline, enrofloxacin, erythromycin and nalidixic acid) were detected, suggesting that the prevalence of antibiotic residues in living avian scavengers in the Pyrenees is very low (Oliva-Vidal et al., in prep.). However, caution should be taken since several authors have found evidence of antibiotic residues in Spanish vultures. For example, Casas-Díaz et al. (2016) found quinolone residues in 65% of 106 griffon vultures analyzed, of which 15.1% had quantifiable amounts of enrofloxacin and 5.7% of ciprofloxacin, although at much lower levels than the concentrations used in the clinical treatment of scavengers and thus probably too low to cause intoxication. Additionally, 92% of nestling griffon and 100% of cinereous vultures have been reported to have been exposed to quinolones (Blanco et al., 2016, 2017). Furthermore, Gómez-Ramírez et al. (2020) analyzed 29 griffon vultures nestlings by applying a novel multi-residue method for quantifying levels of antibiotics and NSAIDs in avian scavengers and found that enrofloxacin (69%) and tolfenamic acid (20%) were among the most commonly detected compounds.

On the other hand, of all the NSAIDs tested for in our Pyrenean samples (ketoprofen, naproxen, meloxicam, flunixin, carprofen, suxibuzone, indomethacin, diclofenac, phenylbutazone and tolfenamic acid), residues of flunixin were detected in only five (n = 5; 7.04%) griffon vultures (Oliva-Vidal et al., in prep.). The mean concentration of this NSAID in plasma was 16.28 ng/ml (range: 15.0–21.0 ng/ml). This finding raises questions about the potential toxicity and lethal exposure of flunixin and other NSAIDs in avian scavengers. Of these five positive vultures, two were equipped with GPS devices when they were sampled in April 2019. We determined that both were Pyrenean breeding birds and neither showed any untypical behaviour (Oliva-Vidal et al., in prep.). However, this lack of effects could be related to the concentrations ingested by these birds since high concentrations in liver and kidneys have been suggested to cause mortality in griffon vultures (Zorrilla et al., 2015).

It is therefore necessary to identify vulture-safe drugs to reduce the use of those that are toxic for vultures. Recently, it has been demonstrated that tolfenamic acid in the concentrations encountered by wild birds is probably safe for *Gyps* vultures and so could be promoted as a good alternative to toxic NSAIDs (Chandramohan et al., 2022). Conversely, Galligan et al. (2022) found that the NSAID nimesulide, which is widely available and commonly used in Asia, has been found in dead vultures displaying signs of renal failure. These authors also found experimental evidence of the lethality of this NSAID in vultures and thus advocate an immediate ban on this drug throughout southern Asia. Finally, more monitoring programs evaluating the long-term impact of veterinary drugs on scavengers are still needed. This is crucial in the case of antibiotics, which could have severe adverse effects on vultures. Exposure to these compounds has been investigated less often than the effects of NSAIDs, despite their potential impact on vulture health and fitness (Plaza et al., 2022). Increasing scientific information on vulture exposure to antibiotics is critical in areas where there is a lack of information (e.g., Asia, the Americas and Africa) in order to further knowledge of this emerging threat (Plaza et al., 2022). Moreover, pharmaceutical industries should test the potential impact of such drugs on non-target wildlife such as scavenging birds (e.g., their lethal, clinical and subclinical effects) before their veterinary use is approved — at least for the most commonly used drugs and those that could be potentially toxic to scavenger birds.

BOX 2

The shadow of diclofenac hangs over European vultures

(Margalida and Oliva-Vidal, 2017)

Of the sixteen Old World vultures, 81% are globally threatened or near-threatened; four vulture species inhabit Europe, of which three are threatened or near-threatened. Since 1993, the EU and various national governments have invested significant financial resources in the conservation of vultures — including at least 76 LIFE projects related to these species — and between 1993 and 2014 spent € 121.9 million, of which € 59.7 million came from European funds. During this period, Spain, home to 90% of all European vultures, invested € 72.8 million (€ 30.8 million received from EU) on 38 projects related to vulture conservation (Life Programme).

However, all these conservation efforts will be of little worth if the use of veterinary diclofenac, authorized in Spain since 2013, spreads. A mathematical model has estimated that annually diclofenac could cause 715–6,389 vulture deaths (Green et al., 2016). Thus, its potential ecological impact on ecosystem services is obvious.

In November 2014 the Conference of the Parties of the UNEP Convention on Migratory Species adopted a resolution aimed at providing the veterinary sector with guidance on how to prevent the poisoning of migratory birds and called for an evaluation of the risks that veterinary medicinal products pose to scavenging migratory bird species (UNEP/CMS, 2014). In December 2014, the European Medicines Agency’s Committee for Medicinal Products for Veterinary Use proposed to the European Commission that the veterinary use of diclofenac in Europe should be regulated (EMVA/CVMP, 2014). In June 2015, the Veterinary Pharmaceutical Committee (VPC) decided not to initiate a withdrawal of marketing authorizations for veterinary products containing diclofenac in Europe since, in the Committee’s opinion, the management measures being applied by member states would be effective in keeping the risk to vultures and other necrophagous birds under control. On 4 July 2016, in a VPC meeting, member states were invited to provide an update of the situation in their territories. Their report indicates that (1) most member states say that they have not yet authorized the use of diclofenac; (2) those that have authorized the use of this product state that appropriate safety warnings are included in the product literature and that fitting measures have been put in place for the safe disposal of fallen stock and to provide birds of prey with carrion; (3) no member states have yet reported any deaths of vultures due to poisoning by veterinary medicinal products in their territories.

Griffon vulture *Gyps fulvus* in the Lleida Pre-Pyrenees, Catalonia (Spain). Photo: Pilar Oliva-Vidal

However, the true situation is somewhat different:

(1) the use of diclofenac has been authorized in at least five member states (Spain, Italy, Estonia, Czech Republic and Latvia) and currently there is a request for a permit in Portugal; (2) the safety warning in the product literature is insufficient and merely recommends "Do not administer to animals susceptible to enter the wild animal food chain," there is no information about the 'appropriate measures' to be applied for the safe disposal of carrion by avian scavengers or any supervision of these measures; (3) until 2016, there was no monitoring of NSAIDs (nonsteroidal anti-inflammatory drugs) contamination of ungulate cadavers available to vultures and other obligate and facultative avian scavengers. Nevertheless, to date at least one griffon vulture has been reported to have died as a result of ingesting flunixin (Zorrila et al., 2015), an NSAID with similar effects to diclofenac. Thus, it is just a question of time before deaths due to diclofenac begin to occur.

Currently, there is no complete ban on diclofenac in Europe and the potential risk it represents is still present (Margalida et al., 2014b). Given that the VPC has no data for anywhere in Europe on veterinary medicine residues in carcasses available to scavengers, the current risk cannot be effectively assessed. Accordingly, the precautionary principle must be applied, which should entail the immediate ban on the use of diclofenac for livestock to avoid undesirable consequences to vulture populations (Swan et al., 2006b) and the promotion of the use of safe alternatives such as meloxicam (Margalida et al., 2014a). The catastrophic decline in Asian vulture populations (Oaks et al., 2004; Green et al., 2004) is sufficient warning of what could happen and, likewise, ought to make it unnecessary to have to wait until more dead vultures begin to appear.

Lead contamination in avian scavengers

Lead (Pb) contamination is a widely recognized conservation problem affecting raptors worldwide (Pain et al., 2009; Plaza and Lambertucci, 2019; Descalzo et al., 2021). The wide-ranging review by Monclús et al. (2020) found that of European raptors, obligate and facultative avian scavengers have the highest concentrations of lead in tissues (e.g., liver, kidney, blood and bone). Indeed, scavengers are generally the most vulnerable birds to lead exposure and poisoning due to their obligate and opportunistic scavenging behaviour (i.e., carrion consumption), and lead contamination due to the ingestion of shot pellets or ammunition fragments embedded in game animals is the most common source of exposure (Hunt et al., 2006; Kelly et al,. 2011; Krone, 2018; Pain et al., 2019). The prevalence of lead poisoning in raptors in Europe varies between countries but correlates with the density of hunters (Green et al., 2022). Therefore, high levels of lead in raptor blood are linked to their feeding habits (i.e., scavenging) and the hunting season (Mateo-Tomás et al., 2016; Monclús et al., 2020). For example, lead exposure related to hunting has been documented for threatened species such as Egyptian (Gangoso et al., 2009) and bearded (Hernández and Margalida, 2009a; Berny et al., 2015) vultures and for endangered *Gyps* vultures in Africa (Naidoo et al., 2017; Garbett et al., 2018). Lead bullets often fragment and become lodged in muscle and soft tissues, thereby remaining available to scavengers that consume viscera or muscle tissue from unrecovered big game. Although vultures can eliminate lead by regurgitating pellets, their digestive processes facilitate rapid dissolution and absorption into the bloodstream and enable lead to reach all organs and tissues including the liver, kidney, bones and growing feathers (Berny et al., 2015; Pain et al., 2019).

The attribution of lead levels to potential sources has been assessed using stable isotope analyses in griffon vultures (Arrondo et al., 2020), condors (Finkelstein et al., 2014) and Eurasian buzzards *Buteo buteo* (Taggart et al., 2020). Mateo-Tomás et al. (2016) found that environmental lead exposure through soil bioavailability (e.g., geological sources) could occur directly as a result of high soil Pb levels. Alternatively, it could occur indirectly by enhancing lead solubility at low soil pH values, when griffon vultures ingest lead biologically incorporated by the large grazing ungulates they scavenge, or via ingestion from the topsoil when feeding on carcasses. Thus, environmental exposure to this toxic metal through natural sources may also be frequent and result in basal exposures that increase when vultures are exposed acutely to lead-base ammunition. Other factors such a lead-based gasoline and solid-waste incinerators, fishing weights and mining activities have also been highlighted as potential sources of lead pollution (Monclús et al., 2020). Accidental lead intoxication in captive vultures have also been reported. For example, eight cinereous and two Egyptian vultures housed in a zoo in the Czech Republic were accidentally exposed to lead contamination in their aviary due to soil contamination from paint debris, with the result of the death of one of the Egyptian vultures (Pikula et al., 2013).

Lead poisoning affects both Old World (Gangoso et al., 2009**;** Hernández and Margalida, 2009a; Naidoo et al., 2012, 2017; Kenny et al., 2015a, b; Mateo-Tomás et al., 2016; Garbett et al., 2018; Arrondo et al., 2020) and New World (Lambertucci et al., 2011; Finkelstein et al., 2012) vultures, although it remains poorly studied in many regions of the world (Plaza and Lambertucci, 2019). Most research has been published in the last two decades, which indicates that knowledge of lead poisoning in vultures only began comparatively recently compared to other avian guilds, especially in developed regions (Pain et al., 2019; Monclús et al., 2020). The review by Plaza and Lambertucci (2019) revealed that current research into vultures only includes information on 13 species from the Old (9) and New (4) Worlds and that 88% of published articles contained data on individuals with lead concentrations above threshold levels, New World vultures being more affected than their Old Word counterparts. This is thus evidence that vultures are exposed to high levels of this contaminant (Fig. 3) but that its potential as a threat to many vulture species remains poorly unknown. In fact, the IUCN Red List does not consider lead contamination a major conservation problem for most threatened species, the exception being California condors and Egyptian vultures. Although it is an important threat to several vulture species worldwide, lead exposure is often undiagnosed and not well recognized in some species and in some geographical areas (Plaza and Lambertucci, 2019).

Sublethal exposure to heavy metals can affect bone mineralization (Gangoso et al., 2009), reduce muscle and fat concentrations (Carpenter et al., 2003), cause organ damage and internal lesions (Pattee et al., 1981), affect oxidative stress biomarkers (Espín et al., 2014) and reduce hatching success (Steidl et al., 1991). When exposure results in acute toxicity birds can die suddenly despite seeming to be in good physical condition (Krone, 2018). However, knowledge is still lacking regarding the effects of lead poisoning on vulture populations and so particular attention should be paid to its potential to cause population declines. A model suggests that lead poisoning may notably reduce European populations of adult raptors even when the annual number of birds estimated to be killed by lead is low since it affects, above all, species with high annual survival rates and delayed ages at first breeding such as griffon and bearded vultures (Green et al., 2022).

In Europe, the species with the highest prevalence of exceeded subclinical (e.g., the point at which deleterious effects could begin to appear) threshold values (i.e., blood > 20 µg/dl, according to Franson and Pain, 2011) include bearded and griffon vultures, as well as other facultative avian scavengers such as the endangered red kite. Griffon vultures exhibit the highest average liver, kidney and blood lead concentrations (Monclús et al., 2020). At species level, the highest proportion of studies reporting the exceeding of lethal (e.g., likely to cause mortality) concentration thresholds or mortality (i.e., blood > 50 µg/dl) included the bearded vulture (for details see Monclús et al., 2020). These results are consistent with those obtained during the active monitoring we carried out in the Pyrenees (Fig. 3). More recently, Green et al. (2022) have suggested that scavenging species including griffon and bearded vultures feeding regularly on the carcasses of game animals tended to have a greater annual probability of death from lead poisoning. However, interestingly, reports of mortalities in griffon vultures are scarce even though this species often has the highest accumulated lead residues of all species (Monclús et al., 2020), which suggests that this species (and possibly others) may have a certain tolerance of lead (Fig. 3; Oliva-Vidal et al., in prep.). Therefore, a multi-species exploration of exposure in avian scavengers not only to lead but also to other heavy metals over large areas (e.g., the entire Pyrenean range) is required to be able identify intra- and inter- specific differences, as well as the potential susceptibility of each species. This will enable us to evaluate the true magnitude of the toxicity of such environmental contaminants.

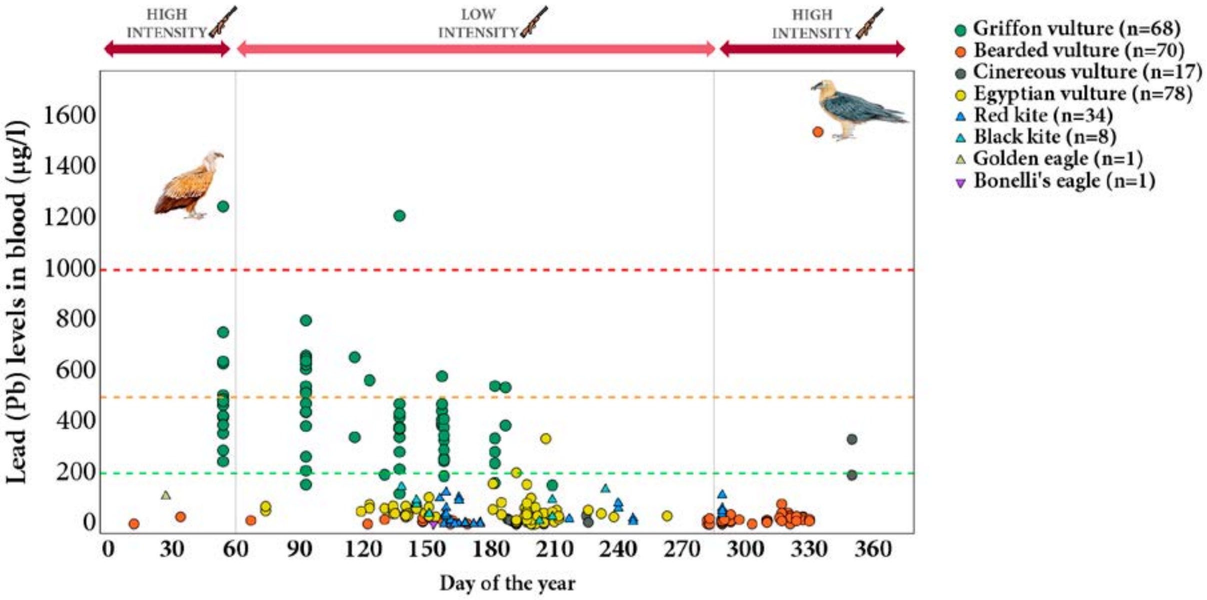


Figure 3. Blood lead concentrations in 277 free-living obligate and facultative avian scavengers sampled throughout the whole Pyrenees in November 2017–July 2021. Discontinuous lines show the thresholds of exposure according to Franson and Pain (2011): green (basal; < 200 µg/l), orange (sub-clinical; 200–400 µg/l), red (clinical; 500–1000 µg/l) and above 1000 µg/l indicate severe exposure (i.e., potentially lethal). The main hunting periods are shown. Griffon vultures had the highest Pb levels in blood (e.g., mean ± SD = 417.90 ± 213.56 µg/l), although the single highest value (1542.6 µg/l) was found in a female bearded vulture equipped with a GPS at the sampling time (November 2017). Interestingly, we did not detect uncharacteristic behaviours in this particular bird and she is still alive. One griffon vulture within the lethal threshold was also equipped with a GPS and we likewise have detected no uncharacteristic behaviours in this breeding bird, which is still alive. Of the other species, except for two Egyptian and one cinereous vultures, blood Pb levels were below the basal threshold (albeit with values of blood Pb taken during the least intensive hunting period). More knowledge of the potential sources of lead in these species, as well as the potential species-specific tolerance to lead contamination (e.g., griffon vulture), is required if we are to improve our understanding of the exposure thresholds that are widely used to examine the toxicological significance of lead contamination in obligate and facultative avian raptor scavengers (Oliva-Vidal et al., in prep.).

Given the ongoing evidence of the environmental impacts of lead, several countries have implemented national regulations banning lead gunshot and replacing it with non-toxic alternatives. For example, in 1991 a nationwide ban was approved on the use of lead gunshot for shooting waterfowl in the USA, while in January 2021 a ban on the use of lead gunshot in and around wetlands throughout the European Union was introduced (Green et al., 2022). In terms of avian scavengers, raptors and other terrestrial birds, there has been less regulatory action aimed at reducing the incidence of poisoning. However, the increasingly critical situation regarding lead poisoning in California condors led to a state-wide ban on the use of all lead ammunition for hunting in California as of July 2019. In 2021 the European Commission requested that the European Chemical Agency (ECHA) produce a proposal to restrict the use of all lead ammunition for hunting throughout the EU to protect wild birds in terrestrial habitats, human health and the environment. If accepted, the proposal will be adopted in 2023 (Green et al., 2022). The next step should be the application of similar regulations in Africa where vulture populations are also affected by lead contamination (Garbett et al., 2018).

Environmental pesticides: the silent threat of rodenticides

Exposure to pesticides is a major threat to scavenging birds that causes high mortality rates worldwide (Margalida, 2012; Botha et al., 2015; Ogada et al., 2016b; Alarcón and Lambertucci, 2018). Nevertheless, the real magnitude of this problem is not fully appreciated and available information is sparse, particularly in the case of vultures (Plaza et al., 2019). Vultures can be exposed to pesticides either accidentally (e.g., when compounds used for approved targets and in correct doses accidentally harm non-target species) or due to deliberate abuse when used to illegally kill predators or herbivores, an activity that can have a drastic impact on vultures either unintentionally or intentionally if they are targeted (Martínez-Haro et al., 2008; Ogada, 2014, Pauli et al., 2018; Fig. 4). Although the exact number of vultures killed by pesticide poisoning is not currently available, the generalized use of these compounds to poison wildlife is producing alarming mortality rates in several endangered vulture species worldwide (e.g., up to 500 individuals have been reported poisoning in a single event, Plaza et al. (2019); see section on poisoning). These deliberate poisoning events are evidence of the impact of non-natural mortality rates and some authors suggest that they represent just the tip of the iceberg (Ogada, 2014; Plaza et al., 2019).

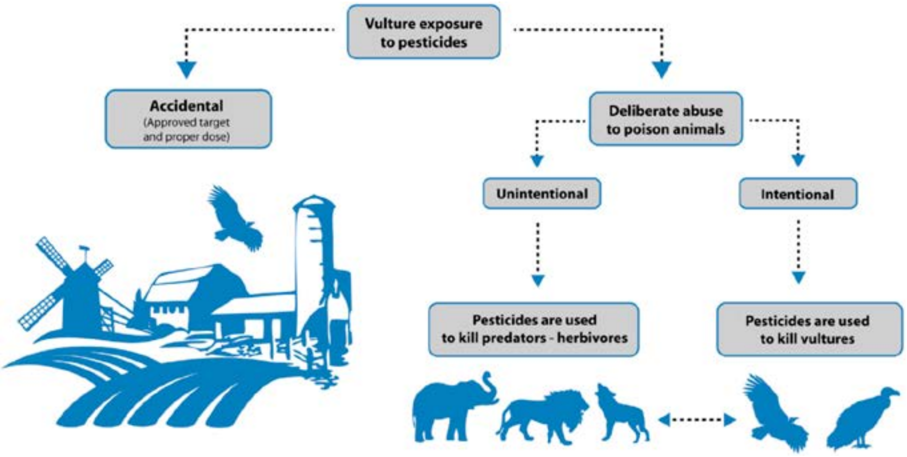


Figure 4. Conceptual scheme showing the main ways in which vultures and other scavenger birds are exposed to pesticides. Source: Plaza et al. (2019).

The most common pesticides implicated in wildlife poisoning are carbamates and organophosphorus that include compounds such as aldicarb, carbofuran, methomyl, monocrotophos, diazinon, parathion and fenthion-ethyl. Indeed, of all these pesticides, carbofuran is the most widely used to kill vulture species around the world (Ogada et al., 2016a; Plaza et al., 2019). In addition, organochlorines, pyrethroids, anticoagulant rodenticides and even strychnine (which was banned worldwide many years ago) are commonly involved in wildlife poisoning and have been reported to poison Spanish vultures (Hernández and Margalida, 2008, 2009b). Although the exposure of vultures to certain pesticides has been documented in some regions (for more details see the review by Plaza et al., 2019), the current impact of anticoagulant rodenticides, despite the growing evidence of secondary exposure to non-target species, has not to date been quantified in living obligate and facultative avian scavengers (but see Chapter 3).

Rodenticides are widely used to control rodents inhabiting human environments that damage different types of goods, and to prevent zoonotic diseases (Buckle and Smith, 2015). The use of such chemical compounds is therefore widespread in towns, landfills, farms and croplands (Shore et al., 2006; Tosh et al., 2011a; Sánchez-Barbudo et al., 2012). Nowadays, the most widely used compounds are anticoagulant rodenticides (ARs) that act with a sufficient time delay to reduce the likelihood that rodents will associate eating the poison with the resulting sickness, thereby avoiding any potential development of learned aversion. The active mechanism is produced by the inhibitive action of the enzyme vitamin K epoxide reductase, which is responsible for the maintenance of adequate hepatic vitamin K levels that produce coagulation factors II, VII, IX and X. Two chemical families of commercial anticoagulant rodenticides, indandiones and coumarines, have this vitamin K-antagonism capacity (Rattner and Mastrota, 2018). The massive development of resistance in rodents to the compounds when the first generation ARs (FGARs; e.g., warfarin and diphacione) were used (Ishizuka et al., 2008) led to the introduction in the 1970s of more toxic and more persistent (i.e., greater bioaccumulative capacity) compounds, the second generation ARs (SGARs; e.g., difenacoum, bromadiolone, broficaoum and flocoumafen), also known as 'super warfarins' (Thomas et al., 2011). Unlike FGARs, which have a relatively short half-life ($t_{1/2}$) and require multiple exposure to kill target species, SGARs are extremely persistent and acutely toxic with a lethal dose delivered by a single feeding event. However, rodents have already developed resistance to some SGARs (Ishizuka et al., 2008; Vein et al., 2011; Meerburg et al., 2014).

The widespread use of ARs in the environment can lead to the poisoning of non-target species, as well as secondary exposure in predators and scavengers that can drive long-term chronic accumulation of SGARs in these species (Olea et al., 2009). Secondary exposure occurs through the consumption of contaminated prey (e.g., animals that suffered a primary exposure/intoxication due to bait ingestion) as it may take several days for a poisoned animals to die, during which time they can be consumed by predators and scavengers. Such contaminated prey (mainly mammals and birds but also reptiles and insects, López-Perea and Mateo, 2018) will contain a significant amount of ARs in their tissues (especially the liver). Secondary exposure has been reported in several raptors worldwide (Berny et al., 1997; Christensen et al., 2012; Langford et al., 2013; Coeurdassier et al., 2014, 2019; Ruiz-Suárez et al., 2014; Huang et al., 2016; Martínez-Padilla et al.,

2017) and evidence of lethal exposure has been found in Europe in species of conservation concern such as the red kite (Berny and Gaillet, 2008; Coeurdassier et al., 2012; Hughes et al., 2013). Although the levels of ARs ingested may not actually kill, persistent SGARs can gradually accumulate in tissues over time. Indeed, the persistence of SGARs in rodent tissue (e.g., the half-life of ARs in liver varies between 0.3–66.8 days for FGARs and 28.1–350 days for SGARs, Horak et al., 2018) does not immediately result in death, which makes these compounds highly dangerous due to bioaccumulation and biomagnification in predators and scavengers (Brakes and Smith, 2005; Tosh et al., 2011b; Sánchez-Barbudo et al., 2012; Rattner et al., 2014; López-Perea et al., 2015, 2019; Shore et al., 2015). The great toxicity of SGARs, as well as the abundance of contaminated prey items in areas where these compounds are employed, make these pesticides/biocides a potential threat for several obligate and facultative scavenging species (Fernandez-de-Simon et al., 2019).

However, compared to other pesticides, there is very little information about the exposure of vultures to ARs (Fig. 5) and the few published articles have only evidenced exposure in tissues of vultures found dead, for example in turkey vultures in USA (Hosea, 2000; Stone et al., 2003; McMillin, 2012; Kelly et al., 2014), in griffon and Egyptian vultures in the French Pyrenees (Berny et al., 2015) and in griffon, Egyptian and bearded vultures in Spain (Sánchez-Barbudo et al., 2012; López-Perea et al., 2019). No assessment comparing the true magnitude of exposure to SGARs in free-living individuals within the avian scavenger guild exists to date. The prevalence and concentration of SGARs we found in both obligate and facultative European avian scavengers in the Spanish Pyrenees, underlines the fact that the exposure of avian scavengers to ARs is an important threat to these species and that further investigation assessing their potential impact at population level is needed, particularly in the case of certain endangered species (Chapter 3).

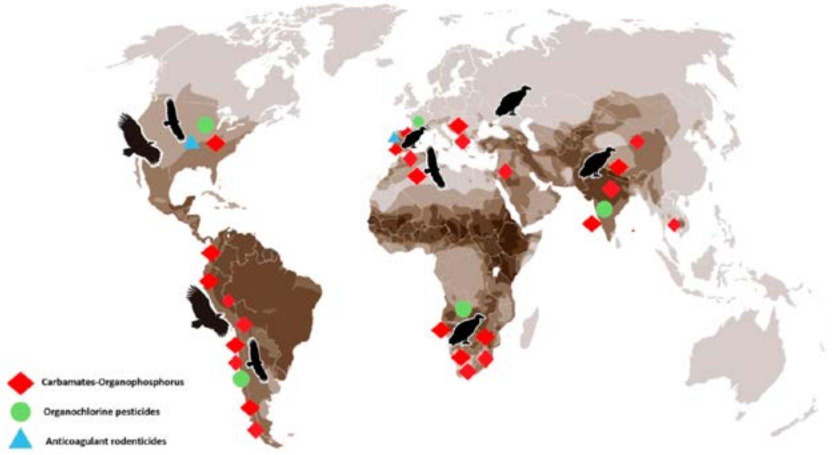


Figure 5. World map showing where different vulture species are exposed to pesticides. Note that exposure to ARs is still poorly investigated. The world distribution of vultures is shown in brown (the darker the color the higher species richness). The map with the world distribution of vultures was obtained from www.nationalgeographic.com. Source: Plaza et al. (2019).

Changes in health policies

Another non-natural threat to the conservation of European vultures and facultative scavenger populations emerged with the mad cow crisis (i.e., bovine spongiform encephalopathy, BSE) at the beginning of the twenty-first century. Historically, carcasses of extensively grazed livestock had been left in the field, resulting in a large amount of unpredictable resources scattered throughout agropastoral landscapes. For example, in the Pyrenees, the estimated proportion of carcasses of wild and domestic ungulates available to avian scavengers ranged between 25 and 80% depending on the habitat occupied by the prey species (forest or open landscape; Margalida et al., 2011a; Margalida and Colomer, 2012). However, in Europe, in light of the outbreak of BSE in 2001 and the appearance of the variant vCJD and the new variant nvCJD of Creutzfeldt-Jakob disease in humans, acquired from cattle infected by BSE, led to the approval of new sanitary legislation (Regulation EC 1774/2002). This legislation greatly restricted the use of animal by-products not intended for human consumption (ABPs) and as a result all carcasses of domestic livestock had to be collected from farms and transformed or destroyed in authorized plants. Many vulture feeding stations associated with stock farms closed down almost overnight, thereby drastically limiting the availability of food for both obligate and facultative scavengers (Margalida and Colomer, 2012). In Spain, home to >90% of European vultures and a wide range of facultative avian scavengers (Margalida et al., 2010), it was estimated that 80% of all sheep and goat and 100% of all cow carcasses were collected for controlled destruction as a result of the new law (Donázar et al., 2009a, b; Margalida et al., 2010). For example, in Navarre and Aragon (NE Spain) it was estimated that 80% of feeding stations were forced to close between 2005 and 2008 (Cortés-Avizanda et al., 2010; Margalida et al., 2014c).

This new scenario led to a decline in the services provided by vultures, which increased the ecological and economic costs. For example, it has been estimated that Spanish vulture populations remove 134–200 t of bones and 5,551–8,326 t of rotten meat every year (Margalida and Colomer, 2012). However, the new law forced farming concerns to invest in the collection of livestock carcasses that traditionally had been consumed by vultures and other scavengers. For instance, in Spain and France the cost of collecting a carcass was estimated at around 20€ per animal (Boumellasa, 2004; Donázar et al., 2009a; Donázar and Margalida, 2009). Moreover, supplanting carrion consumption by scavengers by artificially removing livestock carcasses (e.g., by burning or recycling) led to the emission of over 77,000 metric tons of CO_2 eq. into the atmosphere every year, and annual payment of ca. $50 million to insurance companies by farmers and public administrations (Morales-Reyes et al., 2015).

This sudden reduction in trophic resources provoked shifts in diet (Donázar et al., 2010), behaviour (Zuberogoitia et al., 2010), demographic parameters (Margalida et al., 2014d) and foraging movements of vultures (Donázar et al., 2016). However, the most critical threat to European vulture populations (especially the griffon vulture) was the unexpected behavioural conflict that arose between vultures and livestock. During the 1990s, farmers began to attribute attacks on livestock to griffon vultures and reports of such occurrences increased exponentially during the following decade (Margalida et al., 2014c; see next section). Most complaints took place from 2006 onwards, coinciding with the food shortages provoked by the sanitary policies (Donázar et al., 2009a; Margalida et al., 2010), which facilitated a generalized social perception of this problem – misinterpreted by the media and many people – that discerned a causal relationship between food shortages and vultures attacking livestock (Margalida et al., 2011).

It was patent that the restrictions imposed by this new EU legislation, which deprived scavenger populations of essential resources, flagrantly contradicted the obligations and efforts of member states to conserve these threatened species (Tella, 2001; Donázar et al., 2009a; Margalida et al., 2010). Fortunately, recommendations made by scientists, conservationists and conservation managers led to the approval of new regulations (EC 142/2011) that allowed farmers to leave dead extensive livestock in the field and/or at feeding stations (e.g., Margalida et al., 2012) as well as in the so-called 'Protection areas for the feeding of necrophagous species of European interest' (PAFs, Morales-Reyes et al., 2017). This scenario illustrates how scientific arguments can trigger positive political action and help reconcile conservation challenges and human activities (Sutherland et al., 2004; Margalida et al., 2012).

Human-wildlife conflicts and fake news: an unexpected emerging threat

Humans and vultures have coexisted throughout human history, particularly since the rise of agriculture and animal domestication around 10,500 years ago (Moleón et al., 2014). Indeed, vultures have been allies of farmers as recyclers of carcasses of domestic animals since ancient times. However, an unexpected human-wildlife conflict has emerged in modern times: the purported attacks by vultures on livestock. Therefore, paradoxically, vultures have changed from allies to enemies and the age-old symbiotic relationship between vultures and farmers has been lost. Unfortunately, the media find this conflict attractive and the absence of scientific information has facilitated a growing negative perspective of vultures (Margalida and Donázar, 2020; Ballejo et al., 2021; Lambertucci et al., 2021).

Despite the adaptations in vultures that make them one of the most efficient species at exploiting dead animals and, at the same time, limit their ability to kill live prey, the current social alarm is causing people to perceive vultures as undesirable livestock predators. Vultures have large wings adapted for energetically efficient slow soaring rather than fast flight for hunting prey (Ruxton and Houston, 2004). Their visual adaptations and foraging strategies that rely on cues from conspecifics allow them to detect carcasses randomly distributed over large distances (Jackson et al., 2008; Potier et al., 2016, 2020). Furthermore, their large body size endows them with greater body reserves so that they can cope with periods of food scarcity. Unlike the prey-catching grasping and piercing talons of hawks and eagles, vultures' feet and claws are adapted to walking on the ground and holding carrion whilst eating. Moreover, to facilitate the dismembering of corpses and access to their innards, they generally have long necks, strong beaks and little feather cover on their heads and necks to avoid fouling their heads when inside carcasses (Böhmer et al., 2020). In fact, vultures have evolved a remarkable tolerance to the bacterial toxins present in their diet (e.g., decaying meat) due to physiological adaptations such as low stomach pH and a low diversity gut microbiota (Roggenbuck et al., 2014; Plaza et al., 2020). These anatomical and physiological traits make vultures well adapted to consuming carrion but not to hunting and killing.

However, news items and social media have increasingly depicted both Old and New World vultures as livestock predators undergoing 'behavioural changes' that have led them to attack not only sheep but also large animals such as cows and horses, and even dogs and humans (Duriez et al., 2019; Margalida and Donázar, 2020; Ballejo et al., 2021). This unexpected perception has increased in recent years, associated with a viral spread of partial and biased information through social media, despite limited empirical support for these assertions (Lambertucci et al., 2021). Misinformed and false public opinions ('fake news') suggesting that vulture attacks on livestock are frequent and widespread drive people's negative perceptions, attitudes and tolerance towards them (Margalida and Donázar, 2020; Ballejo et al., 2021). Such misleading items often 'go viral' and reach large audiences (Lazer et al., 2018). For instance, in Spain, a video titled *Vultures attack sheep* consisting of a farmer explaining the sheep losses he attributes to vulture attacks has been watched more than 15K times. However, the video was refuted by the Spanish authorities, who assessed the case and demonstrated that the attack was produced by a carnivore (e.g., dogs) and that the vultures this farmer observed were just scavenging. Several other media items refer to attacks by vultures but just show birds feeding on carcasses (Margalida and Donázar, 2020; Lambertucci et al., 2021). Unfortunately, the fake videos or those providing misinformation remain online for several months and can be seen, commented on and reproduced by millions of people who have the potential to perpetuate negative perceptions of vultures (for more examples, see Ballejo et al., 2021). In response to this social media impact, a new conservation field has emerged, the so-called 'conservation culturomics', that aims to use digital sources to investigate human-nature interactions and provide insights into how to deal with conservation problems (Correira et al., 2021).

Of all conflicts between humans and wildlife, the killing of livestock by vultures is a relatively minor problem. Domestic species such as dogs cause far greater damage to livestock than vultures and no certified case of griffon vultures killing healthy livestock has ever been published in peer-reviewed literature. However, this emerging human-wildlife conflict has led to discontent in some farmers and even vengeance in the form of illegal practices such as lethal control methods (e.g., deliberate poisoning) that threaten the conservation of both Old and New World vulture populations (Margalida et al., 2011; Ballejo et al., 2020). In Europe, farmers attribute these attacks to the food shortages caused by the outbreak of bovine spongiform encephalopathy in 2006–2011 (Donázar et al., 2009a; Margalida et al., 2014c). However, farmers' opinions merely provoke perceived conflicts rather than highlighting any change in vulture behaviour (Duriez et al., 2019; Ballejo et al., 2020). Moreover, in regions where farmers receive economic compensation for livestock losses, the lack of evidence regarding the real causes of mortality may exacerbate the conflict and stimulate the continued publication of false reports.

It is therefore vital to replace the dissemination of speculative and misinterpreted (or even fraudulent) news items that jeopardize vulture conservation efforts with scientific evidence-based information (König et al., 2020). Fake news magnifies existing HWCs and so regulations to prevent its spread are crucial (Ballejo et al., 2021). In addition, it is essential to promote educational campaigns explaining the importance to society of vultures as providers of pivotal ecosystem services such as carcass removal and disease control, greenhouse emission regulation, ecotourism and spiritual values (Markandya et al., 2008; Ogada et al., 2012b; Moleón et al., 2014; Morales-Reyes et al., 2015; DeVault et al., 2016; Donázar et al., 2016; Cortés-Avizanda et al., 2018; Aguilera-Alcalá et al., 2020; García-Jiménez et al., 2021). Henceforth, scientific assessment and interdisciplinary awareness campaigns must be promoted to bring managers, scientists and farmers together, and to move farmers away from a conflict mentality towards one of coexistence with vultures in our changing world.

Favourable trends in Spain: successful conservation actions help restore European populations

Although some regions have reported only a few local successes, vulture populations in Europe are increasing in number and recolonizing their old ranges. For example, griffon vulture populations in Western Europe have increased by > 200% over the past 12 years (mostly in Spain), the continent's cinereous vulture populations have increased by 50% in the last 20 years, and the bearded vulture has been successfully reintroduced into parts of its former range, notably in Andalusia in Spain. Furthermore, in the Balkans, vulture populations are also recovering, despite the persistent threat of poisoning (Safford et al., 2019). These successful trends are attributable mainly to evidence-based conservation actions, robust legislation (e.g., the EU Birds and Habitats Directives) and important funding aimed at implementing solutions (e.g., ~80€ million invested in vulture conservation over the past 15 years) (Safford et al., 2019).

Spain is playing a key role in this encouraging panorama for European vultures. The population of griffon vultures in this country has increased by about 17.5–26.0% since 2008 to reach a total of ~31,000 breeding pairs in 2018 (Del Moral and Molina, 2018a). This trend reflects the fact that Spain is currently home to 90% of the European population of this species, followed by France and Portugal, where the number of breeding pairs reaches or slightly exceeds 1,000 (Margalida et al., 2010; Del Moral and Molina, 2018a). Populations of the cinereous vulture have increased by almost 40% since 2006 and today about 2,500–3000 pairs breed in Spain, second only to Mongolia in the number of breeding pairs of this vulture (Del Moral, 2017). The Spanish Egyptian vulture population is estimated at 1,490–1,567 breeding pairs, about ~92.5% of the European Union population (Del Moral and Molina, 2018b). Despite some local breeding successes, unlike other European species, the overall Egyptian vulture population trend remains stable as by 2008 it was estimated that the breeding Spanish population had reached ~1,500 breeding pairs (Del Moral, 2009). Finally, Margalida and Martínez (2020) report that the current trend in the Spanish bearded vulture population is clearly positive and since the first census carried out in the Pyrenees in 1988 the size of its breeding population has increased from 30 to 146 breeding pairs in 2018 (Margalida et al., 2020). This significant increase – at least during the first years of field monitoring – cannot be attributed to population growth *per se* since a greater monitoring effort probably led to the discovery of territories that had previously been undetected (Margalida and Martínez, 2020).

This favourable scenario has led Spain to actively collaborate with international reinforcement (i.e., the release of individuals into an existing population of conspecifics to enhance population viability) and reintroduction (i.e., when the aim is to re-establish a population in an area after local extinction) projects in many European regions. This type of international collaboration consists of the translocation (i.e., the intentional movement and release of animals to restore populations; IUCN, 2013; Seddon et al., 2014) of individuals from Spain that are released in different countries/regions where vultures are extinct or at risk of extinction in order to restore their populations. For example, such regions include France (cinereous vultures) the Alps (bearded vultures), Bulgaria (griffon and cinereous vultures) and Israel (griffon vultures). Moreover, some endangered facultative avian scavengers such as the red kite have been translocated to other European regions (e.g., United Kingdom, Evans et al., 1999). In recent years national reintroduction projects have mainly focused on the bearded vulture reintroduction and, for instance, reintroduction projects of this vulture are currently underway in Andalusia (started in 2006), Asturias (Picos de Europa National Park, started in 2012) and, more recently, in El Maestrazgo (Castellón, started in 2018). To date, captive bred individuals have been used in the Andalusia and Maestrazgo projects (European Endangered Species Programme), while in Asturias, birds raised from eggs taken from clutches of nests in the Aragonese Pyrenees are being used in this managed reintroduction.

In this context and within an adaptive management framework, an experiment was conducted using the translocation of bearded vultures – taken from the non-breeding adult Pyrenean fraction – to the Maestrazgo region in order to reinforce the hacking of captive-bred birds. This project got underway in 2018 and to date seven adult individuals have been translocated to this region, of which six returned to the Pyrenees and one disappeared (author unpubl. data). The aim was to establish a breeding population to bridge the gap between populations in the Pyrenees and Andalusia. As a part of this project, captive-bred birds are also released in specially constructed hacking sites, where at least two chicks are released annually. This reintroduction management (i.e., hacking) has also been employed in the Picos de Europa National Park, where in 2010–2018 at least 24 individuals were released and today at least two breeding pairs have become established in the area (Margalida and Martínez, 2020).

The role of multidisciplinary approaches for biodiversity conservation

In an ever-changing human-dominated world, policy decisions aimed at guaranteeing biodiversity conservation require multidisciplinary approaches. Over the past two decades, the link between the mainstream conservation biology and the discipline of behavioural ecology has gained ground among conservation biologists given their interest in applying the principles and methods of behavioural ecology to species and habitat conservation (Caro, 1999; Berger-Tal et al., 2011; Bro-Jørgensen et al., 2019). Caro (1998) states that "conservation biology is a theoretical and applied discipline aimed at preventing population extinction, whereas behavioural ecology attempts to understand the way in which behavioural and morphological traits contribute to the survival and reproduction of individual animals and plants under different ecological circumstances". In this context, several studies have highlighted the crucial connection between these disciplines and emphasized the importance of taking into account animal behaviour in conservation practices (Sutherland, 1998). In addition, the growing consideration in recent decades of the behavioural ecotoxicology as an emerging discipline focusing on the study of behavioural responses to anthropogenic environmental toxicants and other stressors – mainly chemical contaminants – in individuals, populations and communities (Dell'Omo, 2002; Peeters et al., 2009; Hellou, 2011) has led to a more comprehensive interdisciplinary framework working to preserve biodiversity in the face of increasing anthropogenic impacts (Peterson et al., 2017; Ford et al., 2021). Finally, the inclusion of social dimensions (i.e., perceptions, values, beliefs and attitudes) in environmental issues has created a novel perspective, often highlighted in research and policy-makers' agendas due to their crucial role in improving conservation efforts (Dickman, 2010; Bennet et al., 2016, 2017).

Given the multiple threats to vulture populations worldwide, future conservation directions need to combine all these disciplines in order to establish a common ground for designing useful conservation management strategies with an evidence-based perspective. Indeed, ignoring some of these fields may in fact skew conservation approaches and lead to the failure of management programs. Integrative research linking all these disciplines may help identify direct and indirect stressors at multiple threat levels (see above) and will allow us to establish priority research lines and optimize the design and application of conservation measures. Therefore, multidisciplinary approaches are needed to provide the information required to better understand conservation issues and obtain all relevant information when searching for effective solutions, which should be established, monitored and evaluated as a part of a broader move toward adaptive management and evidence-based conservation (Sutherland et al., 2004; Pressey et al., 2007; Bennet, 2016; König et al., 2020).

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Thesis outline, objectives and structure

This thesis aims to provide a multidisciplinary approach embracing a series of different disciplines to improve the efforts currently being made to conserve European scavenger birds in a context of an ever-changing human-mediated world. More specifically, it investigates how cascading anthropogenic (e.g., ecological, toxicological, socio-economic and political) changes affect avian scavengers and the ecosystem services they provide. In this context and under a changing environment scenario, this thesis first explores how changes in rural landscapes due to farmland abandonment affect the functioning of scavenger assemblages and scavenging dynamics (Chapter 1). Next, the human-wildlife conflict between vultures and livestock is addressed from a socio-economic ecological perspective (Chapter 2). Then, from an ecotoxicological point of view, this thesis assesses how avian scavengers exhibiting different foraging behaviour are exposed to anticoagulant rodenticides (Chapter 3). Finally, we apply bioinspired computational models to estimate the carrying capacity of an ecosystem on the basis of the availability of trophic resources, to quantify the spatio-temporal distribution of these resources and their relationship to transhumance (Chapter 4) and to forecast the demographic impact of translocations on reintroduction projects targeting the Pyrenean bearded vulture population (Chapter 5). The conceptual framework of this thesis thus combines field studies, active monitoring programs, ecological modelling and adaptive management approaches to provide policy-makers, managers and conservationists with a new set of evidence-based conservation tools.

The present PhD dissertation includes a compilation of various research articles. The information is preceded by a *General introduction* that includes a review of the main threats affecting avian scavengers, with special emphasis placed on the topics addressed throughout the thesis and so there may be some overlap in information between the general introduction and some sections of the following chapters. This thesis then includes a general overview of the *Materials and methods* used for the data collection and analyses, five research chapters (*Chapters 1–5*) presented in the form of scientific research articles, a *General discussion*, and a closing section that synthesizes the main conclusions (*Conclusions*). The supplementary information for each chapter can be found at the end of the thesis, along with an *Appendix* containing five original publications. All chapters are organized following the systematic structure used for scientific works published in scientific journals and conclude with a list of the references cited in their respective text. Since each section closes with a list of references, some references may be repeated in different chapters or sections. Each chapter of this thesis addresses specific objectives, which are outlined briefly in the following section:

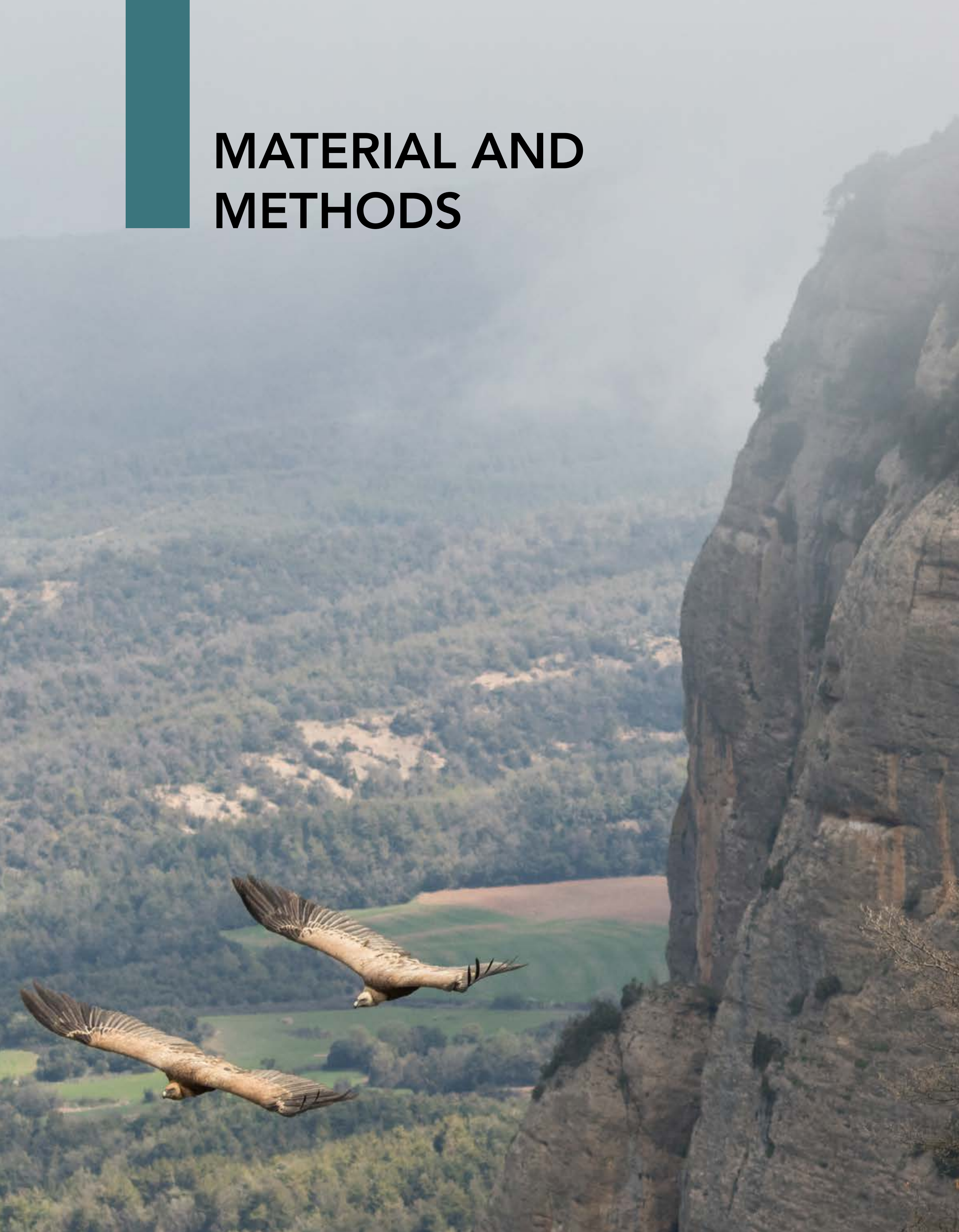
- **Chapter 1** investigates the effects of passive rewilding after farmland abandonment on scavenging dynamics. It assesses differences in 'scavenger assemblage composition' and 'scavenging efficiency' – at both community and species levels – by monitoring a large number of carcasses in three landscape types ('open', 'shrubland' and 'forest') and accounting for the effects of carcass size/type, placement time and carcass consumption by griffon vultures.

- **Chapter 2** explores the vulture-livestock conflict from three different perspectives. It assesses the complaints reported by farmers to wildlife authorities, models the eco-anthropological factors driving the frequency of complaints, and interviews farmers to better understand their perceptions of the conflict.
- **Chapter 3** determines the prevalence and concentrations of different SGARs in the blood of obligate and facultative avian scavengers captured in the field and at the nest, and explores the influence of avian species, age-class and sex, and the most frequent diastereomer forms (i.e., *cis* and *trans*) of the compounds analyzed.
- **Chapter 4** uses a computational bioinspired model to assess population trends in three vulture species in terms of the distribution, quantity and availability of carrion in a region in which traditional livestock practices are maintained. It also explores the carrying capacity of such ecosystems and their relationship with the practice of transhumance.
- **Chapter 5** uses a bioinspired computational model to investigate the demographic effects on the source population of Pyrenean bearded vulture of different strategies involving the removal of individuals (i.e., translocations) for use in reintroductions projects and the trade-offs between various management options.

Owing to the multidisciplinary nature of this thesis, which combines aspects from a variety of research fields and different methodologies to address its objectives, it is divided into five chapters as follows:

Trophic and foraging ecology	**Chapter 1:** Scavenging in changing environments: woody encroachment shapes rural scavenger assemblages in Europe.
Human-wildlife conflicts	**Chapter 2:** Griffon vultures, livestock and farmers: unraveling a complex socio-economic ecological conflict from a conservation perspective.
Environmental pollutants	**Chapter 3:** Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers.
Demography and population viability	**Chapter 4:** Bioinspired models for assessing the importance of transhumance and transboundary management in the conservation of European avian scavengers.
	Chapter 5: Prioritizing among removal scenarios for the reintroduction of endangered species: insights from bearded vulture simulation modeling.

MATERIAL AND METHODS



Griffon vultures *Gyps fulvus* in the Lleida Pre-Pyrenees, Catalonia (Spain)
Photo: Pilar Oliva-Vidal

Study area and focus species

This PhD project was conducted in the Pyrenees, a 430-km long and 65–150-km wide continuous mountainous barrier running from the Atlantic to the Mediterranean (Fig. 6). This range covers around 50,000 km^2 and is the natural boundary between France and Spain (Améztegui et al., 2010). Its northern slopes stretch through the French regions of New Aquitaine and Occitania, while to the south they pass through the Spanish autonomous communities of the Basque Country (province of Gipuzkoa), Navarre, Aragon (Huesca province) and Catalonia (provinces of Lleida, Barcelona and Girona). The principality of Andorra nestles within the mountain range. With its 212 summits over 3,000 m a.s.l., after the Alps, the Pyrenees is the second highest mountain chain in Europe, its highest peak being Aneto at 3,404 m a.s.l., exceeded in height in the Iberian Peninsula only by Mulhacén in Sierra Nevada.

Transversally, the Pyrenees can be divided into three sectors, the Western (or Atlantic), Central and Eastern (or Mediterranean). Latitudinally, the Pyrenees are habitually split into two main morpho-structural regions, the central or *Axial* Pyrenees and the *Pre-Pyrenees*, defined on a basis of their geology and tectonics (Fig. 7 and 8). The *axial Pyrenees* occupy the central and inner zone, gradually decreasing in altitude from west to east and essentially consisting of Palaeozoic and pre-Palaeozoic formations belonging to an ancient Hercynian massif, mainly formed of primary rocks such as granite and gneiss. The *Pre-Pyrenees*, on the other hand, are a series of mountain ranges extending on both the northern and southern sides of the main chain consisting mainly of Mesozoic and Tertiary formations. They are composed of limestone characterized by abrupt relief and deep gorges, alternating with loamy depressions. The southern Pyrenean zone can be further divided into the Interior Ranges (Sierras Interiores), Marginal/Exterior Ranges (Sierras Marginales/Exteriores), Central Depression (Depressión Media Prepirenaica) and the Ebro Basin. The northern Pyrenean zone can be divided into the North Pyrenean zone (Sierras Interiores), Petites Pyrénées and Aquitania Basin (ECORS Pyrenees team, 1988).

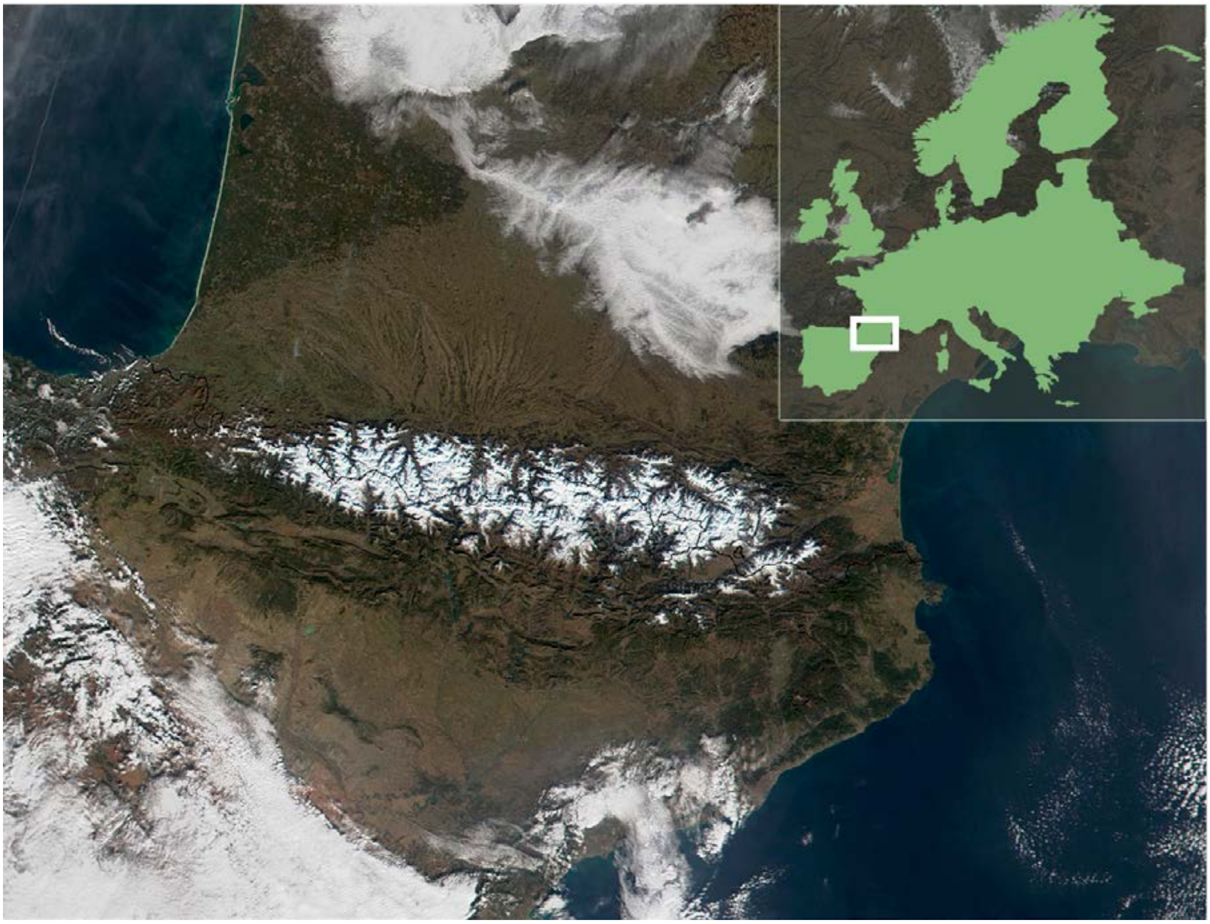


Figure 6. Pyrenees and south-west France in a Moderate-Resolution Imaging Spectroradiometer (MODIS). Image taken 18 January 2002. Modified from MODIS Rapid Response team.

The geographical position of the Pyrenees between the Mediterranean Sea and the Atlantic Ocean, together with its range of altitudinal gradients from sea level to >3,000 m a.s.l., explains its heterogeneous meteorology. Its climate is therefore subject to Mediterranean and Atlantic influences as well as to the effects of macro-relief on precipitation and temperature. In the Central Ebro Depression, the average annual precipitation is ~300 mm, while the average annual temperature ranges from 13 to 15° C. In the mountains, however, annual precipitation exceeds 600 mm and sometimes reaches 2,000 mm at the highest heights. Above 1,600 m a.s.l. most of the annual precipitation falls as snow during the cold season (López-Moreno et al., 2008). Most annual precipitation falls in winter in Atlantic areas but during spring and autumn in Mediterranean regions. The latitudinal location of the Pyrenees within a temperate climate zone leads to extreme seasonal variations: the summer season is relatively dry in the Pyrenees (and very dry in the Ebro Depression) but is moderately humid the rest of the year. Overall, above 1000 m a.s.l., the average annual temperature is < 10° C, while at 2,000 m a.s.l. the mean temperature is ~5°C (López-Moreno and García-Ruiz, 2004).

Figure 7. Punta alta de Napazal in Aísa Valley (Valles Occidentales Natural Park), located in the north-western Aragonese Pyrenees to the south of the axial zone. Photo: Pilar Oliva-Vidal.

Figure 8. Serra de Mont-Roig in the Lleida Pre-Pyrenees, Catalonia. Photo: Pilar Oliva-Vidal.

Vegetation patterns are also strongly influenced by this double topographical and altitudinal gradient. The alpine stage (> 2,300–2,800 m a.s.l.) is highly influenced by the low temperatures and only herbaceous vegetation, dominated by different seasonal plant communities, thrives. The subalpine stage (2,300–1,600 m a.s.l.) is essentially the domain of mountain pine *Pinus uncinata*, which is ubiquitous (reaching the tree line in most of the central and eastern Pyrenees) and forms forests of diverse types, alternating with shrubs and grazing areas. This belt has been heavily deforested over the centuries to increase the surface area of the pastures. In the montane stage (1,600–800 m a.s.l.), Mediterranean mixed deciduous woodland (e.g., oaks) and coniferous forests (mainly natural Scots pine *Pinus sylvestris*) in more continental areas, with extensive secondary vegetation of shrublands and pastures, predominate. Beechwoods of beech *Fagus sylvatica* and silver fir *Abies alba* are the most characteristic montane forests on the Atlantic face and in areas of Atlantic influence. The current abandonment of traditional land uses, combined with rising temperatures, is driving widespread woody encroachment from the remaining forests (mainly from the lower part of subalpine areas) in this stage. Valley bottoms (< 800 m a.s.l.) support most conventional human activities and the highly anthropized floodplains are dominated by cultivated fields or abandoned land (for details see Ninot et al., 2007; Améztegui et al., 2010).

Current Pyrenean landscapes have undergone a long history of human occupation and changes in land cover dating back to at least the Neolithic (Blondel, 2006) or even the Palaeolithic periods provoked by fires and the hunting of large herbivores (González-Sampériz et al., 2017). The human transformation of upland agropastoral landscapes continued until the mid-nineteenth century given the need to preserve large areas of pastures to support vast transhumant herds and feed the growing population (Sancho-Reinoso, 2013). The result of this intense pressure on mountain agroecosystems was the creation of large open landscapes with few vestiges of the former forests and a predominance of herbaceous communities (Fillat et al., 2008). However, the arrival of industrialization in the second half of the nineteenth century drastically changed the traditional model of land use as the ever-increasing rural exodus, which accelerated above all from the mid-twentieth century onwards, began to have important consequences for the dynamics of landscapes and ecosystems (Lasanta et al., 2017; Palacio, 2021). Natural processes of vegetation regeneration involving first shrub and then forest colonization provoked by farmland abandonment (the so-called 'passive rewilding', see Pereira and Navarro, 2015; Nogués-Bravo et al., 2016), were the most significant landscape changes to take place in the Pyrenees in the twentieth century (García-Ruiz and Lasanta-Martínez, 1990; García-Ruiz et al., 1996, 2020a; Poyatos et al., 2003; Lasanta-Martínez et al., 2005; Lasanta et al., 2017; Nuche and Alados, 2018). For instance, more than 70% of historically cultivated farmland was abandoned in the central Spanish Pyrenees in the twentieth century (Lasanta-Martínez, 1988) and nowadays forest is the most abundant landscape feature in these mountains (Poyatos et al., 2003; Lasanta-Martínez et al., 2005).

Like many other European rural areas, the abandonment of farmland throughout the Pyrenees arose as a result of significant rural-urban migration as people sought better economic opportunities as mountain areas became unable to compete with the new industrial economies (Mottet et al., 2006). The result was the collapse of mountain societies due to the socio-economic transformation involving the abandonment of agriculture and the concomitant fall in the rural population (Roura-Pascual et al., 2005; García-Ruiz and Lana-Renault, 2011). However, in some Pyrenean regions, the abandonment of slopes took place at the same time as agricultural intensification occurred in valley bottoms where pasture for cattle fodder could be grown (García-Ruiz and Lasanta-Martínez, 1990; Mottet et al., 2006). At the same time, the expansion of tourism contributed to further spatial disorganization and conflicts over land use. Land in the most favoured locations began occupied for the construction of tourist and recreational infrastructures (Balcells, 1983; García-Ruiz and Lasanta-Martínez, 1993). In some villages this scenario pushed stock-breeding into the background since the new generations were no longer interested in cattle and preferred to devote their time to commerce, hotel management and working in winter resorts. Although tourism may occasionally act as a complement to agriculture by providing extra income for households, it does also compete for the labour force (Bernués et al., 2014). García-Ruiz and Lasanta-Martínez (1993) have shown that the most well-developed tourist areas tend to be less involved with livestock, a finding that highlights the essential incompatibility of these two interests.

Simultaneously, most sheep herds have disappeared and been replaced by beef cattle, which is now the predominant livestock type in the Spanish Pyrenees (García-Ruiz and Lasanta-Martínez, 1993; Lasanta-Martínez et al., 2005; Muñoz-Ulecia et al., 2021). Indeed, during the twentieth century, some regions of the central Spanish Pyrenees lost over 80% of their sheep resulting in minimal grazing pressure over large areas, especially on lower slopes (Vicente-Serrano, 2001). Moreover, the traditional practice of 'transhumance' – the seasonal movement of livestock (mainly sheep and goats, occasionally also cows and horses) to higher ground in summer and to the lowlands in winter – that had been one of the mainstays of the Pyrenees since the twelfth and thirteenth centuries is today undergoing a serious crisis (García-Ruiz et al., 2020b). Transhumance usually implies long-distance movements of livestock that in some cases involve treks along hundreds of kilometres of trails that link uplands to areas far outside the mountains. Conversely, the so-called 'transtermitance' or 'valley transhumance' (i.e., the seasonal movement of livestock over short distances) is nowadays the most common livestock dynamic in which there is no exploitation of grasslands outside mountain areas (Fernández Mier et al., 2013). This type of management is characterized by short livestock movements (about 10–25 km) in summer, with cattle remaining in valley bottoms near villages during the cold season. This movement of thousands of sheep, goats, cows and horses that has taken place in the Pyrenees for hundreds of years is still practiced but is declining fast (O'Flanagan et al., 2011; García-Ruiz et al., 2020b). This loss of traditional livestock practices since the end of the eighteenth century and, in particular, in the twentieth century, has accelerated the re-colonization by woody plants (i.e., rewilding or landscape naturalization) of less accessible and marginal areas once farmland is abandoned (García-Ruiz et al., 2020a).

From an ecological point of view, the main consequences of this rural collapse is the colonization of former fields with shrubs (e.g., *Genista scorpius*, *Rosmarinus officinalis* and *Buxus sempervirens* on basic soils and *Cistus laurifolius* on siliceous soils), the establishment of young forests of *Quercus rotundifolia* and *Q.*

pyrenaica, and intentional reforestation with *Pinus nigra* and *P. sylvestris*, which occurred mainly during the 1960s (García-Ruiz et al., 2020a). By the end of the twentieth century, only a few areas of grasslands were covered by shrubs, which were kept under control by the remaining livestock. Indeed, some stockbreeders indicate that the most important challenge in maintaining or increasing their livestock numbers is the progressive decline of grazing areas due to woody encroachment (Lasanta-Martínez et al., 2013a). For instance, the encroachment of abandoned fields results in dense thorny shrub cover that becomes impossible to graze just 25–30 years after being abandoned (Molinillo et al., 1994). Furthermore, agronomists and forest engineers warn that the risk of fire increases in areas covered with dense shrublands and forest (Lasanta et al., 2019).

Meanwhile, in recent decades, tourism and urbanization (closely related to recreational activities such as skiing Lasanta-Martínez et al., 2013b) have become the main economic activities and major drivers of socioeconomic development in many Spanish Pyrenean regions (García-Ruiz and Lasanta-Martínez, 1993; Vaccaro and Beltran, 2007). As a consequence, many large infrastructures (e.g., roads, hotels, apartments, weekend homes, sport complexes, restaurants and shops) have been built. Although the expansion of tourism was first mostly related to the ski industry, the Pyrenees has become the focus of marketing efforts that offer a wide choice of outdoor recreational activities. This is due to its unique combination of climatic and environmental conditions, which opens the doors to an economic model based on mass tourism that could have an impact on future conservation efforts, as some local people recognize (López-i-Gelats et al., 2009).

This changing rural scenario (decline of livestock and grazing, farmland abandonment and the expansion of tourism) may dramatically affect the preservation of Pyrenean mountain 'cultural landscapes' (i.e., landscapes subject to human influence whose socio-ecological patterns and feedback mechanisms govern biodiversity; Farina, 2000), inherently linked to traditional land use and agropastoral practices. Indeed, many of the rural and/or mountain regions of Europe are areas of highest nature value (HNV). For instance, the European Environment Agency (EEA) recognizes that "Europe's rich cultural and natural heritage is reflected in its traditional agricultural landscapes. A wide variety of natural conditions and farming traditions has created unique landscapes that are not only pleasing to the eye but provide the living conditions for many plants and animals. High nature value (HNV) farmland comprises the hot spot of biological diversity in rural areas and is often characteristic of extensive farming practices (EEA, 2004)". Furthermore, this assessment shows that the prevalence of HNV farmland with inherently high biodiversity values occurs in the least productive areas with semi-natural grasslands, for example in southern Europe and in mountainous regions such as the Pyrenees, where several habitats contain many species of particular conservation concern (EEA, 2004; Paracchini et al., 2008).

The Pyrenees hold a rich diversity of flora and fauna including several endemic species (Pedrocchi, 1997). Charismatic species such as Tengmalm's Owl *Aegolius funereus*, Pyrenean Rock Ptarmigan *Lagopus muta pyrenaica*, western capercaillie *Tetrao urogallus*, bearded vulture and medium- and large-sized mammals such as brown bear *Ursus arctos* and, sporadically, wolf *Canis lupus* inhabit their ecosystems even though most are listed as Critically Endangered. Drastic habitat loss, changes in landscape, human pressure, climate change and low genetic diversity are among the main threats to these species (Chapron et al., 2003; Salvatori and Linnell, 2005; López et al., 2010; García-González et al., 2016; García-Lozano et al., 2020; Margalida et al., 2020; Tobajas et al., 2021). Of the species inhabiting the Pyrenees, scavengers are a crucial guild due to the pivotal ecosystem services they provide and are the focus of this thesis, which mainly discusses obligate and facultative avian scavenger species.

Today, the Pyrenees is inhabited by all four European vulture species (griffon, cinereous, Egyptian and bearded vultures), along with several facultative avian and mammal scavenger species. Of the facultative avian scavengers, the most abundant are the corvids: common raven *Corvus corax*, carrion crow *C. corone*, common magpie *Pica pica* and European jay *Garrulus glandarius*. Raptor scavengers include golden *Aquila chrysaetos* and booted *Hieraaetus pennatus* eagles, red *Milvus milvus* and black *Milvus migrans* kites, northern goshawk *Accipiter gentilis*, common buzzard *Buteo buteo* and western marsh harrier *Circus aeruginosus* as the most frequent species. Mammalian scavengers include, above all, red fox *Vulpes vulpes*, wild boar *Sus scrofa*, pine *Martes martes* and stone *Martes foina* martens, as well as frequent free-ranging shepherds' and hunting dogs.

Trends in the populations of the four European vulture species have varied in the Pyrenees in recent decades, even though their current status seems to be favourable. The overall Pyrenean breeding population of griffon vultures – the most abundant species – was estimated at ~6,000 breeding pairs in 2018 (Del Moral and Molina, 2018a), which represents a positive trend compared to 2008 (~5,500 pairs). A closer look reveals that the trend during this period (2008–2018) was negative in Huesca (-0.69%) and, in particular, in Navarra (-3.7%) but was offset by the highly positive trends in Catalonia (59.64%) and Gipuzkoa (52.25%). There is a single breeding colony of the cinereous vulture in the Boumort National Hunting Reserve (Pallars Jussà, Pre-Pyrenees), in which 14 breeding pairs were recorded in 2017 (Del Moral, 2017). This colony was established by a reintroduction project initiated in 2007 in two neighbouring zones (Boumort and Alinyà) in the Catalan Pre-Pyrenees. Its aim was to restore the former range of this species by establishing a population located halfway between the conspecific Iberian and French populations. Currently, at least 70 individuals and 18 breeding pairs have established themselves as a result of this conservation effort. The recent trend in the breeding population of the bearded vulture is clearly positive throughout the Pyrenees (Spain, France and Andorra) and from 45 breeding territories in 1987 this population evolved to around 182–184 pairs in 2021. In 2021, at least 76, 46, 7 and 1 breeding pairs were reported from Aragón, Catalunya, Navarra and Andorra, respectively. Furthermore, polyandrous formations have increased steadily since the 1980s (e.g., from 29% of territories in 2007–2008 to 38% in 2017–2018) (Margalida and Martínez, 2020). In 2016, the adult breeding fraction was estimated at ~365 individuals, 49% of the adult population and 36% of the overall Pyrenean population (estimated at 1,026 individuals, Margalida et al., 2020). The large number of non-breeding adults probably means that the mean age of first reproduction was higher than previously thought and that a higher proportion of territories are occupied by polyandrous trios (Margalida et al., 2020**)**. Finally, trends in the Pyrenean Egyptian vulture population varied in 1987–2018: its estimated breeding population fluctuated negatively in Huesca (180–142 territories) and Navarra (141–129) but grew in Catalonia (29–75) and Gipuzkoa (3–12) (Del Moral et al., 2009, 2018b).

Data collection and analyses

Chapter 1

To better understand the effects of woody encroachment after farmland abandonment on scavenger assemblages functioning and scavenging dynamics, we monitored 238 carrions in three landscape types (open, shrublands and forests) from 2017 to 2019. Carrions consisted on i) herbivore carcasses: sheep/goat *Ovis aries/Capra hircus* (n = 60, including lambs/kids: n = 31), wild boar (n = 40), roe deer *Capreolus capreolus* (n = 8) and European rabbit *Oryctolagus cuniculus* (n = 15); ii) carnivore carcasses: red fox (n = 6), European badger *Meles meles* (n = 9), domestic cat *Felis catus* (n = 7) and stone marten (n = 2); and iii) viscera (n = 60). In Chapter 1, only animal carcasses (n = 178, including herbivore and carnivore species) ranging in size from 1 to 100 kg were used.

Carcasses were placed between 600 and 1750 m a.s.l., and were randomly distributed among the three landscape types over all altitudes: open areas (n = 70), shrublands (n = 71) and forests (n = 37). We selected each landscape according to the vegetation cover within a 25 m radius from the center of the carcass. For open landscapes, we chose large open areas (e.g., grasslands) without shrub/tree cover (i.e., shrub and tree cover < 5%) (Fig. 9 A–B). For shrublands, we selected sites with abundant vegetation cover (i.e., shrub and tree cover ranging 50–70%), mainly large (e.g., *Buxus sempervirens* and *Juniperus* sp.) and medium-size shrub species (e.g., *Genista scorpius* and *Rosa sp.*), or dispersed trees (mainly oak species) (Fig. 9C). Regarding forest landscapes, we placed carcasses in mature pine and beech forests with moderate canopy closure (i.e., tree cover > 90%) (Fig. 9D). Carcasses were weighed and fixed to the ground using inconspicuous iron stakes to prevent scavengers moving them away from the camera focus (Fig. 9A). Carcass monitoring was carried out using Moultrie motion-triggered remote cameras (M–990i 10MP, M–999i 20MP and S–50i 20MP). Cameras were attached to a tree close to the carcasses (e.g., 5–10 m away, Fig. 9) and were programmed to take a set of three consecutive pictures when motion-activated, with one-second delay, and wait for 15 seconds before taking the next set of pictures (see more details in Chapter 1).

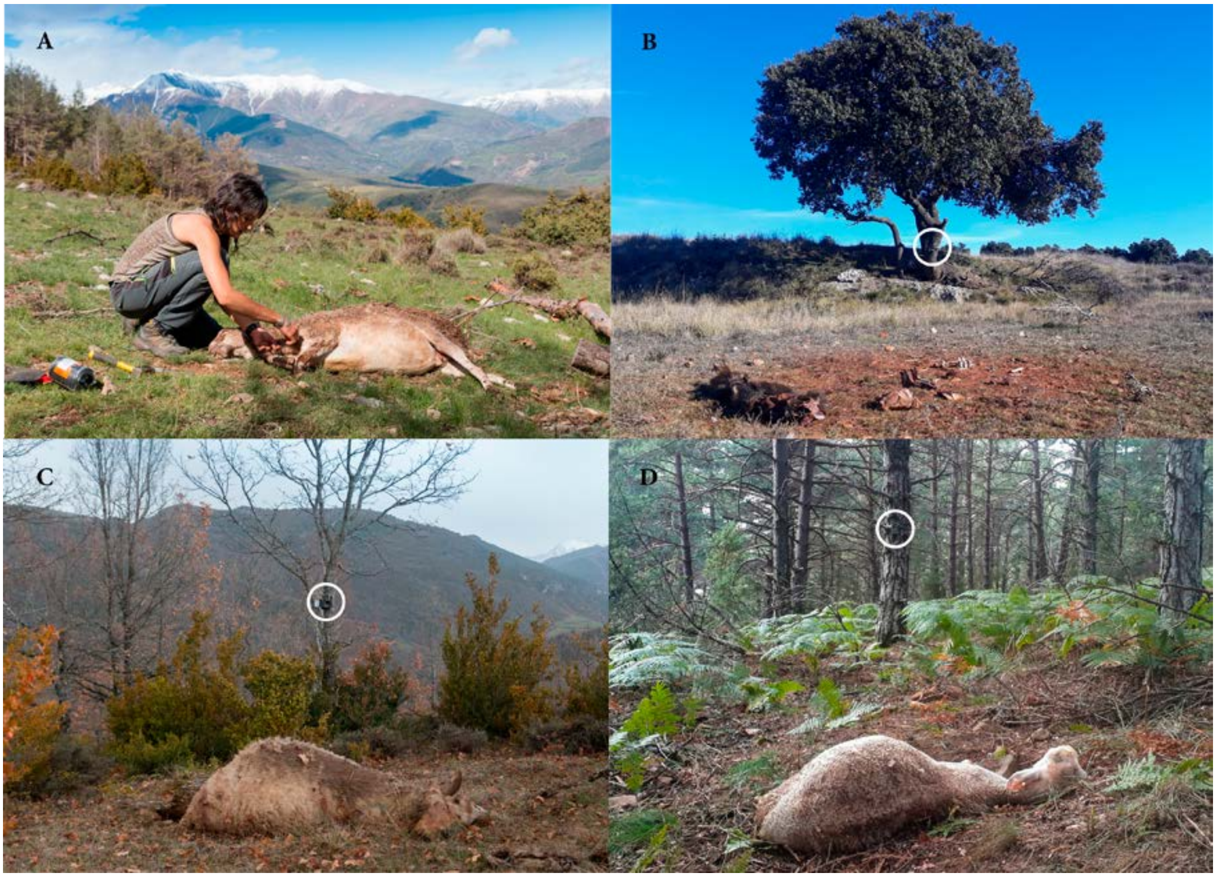


Figure 9. Images of the three landscape types (open, 'A–B'; shrubland, 'C'; and forest, 'D') considered in the carcass monitoring study. The circles show the location of the camera traps.

From the 178 carcasses used in Chapter 1, we obtained ~485,000 photos of which, after deleting those with no relevant information (e.g., empty photos or with no focus species), ~290,000 were used to extract data. Pictures were downloaded from cameras after the conclusion of each trial and were visually inspected to determine: i) arrival date, time, species identification, and consumption time at each scavenging event (e.g., detections were considered independent events if the time between consecutive photos of the same species was > 0.5 hours apart following O'Brien et al., 2003) for all species visiting carcasses and ii) the maximum abundance of each species per carcass. Then, we assessed the vertebrate scavenger assemblage composition and scavenging efficiency by calculating different scavenging measures. First, the 'scavenger assemblage composition' was explored by determining the following variables for each landscape type:

- **Total richness:** number of vertebrate scavenger species recorded at all carcasses
- **Richness:** number of vertebrate scavenger species per carcass. It was also calculated for birds and mammals separately.

- **Scavenger abundance:** maximum number of unequivocally different individuals of each species per carcass. It was calculated by counting the highest number of individuals appearing simultaneously in a picture and by differentiating individuals of different age, sex or individuals features.

Second, the 'scavenging efficiency' (e.g., carcass location and consumption ability) was explored by determining the following variables for each carcass and landscape type:

- **Scavenging frequency:** percentage of consumed carcasses for each species.
- **Detection time:** time elapsed (h) between carcass placement and the arrival of the first scavenger. It was also calculated for birds and mammals separately.
- **Total consumption time:** time elapsed (h) between carcass placement and its complete consumption.
- **Active consumption time:** time elapsed (h) between start of carcass consumption and its complete consumption.
- **Consumption rate:** kilograms of carrion biomass consumed divided by carcass consumption time. Carcass stomach contents and unconsumed parts were excluded from the carcass weight.
- **Unconsumed remains:** weight (kg) of the skin and skeletal remains once the carcass was completely consumed.

The factors influencing both the 'scavenger assemblage composition' and 'scavenging efficiency' were investigated by fitting generalized linear models (GLMs) where 'richness', 'avian/mammalian richness', 'abundance', 'detection time', 'consumption time' and 'consumption rate' were the response variables; and 'landscape type' (open, shrubland or forest), 'carcass type' (herbivore or carnivore), 'placement time' (morning or afternoon) and 'carcass weight' (kg) were predictors. The 'taxon' (avian or mammalian) of the first detector was used as categorical predictor to test for differences in detection time between them using univariate (i.e., single-predictor variable) GLMs, where 'detection time' (h) was the response variable. We used Poisson error distributions and log link functions to model 'richness'; negative binomial error distributions and log link functions for 'abundance'; and Gaussian error distributions and identity link functions for 'detection time', 'total and active consumption times' and 'consumption rate', in all analyses. 'Detection time', 'consumption times' and 'consumption rate' were log-transformed to improve normality assumptions. The unconsumed remains (i.e., skin and skeletal remains, in kg) of medium-size ungulates among landscape types were compared using the Kruskal-Wallis test. GLMs were also used to investigate factors influencing the scavenging frequency at the species level (i.e., response variable) according to the same predictors (see above), using a binomial error distribution. Models were fitted with all possible combinations and subsets of the predictors. We selected the model with the lowest AICc and when there was more than one model with a ΔAICc < 2 relative to the best model, we used a model-averaging function in the 'MuMIn' package (Barton, 2013). Finally, to investigate whether the presence of griffon vultures at carcasses affected scavenging dynamics, we evaluated the relationship between all the composition and efficiency variables and the presence of this species using single-predictors GLMs with a binomial error distribution (see more details in Chapter 1).

Chapter 2

To try to disentangle the factors influencing the occurrence of vulture-livestock conflicts (i.e., the so-called 'vulture attacks'), in Chapter 2 we investigated this human-wildlife conflict (HWC) from three different perspectives: 1) characterizing the complaints reported by farmers, 2) modelling the eco-anthropological factors influencing their frequency and 3) assessing farmers' perception through interviews.

- **Complaints characterization:** we used an official database provided by the Generalitat of Catalunya containing all complaints reported by farmers (n = 683) to wildlife authorities from 1996 to 2000, and explored their temporal trend. Then, we used the complaints reported from 2008–2020 (n = 537, for which more detailed data was available) to characterize the interactions. For each complaint, data included: i) date of the interaction, ii) date of the assessment, iii) municipality and country, iv) livestock type (cattle, horses or sheep/goats), and category ('adult', 'female and new-born' or 'new-born'), v) number of individuals affected, vi) validation (i.e., 'accepted' or 'rejected'), vii) financial compensation (€) and viii) UTM coordinates. To characterize the interactions, we recorded: i) livestock type, ii) livestock category, iii) seasonality (e.g., the month the claim was reported), iv) number of claims 'accepted' (i.e., financially compensated) or 'rejected' and v) total economic (€) compensation by the authorities.
- **Frequency of complaints:** we used the claims reported from 2008–2018 (n = 573, 83.9% of the total) for modelling the determinants of conflicts, as updated information of livestock density was not available for the latest period. Complaints reported during this period were distributed in a grid of 10 x 10 km UTC cells, since each cell was considered as one single statistical observation in the analyses (n = 110 cells accounting for 537 claims). Geoprocessing analysis was performed using the ArcMap 10.5 program. We calculated the following explanatory variables at the cell level (see more details in Chapter 2):
  - Number of griffon vultures breeding pairs
  - Global griffon vulture abundance (breeding and non-breeding fractions)
  - Extensive livestock density (animals/km^2)
  - Distance to nearest Supplementary Feeding Station 'SFS' (km)
  - Distance to nearest landfill site (km)

- **Farmer interviews:** farmers' perceptions toward the vulture-livestock conflict were assessed through face-too-face and telephone interviews from 2018 to 2021. We interviewed 127 farmers from Pyrenean and pre-Pyrenean regions, obtaining information on 166 farms of cattle (53.6%), sheep/goats (30.1%) and horses (16.3%), since 34 respondents had mixed farms. First, we recorded information regarding the farm characteristics (e.g., livestock type) and then, we asked both closed and open questions relating the vultures-livestock conflict. Farmers were also asked if they had experienced vulture interactions and if so, information about the livestock involved as well as if they had recognized the species starting the interaction was also recorded. Furthermore, farmers were asked a range of questions regarding their views on whether vultures could attack livestock, the temporal trend of such interactions, the livestock supervision during birthing times and on the response of authorities as well as any financial compensations. Finally, their perception towards both the coexistence and ecological relationship between vultures and livestock as well as on the measures that could be implemented to mitigate the conflict were also recorded. Some questions were in multiple-choice format (see Supporting information for the full list of questions and for the responses obtained).

The eco-anthropological factors influencing the frequency of vulture-livestock interactions (i.e., number of complaints) were investigated by fitting a GLM where 'number of complaints' was the response variable and 'number of griffon vulture breeding pairs', 'global griffon vulture abundance', 'extensive livestock density', 'distance to nearest landfill site' and 'distance to nearest SFS' were the explanatory variables. We used Poisson error distributions and log link functions. The Akaike's information criterion for small sample sizes (AICc) was used to identify the most parsimonious model (e.g., lowest AICc) and to rank the remaining models. When there was more than one model competing with the best AICc model (i.e., with ΔAICc < 2), a conditional model-averaging across the candidate models was performed (Burnham and Anderson, 2002; Barton, 2013). We used Chi-squared analyses to look for differences in farmers' perceptions on the coexistence and ecological relationships between vultures and livestock based on whether they had suffered attacks (see more details in Chapter 2).

Chapter 3

Since starting work on this thesis, we have carried out important active monitoring (e.g., of blood, feathers and swabs) by sampling different obligate and facultative avian scavenger species in the Pyrenees and adjacent regions (NE Iberian Peninsula). Above all, in 2017–2022 we sampled 350 avian scavengers throughout the whole Pyrenees (i.e., western, central and eastern) and Pre-Pyrenees to improve our knowledge of the extent to which they are exposed to different anthropogenic contaminants (e.g., anticoagulant rodenticides, veterinary pharmaceuticals, lead and other heavy metals). This active monitoring included both obligate (e.g., griffon, cinereous, bearded and Egyptian vultures) and facultative (e.g., red and black kites and golden eagles) avian scavengers, and individuals of all age classes (nestlings, subadults and adults).

Blood samples were collected when birds were fitted with satellite transmitters and/or marked with patagial tags and ringed. Whole-blood samples (3–5 ml, never more than 1% of the body weight) were collected from the brachial vein using a sterile hypodermic needle and syringe (Fig. 10). Samples were immediately transferred into EDTA or heparinized tubes and were carefully shaken to ensure thorough mixing with the anticoagulant medium. Samples were kept frozen (-80ºC) until analysis in the laboratory.

Figure 10. Images showing the blood sampling and bird handling of different avian species: A) bearded vulture, B) red kite, C) cinereous vulture and D) Egyptian vulture.

In Chapter 3, we investigated the exposure of obligate and facultative avian scavengers to different SGARs (brodifacoum, difenacoum, bromadiolone, and flocoumafen) by analyzing 261 blood samples. This study included the four European vulture species (i.e., obligate avian scavengers) in addition to kites and a golden eagle (i.e., facultative avian scavengers) of different age classes and sexes (Table 2). Our main goals were to determine 1) the prevalence and concentration of different SGARs at the species level; 2) the influence of 'avian species', 'age class' and 'sex' in SGAR prevalence and concentrations; and 3) the most frequent diastereomers forms (i.e., *cis* and *trans*) of the compounds analyzed.

Table 2. Species included in this study and number of individuals sampled according to the age class (nestling, juvenile, subadulat and adult). The number of females and males of each species is shown.

Species	Age class					Sex	
	Nestling	Juvenile	Subadult	Adult	Total	Females	Males
Griffon vulture	7	–	11	47	65	20	45
Cinereous vulture	16	–	–	–	16	9	7
Bearded vulture	7	7	19	34	67	44	23
Egyptian vulture	33	–	7	27	67	36	31
Red kite	20	8	–	8	36	17	19
Black kite	–	1	–	7	8	6	2
Golden eagle	–	–	–	1	1	1	–
Bonelli's eagle	1	–	–	–	1	1	–

SGAR analyzes were performed in a Spanish Laboratory of Toxicology (IREC, Ciudad Real), following the extraction method described by Martínez-Padilla et al. (2017) with some modifications (see Chapter 3 for further details). To ascertain whether 'avian species', 'age class' (nestling or non-nestling) or sex (female or male) affected the prevalence and concentrations of SGARs, we fitted GLMs where 'SGARs presence' (positive or negative), 'ΣSGARs concentration' (i.e., sum of concentrations of all detected compounds in each bird) and 'ΣSGARs concentration > LOQ' (i.e., considering individuals with SGARs levels > LOQ) were the response variables; and 'avian species', 'age class' and 'sex' were categorical predictors. Given that we did not sample individuals of all age classes for all species (see Table 2), we lumped the age data into two categories, 'nestling' and 'non-nestling' in all models. We used binomial error distributions and log link functions for SGARs prevalence and Gaussian error distributions and identity link functions for ΣSGARs concentration and ΣSGARs concentration > LOQ. To improve normality assumptions, both ΣSGARs concentration and ΣSGARs concentration > LOQ were log-transformed. When we found significant differences among avian species, we used a post hoc Tukey's HSD test for pairwise comparisons (Sokal and Rohlf, 2012) using the *multcomp* package (Hothorn et al., 2008) to determine where significant differences occurred.

At the intraspecific level, we reported 'SGARs prevalence', 'ΣSGARs concentration' and 'ΣSGARs concentration > LOQ' according to all age class categories (nestling, juvenile, subadult and adult). Given sample size limitations, we did not model SGARs prevalence and concentrations at the species level. We used the Krukal-Wallis test and post-hoc pairwise comparison using the pairwise Wilcoxon rank sum test to test for intraspecific differences in ΣSGARs concentration among age classes for species with sample size ≥ seven individuals and with individuals of at least three age classes (griffon, bearded and Egyptian vultures and red kites; Table 2). To explore differences in the proportion of the most frequent diastereomers of each SGAR, we performed GLMs where 'proportion of *cis* forms' or 'proportion of *trans* forms' were the response variables, and 'avian species', 'age class' and 'sex' were categorical predictors. We used Gaussian error distributions and identity link functions.

Chapters 4 and 5

Ecological modelling (i.e., the modelling of living organisms and their relationships with the environment) has received growing scientific attention given its usefulness in advancing knowledge of ecosystem functioning (Jørgensen, 2009). However, modelling ecosystem dynamics is often a highly complex process given the large number of variables and internal interactions. This complexity has been in the past the main factor limiting the design of models since highly powerful computers were required to execute traditional modelling methods that employed differential equations. In recent decades, however, the increasing power and technical characteristics of computers and the growth of the scientific information available for ecological studies have led to the development of more complex modelling techniques (Reshef et al., 2011) and the emergence of a new family of models known as 'computational models' (Fisher and Henzinger, 2007; Petrovskii and Petrovskaya, 2012). These models are formal models, the semantics of which are operational (i.e., the model prescribes a series of steps or instructions that can be executed by an abstract machine). This modelling system is programmed using a computer operating with a specific programming language. Computational models reach a large number of states in a non-linear and non-deterministic way, which is not possible using mathematical models. The efficiency with which computers can execute instructions is crucial for the execution of highly complex computational models. However, unlike mathematical or statistical models, computational models neither have formal construction or standards nor are easily interpretable, which complicates their use by researchers (Fisher and Henzinger, 2007).

In this context, the discipline of 'natural computing' has emerged as a means of studying and simulating complex dynamic processes. The main characteristic of natural computing is the fact that it is inspired by biological processes (i.e., bioinspired models) and therefore attempts to imitate natural phenomena in order to solve complex problems as an alternative to more classic computing (Pérez-Hurtado, 2010). Different computational models have been developed within the natural computing discipline. In 1998 a new branch of natural computation emerged, the so-called 'cellular membrane computing', which was developed with the purpose of defining computing devices known as P systems that abstract from the structure and the function of living cells (Păun, 1998, 2010).

Păun (1998) proposed an abstraction and graphical representation of the cell that allows the definition of P systems. Briefly, cells are the morphological and functional units of living organisms and are characterized by a membrane system that delimits, defines and organizes intracellular spaces and its compartments. The outer membrane delimits the cell from the outside environment and encloses an interior space, the cytoplasm. Within the cytoplasm, there are a set of cellular structures (organelles such as the nucleus, which contain the genetic material). Some organelles have a double membrane system while other organelles are contained in only one membrane. The main feature of the biological cell membranes is that they do not generate watertight compartments and allow instead for the flow of certain chemical substances, sometimes selectively, sometimes unidirectional. Thus, the cell can be seen as a set of permeable membranes delimiting spaces in which organelles and chemical substances are located that can evolve according to specific membrane reactions. Fig. 11 shows the inside of a eukaryotic plant cell, including the structure that inspired natural computing with membranes.

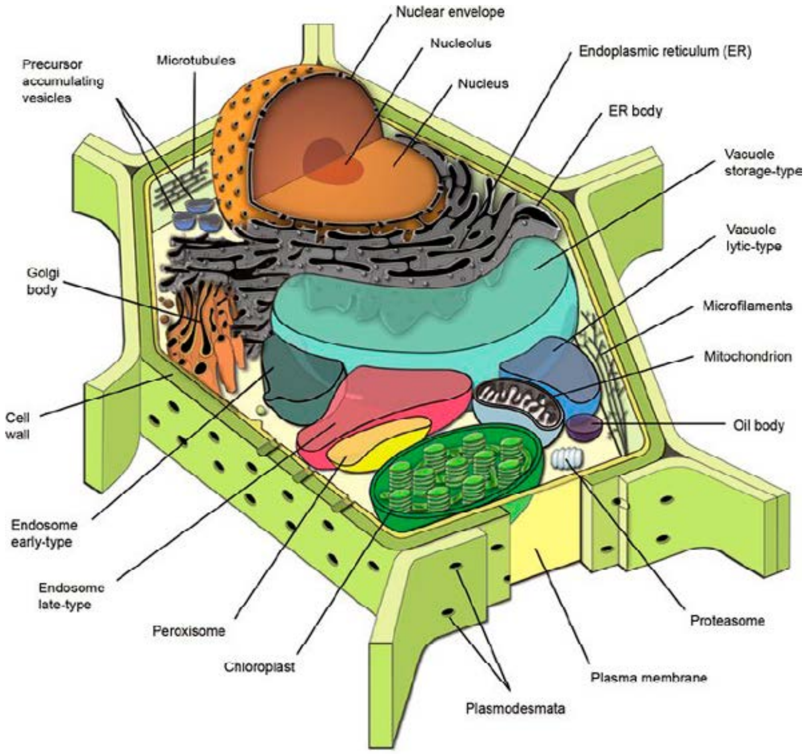


Figure 11. Diagrammatic representation of a eukaryotic plant cell depicting the principal organelles, compartments and structures (Dhanoa et al., 2011). The cell contains a set of organelles, some of which are delimited by different types of membranes (and regions) that evolve according to a series of chemical reactions.

The domain of P systems includes variants that aim to solve complex problems and achieve a better approximation to the true biological models on which they are inspired. One of these variants are the Population Dynamic P System (PDP) models, which consist of probabilistic individual-based models that have been successfully applied to the simulation of ecosystem dynamics (Colomer et al., 2013; Chapter 4 and 5). PDPs are inspired by cell functioning whereby small organelles (i.e., 'objects', see below) grow, evolve, reproduce and die while interacting with their surroundings and other organelles of the same or different types. The main characteristics of PDPs are their ability to work in parallel (simultaneously interrelating different processes), modularity and high computational efficiency, and have become an effective tool for modelling complex systems (Colomer et al., 2013).

A certain analogy can be established between a PDP system and an ecosystem. An ecosystem corresponds to a physical space where there are a number of distinct areas of certain characteristics (e.g., landscape and weather conditions). Within these areas, there are individuals whose development is conditioned by their own particular biological and demographic singularities. Individuals evolve simultaneously and interact and compete with each other and with the environment according to patterns or rules of evolution. It is also possible that individuals move from one area to another based on certain ecological restrictions (e.g., food and carrying capacity; Chapter 4). Each of these areas can be seen as a different spatial environment of a PDP model and its contents can be specified as a cell having its own structure and its own life-style traits (Colomer et al., 2013).

In ecosystems, these processes are carried out simultaneously, in a synchronized and inter-related fashion. Synchronization can be materialized by the biological cycles of the organisms that compose it and so we can assume that there is a global clock operating in the system (Colomer et al., 2013). PDPs have a great potential for modelling apparently complex problems that are difficult to tackle using classical models, for instance when modelling natural ecosystems in which a large number of individuals evolve individually and interact with each other and with the ecosystem. This analogy between PDP systems and real ecosystem has been developed in recent years. For example, PDPs have been used to assess the population dynamics of threatened populations in relation to food availability and climate change (Colomer et al., 2011, 2014; Margalida et al., 2011; Margalida and Colomer, 2012; Kane et al., 2014; Cortés-Avizanda et al., 2015). More recently, PDPs have been used to model the effects of vaccination and the usefulness of effective measures to reduce pandemics (e.g., the recent SARS–CoV–2 outbreak) at population level (Colomer et al., 2021a, b).

PDPs are probabilistic P systems, thus their rules are associated with probabilities. All cells in the PDP system have the same membrane structure, which can be formally described by a rooted tree in which the external membrane is the father of inner membrane. Membranes are identified by labels as subscripts on the membrane and, to simplify the task of designing the model, membranes have electrical charges. A PDP model can be viewed as a collection of environments each of them containing a cell with the same membrane structure (Fig. 12). The basic components of a PDP system are:

- A set of **environments** (i.e., space in which a single cell is located) that are connected among them according to some prefixed relation, and which can be formally described by a network structure.
- A **membrane structure** that provides the hierarchy among the different membranes that constitute the cell contained in each environment. This structure forms compartments in which the multisets of objects evolve according to biochemistry inspired rules. The membranes are organized hierarchically within an outer membrane, called *skin membrane*. Each membrane is identified by a label and is electrically charged (i.e., positive [+], negative [-] or neutral [0]). All cells of the system must have the same membrane structure.
- A **working alphabet** that allows the representation of multiset of **objects** (e.g., individuals, resources, etc.) involved in the system under study. Individuals or objects are represented using a specific alphabet contained in the work alphabet. Depending on the model, two types of objects can be differentiated: those associated with the agents forming part of the model and those associated with the processes that determine their evolution and synchronization. These objects may be located in the areas defined by the membranes or outside (i.e., in the environment) and can appear repeatedly within the same multiset. Objects can evolve and move through membranes by means of evolution rules, just like real cell organelles.
- A set of **rules** (mathematical expressions) that will enable the specification of the evolution of the objects inside and a set of rules for the environments that serve to specify how individuals can move from one environment to another, to generate values for variables that are correlated between environments and to generate objects whose multiplicity will depend on the environment. Each rule consists of a left-hand side in which objects and membranes are found before applying the rules and a right-hand side, where the multisets of objects and membranes appear after the application.

A simulator needs to be designed to execute a PDP model. We used a free software (MeCoSim) developed by the Natural Computing Group of the University of Sevilla (Pérez-Hurtado, 2010). This software consists of a visual interface that allows the configuration of the inputs and the outputs, one of the major advantages of which is the ease with which the input data can be entered into the model. For example, when the purpose is to study the behaviour of the model in a particular scenario, the only change needed is the introduction of the input value in the interface. MeCoSim input files are of two types: *name.xls* and *name.pli*. The first defines the menus and submenus of the simulator, the tables in which data is entered, the values of the parameters used by the model, and the outputs in form of tables and graphs, while the second, written in ASCII code, contains the model and describes the membrane structure, the initial alphabet and the evolution rules. Colomer et al. (2013) have published a detailed protocol describing the functioning of a PDP model in which seven steps are followed: the first four are the same as in other model types, whereas the rest are specific to modelling with PDP systems.

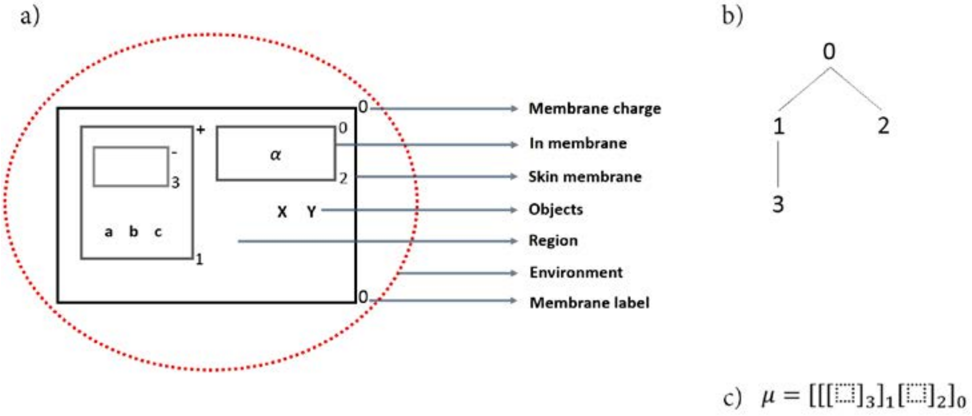


Figure 12. PDP systems structure. a) Representation and components of the cell, b) representation of the membrane structure using a rooted tree, and c) analytical representation of membrane structure. Adapted from Colomer et al. (2013).

In Chapter 4, we assess the role of transhumance as an important food resource for avian scavengers. Additionally, we quantify the carrying capacity and population trend of three vulture species according to the food biomass provided by wild and domestic ungulates, as well as the food provided at supplementary feeding sites (SFSs) using bioinspired PDP models. We employed a dataset provided by the Navarre government describing the spatial and temporal livestock movements throughout the study area and the population dynamics of the three studied avian scavengers (griffon, bearded and Egyptian vultures), as well as the amount of food provided (by farmers and wildlife managers) at the 10 SFSs in the study area. See Chapter 4 for more details of the study area and the model inputs, design and particularities.

In Chapter 5, we used 'Response Surface Methodology' (RSM) consisting of a combination of a Box-Behnken design and a bioinspired PDP model to assess the impact of different removal scenarios of clutches, fledglings and non-territorial adults of bearded vultures in its Pyrenean population. We used data from the long-term monitoring carried out in the Spanish Pyrenees by the Catalan, Navarre and Aragón governments, which provided detailed information on the demographic parameters and population trends in this species. We first estimated the population parameters (e.g., productivity, breeding success, age of first breeding attempt, and survival by age classes) based on historical records. In order to study the effects of various life history parameters and extractions on the population trends, we used a response surface design (Box-Behnken). We also combined the extreme range values of certain demographic parameters (productivity and juvenile, subadult and adult survival) in a second Box-Behnken model to assess the potential effects of changes in these parameters on population dynamics. Then, we constructed a PDP model to evaluate the effects of removals of clutches and floating individuals of different ages. The results of the PDP model were compared with a baseline non-intervention scenario based on the demographic rates considered. See Chapter 5 for more details of the PDP and Box-Behnken model design.

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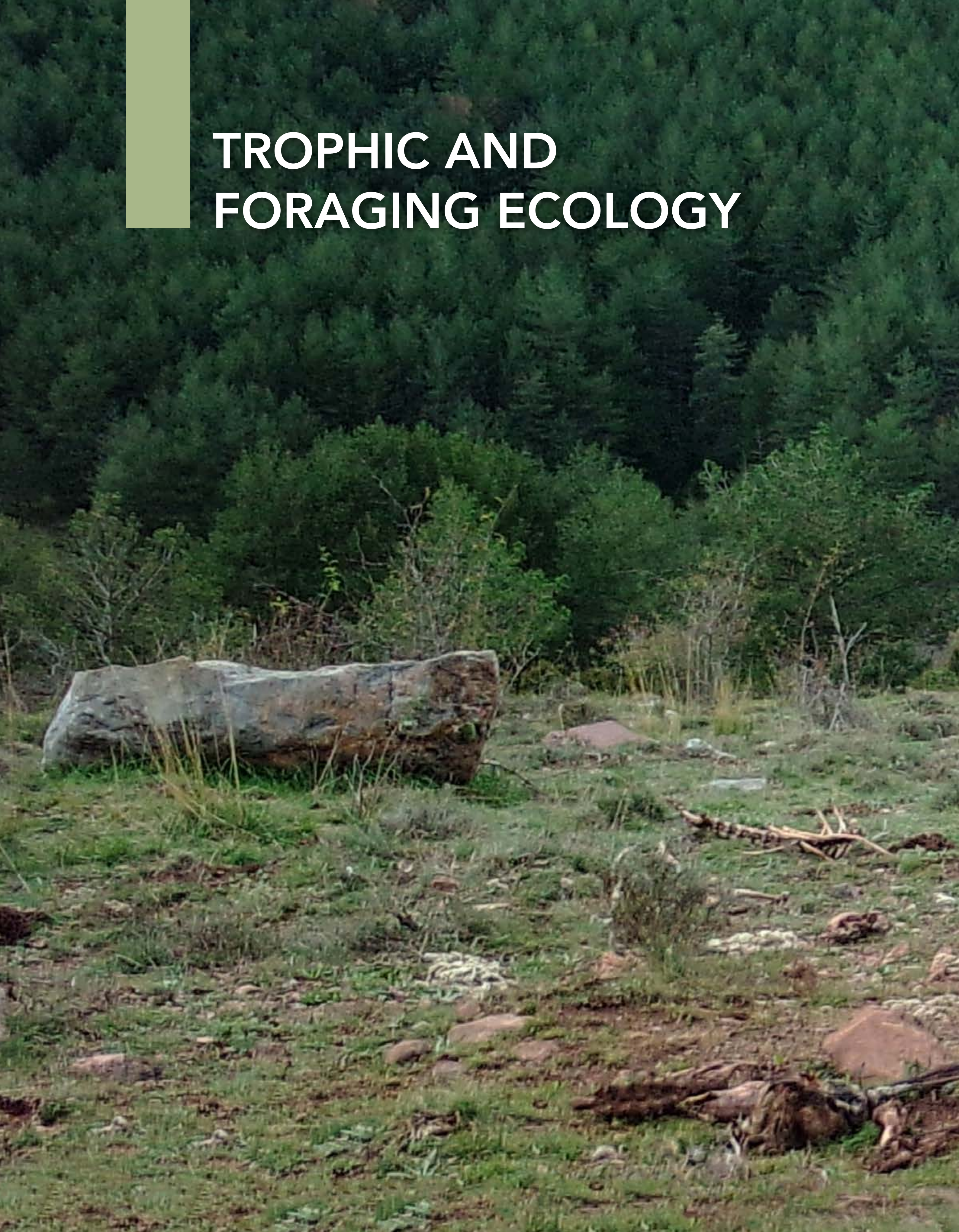
TROPHIC AND FORAGING ECOLOGY



Bearded vulture *Gypaetus barbatus* in the Lleida Pyrenees, Catalonia (Spain)
Photo: Pilar Oliva-Vidal

CHAPTER 1

SCAVENGING IN CHANGING ENVIRONMENTS: WOODY ENCROACHMENT SHAPES RURAL SCAVENGER ASSEMBLAGES IN EUROPE

This chapter corresponds to the article:

Oliva-Vidal, P., Sebastián-González, E., Margalida, A. 2022. Scavenging in changing environments: woody encroachment shapes rural scavenger assemblages in Europe. *Oikos*. https://doi.org/10.1111/oik.09310.

CHAPTER 1

Scavenging in changing environments: woody encroachment shapes rural scavenger assemblages in Europe

Abstract

Rural abandonment and subsequent vegetation regeneration (‘passive rewilding’) are expected to increase worldwide, producing cascades of dynamic socioeconomic, landscape and biological changes. Although landscape characteristics strongly influence the structure and functioning of scavengers, little is known about the ecological consequences of passive rewilding due to woody encroachment (i.e., ‘landscape closure’) on scavenging assemblages. We investigated differences in ‘scavenger assemblage composition’ (species richness and abundances) and ‘scavenging efficiency’ (scavenging frequency, detection and consumption times and consumption rates) in a mountain agroecosystem (Pyrenees) undergoing passive rewilding. We monitored 178 carcasses in three landscapes: ‘open’, ‘shrubland’ and ‘forest’, and evaluated the effects of landscape type on ‘scavenger assemblage composition’ and ‘scavenging efficiency’ at the community and species levels, while accounting for the influences of carcass size, type and placement time. We also examined whether the locally most abundant and efficient scavenger (i.e., the griffon vulture *Gyps fulvus*) affects scavenging patterns. We found that landscape type was the main factor governing scavenging dynamics. Overall and average scavenger richness were similar in open and shrubland landscapes, while forests contained the lowest number of scavengers, mainly comprising mammals. Unlike mammals, avian scavenging frequency decreased as vegetation cover increased, especially for obligate scavengers (i.e., vultures). Scavenger abundances were highest in open landscapes, and carcasses were detected and consumed more rapidly in these landscapes. Carcass size did not influence detection and consumption times, although it did affect average scavenger richness, abundances and consumption rates. Consumption rates were higher in open landscapes and were strongly associated with the presence of griffon vultures. Interestingly, we found that griffon vultures influenced scavenging dynamics via facilitation processes. However, woody encroachment could reduce the scavenging role of this species, while favouring mammalian facultative scavengers. Finally, our findings highlight the pivotal role of griffon vultures, mediated by landscape characteristics, in reducing carcass persistence.

Keywords: agropastoral landscapes, carrion, farmland abandonment, passive rewilding, Pyrenees, rural abandonment, scavengers, vultures

1. Introduction

Rural Europe has been shaped by traditional land-use and encompasses a wide diversity of cultural landscapes, especially in mountain regions (Tieskens et al., 2017). European mountain landscapes are the result of several thousand years of interactions between people and agroecosystems and deliver valuable ecosystem services (Bernués et al., 2014), often with exceptional conservation value (Fisher et al., 2012). Over the centuries, agro-livestock practices have promoted biodiversity by preserving high spatial heterogeneity within forest-agricultural mosaic landscapes (Fahrig et al., 2011). However, socioeconomic changes during the 19th and 20th centuries have led to widespread abandonment of rural areas and the loss of landscape heterogeneity, posing major scientific and policy challenges due to the crucial role of traditional farming for biodiversity and ecosystem service maintenance (MacDonald et al., 2000; Plieninger 2006; Cramer et al., 2008; Lasanta et al., 2017; Ustaoglu and Collier 2018). Understanding the ecological consequences of rural abandonment for ecosystem dynamics is key to the design of biodiversity conservation strategies in such areas.

As in most European mountain regions, important landscape changes occurred in the Spanish Pyrenees during the mid-20th century due to rural depopulation and agricultural modernization (Lasanta-Martínez et al., 2005). Traditional livestock farming systems intensified during this period, resulting in a severe decline in sheep numbers and an increase in cattle. Some valleys in this region saw sheep numbers decline by more than 80%, drastically reducing grazing pressure (Lasanta-Martínez et al., 2005) and livestock biomass over large areas (Margalida et al., 2018). This trend is widespread across Europe, where extensive livestock numbers declined by 25% between 1990 and 2010 (Navarro and Pereira, 2015). Furthermore, the 'transhumance', a seasonal livestock (mainly sheep) migration – usually across long distances – between winter and summer pastures, is in strong decline (García-Ruiz et al., 2020a). Nowadays, livestock are kept in the valleys during the cold season and moved to adjacent pastures during the summer (i.e., 'transtermitance'). This drastic decline in sheep numbers has caused a significant reduction of livestock pressure, accelerating the processes of vegetation succession, especially in marginal areas (García-Ruiz et al., 2020a). These changes have been characterized by generalized farmland abandonment and subsequent vegetation encroachment, modifying the long-standing forest-agricultural mosaic, reducing the extent of open spaces and increasing the extent of woody landscapes (Poyatos et al., 2003; Lasanta-Martínez et al., 2005; Roura-Pascual et al., 2005; Améztegui et al., 2010). The result is passive landscape restoration, or 'passive rewilding', by natural shrub and tree colonization ('landscape closure'), driving important changes in habitat structure and landscape configuration (Pereira and Navarro, 2015; Corlett, 2016; Perino et al., 2019).

Although active rewilding has recently been proposed as a conservation strategy to restore the loss of biodiversity and ecosystem functions (Pereira and Navarro, 2015), there is ongoing debate regarding the associated impacts on species diversity and community dynamics (Queiroz et al., 2014; Nogués-Bravo et al., 2016; García-Ruiz et al., 2020b). For example, depopulation and agricultural abandonment in rural areas of Japan have posed major conservation challenges owing to changes in species distribution, resulting in increased human-wildlife conflicts (Tsunoda and Enari, 2020) and plant encroachment has been associated with losses of mesocarnivore scavenging efficiency in tropical-grassy African savannas (Lima et al., 2021). In Europe, much attention has focused on how natural revegetation leads to an expansion of forest species (Falcucci et al., 2006; Acevedo et al., 2011; Martínez-Abraín et al., 2020), but the effects on species adapted to open-habitats or mountain agropastoral landscapes remain poorly understood (Laiolo et al., 2004; Regós et al., 2014). There is therefore a need to identify the ecological impacts of rewilding, and the resulting changes in landscape configuration, on functional assemblages linked to traditional mountain landscapes. Scavengers (i.e., carrion-eating species), form one such important assemblages. Understanding how scavenger assemblages function in different landscape configurations differing in vegetation structure is critical to inform management priorities dealing with passive rewilding.

Despite the essential role of scavengers in providing regulatory services by efficiently removing carrion from ecosystems (Moleón et al., 2014; DeVault et al., 2016), the effects of rewilding or other changes in habitat configuration on scavenging dynamics have not been extensively investigated (but see Cortés-Avizanda et al., 2015; García-Barón et al., 2018; van Klink et al., 2020). For example, Arrondo et al. (2019) found that in mountainous areas of south-east Spain, sheep carcasses in open pasturelands were detected and consumed faster than wild ungulate carcasses in more heterogeneous areas. Thus, partially abandoned mountain agroecosystems inhabited by both facultative scavengers (species that scavenge opportunistically, e.g., mammalian carnivores, raptors and corvids) and obligate scavengers (species that depend totally on carrion, i.e., vultures) present an ideal opportunity to improve our understanding of how vegetation encroachment affects scavenging dynamics.

Carrion occurs unpredictably in time and space, and provides an ephemeral, valuable food resource. It has high nutritional value and is generally rapidly consumed following the multiple complex interactions that structure scavenger communities and stabilize food webs (Wilson and Wolkovich, 2011; Moreno-Opo et al., 2016; Sebastián-González et al., 2020). However, vegetation cover and structure may influence the ability of scavengers to locate and access carcasses, affecting consumption patterns and efficiency (Selva et al., 2005; Moleón et al., 2019; Pardo-Barquín et al., 2019). An increase in shrub and forest cover may favour carcass detection by scavengers that mainly use odor cues to locate food, such as mammals (DeVault and Rhodes, 2002; Enari and Enari, 2021), to the detriment of avian scavengers that mainly rely on visual cues, such as Old World vultures (Ruxton and Houston, 2004).

To better understand these dynamics, we monitored a large number of carcasses (>170) of several types and sizes, representing the widest carrion diversity appearing in a mountain agroecosystem in which the four European vulture species coexist with important facultative scavenger populations. Our main goal was to describe the 'scavenger assemblage composition' (species richness and abundances) and 'scavenging efficiency' (scavenging frequency, detection and consumption times and consumption rates) at both the community and species levels in three landscape types ('open'; semi-closed or 'shrubland'; and closed or 'forest') where passive rewilding processes occur. We also evaluated whether carcass size, type (herbivore/carnivore) and placement time (morning/afternoon) influenced carcass consumption patterns.

Our general hypothesis was that landscape type, carcass size and type, and placement time influence both scavenger assemblage composition and scavenging efficiency. We predicted: i) that carcasses placed in open areas would be detected and exploited more rapidly, mainly by avian scavengers (i.e., species relying on sight for carcass detection), than those placed in shrublands and forests, which would mainly be consumed by mammals (i.e., species relying on scent for carcass detection); ii) that herbivore carcasses would show higher scavenger species richness, abundance and consumption rates, as carnivore carcasses are known to be avoided by carnivores (Moleón et al., 2017); iii) that carcasses deployed in the morning would be exploited faster, since the more efficient vultures are inactive at night; iv) that larger carcasses would be consumed at a faster rate and by a richer and more abundant scavenger assemblage than small ones, as found by Moleón et al. (2015) in African assemblages; and v) that consumption by griffon vultures would influence the composition and efficiency of the scavenger assemblage, as this species is known to have higher consumption rates and to facilitate carrion location (Cortés-Avizanda et al., 2014). Our research provides a novel approach to the study of the effects of passive rewilding and 'landscape closure' on scavenging dynamics in mountain landscapes.

2. Material and methods

2.1. Study area

The Pyrenees is a mountain range extending over more than 50,000 km^2 in the north-east of the Iberian Peninsula. The study area is on the southern slope and covers around 170,000 ha. This area is characterized by a wide range of altitudes (from ~500 to > 3000 m a.s.l.) and shows significant climatic variation, which offers contrasting landscapes and great vegetational diversity. In the cultivated lands of the lower valleys (< 700 m a.s.l), Mediterranean shrubland and mixed woodlands of oak (*Quercus ilex, Q. faginea*) and Scots pine (*Pinus sylvestris*) predominate. Montane forest zones occur at the middle altitudes (700 – 1600 m a.s.l.), mainly dominated by Scots pine, European beech (*Fagus sylvatica*) and a wide variety of scrublands, open grasslands and pasturelands. In the upper mountain areas (1600 – 1800 m a.s.l. upwards), mountain pines (*Pinus uncinata*) are abundant, and above the treeline (2300 m asl), a mosaic of different types of alpine pastures dominate (Ninot et al., 2007). Historically, these landscapes have been shaped by human activity (e.g., long-term use for crops, livestock and forestry created an increase in open areas at the expense of forest, Roura et al., 2005) but more recent rural abandonment and shepherding decline have led to an increase in natural vegetation cover in previously open areas.

Extensive and semi-extensively reared livestock (cattle, sheep and horses) provide most of the biomass for the scavenger guild (Colomer et al., 2011). Extensive livestock are kept outdoors from April to October, depending on the weather conditions, and some transhumant herds migrate to high summer pastures. Important populations of wild herbivorous ungulates, mainly red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), Pyrenean chamois (*Rupicapra pyrenaica*) and fallow deer (*Dama dama*) are present. Of the obligate scavengers within a 30 km radius of the central study area, almost 900 pairs of griffon, 16 pairs of cinereous (*Aegypius monachus*), 21 pairs of bearded (*Gypaetus barbatus*) and 50 pairs of Egyptian vultures (*Neophron percnopterus*) breed in the area. Regarding facultative scavengers, the study area holds a rich community of avian (e.g., corvids and raptors) and mammal species, while shepherd and free-ranging hunting dogs are also frequent (Supporting information Table 1).

2.2. Carcass monitoring

From October 2017 to August 2019, we monitored 178 carcasses (herbivores, n = 154; carnivores, n = 24) of different species ranging in size from 1–100 kg, placed across three landscape types (open, n = 70; shrubland, n = 71; and forest, n = 37). Herbivore carcasses consisted of sheep/goat *Ovis aries*/*Capra hircus* (n = 60, mean ± SD = 47.65 ± 12.53 kg, including lambs/kids: n = 31, 9.34 ± 6.33 kg); wild boar *Sus scrofa* (n = 40, 53.20 ± 22.47 kg); roe deer (n = 8, 22.04 ± 3.60 kg); and European rabbit *Oryctolagus cuniculus* (n = 15, 1.48 ± 0.29 kg). Carnivore carcasses (mean weight ± SD = 5.71 ± 3.14 kg) consisted of red fox *Vulpes vulpes* (n = 6), European badger *Meles meles* (n = 9), stone marten *Martes foina* (n = 2) and domestic cat *Felis catus* (n = 7). Carcasses were placed between 600 and 1750 m a.s.l., (i.e., the mountain sectors where passive rewilding is widespread, García-Ruiz and Lasanta, 1990), and were randomly distributed among the three landscape types over all altitudes. We selected each landscape type according to the vegetation cover within a 25 m radius from the center of the carcass (Supporting information Fig. 1). For open landscapes, we chose large open areas (e.g., grasslands) without shrub/tree cover (i.e., shrub and tree cover < 5%) (Fig. 1A–D). For shrublands, we selected sites with abundant vegetation cover (i.e., shrub and tree cover ranging 50–70%), mainly large (e.g., *Buxus sempervirens* and *Juniperus* sp.) and medium-size shrub species (e.g., *Genista scorpius* and *Rosa sp.*), or dispersed trees (mainly oak species) (Fig. 1E–G). Regarding forest landscapes, we placed carcasses in mature pine and beech forests with moderate canopy closure (i.e., tree cover > 90%), allowing some visibility of the forest floor (Fig. 1H–J). Most carcasses in shrublands and forests were placed in areas where there was historical evidence of rewilding, e.g., from open landscapes to shrublands, or from shrublands to forests. Scavenging activity was recorded at all carcasses except for one stone marten placed in a shrubland. Carcasses were obtained from legal farms and slaughterhouses or collected as road kills.

Carcasses were monitored using Moultrie motion-triggered remote cameras (M-990i 10MP, M-999i 20MP and S-50i 20MP). Cameras were attached to a tree close to the carcasses (5–10 m away) and were programmed to take three consecutive photos when movement was detected, with a one-second delay between photos, and a delay of 15 seconds before the next set of photos. Carcasses were weighed and fixed to the ground using inconspicuous iron stakes to prevent scavengers moving them away from the camera focus. Carcasses were placed at random between dawn and dusk and separated by at least 1 km to maximize independence (Morales-Reyes et al., 2017). Once a carcass was consumed, we waited at least one month before placing another carcass closer than 1 km from the previous placement. A species was considered a 'consumer' when it was clearly recorded feeding on a carcass. When consumption was suspected, but not clearly recorded, we assumed consumption if that species had already been detected consuming another carcass (Sebastián-González et al., 2019). Carcasses were monitored continuously (24 h/day) until scavenging ended (i.e., only bones and/or skin remained) or the carcass was removed by a scavenger.

Figure 1. Images of some of the most frequent scavengers recorded in the three landscape types studied: 'open' (A–D), 'shrubland' (E–G) and 'forest' (H–J). A) Griffon vultures (*Gyps fulvus*), B) cinereous (*Aegypius monachus*), Egyptian (*Neophron percnopterus*) and griffon vultures, C) bearded vulture (*Gypaetus barbatus*), D) red kite (*Milvus milvus*), E) Egyptian vulture, F) golden eagle (*Aquila chrysaetos*), G) cinereous, bearded and griffon vultures, H) wild boars (*Sus scrofa*), I) common ravens (*Corvus corax*), and J) red fox (*Vulpes vulpes*).

2.3. Scavenging measures

For each landscape type, we calculated the following variables relating to scavenger assemblage composition: a) 'total richness' (number of vertebrate scavenger species recorded at all carcasses) and b) 'richness' (number of vertebrate scavenger species per carcass). Richness was calculated individually for each species and also for birds and mammals separately. For some analyses we grouped scavengers by 'taxon' (avian or mammalian) and as 'obligate' or 'facultative', or at the species level. We also recorded c) the 'abundance' (maximum number of unequivocally different individuals of each species per carcass). This was calculated by counting the highest number of individuals appearing simultaneously in a picture, and by differentiating individuals of different age, sex or individual features (Sebastián-González et al., 2019). Regarding scavenging efficiency (i.e., carcass location and consumption ability), we first calculated the scavenging frequency (i.e., percentage of consumed carcasses) for each species in each landscape type. Then, for each carcass and landscape type we estimated: a) 'detection time' (time elapsed in hours between carcass placement and the arrival of the first scavenger), and separately for birds and mammals; b) 'total consumption time' (time elapsed in hours between carcass placement and its complete consumption); c) 'active consumption time' (time elapsed in hours between start of carcass consumption and its complete consumption); and d) 'consumption rate' (kilograms of carrion biomass consumed divided by carcass consumption time). To calculate consumption rates, we excluded the carcass stomach contents and the unconsumed parts from the carcass weight, so that the consumed biomass was calculated as: (initial weight of the carcass) – (weight of unconsumed remains) – (weight of stomach contents). Unconsumed remains were weighed at the end of each trial (Moleón et al., 2015) and stomach contents were estimated as 10% of the animal weight (Selva, 2004).

2.4. Statistical analyses

We investigated factors affecting scavenger assemblage composition and scavenging efficiency by fitting generalized linear models (GLMs), where 'richness', 'avian/mammalian richness', 'abundance', 'detection time', 'total and active consumption time' and 'consumption rate' were the response variables; and 'landscape type' ('open', 'shrubland', 'forest'), 'carcass type' (herbivore or carnivore), 'placement time' (morning, from dawn to midday; afternoon, from midday to dusk) and 'carcass weight' (kg) were predictors. We also used the 'taxon' (avian or mammalian) of the first detector as a categorical predictor to test for differences in carcass detection time using univariate (i.e., single-predictor variable) GLMs, where 'detection time' (hours) was the response variable. We used Poisson error distributions and log link functions to model 'richness'; negative binomial error distributions and log link functions for 'abundance'; and Gaussian error distributions and identity link functions for 'detection time', 'total and active consumption times' and 'consumption rate', in all analyses. 'Detection time', 'consumption times' and 'consumption rate' were log-transformed to improve normality assumptions. We compared the unconsumed remains (i.e., skin and skeletal remains, in kg) of medium-size ungulates among landscape types using the Kruskal-Wallis test.

We also performed GLMs at the species level to investigate factors influencing scavenging frequency according to the same predictors: 'landscape type', 'carcass type', 'placement time' and 'carcass weight', using a binomial error distribution. For each species, we only used the presence/absence matrix of those landscapes in which the consumption of at least one carcass was detected. We fitted models with all possible combinations and subsets of the predictor variables. We selected the model with the lowest AICc, but when there was more than one model with a ΔAICc < 2 relative to the best model, we used a model-averaging function in the 'MuMIn' package (Barton, 2013). This function averages parameter estimates across all considered models for each dependent variable where the respective parameter appeared, weighted by the relative importance of each model. We also calculated the percentage of deviance explained (i.e., the amount of variability explained) of each model. Finally, because one of our goals was to evaluate whether the presence of griffon vultures at carcasses could affect scavenging dynamics, we evaluated the relationship between all the composition and efficiency variables and the presence of griffon vultures using single-predictor GLMs with a binomial error distribution. We checked the model assumptions using statistical tests for normality and homogeneity of variance, and the fit of all models was visually inspected using residual versus fitted values and Q-Q plots. All analyses were performed using R version 3.6.1 (R Development Core Team 2019).

3. Results

3.1. Differences in scavenger assemblage composition

We analyzed 286,572 photos and detected 19 vertebrate scavenger species (Supporting information Table 1). Total species richness in open and shrubland landscapes was similar (18 and 17 species, respectively), including the four European vultures, while forests showed the lowest scavenger richness (nine species) (Table 1). Mean scavenger richness was dependent on landscape type (open > shrubland > forest, Table 1) although GLM analyses revealed that it was only significantly lower in forests and that it increased with carcass size (Table 2, Supporting information Fig. 2). Mean avian species richness was slightly higher in open landscapes compared with shrublands, and up to eight times higher compared with forests, and also increased with carcass size. In contrast, mean mammalian richness only increased with carcass size (Table 1 and 2, Supporting information Fig. 2). Placement time and carcass type did not influence scavenger richness (Table 2). Mean scavenger abundance was almost double in open compared with shrubland landscapes, and up to 12 times higher compared with forests (Table 1). It increased with carcass size but was not affected by placement time or carcass type (Table 2, Supporting information Fig. 2). The most abundant avian scavengers were griffon vultures, followed by *Corvus* spp. and Egyptian vultures. Wild boars, domestic dogs and red foxes were the most abundant mammals. (Supporting information Table 1).

Table 1. Measures of scavenger assemblage composition recorded in each landscape type: total scavenger richness, scavenger richness, avian and mammalian scavenger richness, and scavenger abundance per carcass. Values represent the mean ± standard deviation and range. The number of obligate and facultative avian and mammalian species and the number of carcasses monitored are shown.

	Open	**Shrubland**	**Forest**	**Total**
Total richness	18	17	9	19
Richness	4.70 ± 1.75 [1–9]	3.97 ± 1.88 [0–8]	2.65 ± 1.25 [1–7]	
Avian richness	2.77 ± 1.36 [0–5]	1.90 ± 1.47 [0–6]	0.35 ± 0.67 [0–3]	
Mammal richness	1.93 ± 1.04 [0–5]	2.07 ± 0.85 [0–4]	2.30 ± 0.94 [1–4]	
Scavenger abundance	51.5 ± 37.7 [2–124]	28.2 ± 24.8 [0–89]	4.3 ± 2.7 [1–12]	
Avian species	12	11	4	13
Mammal species	6	6	5	6
Obligate scavengers	4	4	1	4
Facultative scavengers	14	13	8	15
Number of carcasses	70	71	37	178

3.2. Differences in scavenger efficiency

Avian and mammalian scavenging frequency showed clear differences according to landscape type and carcass size (Fig. 2). Birds scavenged at 91.4%, 73.1% and 27% whereas mammals scavenged at 90%, 97.2% and 100% of the carcasses in open, shrubland and forest landscapes, respectively. Obligate scavengers, except for the Egyptian vulture, consumed carrion significantly more frequently in open landscapes than in shrublands (Fig. 2, Supporting information Table 2). Griffon vultures were the most frequent scavenger, followed by bearded, Egyptian and cinereous vultures (Fig. 2). Facultative avian scavenging frequency did not differ between open and shrubland landscapes, except for the red kite *Milvus milvus* (Supporting information Table 2). Ravens *Corvus corax* and golden eagles *Aquila chrysaetos* were the most frequent facultative avian scavengers (Fig. 2). Only ravens, Eurasian jays *Garrulus glandarius*, golden eagles and griffon vultures consumed carcasses within forests (Fig. 2). Mammals scavenged carcasses in all landscapes to a similar extent, except for the wild boar and *Martes* spp. (Fig. 2, Supporting information Table 2). Foxes and wild boars were the most frequent mammalian scavengers. GLMs confirmed that, except for the Egyptian vulture, kites *Milvus spp.*, badger and *Martes* spp., species visited carcasses more frequently as carcass size increased (Fig. 2, Supporting information Table 2).

Table 2. Models (GLMs) used to assess the effect of landscape type ('open', 'shrubland' and 'forest'), carcass weight (kg), carcass type (herbivore and carnivore) and placement time (morning and afternoon) on the composition of scavenger assemblages (scavenger richness, avian and mammalian species richness, scavenger abundance) and scavenging efficiency (carcass detection time, consumption times and consumption rate). We present the model-averaged coefficients and standard error (SE) for models with AICc < 2. Significant p-values (< 0.05) are highlighted in bold. The percentage of explained deviance of the model (i.e., percentage of the variability explained by each model 'D^2') is shown.

Response variable	Model	Coefficient	SE	p-value	D^2
Scavenger richness	Open	0.133	0.082	0.105	**40.0%**
	Forest	-0.311	0.118	**0.009**	
	Carcass weight	0.224	0.039	**< 0.001**	
	Carcass type (herbivore)	-0.109	0.133	0.417	
	Placement (afternoon)	0.128	0.098	0.195	
Avian richness	Open	0.325	0.113	**0.003**	**43.1%**
	Forest	-1.562	0.291	**< 0.001**	
	Carcass weight	0.308	0.054	**< 0.001**	
	Carcass type (herbivore)	-0.076	0.202	0.707	
Mammalian richness	Open	-0.105	0.120	0.383	**13.1%**
	Forest	0.159	0.138	0.251	
	Carcass weight	0.121	0.053	**0.024**	
	Placement (afternoon)	0.220	0.137	0.110	
Scavenger abundance	Open	0.489	0.115	**< 0.001**	**65.1%**
	Forest	-1.461	0.158	**< 0.001**	
	Carcass weight	0.636	0.060	**< 0.001**	
	Carcass type (herbivore)	0.259	0.179	0.150	
	Placement (afternoon)	0.184	0.131	0.162	
Detection time	Open	-0.852	0.297	**0.004**	**15.4%**
	Forest	0.725	0.358	**0.04**	
	Carcass weight	-0.183	0.136	0.182	
	Carcass type (herbivore)	-0.356	0.407	0.385	
	Placement (afternoon)	0.989	0.321	**0.002**	
Total consumption time	Open	-1.306	0.281	**< 0.001**	**31.3%**
	Forest	1.221	0.338	**< 0.001**	
	Carcass type (herbivore)	-1.190	0.366	**0.001**	
	Placement (afternoon)	1.176	0.300	**< 0.001**	
Active consumption time	Open	-1.761	0.396	**< 0.001**	**31.7%**
	Forest	1.772	0.479	**< 0.001**	
	Carcass weight	0.206	0.202	0.311	
	Carcass type (herbivore)	-2.023	0.552	**< 0.001**	
	Placement (afternoon)	1.570	0.427	**< 0.001**	
Consumption rate	Open	1.270	0.299	**< 0.001**	**49.7%**
	Forest	-1.178	0.363	**0.001**	
	Carcass weight	1.112	0.152	**< 0.001**	
	Carcass type (herbivore)	1.350	0.433	**0.002**	
	Placement (afternoon)	-1.020	0.330	**0.002**	

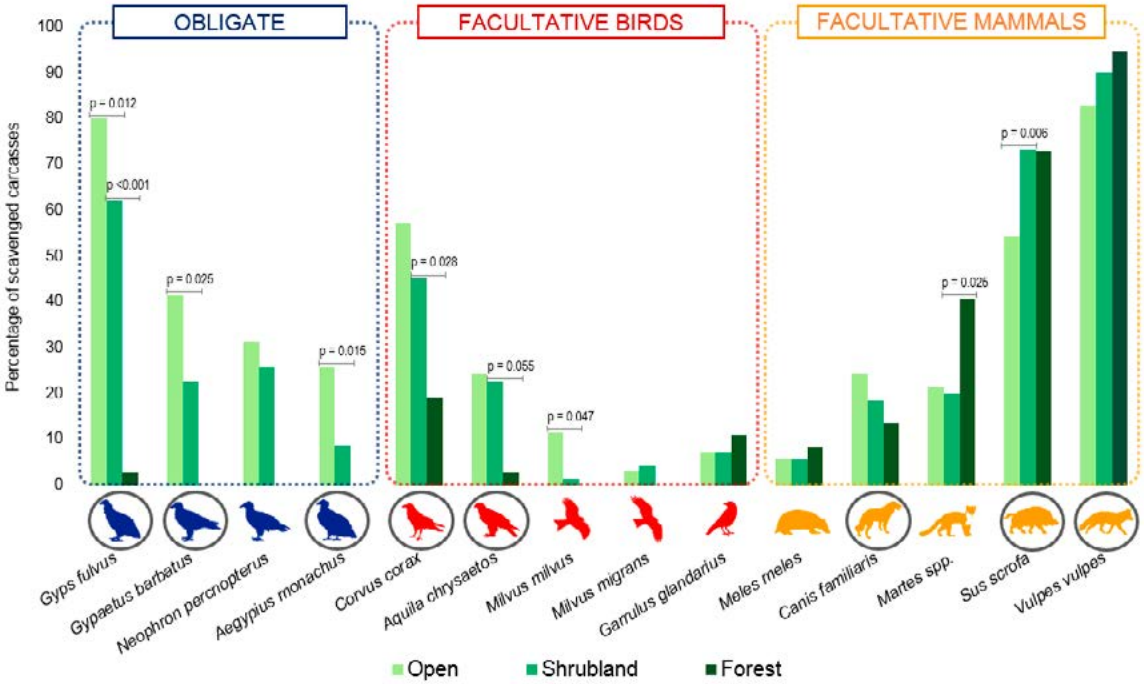


Figure 2. Scavenging frequency (percentage of consumed carcasses) of the main vertebrate scavengers recorded in each landscape type. Significant p-values among landscape types and significant effect (circles) of carcass size (i.e., preference for large carcasses, Supporting information Table 2) are shown for each species. Obligate (blue square), avian facultative (red square) and mammalian facultative (orange square) scavengers are shown.

Landscape type influenced both the main carcass detector species (Fig. 3) and detection time (open < shrubland < forest, Table 2 and 3). Carcasses were detected faster in open landscapes, while detection times increased significantly in shrublands and forests. Carcasses placed in the morning were detected more rapidly, while carcass size and type did not influence detection time (Table 2). In all landscapes, birds detected carcasses significantly faster than mammals (Fig. 4, Supporting information Table 3), although birds discovered only four carcasses within forests. Mean total consumption time was 2.5 times faster in open than shrubland landscapes, and up to five times faster than in forests (Table 3). Regarding carcass type, herbivore carcasses were consumed more rapidly than carnivore carcasses (Table 2). In addition, carcasses placed during the morning were exploited faster, while carcass size did not influence consumption times (Table 2). Active consumption times showed a similar pattern to that of total consumption time (Table 2 and 3). Consumption rate was three times higher in open than in shrubland landscapes, and up to 12 times higher than in forests (Table 3). It increased with carcass size and was higher for herbivore than for carnivore carcasses, decreasing when carcasses were placed in the afternoon (Table 2, Supporting information Fig. 2). The percentage of unconsumed parts of medium-sized ungulates (n = 82, mean ± SD = 51.03 ± 17.8 kg) was less in open (8.1%) than shrubland (10.6%) and forest (14.6%) landscapes (Kruskal-Wallis test, $\chi^2_2 = 10.9$, $p < 0.05$).

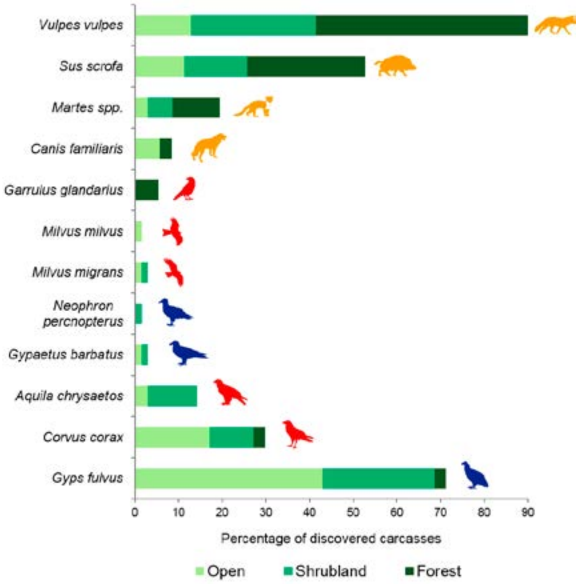


Figure 3. Main carcass detector species (%) in each landscape type. Avian scavengers were the main detectors in open landscapes (44.3% by obligate and 22.9% by facultative species). Both birds (51.4%) and mammals (48.6%) discovered carcasses in shrublands to a similar extent, while mammals were the main detectors (89.2%) in forest landscapes. Griffon vultures (*Gyps fulvus*) and red foxes (*Vulpes vulpes*) were the main detectors in open and forest landscapes, respectively. The percentages were calculated on the basis of carcasses monitored in each landscape type.

3.3. The influence of griffon vultures on scavenging dynamics

GLMs revealed that griffon vultures influenced the scavenger assemblage composition and scavenging efficiency (Fig. 5, Supporting information Table 4). Scavenger richness, avian richness and abundance per carcass were higher when griffon vultures consumed them, although this did not influence mammalian richness. Both birds and mammals arrived faster at carcasses when griffon vultures exploited them. Consumption times decreased when griffon vultures were among the consumers of a carcass (80 vs 362 h with and without the participation of griffon vultures, respectively) and consumption rates were notably higher (7.6 vs 0.06 kg/h with and without the participation of griffon vultures, respectively).

Table 3. Scavenging efficiency measures recorded in each landscape type: overall detection time (h), avian and mammalian detection time (h), total and active consumption time (h), and consumption rate (kg/h) per carcass. Values represent the mean ± standard deviation and range.

Landscape	Detect. Time	Avian Detect. Time	Mammmalian Detect. Time	Total Consum. Time	Active Consum. Time	Consum. Rate
Open	21.90 ± 29.54 (0.02–182.13)	15.4 ± 19.17 (0.02–70.80)	34.91 ± 40.99 (0.16–182.13)	80.91 ± 152.55 (0.22–945.56)	59.0 ± 143.94 (0.10–945.27)	7.89 ± 16.27 (0.004–82.12)
Shrubland	43.20 ± 63.25 (0.10–394.46)	24.56 ± 37.34 (0.1–189.18)	53.93 ± 52.70 (1.87–241.67)	207.12 ± 274.40 (0.90–1234.16)	163.93 ± 244.0 (0.13–1044.98)	2.70 ± 7.82 (0.0005–48.11)
Forest	65.32 ± 67.03 (0.32–297.05)	22.65 ± 33.93 (0.32–72.0)	70.50 ± 68.50 (5.50–297.05)	411.12 ± 336.91 (1.25–1261.98)	345.79 ± 51.30 (0.03–1008.82)	0.66 ± 3.66 (0.002–22.32)

4. Discussion

Allowing passive rewilding has been recognized as a novel conservation strategy in abandoned European agricultural landscapes, where it is estimated that between 2000 and 2030, up to 20 million ha may have been released from agricultural use (Pereira and Navarro, 2015). Rewilding processes may increase carrion availability due to the expansion and increased abundance of wild ungulates (Acevedo et al., 2011), but little is known about the effects of passive rewilding on vertebrate scavenging communities (Mateo-Tomás et al., 2015). Previous studies have highlighted habitat type as an important factor influencing carcass utilization (e.g., Arrondo et al., 2019; Pardo-Barquín et al., 2019; Stiegler et al., 2020). However, this study is the first to assess the effects of woody encroachment or 'landscape closure' due to farmland abandonment and undergrazing on the functioning of scavenging guilds in mountain agroecosystems.

Our findings indicate that landscape type is a major factor influencing scavenger assemblage composition and scavenging efficiency. However, carcass size and the presence of griffon vultures also modulated the functioning of scavenging assemblages. Carcasses were more frequently visited by avian scavengers in open landscapes, while in shrub and forest landscapes mammals dominated at carcasses. In addition, open landscapes were associated with higher scavenger abundances, faster carcass detection and consumption times and higher consumption rates, and favoured rapid carcass location and more efficient consumption by griffon vultures. Furthermore, we found that carcass size influenced the composition of scavenger assemblages (i.e., higher scavenger richness and abundance at larger carcasses). However, regarding scavenging efficiency, carcass size only affected carcass consumption rates (i.e., carcasses with larger biomass were consumed more rapidly), suggesting that landscape type is the main factor determining carrion discovery and consumption times in agroecosystems inhabited by both obligate and facultative scavengers.

Contrary to our expectations, we detected a similar number of obligate and facultative scavenger species in open and shrubland landscapes. Furthermore, the mean number of species per carcass was similar in both. These results differ from those of Arrondo et al. (2019), who found that sheep carcasses in open areas were visited by fewer species but showed higher mean scavenger richness than wild ungulates placed in more heterogeneous sites. This discrepancy could be related to the wide diversity of carcass types we monitored. The provision of more carcass sizes/types may result in greater carrion exploitation by scavenger assemblages. For example, we detected higher scavenger species in both open and shrubland landscapes compared with those recorded in previous studies (Arrondo et al., 2019). We also found higher average avian richness in open landscapes, which could be related to the high scavenging frequency of griffon vultures and ravens at these carcasses, since these species could indicate the location of food to other scavenging birds (Cortés-Avizanda et al., 2012; Orr et al., 2019). On the contrary, our results demonstrate that carcasses within forests were consumed by fewer species, mainly mammals as has been reported in other temperate forests (Inagaki et al., 2019; Pardo-Barquín et al., 2019; Tobajas et al., 2022).

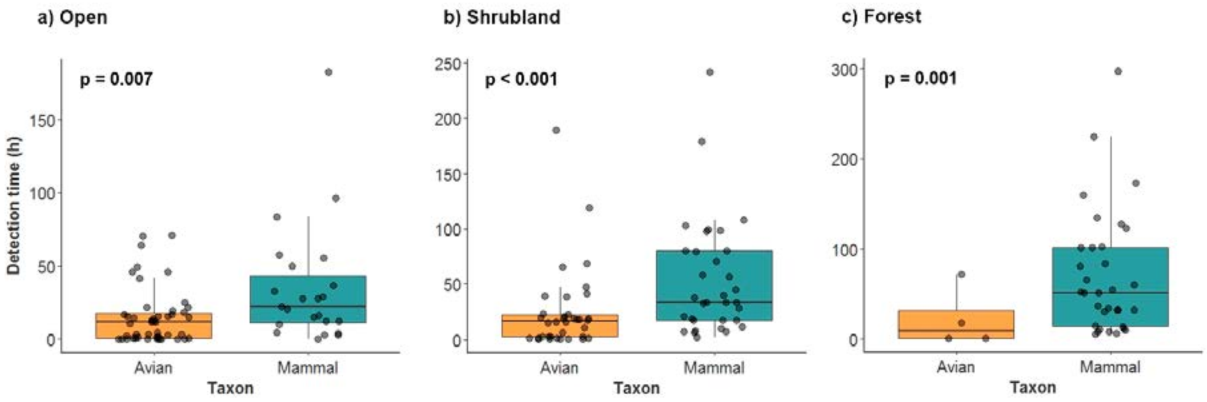


Figure 4. Boxplots and carcass detection times values (hours) at each carcass for birds and mammals in the three landscape types studied. Significant values of the univariate generalized linear models (Supporting information Table 3) are shown.

The adaptive abilities of scavengers to locate and exploit carrion (i.e., visual/olfactory abilities, capacity to break into carcasses and foraging behaviour), determine carcass detection and utilization (Selva et al., 2005). Our findings revealed that birds found carcasses faster than mammals across all landscape types studied. This is probably because birds are generally better adapted to locating food because they can fly and have acute eyesight (Ruxton and Houston, 2004; Potier et al., 2016). However, vegetation cover affects the rate and speed at which carrion is discovered by a species (Ogada et al., 2012). We found that avian scavengers were the main detectors in open landscapes whereas both birds and mammals discovered carcasses in shrublands equally easily. Conversely, mammals detected almost 90% of the carcasses within forests. On the other hand, our results showed that increased vegetation cover led to higher detection times, since scavengers found carcasses in open landscapes up to three times faster than in forests. For example, birds found carcasses more rapidly in open landscapes (~15 h) than shrublands and forests (~25 h). Also, similar to the findings of Lima et al. (2021), mammals discovered carrion more rapidly in open landscapes than forests (~35 h vs. ~70 h).

Unlike mammals, avian scavenging frequency decreased as vegetation cover increased. Overall, obligate scavengers consumed carrion more frequently in open landscapes, while this preference was not observed for the most frequent but less efficient biomass consumers (i.e., facultative avian and mammalian scavengers, Morales-Reyes et al., 2017; Arrondo et al., 2019). Birds, which rely mainly on visual cues to detect food, found carcasses easily in open landscapes and rarely detected them underneath dense forest cover (Moleón et al., 2019). Conversely, the well-developed olfactory sense of mammals plays a larger role in food discovery, making them better adapted to foraging in closed landscapes (Enari and Enari, 2021). Despite this, some birds, such as corvids and eagles, are extremely efficient in locating carcasses, which could explain our observed scavenging frequency of these species within forests (Selva et al., 2005).

In accordance with our predictions, we found that carcass size is an important factor driving scavenging patterns in terms of scavenger assemblage composition (Moleón et al., 2015; Moreno-Opo et al., 2015; Turner et al., 2017; Stiegler et al., 2020). The greater biomass provided by large carcasses (above ~25 kg) helped explain the higher scavenger richness and abundances we observed at these carcasses (Moleón et al., 2015; Turner et al., 2017). However, contrary to our expectations, neither detection time nor total and active consumption times were related to carcass size (Moleón et al., 2015; Turner et al., 2017), suggesting that, in rewilding situations, landscape type is the major factor influencing carcass detection and the time until complete consumption. Despite this, larger carcasses showed higher consumption rates, which could be attributed to exploitation by highly efficient griffon vultures (Sebastián-González et al., 2016). Indeed, our results suggest that griffon vultures play a similar role in terms of consumption rates as that observed for large facultative scavengers in African scavenging assemblages (Moleón et al., 2015), since consumption rates were ~125 times higher when carcasses were consumed by this species.

As expected, carrion removal was faster in open landscapes than within forests, because of the absence of vultures and dominance of mammals in forested areas (Morales-Reyes et al., 2017). In fact, our findings showed that griffon vultures play a crucial role in reducing carcass persistence. Carcasses that were consumed by griffon vultures persisted much less (~3 days) than those that were not consumed by this species (~15 days), highlighting their crucial regulatory service in maintaining healthy ecosystems. Both total and active consumption times increased when carcasses were deployed during the afternoon, since scavenging birds are generally inactive at night. Otherwise, our results support the view that vegetation encroachment leads to longer carcass persistence times. Increased carcass persistence in a landscape has been shown to increase carcass decomposition rates and the likelihood of disease transmission (Markandya et al., 2008; Ogada et al., 2012; Buechley and Şekercioğlu, 2016), while both obligate and facultative scavengers can recycle carrion more efficiently than decomposers (DeVault et al., 2003; Ray et al., 2014).

We found that carnivore carcasses persisted longer and showed lower consumption rates than herbivore carcasses (Oliva-Vidal et al., 2021; Peers et al., 2021). However, contrary to our predictions, we detected no differences in species richness, abundance and detection time between herbivore and carnivore carcasses. Indeed, some avian scavengers such as golden eagles and red kites were more frequent at carnivore carrion. This could be attributed to the smaller size of carnivore carcasses, which may make them harder for griffon vultures to detect. Further, we recorded conspecific necrophagy at all red fox and at 57.5% of wild boar carcasses, where wild boars consumed mainly bone remains from conspecifics. The conspecific necrophagy we observed in red foxes contrasts with the findings of Moleón et al. (2017). They evidenced avoidance of carnivore and conspecific consumption by other carnivores, and attributed this behaviour to a strategy to reduce the probability of disease transmission. However, our observations suggest that conspecific carcasses can provide an alternative resource for red foxes and other scavengers in certain areas or circumstances (Oliva-Vidal et al., 2021).

Overall, griffon vultures consumed 80% of the carcasses in open landscapes and 62% in shrublands. These findings support the idea that their functional traits (e.g, effective foraging strategies and conspecific visual information transfer, Cortés-Avizanda et al., 2014; Gutiérrrez-Canovas et al., 2020) make griffon vultures the most frequent, abundant and efficient scavengers in our study area. However, our results also show that landscape rewilding may jeopardize their high scavenging efficiency because increasing shrub and, especially, tree cover, hinder their ability to locate carrion and reduce both their scavenging frequency and, ultimately, their abundance in more closed landscapes (Ogada et al., 2012; Martin-Díaz et al., 2020). The higher griffon vulture abundances in open areas could also relate to landscape characteristics in other ways, since high vegetation cover may leave insufficient clear space for them to take-off easily (Bamford et al., 2009).

We found strong evidence for the dominant role of griffon vultures in shaping scavenging through conspecific and heterospecific trophic facilitatory processes (Sebastián-González et al., 2016, 2021). First, more avian scavenger species consumed carcasses when griffon vultures exploited them. Mammalian richness was not influenced, perhaps because of the low number of diurnal mammal species in our study area. Second, because of their effective use of social information from conspecifics in their foraging strategy, a greater number of griffon vultures will gather to feed on a carcass (e.g., more than a hundred individuals can be attracted to a carcass once it is discovered). Third, both birds and mammals (mainly foxes, which can forage at the same time as griffon vultures) arrived faster at carcasses discovered by griffon vultures. These findings are consistent with Kane et al. (2017), who found that mammals might find carrion twice as fast when following vultures. Although griffon vultures consumed almost all of the meat biomass, we observed that bone remains provide an essential resource for some species (e.g., bearded vultures) and are valuable resources for mammals (especially red fox and wild boar), because bones are as energetically rich as meat over the long term (Blasco et al., 2019). Thus, in our study area griffon vultures may provide three major trophic facilitatory functions: i) signaling carcass location; ii) facilitating access to the interior of carcasses offering feeding opportunities to less powerful species (i.e., access to small meat pieces, tendons, skin and skeletal remains); and iii) providing resources to more specialist species such as bearded or cinereous vultures (Moreno-Opo et al., 2015).

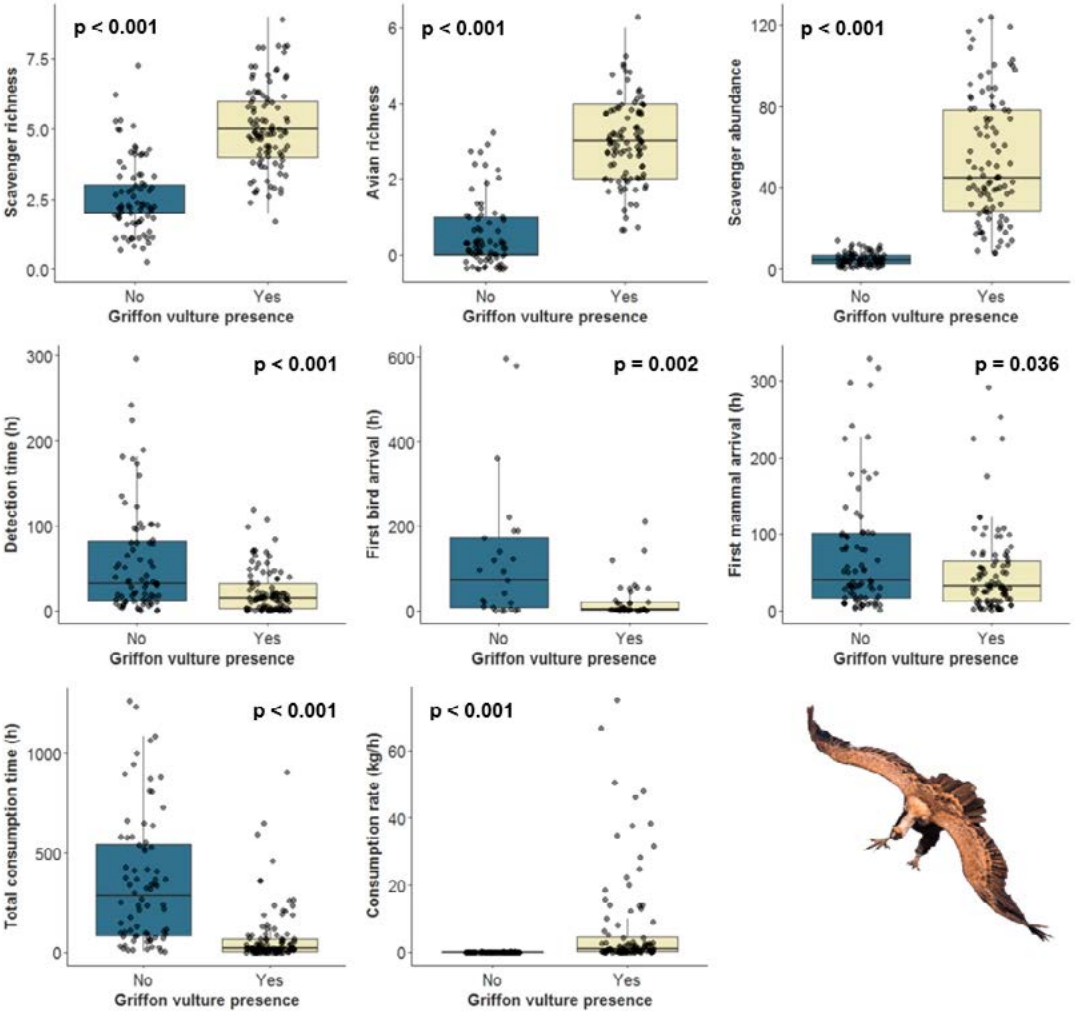


Figure 5. Boxplots showing the significant univariate relationships between the presence of griffon vultures at carcasses and the scavenger assemblage composition, scavenging efficiency and arrival time (h) of the first bird and mammal after griffon vulture appearance (Supporting information Table 4). Dots represent real data.

Concluding remarks

Scavengers are subject to large-scale habitat changes, such as farmland abandonment, which is projected to increase worldwide (Pereira and Navarro, 2015; Tsunoda and Enari, 2020; Lima et al., 2021). The abandonment of rural grazing and farmland practices often results in drastic landscape changes through general processes of vegetation encroachment (passive rewilding) and consequent 'landscape closure' (García-Ruiz et al., 2020b). The effects of habitat type on the functioning of scavenging assemblages have been previously recognized, and understanding the ecological consequences of passive rewilding is necessary to harmonize the coexistence of humans and scavengers. Our findings show that, in rewilding agroecosystems, landscape type is the main factor governing scavenging dynamics through its influence on the composition of scavenger assemblages and their scavenging efficiency. We demonstrate that progressive vegetation encroachment could jeopardize a hugely important functional group (i.e., vultures, which provide crucial ecosystem and scavenging services), reducing the scavenging efficiency of the assemblage (Morales-Reyes et al., 2017). While open landscapes promote carcass removal by the most efficient avian scavengers, in more forested landscapes mammals dominated at carcasses. Moreover, woody encroachment increases carcass persistence, which is known to result in negative ecological, economic and human health consequences (Markandya et al., 2008). We highlight the high consumption rates of griffon vultures as well as their role in driving trophic facilitatory processes, especially to endangered avian scavengers. Our results suggest that future studies should explore the necessity of maintaining open areas (e.g., by supporting traditional pastoral practices) to mitigate the ecological impact of rural abandonment on the functioning of scavenger assemblages.

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Permits

All the work was conducted in accordance with relevant national and international guidelines. Protocols were conducted in accordance to the Regulation EC 1069/2009 and to the guidelines approved by the Catalunya Government following the Law 42/2007, the Legislative Decree 2/2008, and the Decree 148/1992.

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HUMAN-WILDLIFE CONFLICTS

Griffon vultures *Gyps fulvus* in the Lleida Pre-Pyrenees, Catalonia (Spain).
Photo: Pilar Oliva-Vidal

CHAPTER 2

GRIFFON VULTURES, LIVESTOCK AND FARMERS: UNRAVELING A COMPLEX SOCIO-ECONOMIC ECOLOGICAL CONFLICT FROM A CONSERVATION PERSPECTIVE

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CHAPTER 2

Griffon vultures, livestock and farmers: Unraveling a complex socio-economic ecological conflict from a conservation perspective

Abstract

An unexpected human-wildlife conflict between vultures and livestock has emerged in Europe during the last two decades. Farmers attributed changes in vulture behaviour, due to food shortages caused by sanitary regulations, to increasing livestock interactions ('vulture attacks'). To disentangle this conflict, we analyzed 683 farmer complaints between 1996 and 2020 in Catalonia (northerastern Spain) and investigated the eco-anthropological factors driving their frequency. We also assessed farmers' perception through 127 interviews. Most complaints (80%) occurred during the birthing season, mainly involving cattle (76.5%), followed by horses (14.9%) and sheep/goats (8.6%). From 2008 to 2020, vulture-livestock conflicts cost the government €192,000 (~22% of claims compensated). The frequency of complaints was positively associated with extensive livestock density, griffon vulture *Gyps fulvus* abundance (breeding and non-breeding), shorter distances to landfill sites and, to a lesser extent, to supplementary feeding stations. In contrast, there was a negative relationship between complaints and the number of griffon vulture breeding pairs, suggesting that long-distance foraging movements by both breeding and non-breeding individuals may play a major role in determining the occurrence of conflicts. Farmers (88%) said that vultures attack livestock and that attacks had increased in recent years because of significant vulture population increases and food shortages due to sanitary regulations. They considered government policies and compensation ineffective. We highlight the critical need for mitigation in areas with high extensive livestock numbers, particularly during birthing times. Scientific assessments and interdisciplinary awareness campaigns on the coexistence of vultures and livestock are necessary to harmonize biodiversity conservation and agro-pastoral practices in rural economies.

Keywords: human-wildlife conflict, restrictive sanitary regulations, vulture attack, vulture-livestock conflict, wildlife damage compensation

1. Introduction

Human-wildlife conflicts (HWCs) are becoming more common and are of increasing concern to ecologists and managers because of their impact on biodiversity loss (Woodroffe et al., 2005; Treves et al., 2009; Nyhus, 2016). Wildlife attacks on livestock and damage to crops are major causes of HWCs worldwide (Torres et al., 2018) and are projected to increase due to climate change, increasing economic insecurity and retaliations against wildlife in agro-pastoral communities (Abrahms, 2021). Economic losses produced by HWCs, mainly due to endangered predators (carnivores and birds of prey) killing livestock, are considered the main driver of this conflict (Graham et al., 2005; Dickman, 2010; Redpath et al., 2013; Araneda et al., 2021). The resulting direct persecution of predator species can jeopardize conservation efforts (Peterson et al., 2010; Zimmermann et al., 2010). Torres et al. (2018) identified 263 terrestrial vertebrate species involved in HWCs worldwide, of which 53 were listed as threatened, such as vultures. Policy-makers therefore need evidence-based information to design sustainable management actions and policy tools to address this problem (Baynham-Herd et al., 2020).

Old World vulture populations are declining throughout their ranges, mainly due to anthropogenic pressures (Green et al., 2004; Ogada et al., 2012). However, in contrast, European vulture populations are stable, or even increasing. Nonetheless, over the past two decades critical threats to the conservation of European vultures have emerged (Safford et al., 2019). The main current threat, in the shape of new sanitary legislation (EC 1774/2002), appeared after the outbreak of bovine spongiform encephalopathy (BSE) at the beginning of 21st century. This controversial regulation prohibited the abandonment of dead extensively reared livestock in the countryside from 2006 to 2011 (Donázar et al., 2009). A collateral effect subsequently emerged; a widespread view, fueled by public and media misrepresentation, claimed a causal relationship between food shortages for scavengers and presumed attacks on livestock, leading to increasingly negative perceptions of vultures (Margalida et al., 2011).

Negative perceptions of wildlife can promote anthropogenic pressures such as lethal control. Indeed deliberate poisoning is considered one of the major causes of death in wildlife species in Europe (Guitart et al., 2010). Avian scavengers in general, and vultures in particular, are major victims of illegal poisoning, either intentionally or unintentionally (Pfeiffer et al., 2015; Ogada et al., 2016; Santangeli et al., 2016; Plaza et al., 2019; Safford et al., 2019). Laying the blame for livestock killing on Old and New World vultures has recently aggravated this situation (Margalida et al., 2011, 2014; Duriez et al., 2019, Plaza and Lambertucci, 2021). Generalized perceptions of vultures attacking livestock have increased in recent years, associated with the viral spread of partial and biased information through social media, despite the limited empirical support for these assertions (Margalida and Donázar, 2020; Lambertucci et al., 2021). In spite of the long-established mutual relationship between humans and vultures (Moleón et al., 2014), negative perceptions may drive discontent and incite vengeance from some farmers, creating a source of tension and the debate on vulture/livestock conflicts is increasing and remains open and controversial.

The study of public perceptions, "*the way an individual observes, understands, interprets, and evaluates a referent object, action, experience, policy or outcome*" (Bennett, 2016) has received growing attention in conservation biology because of increasing awareness of the critical role of the human dimension (i.e., perceptions, values, beliefs and attitudes) in improving conservation efforts (Bennett et al., 2017) and effectively resolving HWCs (Dickman, 2010). However, perceptions are not always objective since they are mediated by multiple contextual factors, including past experience, personal motivation or the reports of individuals, communities or social media (Naughton-Treves and Treves, 2005; Bennett, 2016; Ballejo et al., 2021). A person's perceptions can also be driven by contradictory governmental policies (e.g., when a species, such as vultures, is listed both as a livestock-predator and a protected species) requiring different mitigation and conservation measures. Therefore, making objective decisions can be challenging because human realities and perceptions may vary widely between and within the stakeholder groups involved (Konig et al., 2020).

A recent example of differing perceptions regarding the relationship between scavengers and livestock relates to farmers. Their perceptions, attitudes and values may change along a species geographical distribution (Ballejo et al., 2019), due to different local legislation (Gigante et al., 2021) or paradoxically, the same species and species within the same guild can be perceived as either beneficial or harmful, or a mixture of both (Morales-Reyes et al., 2018). Moreover, although farmers usually attribute severe damage to livestock as being due to scavenging birds, when the interaction is assessed it is often found to be minimal or even nonexistent (Margalida et al., 2014, Duriez et al., 2019). Indeed livestock losses to bird predation are often found to be less than those produced by other causes, such as disease, injury or poor nutrition (Graham et al., 2005). Recent research on the behavioural conflict between New and Old world vultures and livestock has focused on the human dimension (Ballejo et al., 2019, Gigante et al., 2021; Salom et al., 2021) and to a lesser extent on field observations (Ballejo et al., 2020). However, a complete examination of this phenomenon would require integrating the management/responses of local authorities, complaint assessments, farmer perceptions and broader assessments by technical experts and veterinarians.

Here, we try to disentangle the factors associated with increasing conflicts between vultures and livestock in Catalonia, a region of northern Spain inhabited by the four European vultures. We first assessed the complaints reported by farmers to the authorities and explored their temporal trend. Then, we investigated the influence of ecological and anthropic factors (e.g., global griffon vulture *Gyps fulvus* abundance, number of griffon vulture breeding pairs, extensive livestock density and the distances to the nearest landfill site and supplementary feeding stations 'SFSs') on the frequency of complaints. Finally, we interviewed farmers to analyze their perceptions and concerns regarding this emergent conflict, so as to assess the vulture/livestock interface from an ecological-economic-social perspective. Based on our results, we propose solutions and guidelines to manage this unexpected conflict and to harmonize vulture conservation with agro-pastoral practices.

2. Material and methods

2.1. Study area

Catalonia covers over 32,000 km^2, with altitudes ranging from sea level to > 3,000 m. The landscape is heterogeneous, dominated by forests (34.7% of the total area), crops (30.1%), shrublands (22.4%) and grasslands (4.8%), while urbanized areas cover 5.3% of the surface (González-Guerrero and Pons, 2020). The region is characterized by a high population density (241.8 inhabitants/km^2) and powerful industrial and service sectors, while agriculture is less important (1% of the regional GDP). Intensive farming of pork (54.9%) and poultry (17.9%) are the most important agricultural systems. Cattle (13.0%), sheep/goat (1.8%) and horse (0.4%) rearing are economically less important (DARPA, 2018). Catalonia has at least 5,457 extensive or semi-extensively farms of cattle (37.03%), sheep/goats (34.9%) and horses (28.07%) (http://agricultura.gencat.cat/), which are present throughout the region, but especially in mountainous areas. Extensive livestock in mountainous zones graze on high summer pastures from June to October and remain stabled in the valley bottoms exploiting the surroundings of small hamlets for the rest of the year (García-Ruiz et al., 2020). Rural areas hold important populations of facultative and obligate scavenger species (Oliva-Vidal et al., under review). At least 1628 breeding pairs of griffon, 20 of cinereous *Aegypius monachus*, 88 of Egyptian *Neophron percnopterus* and 49 of bearded vultures *Gypaetus barbatus* occur in the region (Del Moral 2017, Del Moral and Molina, 2018a, b, Margalida and Martínez, 2020).

2.2. Farmer complaints

We used an official database containing all complaints (n = 683) made by farmers from 1996 to 2020 relating to reported vulture 'attacks' on livestock. It is important to note that while farmers reported incidents as 'attacks', our study took the view that the precise nature of the interaction was still to be proven, so we additionally used the term 'interaction' to refer these conflicts. We used the complete database to assess the temporal trend, and the complaints reported from 2008 to 2020, for which more detailed information was available, to characterize the interactions (n = 616, 90.2% of the total). A subset of this data (2008–2018, n = 573 claims, 83.9% of the total) was considered for modelling the determinants of conflicts, as updated information of livestock density was not available for the latest period. For each complaint, the official data included: i) date of the interaction; ii) date of the assessment; iii) municipality and county; iv) livestock type (cattle, horses or sheep/goats) and category ('adult', 'female and new-born' or 'new-born'); v) the number of individuals affected; vi) validation (i.e., 'accepted' when the assessment considered that the animal was still alive when vultures started to consume it, or 'rejected' when no evidence was found or when consumption by vultures was considered to be post-mortem); vii) financial compensation (€); and viii) UTM coordinates. To characterize the interactions, we recorded for each complaint: i) type of livestock involved; ii) livestock category; iii) seasonality (i.e., the month the claim was reported); iv) number of complaints 'accepted' (i.e., financially compensated) or 'rejected'; and v) total economic compensation by the authorities.

2.3. Frequency of complaints

We first recorded the number of complaints reported by farmers in a grid of 10 × 10 km UTM cells during 2008–2018, since each cell was considered as one single statistical observation in the analyses (n = 110 cells accounting for 573 complaints). Next, we calculated the following variables for each cell: i) 'number of griffon vulture breeding pairs'; ii) 'global griffon vulture abundance'; iii) 'extensive livestock density'; iv) 'distance to nearest SFS'; and v) 'distance to nearest landfill site'.

We estimated the 'number of griffon vulture breeding pairs' as the average of two censuses carried out in 2008 and 2018 throughout Catalonia (Del Moral and Molina, 2018a); and the 'global griffon vulture abundance', according to the values obtained by García-Ferré et al. (2011) following the methods described in Herrando et al. (2011). In brief, sampling was carried out through surveys performed in three consecutive winters (2006–2009) over the whole study area, and then was standardized by sampling effort. After that, all the data was processed statistically to achieve a value of abundance for each 1 × 1 km cell. Here, we averaged the 100 1 × 1 km cells to obtain a value of abundance for each 10 × 10 km cell. An advantage of this measure is that it accounts for the abundance of both breeding and non-breeding individuals. It is known that griffon vultures routinely perform movements at large distances from breeding sites (Delgado-González et al., 2021), so they can be abundant in areas located far from breeding sites. Moreover, non-breeding vultures may represent an important fraction of the population, which frequently exploit food resources far from the breeding grounds. Similar information on abundance does not exist for later years, although based on our personal observations we do not expect the spatial pattern of vulture abundances to have changed significantly during the period considered in the analyses.

The 'extensive livestock density' (e.g., extensive, mixed or transhumant farming systems) was estimated considering the potential capacity of the farms (i.e., maximum number of animals that a farm can raise) for each livestock type (cattle, sheep/goats and horses). We assumed that farm capacities are a good proxy of the number of livestock animals (information that was not yearly available) because farmers pay fees on this basis. To estimate the density of extensive livestock at the cell level, we first calculated for each year an average density of livestock heads for each municipality. Then, we calculated a weighted average for each cell according to the municipalities' surface within each cell. Lastly, we averaged the yearly estimates in each cell for the entire period. Farming information was extracted from the Livestock Information System (www.sir.gencat.cat). Geoprocessing analyses were performed using the ArcMap 10.5 program (ESRI, 2016). The distances (km) to the nearest landfill site and SFS from the nearest limit of each cell were estimated assigning a distance equal to zero for those cells containing a landfill site or SFS. Because some SFSs were not active in every year, an average distance value was obtained for the whole study period.

2.4. Farmer interviews

We assessed farmers' perception of vulture conflicts with livestock through face-to-face and telephone interviews from 2018 to 2021 (see Appendix S1 for the full list of questions). We surveyed 127 farmers from Pyrenean and adjacent regions, obtaining information on 166 farms with cattle (53.6%), sheep/goats (30.1%) and horses (16.3%), since thirty-four respondents had mixed farms. We first recorded information regarding the farm characteristics (e.g., livestock type) and then asked both closed and open questions about conflicts between vultures and livestock. Some of the closed questions were in multiple-choice format (Appendix S2). Further, we asked if they had experienced vulture interactions on their livestock and if so, we collected information about the livestock involved (e.g., where and under what circumstances the dead livestock was found) as well as if they had recognized the species starting the interaction and how they detected it. Respondents were also asked a range of questions regarding their views on whether vultures could attack live livestock, the temporal trend of such conflicts, the type of livestock supervision during birthing times and the response of the local authorities and any financial compensations paid. Finally, we recorded farmers' views on the coexistence of vultures and livestock, the ecological relationships between them, and on measures that could be implemented to mitigate conflicts.

2.5. Statistical analysis

We investigated ecological and anthropic factors influencing the frequency of vulture/livestock interactions (i.e., number of complaints) using generalized linear models (GLM), where 'number of complaints' (per 10 × 10 km cell) was the response variable and 'number of griffon vulture breeding pairs', 'global griffon vulture abundance', 'extensive livestock density', 'distance to nearest landfill site', and 'distance to nearest SFS' were the explanatory variables. We used Poisson error distributions and logarithmic link functions. Models considering the main effects of the explanatory variables were evaluated. We used Akaike's information criterion for small sample sizes (AICc) to identify the most parsimonious model (i.e., the one with the lowest AICc) and to rank the remaining models. When there was more than one model competing with the best AICc model (those with ΔAICc < 2), we performed conditional model-averaging across the candidate models (Burnham and Anderson, 2002) using the *MuMIn* package (Barton, 2013). This procedure averages parameter estimates across the set of selected models in which the respective parameter appeared, weighted by the relative importance of each model. The analyses were performed using the R Software for Statistical Computing program (R Development Core Team 2020). We used Chi-squared analyses to look for differences in farmers' perceptions of the coexistence and ecological relationships between vultures and livestock, based on whether they had suffered attacks.

3. Results

3.1. Complaints assessment

We found three temporal trends in the 683 complaints reported from 1996 to 2020. Until 2006, the number of complaints was low and with little variability between years (mean ± SD = 1.8 ± 1.6 claims/year). From 2007 to 2011 complaints increased dramatically reaching a maximum in 2010 (86 complaints). After 2011 the trend diminished progressively until 2020 (Fig. 1). Complaints reported during 2008–2020 mainly involved cattle (n = 471, 76.5%), followed by horses (n = 92, 14.9%) and sheep/goats (n = 53, 8.6%) (Appendix S3). Of these, 78.2% were rejected. The proportion of accepted complaints (n = 134, 21.8%) was similar: 77.6%, 14.2% and 8.2% for cattle, horses and sheep/goats, respectively, and did not differ between species ($\chi^2_2 = 0.13$, $P = 0.94$).

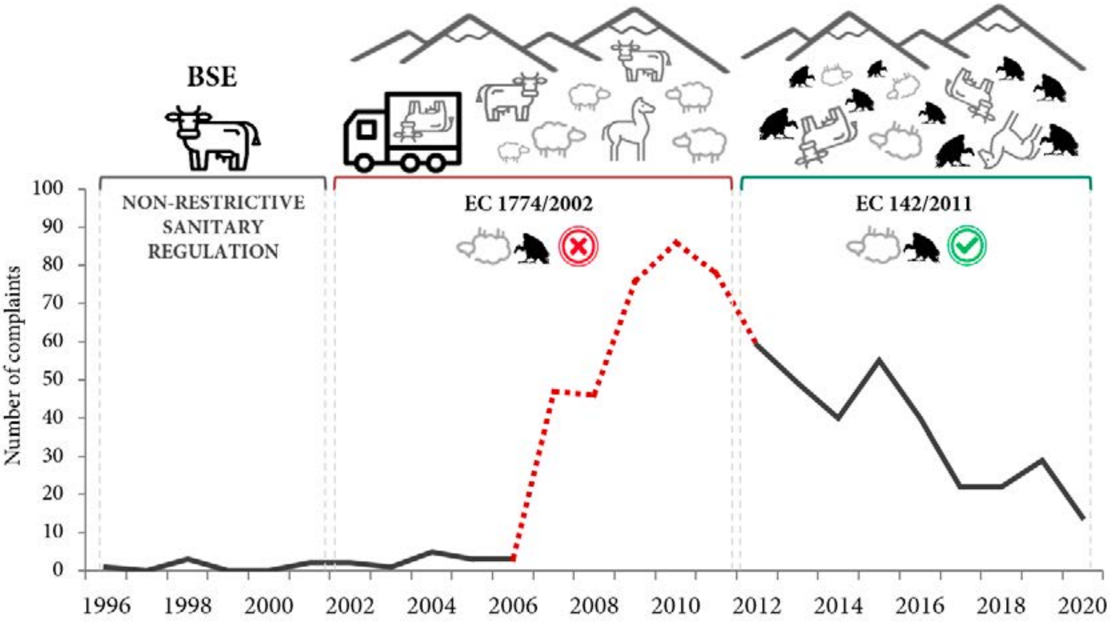

Figure 1. Complaints regarding griffon vulture attacks on livestock from 1996 to 2020 in Catalonia. Three periods are highlighted: a) appearance of bovine spongiform encephalopathy (BSE) in Europe (1996) when no sanitary regulations were in force (e.g., livestock carcasses could be left in the countryside and available for scavengers); b) the approval of restrictive sanitary regulations (EC 1774/2002) prohibiting the abandonment of dead livestock in the countryside (2002–2011); and c) the approval of new regulation (EC 142/2011) allowing farmers to leave livestock carcasses in the countryside.

*The slow adoption of European environmental policies helps to explain the delay between regulation approval EC 1774/2002 and the increase in complaints from 2006 (López-Bao and Margalida, 2018).

We identified a seasonal pattern (e.g., 80% of complaints between February and July) associated with the birthing season: 76.4%, 96.7% and 83% for cattle, horses and sheep/goats, respectively. Further, at least 68.3% of the complaints occurred during birthing times (i.e., involving the female and new-born, or the new-born): 69% for cattle, 83.7% for horses and 35.8% for sheep/goats, respectively (Appendix S3). The period March–June included 69.6% of the total complaints. During this period, on average 8.3 claims were reported per month, whereas for the rest of the year the mean number of monthly complaints was ≤3 (Fig. 2). Most accepted complaints (75.4%) were made during the birthing season (65.7% from March to June). The highest percentage of accepted complaints was in 2008 (56.6%) and 2009 (61.8%) and decreased in 2010 (39.5%) and remained below 10% until 2018. The estimated economic cost during 2008–2020 of the complaints assessed positively was €192,000.

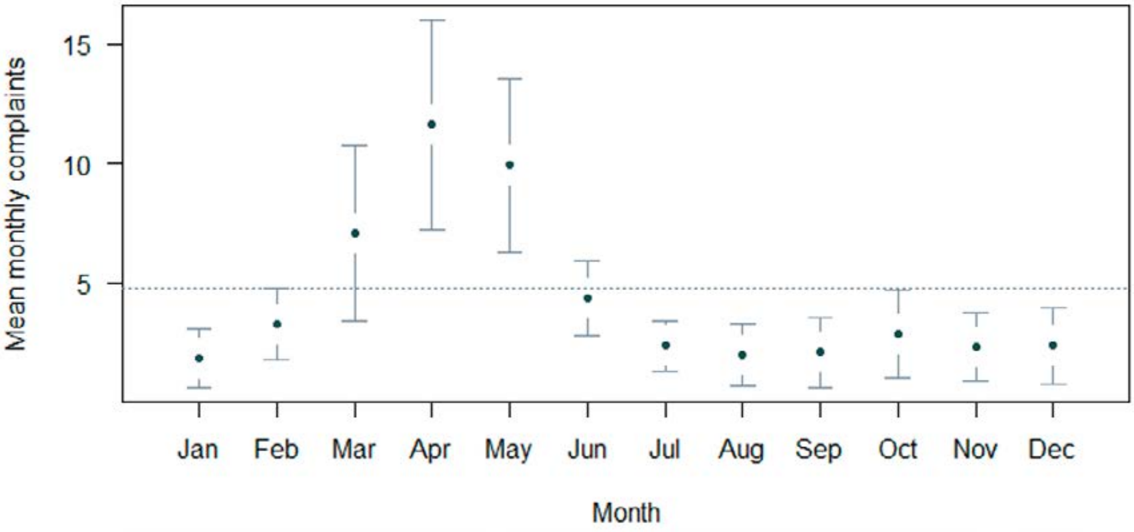

Figure 2. Average number of monthly complaints (n = 616) from 2008 to 2020. The dashed line shows the average number of complaints reported during the entire period. The photograph shows a group of griffon vultures feeding on a dead mare and her new-born foal just after a problematic calving, which could be construed as an 'attack' (photo: Pilar Oliva-Vidal).

3.2. Factors driving the frequency of complaints

We evaluated 32 models to investigate the factors influencing the frequency of complaints (n = 110 cells; Appendix S5). The averaged model included all of the predictors, of which four showed values of relative importance (i.e., > 0.8, Appendix S6). 'Extensive livestock density', 'distance to nearest landfill site', 'number of griffon vulture breeding pairs' and 'global griffon vulture abundance' were the predictors with the highest weights, although the averaged model also included the effect of 'distance to nearest SFS'. We found that higher densities of extensive livestock and shorter distances to both the nearest landfill site and SFS were associated with higher numbers of complaints (Table 1), although the effect of the distance to the nearest SFS was weak.

Table 1. Generalized linear model (GLM) relating the frequency of vulture attack complaints to ecological and anthropic variables. The conditional model-averaged estimates and standard errors (SE) of models with DAICc < 2 are shown.

Variables	**Estimate**	**SE**
Intercept	1.050	0.137
Extensive livestock density (animals/km^2)	0.011	0.001
Distance to nearest landfill site (km)	-0.027	0.005
Number of griffon v. breeding pairs	-0.010	0.003
Global griffon v. abundance	0.872	0.334
Distance to nearest SFS[a] (km)	-0.004	0.005

[a] SFS: Supplementary Feeding Station

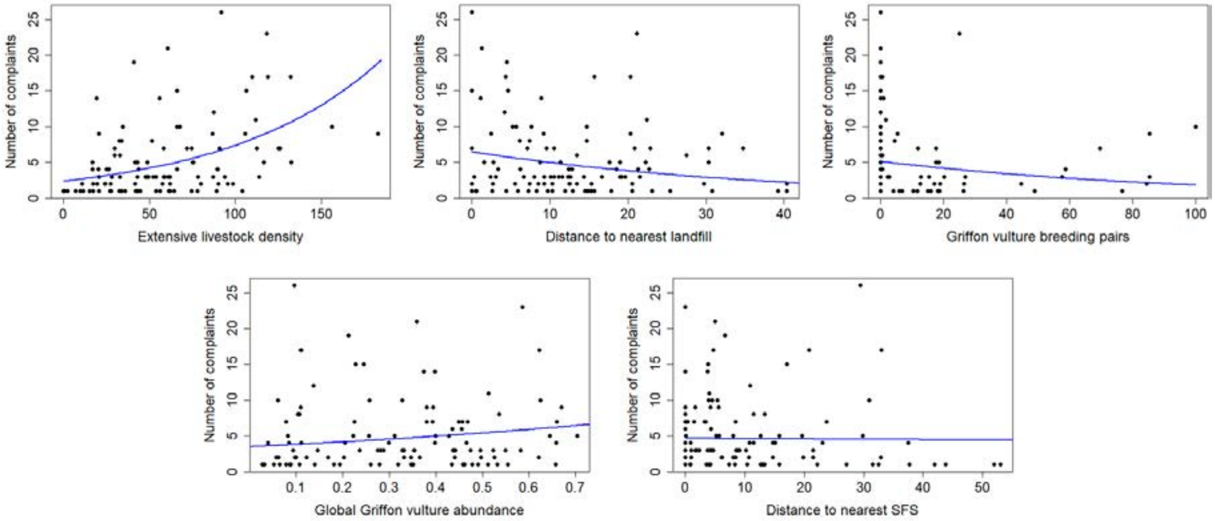

Figure 3. Observed (black spots) and predicted (blue lines) values of the explanatory variables: 'extensive livestock density' (animals/km^2), 'distances to the nearest landfill site and SFS' (km), 'number of griffon vulture breeding pairs' and 'global griffon vulture abundance' considered in the generalized linear model determining the frequency of complaints of vulture/livestock interactions during the period 2008–2018.

In addition, our results showed that a higher abundance of griffon vultures (including both the breeding and non-breeding populations) was associated with higher numbers of complaints, but conversely, the number of griffon vulture breeding pairs was negatively associated with the number of complaints (Fig. 3).

3.3. Farmers' perceptions

Of 127 farmers interviewed, 67 (52.8%) reported having suffered vulture attacks on their livestock, cattle being the most affected (71.9%), followed by sheep/goats (21.1%) and horses (7.0%) (Appendix S4). Most interactions (75.9%) described occurred during the birthing season. However, while most cases involving cattle (91.7%) and horses (54.6%) were associated with calving, interactions with juveniles and adults unrelated to parturition were more important for sheep (66.7%) (Appendix S4). Farmers associated 72.2% of the interactions on adult livestock with old, sick or dying individuals. The attacks described mainly occurred while livestock remained in open fields (48.6%) or fields protected with electric shepherds (44.4%), compared with cases in semi-open (5.6%) or closed (1.4%) farms. Most respondents (76.5%) thought that griffon vultures had initiated the attack, followed by 'don't know' (10.5%), ravens *Corvus corax*/foxes *Vulpes vulpes* (3.9%) and cinereous vultures/bears *Ursus arctos* (2.6%). However, none claimed to have seen the actual start of an attack. Farmers detected attacks because they were in the area and observed griffon vultures (42.9%) while supervising livestock at the time (32.1%), by their noticing a known (21.4%) or unknown (1.2%) person, or while supervising livestock on the following day (2.4%).

Overall, farmers believed that vultures can attack live livestock (88.2%) and most of them (77.2%) said that attacks had increased in recent years, attributing them mainly to the population increase of griffon vultures and to the lack of food in the field due to sanitary regulations (Fig. 4A). Regarding farmers declaring attacks, 68.7% (n = 46) had reported it to the authorities. Of these, 30.4% were financially compensated and half had considered the economic compensation to be sufficient. However, considering all respondents, 86.1% thought that the response of the authorities had not been favourable and 82.6% believed that financial compensation did not satisfy farmers suffering attacks. Few respondents (5.4%) considered that current economic compensations were sufficient. Farmers reporting attacks had a more negative perception of the coexistence of vultures and livestock (Fig. 5A) and the ecological relationships between them (Fig. 5B) than those who had not reported attacks. Further, 91.3% of respondents said that it was necessary to apply management measures to mitigate the conflict (Fig. 4B) and 73.8% believed that interactions would increase further otherwise.

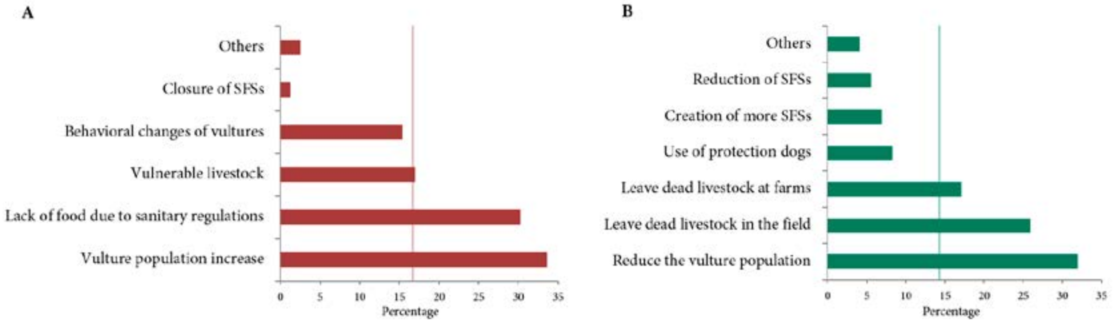

Figure 4. A) Main causes of the increase in vulture/livestock interactions reported by the farmers interviewed (n = 241), and B) main management measures proposed to reduce this conflict (n = 216). Some farmers proposed multiple responses (Appendixes S1 and S2). The vertical line indicates the percentage of responses expected if the responses occurred equally frequently.

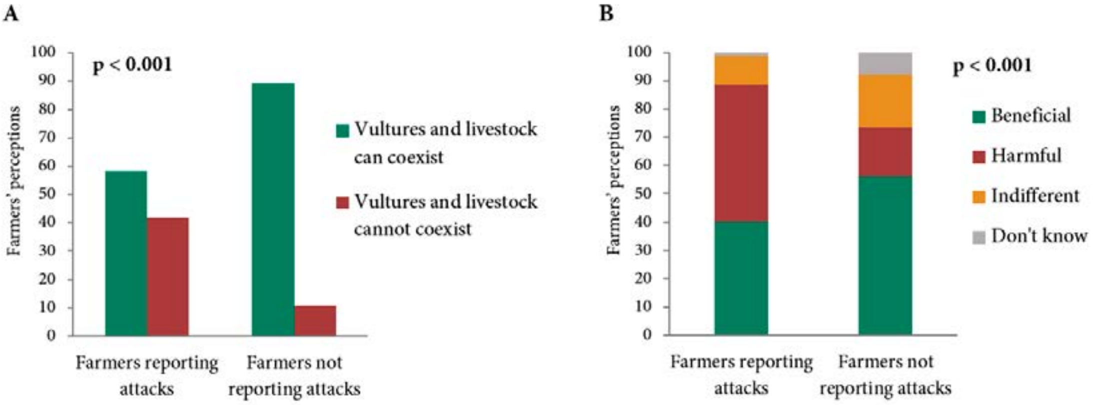

Figure 5. Farmers' perceptions (in %) regarding the coexistence of vultures and livestock (A); and the ecological relationships between them (B). The significance of the Chi-squared tests are shown.

4. Discussion

Livestock and wildlife conflicts have occurred for millennia, mainly involving carnivores and birds of prey (Torres et al., 2018). However, recent widespread perceptions that vultures attack livestock threaten Old and New World vulture populations (Lambertucci et al., 2021). In southern Europe, claims that vultures attack livestock began in the mid-1990s but have increased since the mid-2000s, notably in Spain and southern France (Margalida et al., 2014, Duriez et al., 2019) and an unprecedented and complex socio-economic ecological conflict has emerged.

We identified three temporal trends in reported vulture attacks on livestock. From 1996 to 2006 the number of annual complaints was low but rose significantly from 2007 to 2011, followed by a progressive decline from 2012 to 2020. Certain policy decisions could explain these trends. First, changes in sanitary legislation (Regulation EC 1774/2002) prohibited the abandonment of livestock carcasses in the countryside, provoking general alarm among farmers and establishing a perceived causal relationship between food shortages and presumed vulture attacks (Donázar et al., 2009; Margalida et al., 2010, 2011). Second, the approval in Catalonia of a regulation (Decret 176/2007) including griffon vultures among potential livestock predators (listing them as compensating species with wolves *Canis lupus* and bears). This regulation coincided with a period of perceived vulture food shortage and growing alarm among farmers, leading to government compensation for most complaints (e.g., ~60% of those reported in 2008 and 2009). In 2011, coinciding with the highest frequency of complaints, more flexible regulations were approved (EC 142/2011) allowing livestock carcasses to be left in the field to feed scavengers (Margalida et al., 2012). However, to date few farmers have requested permission to leave livestock carcasses in areas designated for this purpose. In fact, only 9.4% of the farmers interviewed reported leaving carcasses in the field. Official collection and transport of dead livestock from farms to authorized processing plants is still the main means of dealing with carcasses following the BSE outbreak. Our results highlight a paradoxical lack of awareness since 'being able to leave carcasses in the countryside' was the second most frequent measure proposed to mitigate the conflict by the farmers interviewed. The steady fall in farmer complaints since 2011 may result from more rigorous assessments (e.g., accurate necropsies) verifying that livestock deaths were due to other causes (Dalmasso et al., 2012), increasing the percentage of rejected complaints and suggesting that the conflict had been overestimated.

Presumed vulture attacks were highest during the period of severest food shortage. However, Spanish griffon vulture populations increased 20.5% during 2008–2018 (Del Moral and Molina, 2018a), suggesting that the severest food shortage had no demographic effects. Despite this, farmers continue to believe that vultures attack livestock when food is in short supply. Our findings showed that almost 90% of farmers believe that vultures attack livestock and most of them thought that attacks have increased in recent years, mainly because of the drastic increase in the vulture population and a lack of food due to sanitary regulations. Nevertheless, no spatial relationship was found between the food availability and the frequency of complaints (Margalida and Campión, 2009). Further, 70% of complaints in Spain and France were associated with post-mortem consumption and French shepherds were not present at 95% of livestock deaths, so could not confidently attribute the death to vulture attack, suggesting that farmers' biased opinions led to perceived conflicts rather than any change in vulture behaviour (Margalida et al., 2014; Duriez et al., 2019).

Our results showed that complaints were strongly associated with high extensive livestock densities and were influenced by shorter distances to landfills and, to a lesser extent, to SFSs. Griffon vultures and other scavenging birds commonly congregate at landfill sites to forage (Donázar et al., 2010; Tauler-Ametller et al., 2017). The weaker effect of the distance to SFSs could be related to the type and management of the SFSs in our study area, most being specifically designed for target species such as the bearded vulture. The specialized resources provided at these SFSs may be less attractive to griffon vultures (Moreno-Opo et al., 2015). Although griffon vultures may visit SFSs, they seem to be less attractive than landfills, where both breeding and floating non-breeding individuals often gather (Arévalo et al., 2022; Fernández-Gómez et al., 2022). Griffon vulture abundance was positively associated with the number of complaints while the number of breeding pairs showed the opposite trend. However, while most cells with higher complaint numbers harbored few or no breeding pairs, some cells with greater numbers of breeding pairs were associated with a high number of complaints. This suggests that the effect of vulture abundance on the number of complaints may be complex. In this sense, reports of conflict could vary greatly in time and space due to vultures' long distance foraging habits (Spiegel et al., 2015; Gutiérrez-Canovas et al., 2020). The foraging strategies of vultures (Ruxton and Houston, 2004) may explain why griffon vultures find it easier to detect vulnerable livestock (e.g., injured, sick, weak, or those suffering parturition problems) in rural and anthropogenic (e.g., close to landfills) landscapes, resulting in increased conflicts compared to areas less attractive to vultures. Conversely, the negative relationship between complaints and the number of vulture breeding pairs may be because a significant number of complaints were reported in areas far from their breeding sites, suggesting that breeding birds may perform longer foraging movements (Delgado-González et al., 2021), or that floating populations may play a major role in determining the appearance of conflicts.

We identified a strong seasonality in complaints associated with the birthing season, particularly from March to June (~70% of the claims). However, while most complaints involving cattle and horses were associated with calving, most interactions involving adult sheep were not related to parturition. Similarly, farmers reporting attacks (52.7%), declared that cattle were most affected and that attacks were mainly associated with calving. Further, farmers reported that attacks on sheep mainly concerned adult individuals. In our study area, cattle were most often reported as affected and interactions were more frequent in spring, demonstrating a seasonal, birthing time, pattern of vulture/livestock encounters. However, because of the patchy nature of the official data, cases involving calving cattle were probably underestimated. Therefore, it is imperative to establish a standardized protocol, supervised by qualified personnel (e.g., veterinarians and biologists), to assess and decide on official complaints.

Livestock management could explain the seasonal pattern we found regarding both official complaints and attacks described by farmers. In spring during birthing times, livestock are kept extensively in grazing pasturelands, often with minimal supervision. For instance, almost 37% of the farmers interviewed recognized that livestock supervision during the birthing season is insufficient, regardless of whether they had suffered attacks. On the other hand, while sheep usually graze accompanied by shepherds and/or sheepdogs, and most herds spend the night at farms or remain protected by electric shepherds, cattle and horses usually remain at large for longer periods with minimal supervision. This could help explain why most interactions involving sheep were unrelated to parturition, since lambing difficulties are much less frequent compared to cattle (Jacobson et al., 2020). In fact, > 90% of vulture/cattle interactions described by farmers were associated with calving.

Although scavenging birds may cause some harm, especially during parturition, evidence of actual predation is exceptional (Ballejo et al., 2020). However, farmer's perceptions are different, and they usually attribute livestock death to vulture attack, often prompted by increasing numbers of false reports, even if they have never experienced this conflict (Margalida and Donázar, 2020, Ballejo et al., 2021). Nevertheless, media reports just suggest attacks showing vultures feeding on a carcass (Fig. 2) or people claiming livestock losses due to vultures without actual evidence (Lambertucci et al., 2021). Highly visible and potentially dangerous species are especially likely to generate disproportionate antagonism, being perceived as innately evil or harmful, so that even low levels of damage can still elicit harsh responses and even where conflicts are entirely mitigated, negative perceptions can perpetuate (Dickman, 2010). Some people consider vultures 'disgusting birds', associated with death and decay due to their obligate scavenging habits (Hla et al., 2011), despite their having cultural significance in some religions and the valuable contributions they make to public health and ecosystem services (DeVault et al., 2016). Education can reduce these negative perceptions, but such deep-seated preconceptions are difficult to overcome and must be considered in HWC assessments (Dickman, 2010).

We identified certain social factors that may contribute to the conflict. First, most farmers declaring attacks believed that vultures initiated the attack, but only because they had observed vultures or their signs (e.g., feathers). However, none confirmed observing the start of an attack. Second, 68.7% of farmers reported interactions, and half of those compensated thought that the economic compensation was enough (Bauer et al., 2017). However, compensatory measures may not alleviate the problem because they rarely cover the total costs, are open to corruption, and are often expensive to administer (Sillero-Zubiri et al., 2004). Third, we found that sheep farmers complained much less often (8.6%) than cattle (76.5%) or horse (14.9%) farmers. Complex bureaucratic procedures and livestock values can help explain these differences (e.g., in Catalonia, financial compensation for vulture interactions were ~€1200 for cattle, ~€1300 for horses and ~€150 for sheep; Decret 176/2007). Furthermore, 86% of all farmers thought that the authorities' response was ineffective; 83% said that financial compensations did not satisfy farmers suffering attacks; and only 5% believed that compensations were enough – even though the compensation was considered fair by some farmers actually receiving it. Addressing these perceptions is crucial to alleviating this conflict. It is vital to improve administrative processes, and develop awareness campaigns and broader interdisciplinary approaches (e.g., to bring managers, scientists and farmers together) in order to move farmers away from a conflict mentality towards one of coexistence with vultures. The fact that we were able to interview 127 farmers on this topic highlights the opportunities to work together in future, and effectively resolve this complex conflict in the long term.

Farmers declaring attacks on their livestock had a more negative attitude towards the coexistence of vultures and livestock and considered vultures more often harmful than beneficial. This could be due to their lack of knowledge of vultures' behaviour and the pivotal ecosystem services they provide, particularly in areas where vultures are scarce. In fact, we found that many interactions were reported in areas without breeding vulture pairs, suggesting that people's negative perceptions could be influenced by reports of others or the media (Margalida and Donázar, 2020). Finally, our findings illustrate that most farmers considered that reducing griffon vulture populations was necessary to mitigate the conflict. We argue that it is necessary to promote awareness campaigns to rural people that explain that if vulture numbers are reduced, may result in an increase in mesopredators and the spread of pathogens among livestock, wildlife and humans (Markandya et al., 2008; O' Bryan et al., 2019).

Management implications

To mitigate 'vulture attack' conflicts, ~22% of claims were compensated between 2008 and 2020. However, financial compensation is not always the best way to minimize HWCs (Sillero-Zubiri et al., 2004). Instead, broader assessments of environmental and social risk factors affecting the frequency of interactions are critical to better understand the nature of this conflict and how it can be addressed (Dickman, 2010). Damage verification by veterinarians is crucial to clarify the real cause of death, avoid waste of public resources and obtain rigorous data to identify the real magnitude of the conflict (Dalmasso et al., 2012). Further, improving livestock welfare is essential. This is critical in areas with high extensive livestock density, especially those with recurrent conflicts. Increased livestock protection is crucial to reduce livestock vulnerability, especially during the birthing season. As recognized by some farmers "*livestock calving has to be at home and not in the mountains, as was traditionally done*". Governments should enact regulations integrating livestock welfare and hold livestock owners accountable for providing adequate protection (López-Bao and Mateo-Tomás, 2021). The European Directive 98/58/EC on the protection of animals kept for farming purposes, states that "*all animals kept in husbandry systems in which their welfare depends on frequent human attention shall be inspected at least once a day*". Some farmers stated that livestock supervision was insufficient, and that regular inspections are essential, enabling farmers to recognize vulnerable or dead livestock, thus reducing the likelihood of an 'attack' or the uncertainty in verifying suspected attacks (López-Bao et al., 2017). Many complaints occurred in areas where no vultures breed, due to the large numbers of vultures congregating at nearby predictable food sources such as landfills (Arévalo et al., 2022). Clearly, proper management of landfills is essential, particularly taking into account the expected drastic decrease of food available to wildlife at these sites over the coming years (Landfill Waste Directive 2008/98/EC; Circular Economy Action Plan). In addition, it is necessary to allow carcasses to be disposed of more widely than in the currently permitted high altitude (i.e., >1400 m) areas. Finally, because farmers perceive the arrival of vultures in newly colonized areas as a novel threat, it is crucial to implement educational campaigns to explain the importance of the ecosystem services they provide to society. Our findings demonstrate that scientific assessments and interdisciplinary awareness campaigns are urgently required to reconcile biodiversity conservation with rural economies.

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ENVIRONMENTAL POLLUTANTS

Egyptian vulture *Neophron percnopterus* in the Lleida Pre-Pyrenees, Catalonia (Spain)
Photo: Pilar Oliva-Vidal

CHAPTER 3

SECOND-GENERATION ANTICOAGULANT RODENTICIDES IN THE BLOOD OF OBLIGATE AND FACULTATIVE EUROPEAN AVIAN SCAVENGERS

This chapter corresponds to the article:

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CHAPTER 3

Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers

Abstract

The widespread use of second-generation anticoagulant rodenticides (SGARs) and their high persistence in animal tissues has led to these compounds becoming ubiquitous in rodent-predator-scavenger food webs. Exposure to SGARs has usually been investigated in wildlife species found dead, and despite growing evidence of the potential risk of secondary poisoning of predators and scavengers, the current worldwide exposure of free-living scavenging birds to SGARs remains scarcely investigated. We present the first active monitoring of blood SGAR concentrations and prevalence in the four European obligate (i.e., vultures) and facultative (red and black kites) avian scavengers in NE Spain. We analysed 261 free-living birds and detected SGARs in 39.1% (n = 102) of individuals. Both SGAR prevalence and concentrations (ΣSGARs) were related to the age and foraging behaviour of the species studied. Black kites showed the highest prevalence (100%), followed by red kites (66.7%), Egyptian (64.2%), bearded (20.9%), griffon (16.9%) and cinereous (6.3%) vultures. Overall, both the prevalence and average ΣSGARs were higher in non-nestlings than nestlings, and in species such as kites and Egyptian vultures foraging in anthropic landscapes (e.g., landfill sites and livestock farms) and exploiting small/medium-sized carrions. Brodifacoum was most prevalent (28.8%), followed by difenacoum (16.1%), flocoumafen (12.3%) and bromadiolone (7.3%). In SGAR-positive birds, the ΣSGAR (mean ± SE) was 7.52 ± 0.95 ng/ml; the highest level detected being 53.50 ng/ml. The most abundant diastereomer forms were *trans*- bromadiolone and flocoumafen, and *cis*- brodifacoum and difenacoum, showing that lower impact formulations could reduce secondary exposures of non-target species. Our findings suggest that SGARs can bioaccumulate in scavenging birds, showing the potential risk to avian scavenging guilds in Europe and elsewhere. We highlight the need for further studies on the potential adverse effects associated with concentrations of SGARSs in the blood to better interpret active monitoring studies of free-living birds.

Keywords: anticoagulant rodenticides, bioaccumulation, non-target species, raptors, scavengers, vultures

1. Introduction

The contamination of food webs with anticoagulant rodenticides (ARs) is currently of major concern to environmental toxicologists and wildlife ecotoxicologists (Rattner et al., 2014; Lohr and Davis, 2018; van den Brink et al., 2018; Ravindran et al., 2022). Rodents comprise the largest mammalian order with > 2,500 species (Kay and Hoekstra, 2008) and human-rodent conflicts occur worldwide (e.g., by consuming and spoiling crops and stored grain, damaging infrastructure, predating endemic species, and spreading human and livestock diseases; van den Brink et al., 2018) and cost several billion Euros each year (Jacob and Buckle, 2018). The multi-faceted nature of these conflicts requires increasing continuous anthropogenic controls, and the use of ARs has been the most frequent lethal method since the 1950s. However, the rapid development of rodent resistance to the early forms of rodenticides (i.e., first generation ARs or FGARs) has led to the development of more toxic and bioaccumulative second generation ARs (SGARs), also so-called "super-warfarins" (Thomas et al., 2011). The bioaccumulation of SGARs after repeated secondary exposure, negatively affects the vitamin K-dependent coagulation pathway in birds of prey, leading to bleeding in or around critical organs (e.g., brain, heart, lungs) and causing sublethal impairments or death (Murray, 2018).

Although the use of ARs may be necessary for rodent control, the well-known risks of primary and secondary poisoning of non-target species necessitates debate on the need to require measures to mitigate the risks of ARs use (Buckle and Prescott, 2018; Eisemann et al., 2018; Witmer, 2018; Thornton et al., 2022). Indeed, the Convention on the Conservation of Migratory Species of Wild Animals (the CMS, or Bonn Convention) highlighted secondary exposure to ARs as one of the most important toxicological hazards for migratory birds (CMS, 2014). The high acute toxicity and persistence of ARs in animal tissues, especially of SGARs, has led to their becoming ubiquitous in rodent-predator-scavenger food webs (López-Perea and Mateo, 2018; Pay et al., 2021; Elliott et al., 2022; Cooke et al., 2022). Baits containing ARs may be consumed by a number of non-target primary consumers (e.g., invertebrates, fish, wild birds and mammals), increasing the risk of exposure across the entire food web (Shore and Coeurdassier, 2018; Regnery et al., 2020; Alabau et al., 2020).

Predator species that are highly specialized rodent-feeders, such as snakes, kestrels, owls and mustelids, are at a high risk of SGAR exposure (Lettoof et al., 2020; Roos et al., 2021; Elliott et al., 2022). However, generalist predators and scavengers may be exposed to secondary SGAR poisoning at similar or even higher levels than specialist predators (López-Perea and Mateo, 2018). This could be because clinically poisoned (e.g., sick) animals containing high SGAR concentrations can be easily caught and consumed by predators such as raptors (e.g., eagles and kites) and mammalian carnivores. They could also be eaten by obligate scavengers (i.e., species that depend entirely on carrion, such as vultures) and facultative scavengers (i.e., species that exploit carrion opportunistically, including raptors, corvids and mammalian carnivores) (Hindmarch and Elliott, 2018). In addition, avian scavengers frequently exploit food sources in urban landfill sites (Tauler-Ametller et al., 2017, 2019; Plaza and Lambertucci, 2018; Arévalo et al., 2022; Fernández-Gómez et al., 2022), where SGARs are constantly deployed (Coeurdassier et al., 2018). Despite these risk factors, few studies have investigated the exposure of avian scavengers to SGARs, particularly vulture species (Sánchez-Barbudo et al., 2012; Mateo et al., 2015; Plaza et al., 2019; Rial-Berriel et al., 2021; Moriceau et al., 2022).

The prevalence of wildlife exposure to SGARs has usually been assessed by passive monitoring of animals found dead in the field or admitted to wildlife rehabilitation centres. However, this method is probably biased towards higher prevalence values due to the inherent over-representation of poisoned animals in the samples. Active monitoring (i.e., by sampling live animals in the field) may therefore provide more accurate data on the prevalence of contaminant exposure than passive monitoring (Descalzo et al., 2021). However, active monitoring studies of wild bird exposure to SGARs are much less common. Regarding raptors, Martínez-Padilla et al. (2017) detected bromadiolone in the blood of 16.9% (n = 112) of common kestrel (*Falco tinnunculus*) fledglings with an average (± SE) concentration of 0.25 ± 0.02 ng/ml, in a region of central Spain during a common vole (*Microtus arvalis*) population outbreak. Badry et al. (2022) analysed ARs in the blood of nestling raptors from Germany and detected ARs in 22.6% (n = 53) of red kites (*Milvus milvus*) and 8.6% (n = 35) of common buzzards (*Buteo buteo*), with the highest median concentration (of brodifacoum at 13 ng/ml) observed in red kites, evidencing this species' risk of AR exposure. In France, Powolny et al. (2020) detected SGARs in the blood of 30% (n = 47) of red kite nestlings, with a median (and range) of 6.1 ng/ml (0.2–29.4). It should be noted that, as with other chemicals, blood samples may show lower SGAR concentrations than other tissues in which these compounds tend to bioaccumulate and persist for longer periods (i.e., the liver; see Horak et al., 2018), limiting their detectability in active monitoring studies relying on blood SGAR levels. For example, Murray (2020) detected SGARs in the liver of 100% (n = 43) of red-tailed hawks (*Buteo jamaicensis*) admitted to a wildlife clinic in the north-eastern United States, while only 32.6% showed SGARs in their serum. Another current limitation of blood SGAR levels is the lack of reference toxicity thresholds in blood, contrary, for example, to the established toxicity threshold in the liver (i.e., > 0.1 μg/g in wet weight) (Tomas et al., 2011; Rattner et al., 2014).

We carried out an active monitoring program in NE Spain to determine the prevalence and concentration of different SGARs (difenacoum, bromadiolone, brodifacoum and flocoumafen) in the blood of the four European obligate scavengers (griffon *Gyps fulvus*, cinereous *Aegypius monachus*, bearded *Gypaetus barbatus*, and Egyptian, *Neophron percnopterus* vultures) and facultative avian scavengers (black and red kites, *Milvus migrans* and *M. milvus*) of different age classes and dietary preferences. Our general hypothesis was that avian species, age class, and trophic behavior influence SGAR exposure. We predicted: (1) that a higher proportion of rodents in the diet may increase the exposure to SGARs, because rodents are the target species for these biocides; (2) that non-nestlings bioaccumulate more SGAR residues than nestlings, because of their longer time at risk of exposure; (3) that certain diastereomer (*cis* vs *trans*) forms may bioaccumulate more in predator/scavenger species because of their different half-lives in animal tissues; (4) that species foraging in anthropic habitats would suffer greater exposure to these compounds because of the potential association between the exploitation of anthropogenic food resources found on landfill sites and livestock farms; and (5) that the SGAR prevalence observed in living individuals would be lower than that described in animals found dead in other studies in the same geographical area, because SGARs bioaccumulate and persist for longer periods in liver tissue.

2. Material and Methods

2.1. Study area

The study was carried out in the Pyrenees (NE Spain) and adjacent regions (Fig. 1). This mountain range covers around 50,000 km^2 and runs from east-west forming a natural boundary between France and Spain (Améztegui et al., 2010). It is characterized by a strong altitudinal gradient ranging from sea level to > 3,000 m a.s.l. and encompasses a great diversity of vegetation types (Ninot et al., 2007). The area is characterized by extensively and semi-extensively grazed livestock (cattle, sheep and horses) and holds important populations of wild herbivorous ungulates, which provide most of the biomass for the scavenger guild (Colomer et al., 2011, Margalida et al., 2018). The scavenger assemblage (mainly facultative avian species) also exploits carnivorous mammal carcasses (see Fig. 2).

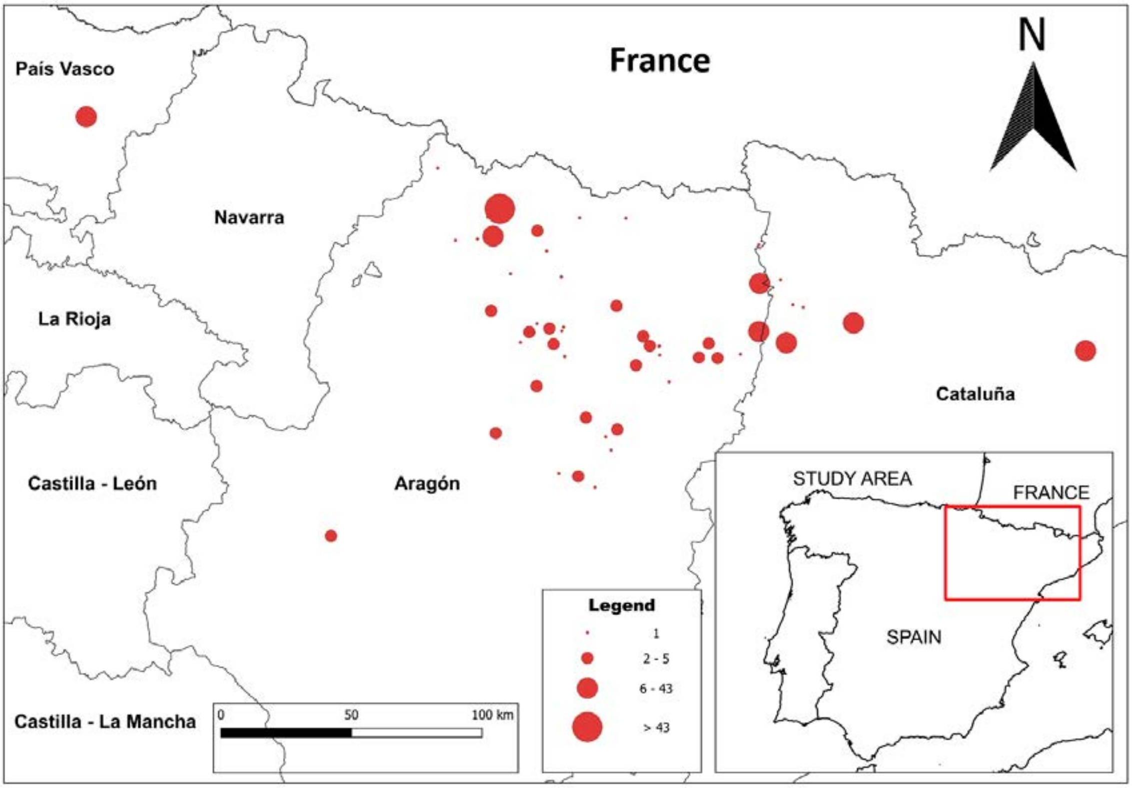

Figure 1. Map of the study area showing the Pyrenees and adjacent regions (NE Spain) that were sampled from 2017 to 2021. The sampling points are shown according to the number of birds sampled at each location.

The Pyrenees holds breeding populations of the four obligate avian scavengers (Table 1) and a rich community of facultative avian (e.g., raptors and corvids) and mammalian scavenger species (e.g., red fox *Vulpes vulpes*, wild boar *Sus scrofa*, *Martes* spp. and badgers *Meles meles*) (Oliva-Vidal et al., 2022).A network of active supplementary feeding stations (SFS) for avian scavengers is present throughout the Pyrenees (Moreno-Opo et al., 2015) and wildlife, mainly avian species, frequently exploit anthropogenic food resources at the urban open-air landfills that occur throughout the study area (Arizaga et al., 2018; Tauler-Ametller et al., 2018, 2019).

2.2. Studied species

We collected blood samples from 261 free-living obligate and facultative avian scavengers, from 2017 to 2021. Our study included the four European obligate avian scavengers (griffon, cinereous, bearded and Egyptian vultures). As for the facultative avian scavengers, we sampled red and black kites, one golden eagle (*Aquila chrysaetos*) and one Bonelli's eagle (*Aquila fasciata*) (Table S1), the latter being the only one that scavenges only rarely. The Spanish breeding population sizes and overall population trends of these species, and their main biological traits (e.g., breeding behavior and principle feeding habits) are detailed in Table 1.

Figure 2. Main avian scavenger species included in this study consuming carcasses of different mammalian carnivores in the study area. The consumption of carnivore carrion could help explain tertiary SGAR exposure or poisoning routes to scavenging species. A) red kite consuming a red fox; B) black kite consuming a badger; C) Egyptian vulture, black kite and common raven consuming a domestic cat (*Felis catus*); D) golden eagle consuming a domestic cat; E) bearded vulture consuming a stone marten (*Martes foina*); and F) griffon vulture consuming a red fox (see Oliva-Vidal et al., 2022).

2.3. Sampling

Blood samples were collected when birds were handled to be fitted with satellite transmitters or ringed and marked with patagial tags. Both nestlings and non-nestling individuals (juveniles, subadults and adults, Table S1) were sampled. Each individual's age class was identified according to its moult pattern and plumage characteristics (Forsman, 2016). All individuals were handled by trained and authorized personnel. Specialized climbers visited nests when nestlings were feathered but not yet ready to fly. Non-nestling individuals were captured using a variety of methods (e.g., baited traps) according to the species concerned (Table S2). Whole-blood samples (3–5 ml, and never exceeding 1% of the body weight) were collected from the brachial vein, placed into EDTA or heparinized tubes and stored at -80ºC until analysis. Two drops of blood were used for sexing the individuals using molecular procedures.

Table 1. Number of breeding pairs, population trend, breeding behaviour, main feeding habits and migratory status in Spain of the four obligate avian scavengers (vultures) and the main facultative avian scavenger species included in this study. The percentage of the European population represented by the Spanish populations of the four European vulture species are shown.

Species	Scavenger group	Breeding pairs	Population trend	Breeding behaviour(8)	Main feeding habits (9, 10, 11)	Migratory status
Griffon vulture	Obligate	30,946(1) [90%]	+	Colonial	Medium-sized and large carrion, garbage	Sedentary (adults) Migrant (juveniles)
Cinereous vulture	Obligate	2,548(2) [> 90%]	+	Colonial and territorial	Medium-sized and large carrion remains (tendons, muscle), small carrion	Sedentary
Bearded vulture	Obligate	140(3) [63%]	+	Territorial	Bone remains of medium-sized and large carrion, small carrions	Sedentary
Egyptian vulture*	Obligate	1,490(4) [32.2%]	-	Territorial	Small pieces of medium-sized and large carrion, small carrions, garbage	Migrant
Red kite	Facultative	1,994(5)	-	Territorial	Small carrion, garbage	Sedentary
Black kite*	Facultative	2,061(6)	+	Territorial	Small carrion, garbage	Migrant
Golden eagle+	Facultative	1,553(7)	+	Territorial	Small and medium-sized prey/carrion	Sedentary

(1) Del Moral and Molina (2018a); (2) Del Moral (2017); (3) Margalida and Martínez (2020); (4) Del Moral and Molina (2018b); (5) Cardiel (2006); (6) Palomino (2006); (7) Del Moral (2009); (8) van Overveld et al. (2020); (9) Moreno-Opo et al. 2015; (10) Tauler-Ametller et al. 2019; (11) Oliva-Vidal et al. 2022 (See references in Supplementary material).

Medium-sized and large carrion items mainly represent domestic and wild ungulate carcasses. Small carrion items represent rodents, birds, invertebrates and small/medium-sized mammals. Garbage refers to food sources found at open-air landfill sites.

*Species with a landfill-based diet (authors unpublished data).

+Apex predator

2.4. SGAR analysis

SGAR extraction was performed following the method described by Martínez-Padilla et al. (2017) with some modifications. Briefly, whole blood samples were thawed, and 400 µl were placed in a 10 ml-glass tube with 3 ml of dichloromethane:acetone (70:30), 2 g of sodium sulphate and 50 µl of internal standard (brodifacoum-d4 at 0.1 ng/ µl) in methanol. Then, the sample was vortexed (10 min), sonicated (5 min) and centrifuged (1048 rcf, 5 min). The organic phase was transferred to another glass tube. This extraction was repeated again with another 3 ml of dichloromethane:acetone (70:30) and the extract obtained was pooled with the first one. The extract was then evaporated to dryness under a stream of N2, resuspended in 200 µl of methanol, filtered through a 0.2 µm mesh nylon filter, and collected in a chromatography vial for analysis using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). The analytical equipment consisted of a liquid chromatograph (Agilent UHPLC Series 1290 Infinity II) coupled to a triple quadrupole mass spectrometer (Agilent 6470 LC/TQ). The chromatographic separation was performed using a reverse-phase column (Agilent InfinityLab Poroshell 120 EC–C18, 2.1 × 150 mm, 2.7 µm) in an oven at 40ºC. The injection volume was 10 µl. The mobile phase comprised a gradient elution of two solvents (A: ammonium acetate10 mM, pH: 6.03; B: methanol). The initial conditions were 50% A and 50% B, reaching 100% B at min 13. This was maintained until min 14, returned to the initial conditions at min 15, and then left to stabilize until min 20 before the next sample injection. The flow rate was 0.250 ml/min. Ionization was performed using an electrospray ionization source (ESI) in negative mode with an Agilent Jet Stream. The conditions were as follows: gas temperature 300ºC; gas flow 8 l/min; nebulizer at 40 psi; sheath gas temperature 300ºC; sheath gas flow 11 l/min; capillary at 4000 V; and nozzle voltage/charging 1750 V. Fragment ion spectra were obtained in a dynamic multiple reaction monitoring (dMRM) scan. The fragmentor voltage, collision energy and ion fragments for quantification or qualification were optimised for every compound (Table S3). Up to four dMRM transitions were performed for each SGAR and identifications were considered positive when all the ratios between qualifier and quantifier ions in the sample differed by less than 20% compared with the standard ratios. *Cis* and *trans* diastereomers of the SGARs (with indistinguishable mass spectra) were identified according to the elution order of each form given by Fourel et al. (2017a). Data collection and processing were performed using the Masshunter™ Work-station from Agilent Technologies.

Matrix-matched calibrations were performed using partridge whole-blood extracts at concentrations of 6.25–250 ng/ml in a final volume of 200 µl, including 25 ng/ml of internal standard. Procedural blanks were analysed for each sample batch. Precision and accuracy were calculated using partridge whole-blood fortified with 31.25, 62.5 and 125 ng/ml of SGAR standards. The recovery percentage ranged between 89.0 and 95.8% and the relative standard deviation (RSD) was between 3.3 and 9.9%. The limits of quantification based on the minimum detected concentrations followed the identification criteria described above and ranged between 0.04 and 0.5 ng/ml with a response to noise ratio greater than 10 (Table S3).

2.5. Statistical analysis

We first calculated the blood concentration (ng/ml) of each SGAR (brodifacoum, bromadiolone, difenacoum and flocoumafen) in SGAR-positive birds (n = 102 individuals). Then, to ascertain whether avian species, age class (nestling or non-nestling) or sex (female or male) affected the prevalence and concentrations of SGARs, we fitted generalized linear models (GLMs) where 'SGARs presence' (positive or negative), 'ΣSGARs concentration' (i.e., sum of the concentrations of all the compounds detected in each bird) and 'ΣSGARs concentration > LOQ' (limit of quantification) were the response variables; and 'avian species', 'age class' and 'sex' were categorical predictors. Given that we did not sample individuals of all age classes for all species (see Table S1), we lumped the age data into two categories, 'nestling' and 'non-nestling' in all models. We used binomial error distributions and log link functions for SGARs prevalence, and Gaussian error distributions and identity link functions for ΣSGARs concentration and ΣSGARs concentration > LOQ. To improve normality assumptions, both ΣSGARs concentration and ΣSGARs concentration > LOQ were log-transformed. When we found significant differences among avian species, we used a post hoc Tukey's HSD test for pairwise comparisons using the *multcomp* package with the Bonferroni correction. At the intraspecific level, we reported 'SGARs presence', 'ΣSGARs concentration' and 'ΣSGARs concentration > LOQ' for all age class categories (nestling, juvenile, subadult and adult). Given sample size limitations for some age class categories (see Table S1), we did not model SGARs prevalence and concentrations at the species level. We used the Kruskal-Wallis test and post hoc pairwise comparisons using the pairwise Wilcoxon rank sum test with Bonferroni adjustment to test for intraspecific differences in ΣSGAR concentrations among age classes for species with both sample sizes ≥ seven individuals and with individuals of at least three age classes (griffon vultures, bearded vultures, Egyptian vultures and red kites; Table S1). To explore differences in the proportions of the most abundant *cis* or *trans* diastereomers of each SGAR, we performed GLMs where 'proportion of *cis* forms' or 'proportion of *trans* forms' were the response variables, and 'avian species', 'age class' and 'sex' were categorical predictors. We used Gaussian error distributions and identity link functions, and only birds with residues of each SGAR were considered. Analyses were performed using R version 3.6.1 (R Development Core Team 2019).

3. Results

SGARs were detected in 102 (39.1%) of the 261 avian scavengers analysed, including every species studied except Bonelli's eagle (Table 2). The highest prevalence of SGARs was observed in black kites (100%), followed by red kites (66.7%), and Egyptian (64.2%), bearded (20.9%), griffon (16.9%) and cinereous vultures (6.3%) (Table 2). SGARs prevalence differed among species with sample sizes ≥ 7 ($p < 0.001$), with higher values in black kites, red kites and Egyptian vultures than in the other species (Tables 2 and 3). SGARs prevalence was lower in nestlings compared with individuals of other age classes ($p = 0.003$), whereas sex had no influence (Table 3). Regarding the specific SGARs, brodifacoum showed the highest overall prevalence in the birds studied (28.8%), followed by difenacoum (16.1%), flocoumafen (12.3%) and bromadiolone (7.3%). We found that 18.4% (n = 48) of all birds analysed contained residues of multiple (> 1) SGARs, representing 47.1% (48/102) of the SGAR-positive birds. Of all SGAR-positive birds, 30.4% contained residues of two SGARs, 15.7% of three SGARs, and four different SGARs were detected in one individual (Fig. S1).

Considering concentrations in SGAR-positive birds (n = 102) only, ΣSGARs showed a mean ± SE value of 7.52 ± 0.95 ng/ml, with a maximum level of 53.50 ng/ml. Considering all individuals analysed (n = 261), the mean total ΣSGARs was 2.94 ± 7.03 ng/ml. Brodifacoum was the compound found with the highest levels in birds with any SGARs present (5.95 ng/ml), followed by bromadiolone (0.72 ng/ml), difenacoum (0.72 ng/ml) and flocoumafen (0.13 ng/ml) (Fig. 3). The maximum blood concentration of brodifacoum (51.66 ng/ml) was found in a golden eagle without evident signs of toxicosis; the 27-year-old female eagle had been equipped with a GPS transmitter since the time of sampling and died by electrocution two years later without showing any uncharacteristic behaviours.

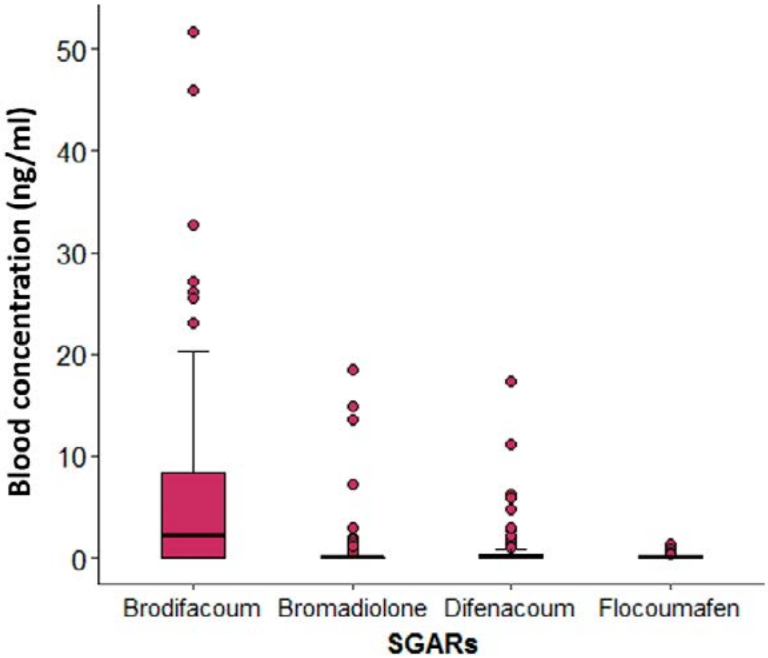

Figure 3. Boxplots showing the blood concentration (ng/ml) of each second-generation anticoagulant rodenticide (SGAR) analyzed in the avian scavenger species sampled in the Pyrenees and adjacent regions (NE Spain). Only SGAR-positive birds are shown. Boxes encompass the 25–75th quartiles from the median (thick line); vertical lines represent the maximum and minimum values, excluding outliers, shown as circles and defined as values further than 1.5 times the interquartile range.

In all of the species studied with sample sizes ≥ 7 individuals, the mean individual concentration of ΣSGARs considering all individuals (n = 259) was significantly higher in black kites, red kites and Egyptian vultures than in bearded, cinereous and griffon vultures ($p < 0.001$; Fig. 4, Table 3). ΣSGARs concentration was lower in nestlings than in non-nestlings (p < 0.001; Table 3), particularly in Egyptian vultures and red kites (Fig. 5). The mean concentration of ΣSGARs differed between species ($p < 0.001$) in individuals with SGARs concentration > LOQ, with higher concentrations in red kite, black kite and Egyptian vultures than in bearded and griffon vultures, and showed no significant differences related to age class (nestling vs. non-nestling) or sex (Tables 2 and 3).

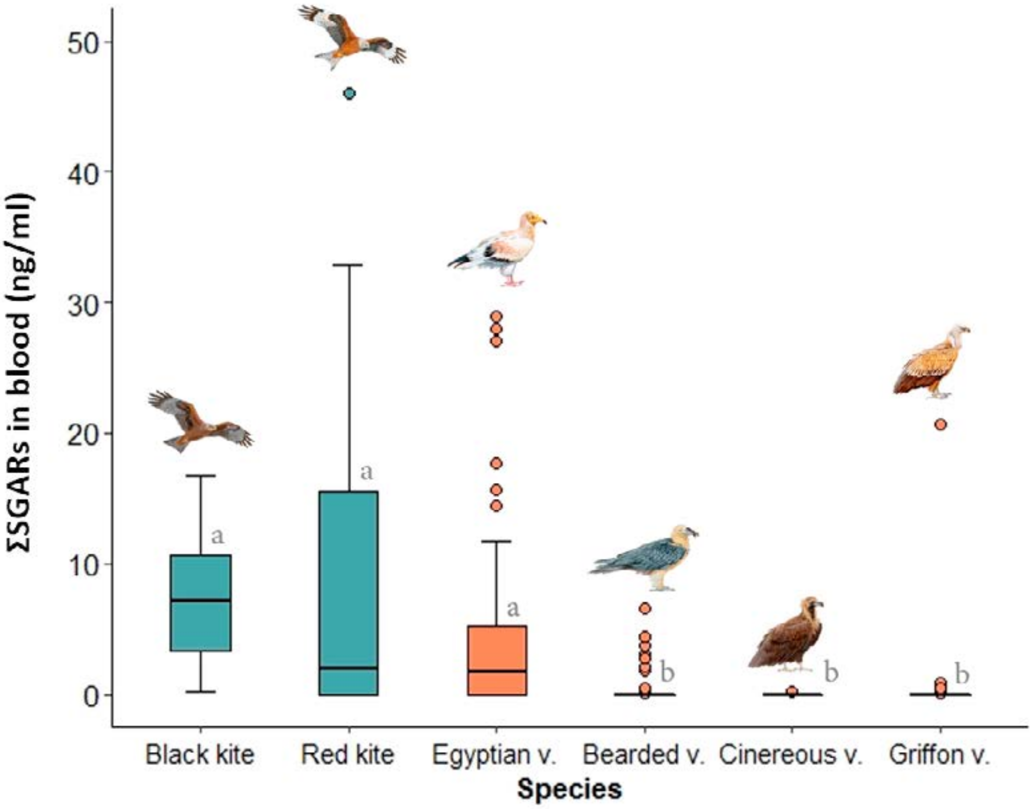

Figure 4. Boxplots representing the blood concentration of the sum of second-generation anticoagulant rodenticides (ΣSGARs) in obligate and facultative avian scavengers with sample sizes ≥ 7 individuals sampled in the Pyrenees and adjacent regions (NE Spain). Boxes encompass the 25–75th quartiles from the median (thick line); vertical lines represent the maximum and minimum values, excluding outliers, shown as circles and defined as values further than 1.5 times the interquartile range. Different letters show significant differences between species as indicated by post hoc Tukey's tests after Bonferroni correction of ΣSGARs concentration generalized linear model (GLM; see Table 3). Facultative (green boxes) and obligate (orange boxes) avian scavenger species are shown separately.

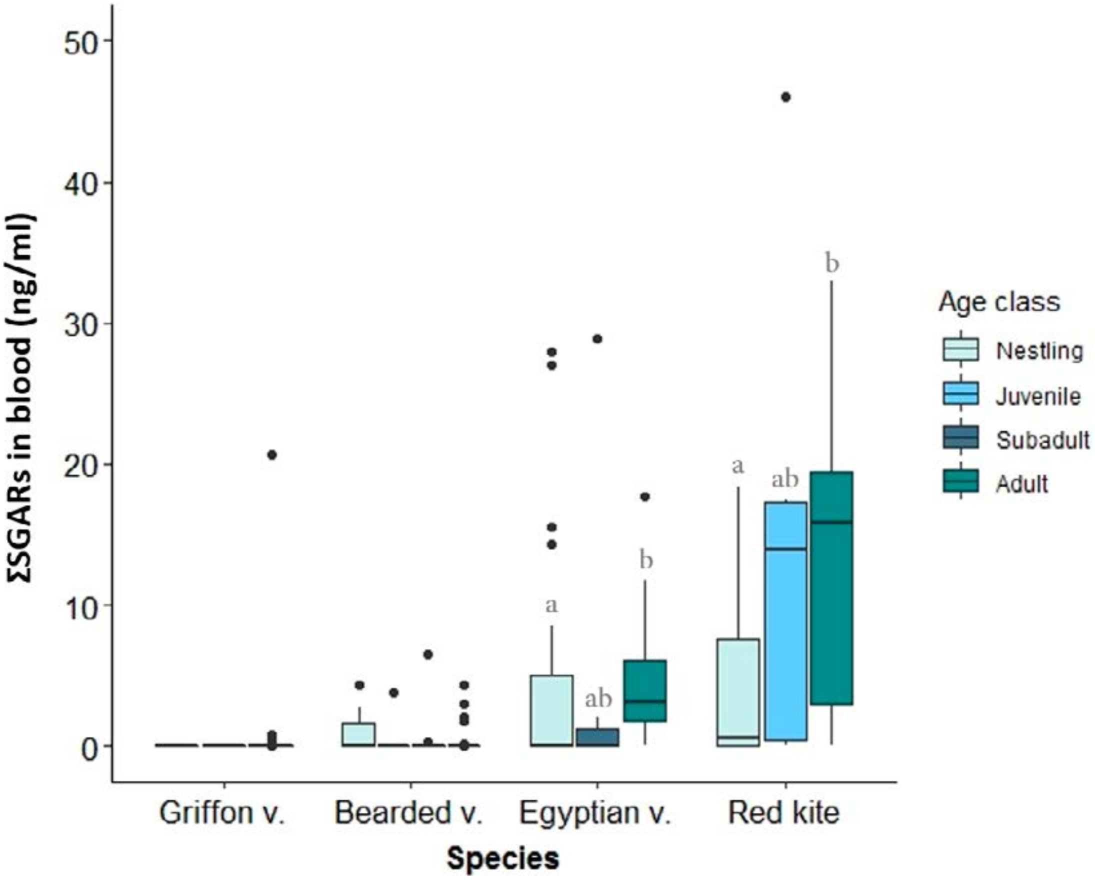

Figure 5. Boxplots representing differences in blood concentrations of the sum of second-generation anticoagulant rodenticides (ΣSGARs) in the obligate and facultative avian scavengers studied in the Pyrenees and adjacent regions (NE Spain) according to age class (i.e., nestling, juvenile, subadult and adult; also see Table S4). Boxes encompass the 25–75th quartiles from the median (thick line); vertical lines represent the maximum and minimum values, excluding outliers, shown as circles and defined as values further than 1.5 times the interquartile range. Different letters show significant intraspecific differences between age classes as indicated by the Kruskal-Wallis test with Bonferroni adjustment (see Methods).

The proportions of SGAR diastereomers did not differ between species, sexes or age classes. The *trans* diastereomer was the most frequent form of bromadiolone and flocoumafen, whereas the *cis* diastereomer was the most frequent form of brodifacoum and difenacoum. The highest difference between diastereomer forms was found for bromadiolone, in which the *cis* form was almost absent (Fig. 6).

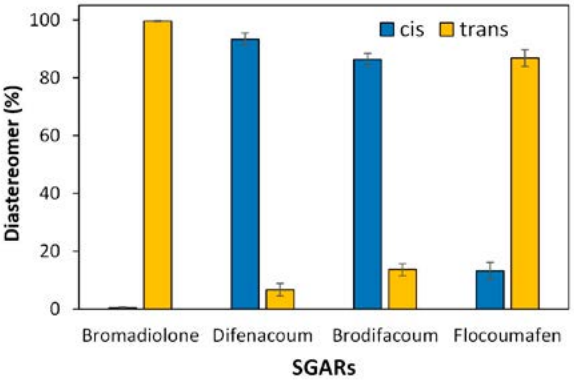

Figure 6. Proportion (mean ± SE) of *cis* and *trans* diastereomer forms of each SGAR detected in the obligate and facultative avian scavengers studied in the Pyrenees and adjacent regions (NE Spain).

4. Discussion

Our active monitoring indicated that medium-sized facultative avian scavengers, such as red and black kites, and Egyptian vultures (an obligate scavenger) were the species most exposed to SGARs, with a prevalence > 64% in red kites and Egyptian vultures, and up to 100% in black kites. Prevalence values were lower for the larger obligate avian scavengers (bearded, griffon and cinereous vultures) but still ranged between 6.25% and 20.9%. The average blood SGAR concentrations were similar in kites and Egyptian vultures (ranging between ~7–13 ng/ml) while among large vultures blood levels were lower (< 2.5 ng/ml). The highest concentration was detected in the only golden eagle (i.e., an apex predator) we sampled, being 53.50 ng/ml. These results showed that bioaccumulation of SGARs presents a risk not only to top predators, but also to the European avian scavenger guild as a whole.

Table 2. Prevalence (%) and concentration (ng/ml) [1] of second-generation anticoagulant rodenticides (SGARs) in the blood of free-living obligate and facultative avian scavengers sampled in the Pyrenees and adjacent regions (NE Spain).

	SGARs				Brodifacoum		
	N	N+	%	Mean ± SE [min-max]	N+	%	Mean ± SE [min-max]
Obligate							
Egyptian v.	67	43	64.18^{A}	6.74 ± 1.1^{A} [0.13-28.87]	39	58.21	6.54 ± 1.11 [0.37-27.15]
Bearded v.	67	14	20.90^{B}	2.29 ± 0.5^{B} [0.1-6.53]	12	17.91	2.48 ± 0.53 [0.17-6.53]
Griffon v.	65	11	16.92^{B}	2.23 ± 1.85^{B} [0.04-20.73]	3	4.62	7.15 ± 6.53 [0.53-20.21]
Cinereous v.	16	1	6.25^{B}	0.17	ND	-	-
Facultative							
Black kite	8	8	100^{A}	7.61 ± 2.20^{A} [0.20-16.71]	7	87.50	7.60 ± 2.28 [0.14-16.71]
Red kite	36	24	66.67^{A}	12.75 ± 2.29^{A} [0.49-45.95]	13	36.11	15.08 ± 3.62 [0.49-45.95]
Golden e.	1	1	100	53.50	1	-	51.66
Bonelli's e.	1	0	-	-	ND	-	-

[1] Concentrations in birds with values > LOQ.

[2] Percentages with different letters are significantly different.

Bromadiolone			Difenacoum			Flocoumafen		
N+	**%**	**Mean ± SE [min-max]**	**N+**	**%**	**Mean ± SE [min-max]**	**N+**	**%**	**Mean ± SE [min-max]**
8	11.94	1.48 ± 0.25 [0.85-2.95]	18	26.87	0.85 ± 0.18 [0.13-2.84]	16	23.88	0.47 ± 0.08 [0.12-1.39]
1	1.49	0.50	1	1.49	0.15	5	7.46	0.35 [0.04-1.31]
ND	-	-	4	6.15	0.19 ± 0.08 [0.1-0.43]	7	10.77	0.33 ± 0.13 [0.04-0.90]
ND	-	-	1	6.25	-	ND	-	-
ND	-	-	5	62.50	1.44 ± 0.85 [0.07-4.77]	1	12.50	0.50
10	27.78	6.08 ± 2.20 [0.58-18.44]	12	33.33	4.02 ± 1.56 [0.23-17.38]	3	8.33	0.29 ± 0.02 [0.26-0.32]
ND	-	-	1	-	1.84	ND	-	-
ND	-	-	ND	-	-	ND	-	-

Table 3. Generalized linear models (GLMs) used to assess the effects of 'avian species' (red kite, black kite, Egyptian, bearded, griffon and cinereous vultures), 'age class' (nestling or non-nestling) and gender (female or male) on SGARs prevalence, ΣSGARs concentration (i.e., sum of concentrations of all compounds detected in each bird) and ΣSGARs concentration > LOQ (i.e., considering only individuals with SGARs levels > LOQ). The coefficients and standard errors (SE) of all models are shown. Significant p-values (< 0.05) are highlighted in bold.

Response variable	Explanatory variable	Coefficient	SE	p-value
SGARs prevalence	Black kite	15.073	-	0.986
	Egyptian vulture	-0.206	0.453	0.649
	Bearded vulture	-0.716	0.542	**< 0.001**
	Griffon vulture	-2.867	0.552	**< 0.001**
	Cinereous vulture	-2.962	1.105	**0.007**
	Age class ~ non-nestling	1.1966	0.408	**0.003**
	Sex ~ male	-0.302	0.319	0.344
ΣSGARs concentration	Black kite	0.678	0.837	0.418
	Egyptian vulture	-0.596	0.430	0.167
	Bearded vulture	-3.645	0.459	**< 0.001**
	Griffon vulture	-3.905	0.458	**< 0.001**
	Cinereous vulture	-3.251	0.642	**< 0.001**
	Age class ~ non-nestling	1.220	0.337	**< 0.001**
	Sex ~ male	-0.274	0.268	0.308
ΣSGARs concentration > LOQ	Black kite	-0.482	0.558	0.390
	Egyptian vulture	-0.530	0.337	0.119
	Bearded vulture	-1.735	0.449	**< 0.001**
	Griffon vulture	-3.136	0.514	**< 0.001**
	Age class ~ non-nestling	0.095	0.309	0.758
	Sex ~ male	0.396	0.283	0.164

Exposure of avian scavengers to SGARs has previously been investigated mainly by analysing the livers of animals found dead, but such passive monitoring method may introduce significant bias in the observed concentration and prevalence values. For example, Sánchez-Barbudo et al. (2012) detected ARs in 13% of griffon vultures throughout Spain (n = 23) and López-Perea et al. (2019) found that 19% of griffon vultures were affected (n = 42) in NE Spain. In griffon vultures in France, Berny et al. (2015) detected ARs in 4.2% of the liver samples examined (n = 119) and Moriceau et al. (2022) in 32.2% of livers (n = 90). Regarding bearded vultures, López-Perea et al. (2019) detected ARs in 22.2% of the birds examined (n = 9) in NE Spain. In France, Berny et al. (2015) did not detect residues in bearded vultures (n = 8), but Moriceau et al. (2022) found AR residues in 33.3% of birds (n = 9). Concerning cinereous vultures, Moriceau et al. (2022) detected ARs in 76.5% of the birds examined (n = 17). For Egyptian vultures, Berny et al. (2015) detected ARs in 22.2% of the individuals examined (n = 9) while López-Perea et al. (2019) found residues in two out of three birds in NE Spain. In the Canary Islands (Spain), Rial-Berriel et al. (2021) detected ARs in 29.9% of the Egyptian vultures analysed (n = 67).

Considering the number of birds studied by Berny et al. (2015) and López-Perea et al. (2019), in areas which overlap or are close to our study area, we estimate an overall prevalence of AR occurrence of 8.1% (13/161) for griffon, 11.8% (2/17) for bearded and 33.3% (4/12) for Egyptian vultures. The values estimated from birds found dead were half of those we obtained by analysing living individuals (i.e., griffon vulture: 16.9%, n = 65; bearded vulture: 20.9%, n = 67; and Egyptian vulture: 64.2%, n = 67), highlighting the need for further studies on the relationship between AR levels in blood and liver tissues. Assuming similar limits of detection in the studies mentioned, we observed that, contrary to our expectations, the prevalence of ARs discovered in birds found dead may underestimate those we observed in living individuals. This fact could be due to significant bias in the sampling method. Most birds found dead could have died from other non-natural causes, such as those related to anthropogenic infrastructures (e.g., trauma or electrocution due to collision with energy infrastructures; Pérez-García et al., 2022) and their carcasses could be more easily detected at the site of death (González et al., 2008). In contrast, clinically poisoned birds tend to move less and remain in safer places (e.g., roosting sites) before dying in the field, making them much harder to find (Peshev et al., 2022). Indeed, there are significant differences in the reported causes of mortality of dead vultures discovered by chance (e.g., 75% relating to shootings and collisions with power lines) compared with dead radio-tagged vultures (e.g., 86% relating to intentional and unintentional poisoning), demonstrating a methodological sampling bias (Margalida et al., 2008). Thus, assessment of threats to avian scavengers by analysing untagged individuals found dead may lead to important biases in the accurate calculation of epidemiological parameters (Franson et al., 1996, González et al., 2008).

Regarding New World vultures, Hosea (2000) found AR residues in one of the two turkey vultures (*Cathartes aura*) examined between 1997 and 2010. McMillin (2012) reported death due to AR poisoning in four turkey vultures in California (USA). Stone et al. (2003) detected AR residues in the liver of both of two turkey vultures in New York state (USA), while Kelly et al. (2014) detected ARs in the liver of seven out of 23 (30.4%) turkey vultures in California (USA). Turkey vultures therefore show a prevalence of exposure to ARs similar to that of Egyptian vultures in Europe. The dietary habits of these opportunistic avian scavengers are similar, feeding on substantial quantities of garbage and the carrion of wild and domestic mammals, ranging from small rodents to small/medium-sized mammals, large ungulates, birds, reptiles, amphibians and invertebrates (Margalida et al., 2012, Hill et al., 2022).

Exposure to ARs in facultative avian scavengers such as kites or large eagles has been more frequently studied. Regarding kites, Badry et al. (2021) detected ARs in the liver of 80.5% of the birds (n = 41) in Germany. In England, SGAR poisoning was diagnosed as the cause of death in 17.3% of reintroduced red kites found dead (n = 110) (Molenaar et al., 2017). In Scotland, 70% of red kites found dead (n = 114) contained ARs in their liver and in 10% of the individuals AR poisoning was the cause of death (Hughes et al., 2013). In France, Coeurdassier et al. (2014) sampled in an area where bromadiolone has been intensively used to control water voles (*Arvicola terrestris*) and poisoning by this compound was confirmed (or highly suspected) in all red kites found dead (n = 28). More recently, Moriceau et al. (2022) detected AR residues in the liver of 100% of red kites (n = 16) and in one black kite (n = 1) found dead in France. In Spain, ARs have been detected by López-Perea et al. (2019) in the liver of red kites (77%, n = 13) and black kites (33%, n = 6) and Sánchez-Barbudo et al. (2012) found a similar situation (red kites, 88%, n = 8; black kites, 60%, n = 5). In Asia, Hong et al. (2018) detected SGARs in the liver of 42.9% of black kites (n = 7) in Taiwan.

Regarding large eagles, Badry et al. (2021) detected ARs in 38.3% of white-tailed sea eagles (*Haliaeetus albicilla*) (n = 60) in Germany. Sell et al. (2022) detected SGARs in 100% of white-tailed sea eagles found dead with suspected poisoning (n = 40) in Poland. In France, Moriceau et al. (2022) detected ARs in the liver of 100% of golden eagles found dead (n = 7). In Norway, Langford et al. (2013) detected SGARs in the liver of 68.8% of golden eagles (n = 16). In Spain, López-Perea et al. (2019) found no ARs in the liver of golden eagles (n = 5) but Sánchez-Barbudo et al. (2012) detected SGARs in one individual (25%, n = 4). In the USA, Viner et al. (2022) detected ARs in the liver of 38.7% of golden eagles (n = 62) found dead under power lines or wind turbines, with no significant differences in AR prevalence between these infrastructures, and Niedringhaus et al. (2021) detected ARs in the liver of 83% of bald eagles (*Haliaeetus leucocephalus*) (n = 96) and 77% of golden eagles (n = 17). AR poisoning was also previously detected in bald and golden eagles by Stone et al. (1999). In Australia, Pay et al. (2021) detected AR residues in 74% of Tasmanian wedge-tailed eagles (*Aquila audax fleayi*) found dead (n = 50). We detected SGARs in the only golden eagle we analysed, but every previous study of large eagles has indicated significant exposure levels varying from 25% to 100%, depending on the sampling method or location.

Our findings evidence that facultative avian scavengers (black and red kites) showed the highest prevalence of SGARs (100% and 66.7%, respectively) although Egyptian vultures (an obligate scavenger) also showed a high SGAR prevalence (64.2%). Moreover, we found that both kites and Egyptian vultures showed a high prevalence of individuals containing multiple different SGARs (four compounds were identified in a single Egyptian vulture). These exposures could relate to the foraging and dietary habits of these species, which comprise the carrion of small and medium-sized mammals, such as rodents, as well as carnivores (Oliva-Vidal et al., 2022) and large quantities of garbage and food items gleaned from landfill sites and livestock farms (Margalida et al., 2012; Tauler-Ametller et al., 2017, 2018; Arévalo et al., 2022; Fernández-Gómez et al., 2022). In the specific case of the red kite, a diet based on small rodents is more likely to result in bioaccumulation of ARs (Coeurdassier et al., 2014) although this species is well-known for frequently exploiting garbage dumps, slaughterhouses and agricultural areas (Seoane et al., 2003; García-Macía et al., 2022). The widespread use of ARs at landfills and farms could result in a high number of contaminated rodents around these areas, which could be consumed by predators and scavengers foraging in such anthropogenic landscapes. Thus, species which exploit anthropogenic habitats are very likely to suffer greater exposure to SGARs through secondary or even tertiary pathways due to their consumption of contaminated rodents or their predators (López-Perea and Mateo, 2018).

Large obligate avian scavengers (griffon, cinereous and bearded vultures) showed lower prevalence and concentration values than Egyptian vultures and kites. However, our results showed that the endangered bearded vulture suffered the highest prevalence (20.9%) among the large vultures. The consumption of small carrion items (e.g., birds, rodents and small/medium-sized mammals), which represent 14% of their diet (Margalida et al., 2009), could explain the vulnerability of this species to ARs and the prevalence that we found. Their greater dietary plasticity could also explain the high AR exposure observed in Egyptian vultures, since small to medium-sized vertebrate carrion items are frequent in their diet during the breeding season (Margalida et al., 2012). Direct and indirect poisoning is the most widespread non-natural mortality factor for Egyptian vultures and other avian scavengers in Spain (Hernández and Margalida, 2009; Mateo et al., 2015) and our findings demonstrate the critical vulnerability of these threatened species to ARs.

We found that both SGAR prevalence and average concentration were related to the age of an individual. Overall, our results showed that nestlings exhibited lower prevalence and concentration values than non-nestling individuals. Badry et al. (2022) detected ARs in the blood of 22.6% of red kite nestlings in Germany, and Powolny et al. (2020) in the blood of 30% of red kite nestlings in France, much lower prevalence values than we found in red kite nestlings (55.0%, 11/20), juveniles (75%, 6/8) and adults (87.5%, 7/8). Several factors may explain the age class differences we found. There may be dietary differences in the trophic spectrum between chicks and adults. In some species, variations in the diet during the breeding period are related to specific energetic requirements or food quality. However, the high bioaccumulative capacity of SGARs could help explain this age-related exposure, resulting in lower concentrations in nestlings than in adults because of the shorter time nestlings have spent at risk. SGARs have long half-lives in the liver of an exposed animal (e.g., 91.7–307.4 days), which could explain the higher concentrations we observed in adults compared with nestlings (Shore and Coeurdassier, 2018). As for differences in SGAR diastereomer concentrations, our results are in line with other studies in which the prevalence in birds of prey of the *trans* forms of bromadiolone and flocoumafen and the *cis* forms of brodifacoum and difenacoum were greatest because of their higher persistence in animal tissues (Fourel et al., 2017b, 2021). This is a relevant finding and highlights the need for developing SGAR formulations with a lower impact on non-target wildlife (Damin-Permink et al., 2016). Currently, commercial SGAR formulations have varying proportions of *cis*:*trans* forms, ranging between 35:65 and 55:45 (Alabau et al., 2020), and the proportions observed in free-living avian scavengers clearly show that the different diastereomer forms bioaccumulate differently across the food chain.

From a conservation and management point of view, our results suggest that scavenging birds foraging in anthropogenic landscapes (e.g., landfill sites and livestock farms) are more vulnerable to SGAR exposure, as reported in other raptor and scavenger species (López-Perea et al., 2019; Badry et al., 2021) and for other pollutants in Egyptian vultures (Ortiz-Santaliestra et al., 2019). The exploitation of predictable food resources at landfills has increased coinciding with the changes in the Spanish sanitary regulations between 2006–2011, which have resulted in a sudden reduction in food availability by restricting the availability of livestock carcasses in the field, causing changes in the foraging behaviour and dietary habits of avian scavengers, particularly griffon vultures (Donázar et al., 2010; Fernandez-Gómez et al., 2022). As with other chemical pollutants, the risk of avian scavenger exposure to SGARs could be mitigated by securing food availability using managed SFSs where livestock and game animal carcasses can be provided regularly under strict controls to prevent wildlife exposure to toxic compounds (e.g., veterinary pharmaceutical residues or lead).

Our study highlights the problems associated with widespread use of ARs to control commensal rodents, which can affect both facultative avian scavengers, such as kites and eagles, and obligate avian scavengers that frequently include rodents and small/medium-sized carrion items in their diet (e.g., bearded and Egyptian vultures). Oliva-Vidal et al. (2022) found that some facultative avian scavengers, such as golden eagles and red kites, exploit carnivorous mammal carcasses more frequently than herbivore carcasses, although other avian and mammalian scavengers can also consume carnivore carrion (Fig. 2), so increasing the risk of secondary or tertiary AR exposure routes across the scavenger guild. The use of bromadiolone against field voles (*Arvicola terrestris*), coypu (*Myocastor coypu*) and musk rat (*Ondathra zibethicus*) in France has been associated with AR exposure and poisoning of both predators and scavengers (Berny et al., 1997; Fournier-Chambrillon et al., 2004; Coeurdassier et al., 2014). A similar scenario could occur in other countries and affect facultative and obligate avian scavengers due to the current underestimation of the potential problems. Future research to reduce biases in the study of SGAR exposure could focus on sampling individuals tagged with GPS transmitters in order to provide temporal and spatial information to identify conflict areas that should be monitored by managers and policy-makers to reduce the negative impact of SGARs on scavenger populations.

5. Conclusions

We performed the first ever active monitoring of SGAR concentrations and prevalence in the blood of the four European obligate (vultures) and facultative (red and black kites) avian scavengers in the Pyrenees and adjacent areas (NE Spain). SGARs were detected in 39.1% of the birds sampled, with variations in prevalence and concentrations among species that could be explained by differences in their foraging and trophic behaviours. Red and black kites and Egyptian vultures, which mainly feed on small/medium-sized carrion items (e.g., rodents and mammals, including carnivores) and frequently forage in anthropic areas (e.g., landfill sites and livestock farms), showed the highest prevalence of SGARs (64% to 100%). In contrast, large vulture species mainly exploiting medium-sized and large domestic and wild ungulate carcasses, such as griffon, cinereous and bearded vultures, showed the lowest prevalence (< 21%), the highest being found in bearded vultures. By analysing the blood of free-living vultures, we found prevalence values higher than those previously described from the analysis of liver samples from individuals found dead in the same area, which may point to methodological sampling biases. Both prevalence and ΣSGAR concentrations showed differences between age classes, being higher in adult individuals than in nestlings, which could be explained by their longer exposure to ARs leading to greater levels of bioaccumulation. The most abundant SGAR was brodifacoum, followed by difenacoum, flocoumafen and bromadiolone, and in all of these compounds one diastereomer form was clearly more bioaccumulated than the other. These results highlight the need to monitor and regulate the use of anthropogenic compounds such as SGARs. The current exposure levels in scavenging birds could have negative impacts on conservation efforts, particularly for the most endangered European avian scavengers. Further studies on the potential adverse effects associated with blood SGAR levels are necessary to better interpret the concentrations found in the blood of free-living birds and to better understand their potential population effects.

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DEMOGRAPHY AND POPULATION VIABILITY

Griffon vultures *Gyps fulvus*, cinereous vulture *Aegypius monachus* and bearded vulture *Gypaetus barbatus* in a supplementary feeding station in the Lleida Pyrenees, Catalonia (Spain).

Transhumant sheep in the Port de Salau, Lleida Pyrenees, Catalonia (Spain)

Photos: Jordi Bas

CHAPTER 4

BIOINSPIRED MODELS FOR ASSESSING THE IMPORTANCE OF TRANSHUMANCE AND TRANSBOUNDARY MANAGEMENT IN THE CONSERVATION OF EUROPEAN AVIAN SCAVENGERS

This chapter corresponds to the article:

Margalida, A., Oliva-Vidal, P., Llamas, A., Colomer, M. À. 2018. Bioinspired models for assessing the importance of transhumance and transboundary management in the conservation of European avian scavengers. *Biological Conservation* 228, 321–330. https://doi.org/10.1016/j.biocon.2018.11.004.

CHAPTER 4

Bioinspired models for assessing the importance of transhumance and transboundary management in the conservation of European avian scavengers

Abstract

The assessment of temporal and spatial availability of food resources is an important prerequisite in developing improved management tools for effective conservation action. It is especially useful in the conservation of avian scavengers inhabiting regions where livestock move on a regular basis (transhumance). Important management decisions can be taken on the basis of theoretical analyses that need to be regularly checked. In this case study, we consider models of griffon vulture *Gyps fulvus*, Egyptian vulture *Neophron percnopterus* and bearded vulture *Gypaetus barbatus* populations in a part of Spain with one of the highest densities of scavenging birds, and where traditional farming practices remain. We applied bioinspired Population Dynamic P System models (PDP) to assess these species' population trends against the distribution, quantity and availability of carrion for food. We show asymmetries in the availability of food resources, which are substantially higher in summer due to transhumant movements. In the study area, a lack of food resources in winter leads to a seasonal reduction in food supplies to levels unable to meet the energetic requirements of the most abundant vulture species, the griffon vulture. Our results suggest that regardless of active management (e.g., supplementary feeding sites) and the birds' use of other potential food resources not included in the model, griffon vultures are able to find important alternative food resources in more remote areas. We show the importance of variations at spatio-temporal scales in the objective forecasting of population trends, and in the correct application of management actions. Because of the importance of robust assessments for management applications, we discuss the advantages and limitations of ecological modelling for avian scavengers, highlighting the importance of transhumance processes and transboundary approaches.

Keywords: livestock movements, obligate scavengers, food requirements, PDP models, population dynamics, simulation model

1. Introduction

Successful conservation action rests upon harmonizing the best available knowledge with management actions appropriate to the prevailing political and economic situation (Linnell et al., 2016). To this end, conservationists and managers must adapt their activities to administrative and regional scales, and be increasingly aware of the importance both of large scale ecological processes and transboundary cooperation (Rands et al., 2010; Wiens and Bachelet, 2010; Rüter et al., 2014, Lim, 2016; Linnell et al., 2016). The need for, and the benefits arising from, transboundary cooperation in managing wildlife populations beyond simple administrative and jurisdictional limits has led to the emergence of wider scale approaches as a major conservation paradigm, and these are being increasingly applied in many locations (Chapron et al., 2014; Fleurke and Trouwbors, 2014).

Because large avian scavengers have extensive foraging ranges, their management and conservation requires transboundary approaches (Margalida et al., 2013; Lambertucci et al., 2014; Arrondo et al., 2018). Because of their specialized diet based mainly on the carcasses of domestic and wild ungulates, the assessment of food resource availability for these species, and its spatio-temporal distribution, is key and provides an important management tool in improving their conservation status (Margalida and Colomer, 2012; Cortés-Avizanda et al., 2016; Kane et al., 2015). The health of vulture populations is good indicators of habitat modification and unsustainable land management at large spatial scales. Therefore, quantitative assessments of trophic availability, in conjunction with information regarding vulture food preferences and selection (Moreno-Opo et al., 2015, 2016) provide useful information about territory quality and can be used to estimate carrying capacity. Such information can also help to develop guidelines regarding the need for, and form of, supplementary feeding programs and the suitability of particular habitats for vulture reintroduction projects.

Spain is home to 90% of the European Union’s avian scavenger population, and conservation management actions carried out there are fundamental for the Europe-wide scavenging bird metapopulation. In contrast with other scavenging bird populations worldwide (e.g., Ogada et al., 2016), Spanish vulture populations have shown moderate and/or important increases in recent years (Donázar et al., 2009a, 2009b). Transhumance is characteristic of many Spanish ecosystems. It is a traditional farming practice whereby livestock are regularly moved between winter and summer pastures, maximizing the exploitation of grazing resources (Ruiz and Ruiz, 1986; Fernández-Giménez and Fillat, 2012). This was a common practice in many European countries, but is now in decline elsewhere (Vicente-Serrano et al., 2004; Oteros-Rozas et al., 2013). Vultures make use of the food resources from transhumant livestock during at least a third of the year, and this is particularly useful for griffon vulture management (Olea and Mateo-Tomás, 2009). Because larger scale ecosystem management is important for biodiversity conservation, political or administrative boundaries, which divide ecosystems and apply different rules and guidelines, pose special problems for ecological processes and conservation efforts (Zbicz, 1999; Papadopoulou and Sitsoni, 2012). These issues were highlighted in Spain during the outbreak of bovine spongiform encephalopathy in 2001, when changes in sanitary regulations suddenly reduced the food available to vultures provided by livestock carcasses (Tella, 2001, Donázar et al., 2009a, 2009b; Margalida et al., 2010). The various Spanish regions applied different sanitary policies, each affecting the distribution and availability of animal carrion biomass. After this, a network of protection areas for the feeding of scavengers in Spain was designated, but the criteria adopted to manage carrion resources differed among regions (Morales-Reyes et al., 2017).

Clearly, it is therefore important to determine whether the available food resources are sufficient to cover the energetic requirements of an avian scavenger assemblage and whether spatio-temporal variations in food availability may affect their population levels and trends. This information allows managers and policy-makers to anticipate and forecast the effects of food shortages, or changes in their spatio-temporal distribution, on scavenger populations, and enable management and conservation measures to minimize such effects (Margalida and Colomer, 2012). The use of bioinspired models (PDP Systems) allows the assessment of the influence of spatio-temporal changes in food availability on the population dynamics (e.g., Colomer et al., 2011; Kane et al., 2015; Cortés-Avizanda et al., 2016). These estimates, based on data collected in the field, allow the modeling of hypothetical scenarios that can enable managers to anticipate decision-points regarding conservation measures such as the provision of Supplementary Feeding Sites (SFS) or "vulture restaurants". However, the different scenarios provided by modeling approaches are subject to degrees of uncertainty, and to minimize bias in the results it is necessary to estimate the sensitivity of any model to changes in the parameters involved, and the degrees of causality between them.

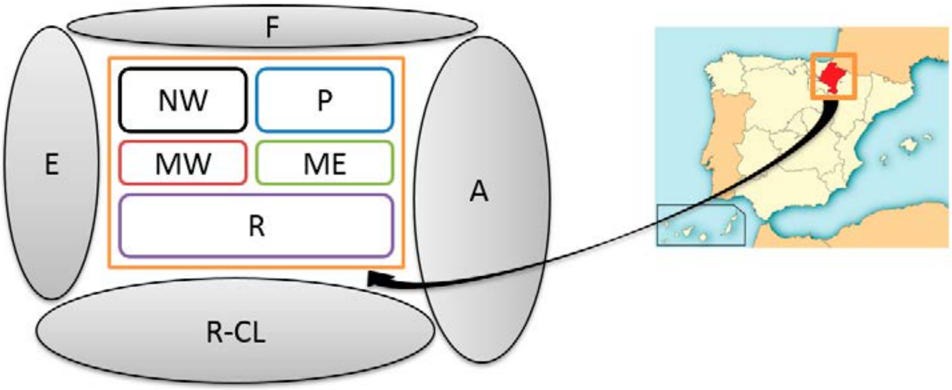


Figure 1. Location of the study area in northern Spain showing the five zones considered within the study area (surrounded by orange) and the peripheral zones (surrounded by grey) in which scavengers can obtain alternative resources. The regular foraging ranges were estimated based on the maximum distance that a bird will fly in a straight line from the nest in search of food (see Material and Methods).

Considering the important ecosystem services provided by vultures (Dupont et al., 2012; Moleón et al., 2014; Morales-Reyes et al., 2015) and the lack of empirical data on the influence of transhumance effects, we undertook a case study on three vulture species (Egyptian vulture *Neophron percnopterus*, Eurasian griffon vulture *Gyps fulvus* and bearded vulture *Gypaetus barbatus*), all of which are obligate scavengers, in a part of Spain with one of the highest avian scavenger population densities (Navarra, N Spain). Our goals were: i) to estimate the carrying capacity of the ecosystem based on the availability of trophic resources; ii) to quantify the spatio-temporal distribution of these resources and their relationship to transhumance practices, in order to determine how the distribution of food impacts vulture population dynamics; iii) to examine the advantages and limitations of ecological modelling in the management of carrion and its effects on ecosystem services provided by vultures, to assess the usefulness of modelling as a decision making tool for managers and policy-makers.

2. Material and methods

2.1. Model building and assumptions

Using a Population Dynamic P System (see Supporting information), we built a model to study the ecosystem dynamics in an area subdivided into five zones and four peripheral zones surrounding the main study area (Fig. 1). PDP models are computational methods that are analogous to the machinery of cells (Colomer et al., 2013). The cells of the model correspond to the physical space of the environment. Animals (which along with things such as resources, are represented by model 'objects') will feed, reproduce, develop, etc. within an environment which is accounted for by a set of mathematical rules describing these behaviours in the model (Colomer et al., 2011). The application of PDP models constitute an effective computational tool to model a complex problem, because these bioinspired models are characterized by the ability to work in parallel (simultaneously interrelating different processes, for example combining demographic parameters with energetic requirements), being modular and with a high computational efficiency.

The subdivision of the study area has been based on climatic, topographic, landscape and ecological criteria (see Elósegui and Pérez Ollo, 1982). This area of 10,391 km^2 is inhabited by three avian scavenger species: seven bearded vulture pairs, 129 Egyptian vulture pairs, and 2798 Eurasian griffon vulture pairs.

Regarding carrion provided by wild species, we considered the Pyrenean chamois (*Rupicapra pyrenaica*), the red deer (*Cervus elaphus*), the roe deer (*Capreolus capreolus*) and the wild boar (*Sus scrofa*). Carrion provided in the study area by domestic ungulates, mainly comprises sheep (*Ovis aries*), cows (*Bos taurus*) and horses (*Equus caballus*), occurring either naturally after death (*in situ*) or artificially at the network of supplementary feeding sites (SFS) (Table 1, Table S1). The study area contains 10 SFS, where farmers and administrators provide carcasses and bone remains (Table S1). In addition, we added the contribution of alternative carrion from other species such as birds, small mammals, rodents, and lagomorphs (see Supporting information). These constitute a very important part of the diet of the Egyptian vulture (see Donázar, 1993; Margalida et al., 2012) and complement the trophic spectrum of the bearded vulture (Margalida et al., 2005; Margalida et al., 2009).

Table 1. Population of domestic and wild ungulates (individuals) in each zone in the study area. Non-transhumant animals are those that are not moved by farmers and remain in the same place year round. R.p. *Rupicapra pyrenaica*; C.e. *Cervus elaphus*; C.c. *Capreolus capreolus*; S.s. *Sus scrofa*; O.a. *Ovis aries*; B.t. *Bos taurus*; E.c. *Equus caballus*.

Zones	R. p.	C. e.	C. c.	S. s.	O. a.	B. t.	E. c.
NW							
No transhumants	0	153	4668	2564	130,429	14,893	7982
Summer	0	0	0	0	0	0	0
Breeding	0	0	0	0	10 800	700	975
MW							
No transhumants	0	0	1556	2177	38,651	1291	2662
Summer	0	0	0	0	15,800	1250	1520
Breeding	0	0	0	0	0	0	0
Pyrenees							
No transhumants	260	4132	3917	4456	71,099	8697	2660
Summer	0	0	0	0	28,000	0	0
Breeding	0	0	0	0	0	0	0
ME							
No transhumants	0	41	3908	5252	58,176	4005	1858
Summer	0	0	0	0	0	0	0
Breeding	0	0	0	0	3200	550	545
Ribera							
No transhumants	0	0	0	0	221,972	7588	5820
Summer	0	0	0	0	0	0	0
Breeding	0	0	0	0	20,800	0	0

The study area is characterized by husbandry related seasonal movements of livestock (transhumance). Two periods are defined annually according to the use of the grasslands and the variations in certain biological parameters throughout the year: ‘summer or non-reproductive period’ (hereafter *summer* – the months between June and September), and ‘winter or reproductive period’ (hereafter *breeding* – the period between October and May). Since livestock are man-managed, we considered their spatio-temporal distribution to be seasonally fixed and the model takes this effect of transhumance into account. Humans also partially manage the feeding of scavengers artificially by placing bones and meat at the SFS.

Some of these SFS are specifically targeted at the bearded vulture (for which lamb carrion is the only food offered), while others are more generic, in which any type of carcass is provided and the entire scavenging vulture guild can feed at them. In addition, some wild ungulates are hunted (some selectively for trophies), so that humans manage the number of animals killed through decisions based on the quarry population sizes or through the issue of hunting permits.

2.2. Annual energetic requirements

To estimate regular foraging ranges, we constructed circular areas around the nesting site based on the maximum distance that a bird will fly in a straight line from the nest in search of food: griffon vulture 90 km, bearded vulture 40 km, Egyptian vulture 15 km (for more details see Margalida and Colomer, 2012). We used an extension of the central place forager theory known as the foraging radius concept at which every individual is energetically constrained in terms of the spatial range they can cover while foraging (Sinclair and Norton-Griffiths, 1995). As central place foragers, breeding individuals must return to their breeding sites after they forage every day. The energetic requirements of the three avian scavengers according to food type (bones and meat) and period (summer vs breeding) were estimated following Donázar (1993). Eurasian griffon vultures need 404 kg/pair/year, Egyptian vultures 100 kg and bearded vultures 308 kg (Table S7).

In addition to including the natural and non-natural mortality rates of ungulates, the model assumes that an animal dies of starvation when it exceeds the carrying capacity of the habitat. In defining the model, a directed network table of avian scavenger movement was specified following the rationale given in Table S2. The model predicts that as a mean foraging range (Table S2), a species will move to a nearby zone if food resources become insufficient at its current location, and that it returns to the starting point (nesting area) if there are food limitations but no space (density) limitations. In this sense, we do not consider the large metabolic cost that result from the requirement to move greater distances. Accordingly, an individual colonizes a new area if insufficient space is available at its current location. Scavengers can choose between more than one available destination if they need to move, and the model assumes that they select one at random. If the new area selected also lacks resources, this random sampling continues until resources are found. If an individual cannot find sufficient resources after the process of random sampling, it will move to another area subject to the maximum density of each species in each zone. If space is not a limiting factor, it will return to its original location, or otherwise colonize a new area.

When feeding resources are insufficient in an avian scavenger's usual home range, birds move to the peripheral zones (A, F, E, R-CL) in search of food, assuming they can obtain the same food resources found in the neighboring areas (Table S3). The model assumes that floater individuals can obtain a part of the resources available in the study area. However, the model does not take into account the use of the resources by neighboring individuals, including obligate and facultative scavengers.

The model takes into account the fact that each species uses the resources closest to their nesting area first, and then widens the radius of search as these deplete. The amount of meat and bones consumed by scavengers depends on the season. Excess meat disappears from the ecosystem at the end of each period (breeding or summer). The model assumes that 20% of the unconsumed bones remain available in the ecosystem in spring, as a consequence of bone preservation (Margalida and Villalba, 2017). In addition, because not all carrion remains available due to its location (i.e., it lies in forested areas), we reduced the food actually available for scavengers by applying a correction factor (Tables S8, S9).

The bone remains of large bovine ungulates and equines are rarely consumed by bearded vultures (Margalida et al., 2009) and simply counting them would therefore overstate their importance, so we reduced their quantification by applying a factor according the size of the various bones (Table S9).

Population growth is restricted due to limitations on physical space imposed by each species foraging range and the food available to cover their energetic requirements. This informs the habitat carrying capacity used in the model (Table S4). Running the model requires input of initial parameters (such as reproduction, mortality and feeding), which are entered before generating the output. The model is first run for each individual and then again simultaneously for all individuals. Therefore, the system operates in parallel, allowing for competition when birds of the same or different species share resources. In this regard, the bearded and Egyptian vultures are the first to arrive at the carrion and/or to feed with respect to the griffon vulture (see Supporting information).

The values of the parameters used in the model were derived from published sources (for more details see Colomer et al., 2011; Margalida et al., 2011a, 2011b; Margalida and Colomer, 2012; see also Supporting information).

2.3. Food availability scenario

Three possible scenarios were studied to test the impact of different food availability regimes based on different livestock mortality rates, to examine their potential effects on population projections over time. The *Medium food availability scenario* represents the estimated food available in a normal year based on average domestic ungulate mortality rates (Table 2). The *High* scenario models the situation where available food increases relative to an average year (optimistic scenario). The *Low* scenario simulates an ecosystem where the food available is less than average (conservative scenario). The scavenger population trends were simulated on the basis of the demographic parameters typical of each species (Table S5) and the availability of biomass provided by the different domestic ungulate mortality scenarios, plus the biomass provided by wild ungulates and feeding stations (see Supporting information).

Table 2. Annual mortality of domestic ungulates according to the different food availability scenarios, age classes and the two temporal periods. Scenario variation indicates the annual mortality variation in the low and high scenarios regarding the medium scenario.

		Annual	Summer	Breeding	Scenario variation
Juveniles					
Low	*O. aries*	0.100	0.067	0.033	−0.05
	B. taurus	0.050	0.033	0.017	−0.01
	E. caballus	0.020	0.013	0.007	−0.01
Medium	*O. aries*	0.150	0.100	0.050	0
	B. taurus	0.060	0.040	0.020	0
	E. caballus	0.030	0.020	0.010	0
High	*O. aries*	0.200	0.133	0.067	0.05
	B. taurus	0.070	0.042	0.028	0.01
	E. caballus	0.040	0.040	0	0.01
Adults					
Low	*O. aries*	0.020	0.013	0.007	−0.01
	B. taurus	0.040	0.024	0.016	−0.01
	E. caballus	0.009	0.009	0.000	−0.01
Medium	*O. aries*	0.030	0.02	0.01	0
	B. taurus	0.050	0.03	0.02	0
	E. caballus	0.010	0.01	0	0
High	*O. aries*	0.040	0.027	0.013	0.1
	B. taurus	0.060	0.036	0.024	0.1
	E. caballus	0.020	0.020	0	0.1

2.4. PDP model

We used PDP models to build the ecosystem model. These are probabilistic computational models inspired by studies of cell function, and can perform a high number of simultaneous and perfectly-synchronized processes. These models resemble multi-agent models, although they have some special characteristics which enable them to model complex processes (see Colomer et al., 2013; Colomer et al., 2014). The integrated data on food availability, food requirements and population dynamics of the avian scavenging guild and the ungulate populations of the study area (Fig. 1 and Supporting Information) try to determine if carcass availability could meet the demands of the avian scavenger population over a 20-year period. The conceptual bases of the models are given in Fig. 2.

The parameters used are defined in Table S10 and the model is thoroughly described in the Supporting information. The model was executed using MeCoSim (a free software under license) developed by the Computation Group at the University of Sevilla (GNU GPL; http:// www.p-lingua.org).

3. Results

3.1. Food availability

The availability of animal biomass (meat and bones) was estimated for the three scenarios considered (low, medium and high availability of food resources), during two periods (summer and breeding). In all scenarios, the meat and bone biomass available to scavenging birds was higher in summer than during breeding (low-meat: 787,823 vs 325,854 kg; low-bones: 144,448 vs 34,377 kg; medium-meat: 1,005,630 vs 416,021 kg; medium-bones: 195,563 vs 46,024 kg; high-meat: 1,339,084 vs 585,049 kg; high-bones: 247,220 vs 58,292 kg), with sheep providing the most domestic meat and bone biomass in all the scenarios considered (meat range: 34.80%–42.11%; bones range: 65.40%–77.39%). During the breeding period, the sheep is also the domestic ungulate that provides the most amount of meat and bone biomass (meat range: 34.57%–39.96%; bones range: 60.35%–74.05%) (Fig. 3).

Regarding the wild species, wild boar and red deer provided in a similar way the highest amount of meat biomass (summer meat range: 9.87%–5.80% and 7.76%–4.61% for wild boar and red deer respectively; breeding meat range: 6.09%–3.38% and 5.91%–3.27% for wild boar and deer respectively) while deer provided the most amount of bones biomass (summer bones range: 13.70%–8.01%; breeding bones range: 17.19%–10.14%) (Fig. 3).

3.2 Temporal availability of food resources to cover energetic requirements under different scenarios

Comparing the total meat trophic resources available (green bars) with the total energy requirements of the avian scavenger species (red bars), the food available is substantially greater than requirements in the summer, in the medium and high scenarios (Fig. 4). On the contrary, during breeding in all three scenarios, the food available is insufficient to cover the energy requirements of the scavenging species assemblage.

When the total bone trophic availability (green column) is compared with the energy requirement (red column), the availability of bones is clearly much higher than needed to cover the energy requirements of the breeding bearded vulture population, even in the low food availability scenario (Fig. 5).

3.3 Population trends in the Eurasian griffon, bearded and Egyptian vultures according to the carrying capacity

In the case of the griffon vulture, the model forecasts significant differences among scenarios ($F_{2,\ 60} = 19.45$, $P < 0.0001$). The differences were found between the low and the rest of scenarios. In the high and medium food availability scenario the population grows and stabilizes at 3900 pairs after 20 years, whereas in the low food availability scenarios the trend is different. In this scenario, the model predicts that the population stabilizes at 3500 pairs after 20 years (Fig. 6).

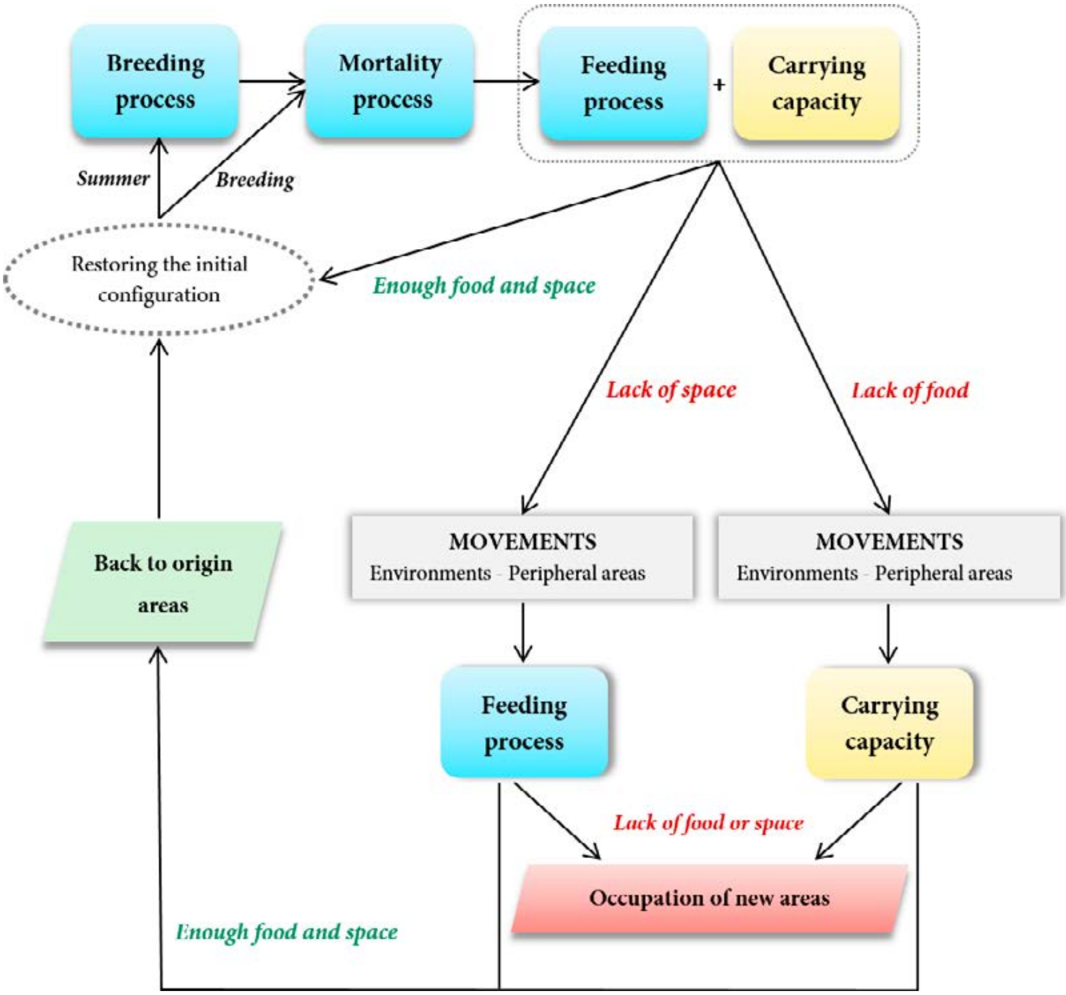


Figure 2. Conceptual basis and sequencing of the processes considered in the PDP model. The model takes into account two periods (summer and breeding) and the processes of reproduction, mortality, feeding and the carrying capacity. When food is insufficient in the foraging area, the scavenger birds forage in peripheral areas. If they find food, they return to their nesting site. On the contrary, when food is also insufficient in peripheral areas, the individual disappear from the study area. Two executions of a loop are equivalent to the passage of one year in the ecosystem.

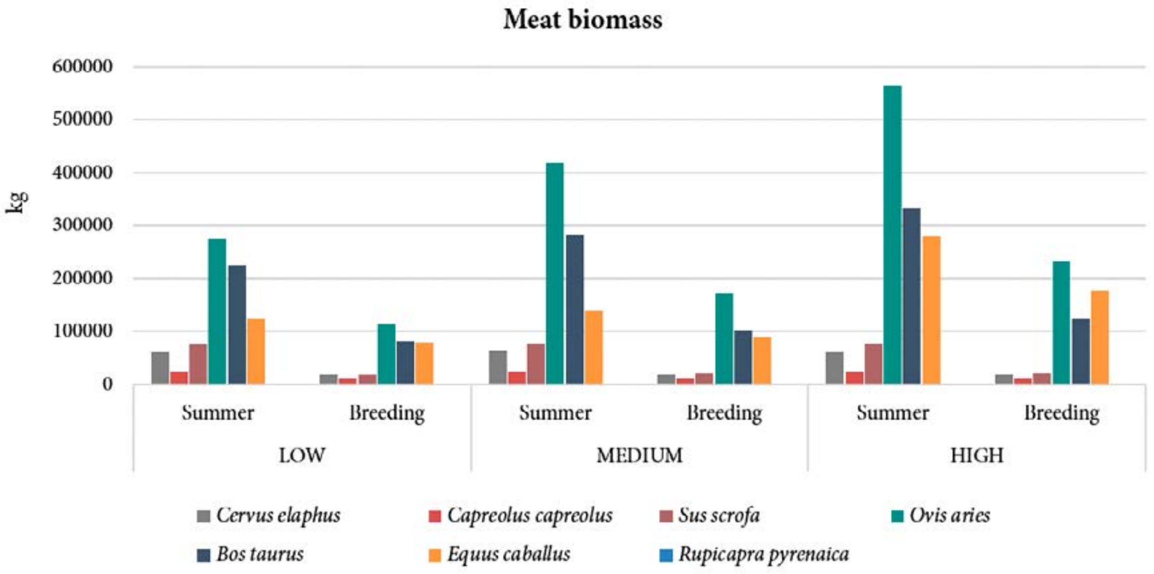
Regarding the bearded vulture, the results suggest oscillations between the current seven pairs and a maximum of 8, stabilizing over the following 20 years, with no significant differences between years ($F_{2,60}$ = 1.126, P = 0.331, Fig. 6).

Considering the Egyptian vulture, the results show a positive population trend in all three scenarios with no significant differences between years ($F_{2,60}$ = 0.149, P = 0.862, Fig. 6). The model predicts an increase of c. 11 pairs in the first six years, before rising later and stabilizing at 143 pairs after 20 years, in all three scenarios with no significant differences between them.

4. Discussion

The availability of resources limits the population size of an animal species, and sets the carrying capacity of an area (Hanski et al., 1993; Turchin, 2001). In the case of avian scavengers, the availability of food provided by wild ungulate carcasses has gradually decreased as a result of their replacement by domestic ungulates (Lambertucci et al., 2009; Margalida et al., 2011a, 2011b; Ogada et al., 2012a, 2012b). Livestock is man-managed and this makes it easy to obtain accurate data on numbers of animals and their demographic parameters, as well as their spatio-temporal distribution and its effect on the amount of food that they provide for scavengers. This quality of information makes it possible to assess the precise carrying capacity of an environment and to forecast scavenger population trends based on estimates of food availability. Modeling this information can help managers and policy-makers to make decisions regarding reintroduction projects, conservation measures, and to assess the impact of policy decisions regarding health and sanitation regulations on scavenger population dynamics (Sarrazin and Legendre, 2000; Hirzel et al., 2004; Margalida and Colomer, 2012). As this study shows, it is imperative to have good datasets in order to model population trends or assess carrying capacity because the sensitivity of some demographic parameters can have a significant impact on the results obtained.

As we show, even a 1–2% change in livestock mortality can substantially modify the assessment of carrion available and the effects on scavenger population dynamics. For example, with respect to the differences between the low vs medium food availability scenario, in the case of the griffon vulture the model forecasts a difference of 384 pairs after 20 years (Fig. 6). Therefore, even small errors in livestock mortality estimates could lead to serious mistakes in management measures, with important conservation repercussions. However, it is important to remark that predictions were only different significantly among the three scenarios for the griffon vulture but not for Egyptian and bearded vultures.



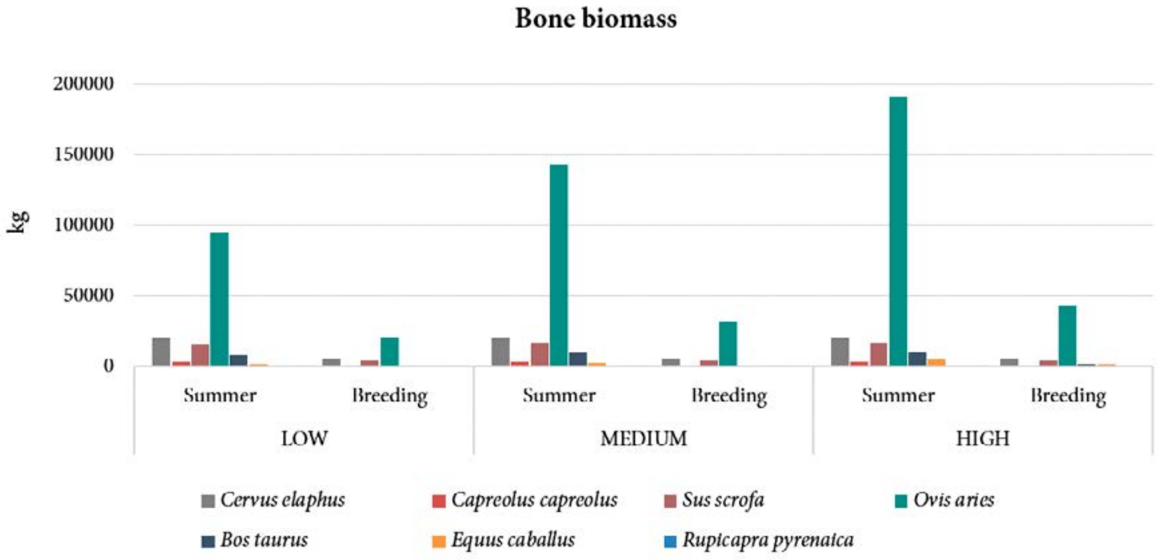


Figure 3. Food availability (meat and bone biomass) provided by the different domestic and wild ungulates in the study area.

We show that asymmetries exist in the availability of food resources during the year, emphasizing the importance of estimating food availability over the full annual cycle (Marra et al., 2015; Zupo et al., 2017). According to our results, although the overall annual availability of carrion for the scavenger populations studied is enough to cover their energetic requirements, when we separate the breeding from the summer period (when transhumance occurs) the results suggest that seasonal food shortages do exist in our study area (Fig. 4), at least for the most abundant species, the griffon vulture. An additional issue which suggests that results are conservative is that some facultative scavengers and breeding pairs inhabiting outside the study area can take advantage of the carrion present (Moreno-Opo et al., 2016). The quantification of the impact of biomass consumption by these facultative carrion eaters (birds and mammals) is difficult and will require future approximations to improve the models. However, the progressive increase in the griffon vulture population size (from 312 pairs in 1979 to 2783 in 2009, Del Moral, 2009), suggests that an important proportion of their food is obtained from zones peripheral to the core area, or even from more distant areas, possible because of the high mobility of these species (Monsarrat et al., 2013). Therefore, as has been shown for other large birds of prey, home ranges vary according to prey density and individual reproductive status, with habitat quality serving to regulate their use of space (Fernández et al., 2009; Pérez-García et al., 2013). Spatial scales are therefore important to assess correctly available feeding resources and to understand the relevance of transboundary agreements between regional administrations to develop, coordinate and apply conservation measures for species with extensive foraging areas (Margalida et al., 2013; Lambertucci et al., 2014; Margalida et al., 2016; Morales-Reyes et al., 2017; Arrondo et al., 2018). This is the case of our study area that, as the results show, do not provides enough resources to cover the energetic requirements of the griffon vulture population being dependent of the availability and management of food in neighbouring areas. Accordingly, species with large foraging areas, like griffon vultures, are difficult to implement on computational models as a consequence of the use of alternative food resources from far-away areas. As a result, the coordination between different Spanish administrations and countries (France and Portugal) regulating health policies are necessary.

From a temporal perspective, we show that the availability of carrion is substantially higher in summer because of the increased numbers of livestock in mountain pastures. Mountain areas are the main zones which benefit from livestock seasonal movements, as occurs in the Pyrenean and MW regions. Sheep provide most of the carrion biomass, and constitute 50% of the food available. Therefore, transhumance, mainly of sheep, is important in increasing food availability in mountain ecosystems and this practice plays an important role in the conservation of avian scavengers and other wild species (Olea and Mateo-Tomás, 2009; Bernués et al., 2011; López-Santiago et al., 2014; Tyrrell et al., 2017). However, transhumance practice is suffering a progressive decline (Olea and Mateo-Tomás, 2009) that can have important consequences for biodiversity conservation (Mateo-Tomás and Olea, 2010, Carmona et al., 2013; Oteros-Rozas et al., 2014). Therefore, any conservation measure which facilitates extensive sheep husbandry should be a priority from a conservation point of view. However, this is compromised by the fact that the critical energetic shortfall for this avian scavenger guild occurs during the breeding period (winter–spring). At this time, the constraints of breeding limit the foraging movements at a time of reduced hours of daylight, adverse weather conditions, and the increased energetic requirements due to feeding chicks. We show that evident food shortages exist during the breeding period. The network of special feeding sites for avian scavengers (ZPAEN) recently established by Spanish administrations (Morales-Reyes et al., 2017) therefore play an important role for breeding birds inhabiting regions with limited food resources, or which are affected by sanitary regulations which remove carcasses from the landscape (Donázar et al., 2009a, 2009b). From a management perspective, the location of SFS should be related to the spatio-temporal distribution of natural resources and the avian scavenger population. Only by taking these into account can managers optimize the value of SFS, always considering the controversial pros and cons of this widespread conservation management tool (Moreno-Opo et al., 2015; Cortés-Avizanda et al., 2016).

Figure 4. Temporal availability of food resources (green column) compared with the energetic requirements of the scavenging species assemblage (red column) in the three trophic availability scenarios: a) low, b) medium and c) high.

Given that all the scenarios modelled show a deficit of food resources in the breeding season, our results suggest that a high proportion of the trophic resources available to the nesting population in the study area are obtained from: i) peripheral zones outside the study area; and/or ii) the exploitation of other sources of food not considered in this study (e.g., landfills, intensive farms, e.g., Plaza and Lambertucci, 2017, Tauler-Ametller et al., 2017). The first explanation can be confirmed by satellite tracking results obtained from several breeding individuals that exploited resources located far from nesting sites, such as certain areas in Extremadura, located 600 km from their breeding colony (C. Fernández, unpubl. rep.). Regarding the second explanation, griffon vultures have been observed exploiting other resources such as garbage dumps following food shortages (Donázar et al., 2010; Plaza and Lambertucci, 2017). Therefore, models which assess food resource availability should consider larger spatial areas and every possible source of scavenger food. In contrast, specialized and less abundant species such as the bearded or Egyptian vulture do not seem limited by food resources. This is probably due to the small size of their breeding populations and the diet plasticity of both species, suggesting that trophic availability is not a limiting factor either for the establishment of new territories or the geographic expansion of these species. This agrees with previous studies (Margalida and Colomer, 2012; Margalida et al., 2017) suggesting that, quantitatively, food is not a limiting factor for Egyptian and bearded vultures. The results suggest that the available food is still substantially more than is needed by the breeding birds. For example, in the case of bearded vultures, while we included the smaller items of carrion from horses and cows, these are only rarely selected by this species (Margalida et al., 2009), and even considering only sheep remains, the potential food available each year in a medium food availability scenario is 175,000 kg, sufficient to sustain 568 bearded vulture breeding pairs. Therefore, the geographical expansion of bearded vultures does not seem to be limited by food resources, and limiting factors are more likely to be other aspects of habitat quality (e.g., disturbance, habitat modification), non-natural mortality factors (i.e., illegal poisoning) and the potential overcrowding with conspecifics attracted to the supplementary feeding sites established in the Pyrenees (Carrete et al., 2006; Margalida et al., 2009; Margalida et al., 2017).

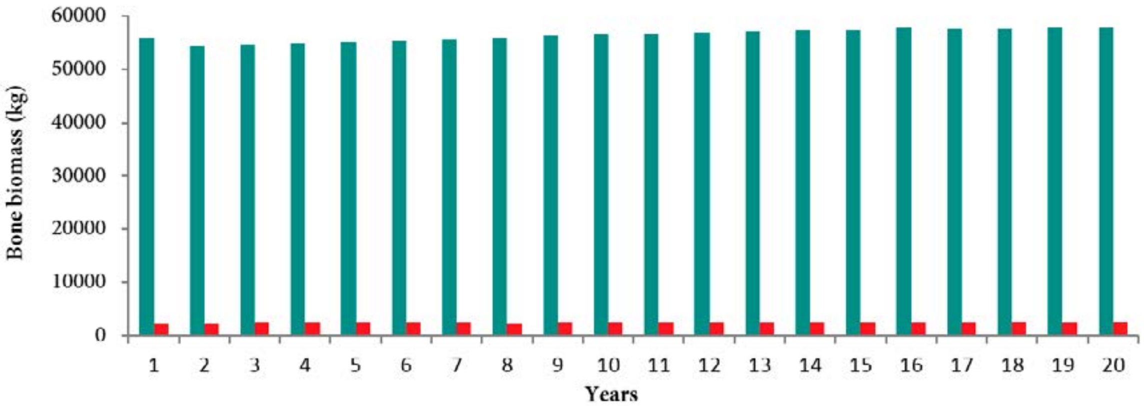


Figure 5. Bone biomass available (green column) in the study area with respect to the energetic requirements necessary (red column) for the breeding bearded vulture population in a low trophic availability scenario.

Among the 23 Old World vulture species, 81% are globally threatened or near threatened and most of these species are declining, particularly in Africa and Asia, as a consequence of anthropogenic activities such as the illegal use of poisons, landscape transformation, health policies and ingestion of toxic veterinary drugs (Ogada et al., 2012a, 2012b). These threats persist and continue to increase, despite the fact that vultures provide important ecosystem services (Moleón et al., 2014). Regarding the projected population estimates, changes in spatial dynamics and distribution of the different species will be governed by factors such as: i) the maximum carrying capacity of the region (density); ii) the availability of suitable nesting sites; iii) the availability of food; and iv) to a lesser extent, longer distance movements of individual birds. Data regarding the increase in the breeding population of the Spanish griffon vulture suggests that there is no density-dependent regulation of numbers, since the population grew more steadily in the more densely occupied provinces (Parra and Tellería, 2004). However, this large-scale result does not preclude the possibility of local regulatory processes in more densely occupied zones, where decreased breeding success has been observed (see Fernández et al., 1996). In the case of the bearded vulture, its population dynamics will depend fundamentally on management measures carried out in the rest of the Pyrenean range. During the last 20 years, there has been hardly any geographic expansion and this is attributable to the effect that supplementary feeding points have in attracting this species (Margalida et al., 2013). The large concentrations of individuals at these sites probably reduce geographic expansion of this species westwards, as evidenced by the lack of movement of pre-adult individuals beyond these areas (Margalida et al., 2013; Margalida et al., 2016). There is an abundant trophic supply and limiting factors could be the quality of available nesting sites and the population density (Donázar et al., 1993; Margalida et al., 2009). With respect to griffon vultures, following an exponential growth in numbers during the last 30 years (Del Moral, 2009), the indications are of reduced growth leading to a possible stabilization of the population. Recent changes in sanitary policies have modified the behaviour and diet of this species (Donázar et al., 2009a, 2009b, 2010; Margalida et al., 2011a, 2011b) and probably affected demographic parameters in similar ways to those noted for bearded vultures (Margalida et al., 2014). Finally, with respect to Egyptian vultures, the models suggest a population increase because its dietary plasticity allows it to utilize a wide spectrum of different prey. Consequently, the primary factor limiting the population viability of this species appears to be illegal poisoning (Hernández and Margalida, 2009; Ogada, 2014; Sanz-Aguilar et al., 2015).

Because each region/country may make independent decisions and work according to their own specific interests and conservation policies, approaches based on large spatial scales are essential to generate effective conservation measures based on transboundary approaches (Bischof et al., 2015). This approach is also required for avian scavengers where management centers on the provision of SFS or "vulture restaurants" at which surplus resources modify the quality of a habitat and provide predictable food resources that might affect spatial distribution and breeding density. Accordingly, the economic costs of providing SFS and their effects on ecological processes (see Donázar et al., 2009a, 2009b; Cortés-Avizanda et al., 2010; Dupont et al., 2012; Cortés-Avizanda et al., 2016) should force managers and policy-makers to assess the natural food provided by the ecosystem and to carefully evaluate the usefulness of supplementary feeding sites (Kane et al., 2015). The identification of optimal areas (i.e., those with abundant food resources and nesting-sites) could provide conservation tools to identify priority areas for reintroduction projects. It is important to consider large spatial scales in order to manage species with large foraging areas and to apply the correct management and conservation measures.

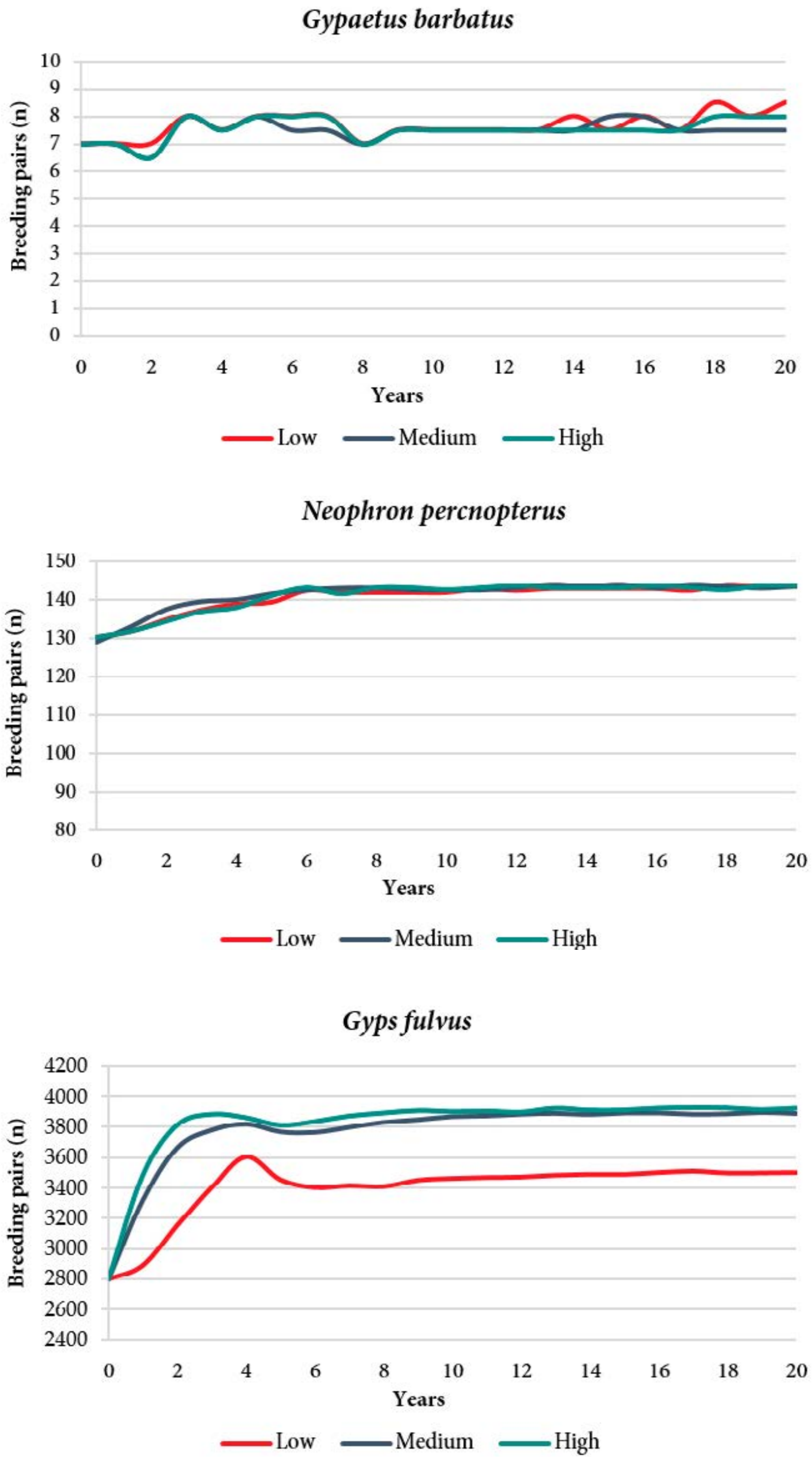


Figure 6. Predicted population trends for the three avian scavengers in the study area, for each of the three scenarios tested (low, medium and high), expressed as the percentage of domestic and wild ungulate carcasses available in the ecosystem. Note the different y-axis scales.

Pros and cons of computational models in ecology

Bioinspired models, such as PDP systems that work in parallel, are more flexible and enable the consideration of the heterogeneity of the population and the environment. These models allows to capture the randomness of the natural environmental processes using stochastic strategies based on Gillespie's kinetics (Gillespie, 1976) and the semantics defined by using probabilistic functions (Colomer et al., 2011; Colomer et al., 2013). However, modeling complex systems in which several environments and species interact competing for resources requires experienced researchers familiarized with these models. Although their complexity could limit the use of this tool, PDP systems allows modelling of demographic parameters with regard to food resources, and provides an effective tool in conjunction with other considerations (Colomer et al., 2013). PDP models are a complementary approach to be used when the classical modeling approaches fail (Colomer et al., 2011), and can aid in conservation planning for species of concern where available trophic spectra can be assessed objectively, and should be used to combine trophic resource measurements with demographic parameters to improve the effectiveness of conservation management. However, as a result of the limitations in computational models, replication of the models seems necessary to increase credibility and efficiency to facilitate theory development (Thiele and Grimm, 2015) and, as occurs in the case of threatened species, to optimize management and conservation actions.

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Bearded vultures *Gypaetus barbatus* in the Huesca Pyrenees, Aragón (Spain)
Photo: Pilar Oliva-Vidal

CHAPTER 5

PRIORITIZING AMONG REMOVAL SCENARIOS FOR THE REINTRODUCTION OF ENDANGERED SPECIES: INSIGHTS FROM BEARDED VULTURE SIMULATION MODELING

This chapter corresponds to the article:

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CHAPTER 5

Prioritizing among removal scenarios for the reintroduction of endangered species: insights from bearded vulture simulation modeling

Abstract

Translocations are an increasing feature of threatened species conservation plans, but the impact of removal of individuals on the source population is seldom studied. Using computational Population Dynamics P System models and the Pyrenean bearded vulture population as a case study we looked at: the effect on the source population of alternative strategies for removal of individual birds for use in reintroduction projects; and the trade-offs between the various management options. According to our models (over a 30 year prediction horizon) the removal of one clutch, juvenile or non-territorial adult each year over an 11 year period, results in an annual loss of 1.57, 3.71 and 0.97 territories, respectively. We forecast the impact of a plausible removal scenario for the Pyrenees source population (the removal of five clutches and five non-territorial adults each year over 11 years), leading to a predicted loss of 16 breeding territories. Nevertheless, changes in demographic parameters, mainly in productivity and adult survival, could substantially affect these predicted results. With the current demographic parameters, the removal scenarios that were estimated to not affect population size after 30 years (95% CI) are limited to: (1) the removal of five clutches and five non-territorial adults during a single year; (2) the annual removal of five non-territorial adults during a 6 year period; and (3) the annual removal of five clutches during a 6 year period. Our results suggest that removals from the Pyrenean bearded vulture source population should be performed with caution due to uncertainties arising from stochastic changes in survival and productivity.

Keywords: adaptive management, demographic effects, long-lived species, population viability, reintroductions, vultures, simulation modeling, translocations.

1. Introduction

The search for innovative and effective methods to improve and optimize species conservation programs is a key issue for managers, conservationists and policy-makers responding to biodiversity loss. The translocation of individuals to formerly occupied habitats is a widespread and increasingly used tool in conservation programs to restore wild populations of endangered species (Seddon et al., 2007, 2014; Pérez et al., 2012; Brichieri-Colombi and Moehrenschlager, 2016; Swan et al., 2018). Because the economic and time costs of population replenishment and reintroduction projects can be limiting factors (Smith et al., 2011), reliable assessments of the effectiveness of various management options are essential in choosing the best course of action (McCarthy et al., 2012). In the case of threatened species, population size of donor population can be an additional limiting factor. As a result, researchers and practitioners need to evaluate the likely trade-offs among the ex situ (e.g., captive breeding) and in situ (e.g., threat management) options available to improve reintroduction outcomes (Dolman et al., 2015).

There are many studies of translocation projects and the success of reintroductions, including settlement, survival and reproduction of translocated individuals and their effects on the viability of the reintroduced population (e.g., Sarrazin and Legendre, 2000; Armstrong and Seddon, 2008; Le Gouar et al., 2008; Mihoub et al, 2013; Robert et al., 2015; Bertolero et al., 2018). However, there is less information regarding the impacts of translocations on the source or donor population (see McCleery et al., 2014; Margalida et al., 2015). This is especially relevant when translocated individuals are extracted from endangered populations, as in the threatened bearded vulture *Gypaetus barbatus*.

Bearded vulture reintroduction projects have been carried out in several European countries since the mid-1980s to counter the population declines and local extinctions of this species during the 19th and the beginning of the 20th centuries. The ambitious and successful reintroduction project conducted in the Alps is the most noteworthy (Schaub et al., 2009). Birds have been bred in captivity and subsequently released not only in the Alps (France, Italy, Austria, Switzerland), but also in the Massif Central (France), Andalucia (Spain) and elsewhere, specifically in Corsica (5 pairs) and Crete (5 pairs) to reintroduce the species or to reinforce threatened subpopulations.

The Pyrenees (Spain, France and Andorre), with 164 breeding territories, holds the most important European population of bearded vultures. There are currently three active bearded vulture reintroduction projects in Spain: Andalusia (started in 2006), Asturias (Picos de Europa, started in 2012) and, most recently, Maestrazgo (Castellón, started in 2018). To date, captive bred individuals have been used in the Andalusia and Maestrazgo projects (European Endangered Species Programme) while the Asturias project has used birds raised from eggs taken from clutches removed from nests in the Aragonese Pyrenees.

There is also a new proposal for an experimental reintroduction project involving the translocation and release of non-territorial adults taken from the Pyrenean population. To date, two non-territorial Pyrenean adults have been captured and released in November 2018 as part of the Maestrazgo project to reinforce the hacking of captive reared birds, but one returned to the Pyrenees after 6 days (authors unpubl. data).

Because the size of the Pyrenean bearded vulture population is regulated by a density-dependent effect on fecundity and its geographic expansion is slow (Carrete et al., 2006; Margalida et al., 2008) managers and policy-makers have proposed that more individuals be extracted from the non-territorial population to strengthen and/or reintroduce new populations elsewhere. It is therefore imperative to assess the effects of translocation on the population dynamics of the source population to establish future guidelines for reintroduction or population reinforcement projects. So far, the only study to forecast the impact of different translocation management scenarios (removal of eggs, chicks or fledglings) on Spanish bearded vulture populations (Margalida et al., 2015) was based on only a part of the Pyrenean population (Spanish Pyrenees) and used demographic records up to 2006 only (see Oro et al., 2008). Any quantitative study of the entire Pyrenean chain (Spain, France and Andorra) on the effects of different extraction scenarios on the source population dynamics will ideally use computational Population Dynamics P System (PDP) models: an important advance in estimating the population dynamics of threatened species in relation to climatic, energetic (trophic availability) or anthropogenic variables that provides a more rigorous and objective alternative to traditional models of population viability (Colomer et al., 2011; Margalida and Colomer, 2012; Margalida et al., 2018).

We applied PDP models to estimate the effects of different management actions on the bearded vulture population trend using data from the long-term monitoring of the entire Pyrenean bearded vulture population (1987–2016) and updated demographic parameters for the whole Pyrenean chain (Margarida et al., in prep). Specifically, we modelled the population impact (number of breeding territories) over a 30-year prediction horizon of different extraction scenarios for reintroduction purposes: removal of clutches; first year juveniles; and non-territorial adults >10–20 years old. In addition, we modelled the effects of changes in demographic parameters on the simulation forecasts. Our goal was to provide accurate information for managers and policymakers in order to establish conservation guidelines for translocation management, which would not compromise the source Pyrenean bearded vulture population.

2. Materials and methods

2.1. Study species

The bearded vulture is the most threatened European vulture species. They can breed from 6 years of age, although most start breeding at between 9 and 12 years old (Antor et al., 2007; López-López et al., 2013; Margalida et al., unpubl. data). One or two eggs are laid per nest but only one chick ever fledges as a result of siblicide (Margalida et al., 2004). The chick-rearing period is about 121 days (Margalida et al., 2003) and after fledging the young enter a dispersal phase until they settle as territorial adults at between 5 and 7 years old (Antor et al., 2007).

In the Pyrenean study area, breeding occurs in about 60% of territories each year (each occupied either by a pair or a polyandrous trio) and the annual productivity ranges between 0.30 and 0.40 chicks/pair/year (Margalida et al., 2014). However, this population is regulated according to the site-dependency hypothesis: as the population increases, average productivity decreases as progressively poorer quality territories are used. In addition, productivity is negatively correlated to the proximity to the nearest conspecific breeding pair (due to crowding) and to the proximity to the nearest supplementary feeding point where floaters congregate (Carrete et al., 2006).

2.2. Population modeling

Our methodology first estimated the population parameters based on historical records. Next, a PDP model was constructed to perform 'virtual experiments' to evaluate the effect of removals of clutches and floating individuals of different ages. In order to study the effects of various life history parameters and extractions on the population trends, we used a surface response model (Box-Behnken). We combined the extreme range values of some demographic parameters (productivity and juvenile, subadult and adult survival) in a second Box-Behnken model to assess the potential effects of changes in these parameters on the population's dynamics.

2.3. Data collection and parameter estimates

The Pyrenean (Spain and France) bearded vulture population has been intensively monitored since the 1980s, including field surveys to monitor population trends, breeding parameters, and survival rates (including a specific capture-mark-resighting sub-programme) (Oro et al., 2008; Margalida et al., 2014, 2015). Between 1987 and 2016, all known breeding Pyrenean territories were visited several times each month to record reproductive parameters. Productivity (number of fledglings per pair/trio per year) and breeding success (number of fledglings per territory with egg-laying) were estimated (Margalida et al., 2014). The ranges of these observed parameters were used to populate our models, assuming an even sex ratio at birth (Bretagnolle et al., 2004). From 1987 to 2016, a total of 151 individuals of known age were identified using rings and wing-marks and were radio- or satellite-tracked in the study area. This has allowed the estimation of demographic parameters such as age of first reproduction, survival and age structure using Integrated Population models (IPM) which integrate capture and recapture methods and counts in a multistate Bayesian approach (Oro et al., 2008; Margalida et al., 2014, 2015; Margalida et al., unpubl. data). We distinguished three age classes according to the most parsimonious age-model obtained in previous analyses (Oro et al., 2008; Margalida et al., 2014): juveniles (1–2 year old), subadults (3–5 years old) and adults (>6 years old). Given that productivity and survival rates have decreased over the years (Margalida et al., 2014), we used the range of values obtained during the last 5 years (2012–2016), gathered from various demographic studies (Table 1, Supporting Information Table S1). Maximum carrying capacity of the region has been estimated at 1000 breeding individuals (500 breeding territories) based on estimates of natural (i.e., without artificial feeding) food biomass availability, which is more than twice the current population size (Margalida and Colomer, 2012; Margalida et al., 2017a).

2.4. PDP model

Population Dynamic P Systems are computational models inspired by the functioning of cells that operate in parallel at both the individual and process levels, and are capable of modeling processes that interact with each other and animals that compete for resources of different types. The components of a PDP are: environments, membrane structure, initial work alphabet and evolution rules (Colomer et al., 2013). The number of environments is usually associated with the number of different zones in the ecosystem to be modelled (i.e., subpopulations inhabiting different regions). The initial alphabet is the input of the model (in our case the objects associated with each individual at the start of the model run, for example, population size, demographic parameters). The rules of evolution use some parameters (e.g., demographic parameters) and aim to describe the processes and process changes that each individual will undergo.

Here we use a PDP model related to the population's dynamics, the density-dependent reproductive process, and the possible effects of extraction of clutches, juveniles >1 year old (hereafter juveniles), and non-territorial adults of ≥10–20 years old (hereafter adults). The results of the model were compared with a baseline non-intervention scenario, based on the demographic rates considered. In the density-dependent model, we take into account the variations in fecundity according to the population size. Our model incorporated negative density-dependence effects on fecundity (Carrete et al., 2006), applying the equation used in *Vortex*:

$$P(N) = \left(P(0) - \left(P(0) - P(K)\right) \cdot \left(\frac{N}{K}\right)^{B}\right) \cdot \frac{N}{N + A}$$

where *P(N)* is the percentage of females that breed at population size *N*, *P(K)* is the percentage of females that breed when the population is at carrying capacity *(K)* and *P(0)* is the percentage of females that breed when the population is close to zero. In the extractions model, we subdivided the fecundity variables into subcategories (the percentage of pairs that lay eggs and both the hatching and reproductive success) to explore the population effects of the different intervention scenarios (clutches, juveniles and adults) on productivity and population size (breeding fraction).

Table 1. Values of demographic parameters obtained from our own empirical data (for details, see Methods) used to calculate the viability of the Pyrenean bearded vulture population.

Parameter	Value
End juvenile stage	2
End sub-adult stage	5
Life expectancy	30
Age first breeding attempt	6
Number of descendents	1
Productivity ratio low density[a]	0.45
Productivity ratio high density[a]	0.25
Maximum density (pairs)	500
Annual mortality age 1–2 year	0.066
Annual mortality age 3–5 year	0.035
Annual mortality age ≥ 6 year	0.036

[a] Values are taken from the high and low end of the productivity distribution that was estimated across all densities, and assigned to low- and high-density, respectively.

2.5. Description of the PDP model

The model takes into account all the items described previously such as: the population density and the processes of mortality; reproductive parameters; and the removal interventions (clutches or individuals of different age classes) to be performed (Fig. 1) each year. These items were sequenced in order to simplify the model, although this simplification does not affect the final results. The PDP defined is formed by three membranes with the following structure:

$$\mu = [[\quad]_1[\quad]_2]_0$$

(see Colomer et al., 2013). The main, or skin membrane (labelled 0), contains two inner membranes labelled 1 and 2, respectively. Most processes are carried out between the skin membrane and membrane 1. Membrane 2 serves to save the information on the removals that will be extracted from the ecosystem each year and to restore the initial configuration at the end of the cycle, to reset the model at beginning of the following year.

The objects that appear in the initial configuration in the membrane labeled by 0, that is, the model inputs, are as follows:

$$\mu_0 = \left\{XS_j^{qs_j}, 1 \le j \le g_3,\right\} \cup \left\{XP_j^{qp_j}, g_4 \le i \le g_3\right\} \cup \left\{XT_j^{qt_j}, g_4 \le i \le g_3\right\}$$

$$\cup\ \left\{NES^{Eg_1}, CHI^{Chi_1}, D, ANY_1, ANY'^{g_3}_1\right\} \cup \{b_i, 1 \le i \le g_3\}$$

A XS_j object is associated to each non-territorial animal of age j, while territorial animals are associated to a *XPj* or *XTj* object, depending on whether they form a pair or trio, respectively. qsj is the number of objects *XSj* (number of non-territorial individuals of age *j*), *qpj* the number of objects *XPj* (territorial individuals that form pairs of age *j)* and *qtj* the number of objects XT_j (territorial individuals that form trios of age *j*). The model allows the removal of clutches, chicks and floating individuals of different age-classes. In this study, we only considered the removal of clutches and non-territorial individuals (juveniles and adults). The number and type of extraction depend on the year. For each year, the quantity of *NES* objects that are generated equals the number of clutches that are extracted from nests that year. The number of *CHI* objects is the number of chicks to be removed from nests (not used in the current model). *D* is an object that generates a counter (R_1) in the first step, which allows control of the model and avoids inconsistency in the application of the rules. In addition, *D* generates other objects that allow control of the maximum carrying capacity in the study area. The object ANY_i stores the year that is being simulated in its index. Finally, the objects ANY'_i and b_j allow the generation of FLY_j objects, which are used to extract *i* non-territorial individuals of age *j* in the simulation.

The starting point is the census of animals of each age estimated through the IPM. The definitions of the parameters of the model are shown in Table 1. The model assumes that productivity varies according to the number of breeding territories as a result of density-dependent effects on fecundity (Carrete et al., 2006; Oro et al., 2008). The probability that an individual mates and reproduces depends on its age. The reproductive success is density-dependent and depends on the size of the population (Carrete et al., 2006). The probability of mortality depends on the age of an individual, and the probability of survival was estimated using observations during the last 5 years for the three age classes considered.

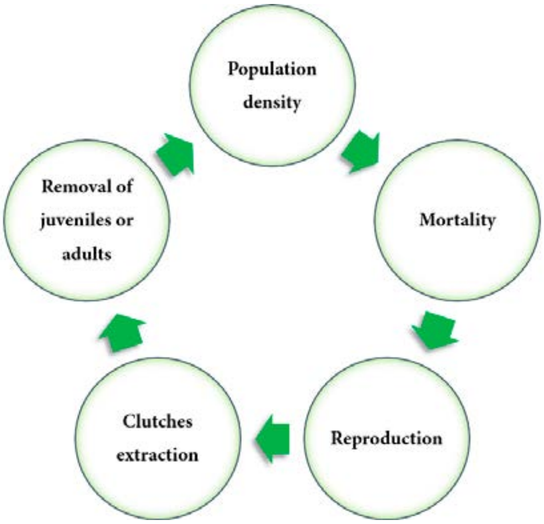

Figure 1. Representation of the modeling process carried out with the Population Dynamics P System. The input of the model is the population size at the start of the process (0) and the output is the population size in the year *t* using the parameters showed in Table 1.

2.6. Box-Behnken design

We used a response surface (number of breeding territories) approach to estimate the size of the bearded vulture population in the Pyrenees over a 30-year period, depending on the mean values of the parameters considered (Table 1) as well as a range (maximum and minimum) of demographic parameter values and removal timeframes (Table 2). The response surface designs are a subset of the experimental designs used to model the relationship between the independent variables or factors and the response variable (Box and Behnken, 1960), using linear models and quadratic or higher order models.

To study the effect of removals on bearded vulture population dynamics we constructed a Box-Behnken design using four factors: clutch extractions (range 0–10); removal of 1 year old juveniles (range 0–10); removal of non-territorial adults ≥10–20 years old (range 0–10) and the time of extractions (range 1–11 years). This results in a total of 28 experiments examining 25 different scenarios and four repetitions.

Since some demographic parameters, such as productivity and survival are subject to progressive decreases (in productivity) or annual fluctuations (in survival), we subsequently created another Box-Behnken design to consider the possible variations (Table 2). In this case, the number of experiments was 44 with a total of 41 different scenarios with four repetitions. For the design and the statistical analyses we used DoE.base, a package in the R program (R 3.5.2.) R Core Team, 2018.

Table 2. Range (minimum–maximum values) of demographic parameters and extraction timeframes used to calculate the viability of the Pyrenean bearded vulture population.

Factor	Low Level	High Level
Productivity	0.25	0.45
Juvenile mortality (age 1–2 year)	0.046	0.086
Subadult mortality (age 3–5 year)	0.015	0.055
Adult mortality (age ≥ 6 year)	0.015	0.055
Years extraction	5	11

3. Results

All of the four factors considered (clutches, juveniles, non-territorial adults and years) had a significant effect on the number of breeding territories (Fig. 2; Table 3). In addition, the interactions among clutches, juveniles and adults and the timing of extractions were also significant. The removal of one clutch, juvenile or non-territorial adult each year over an 11-year period, resulted in an annual loss of 1.57, 3.71 and 0.97 territories, respectively. Therefore, an annual extraction of five clutches lead to the loss of 10 breeding territories over 11 years, compared with 20 territories lost due to an annual extraction of five juveniles and seven territories lost due to an annual extraction of five adults. The annual extraction of five clutches and five adults over an 11-year period is a plausible scenario in the Pyrenees and would lead to the loss of 16 breeding territories after 30 years.

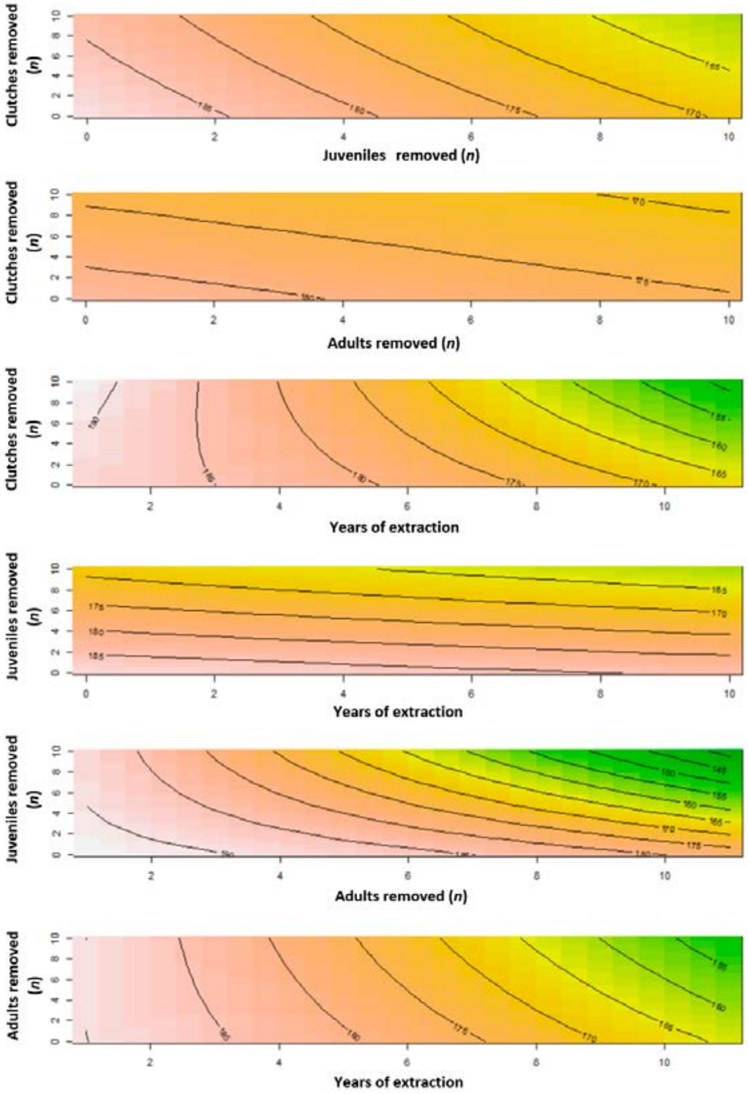

Figure 2. Response surface (number of breeding territories) obtained using the current estimates of the population demographic parameters. The colours represent the number of breeding territories: brown represents the higher values and green the lower ones. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.].

As shown in Fig. 3 and Table 4, there were nine removal scenarios that did not result in a significant change (95% CI) in the population trend over 30 years compared to the non-intervention scenario (Fig. 3). The only combinations lying inside this confidence interval were extractions performed during 1 year (7 scenarios) and 6 years (2 scenarios) (Table 4).

Juvenile refers to individuals 1-year-old and adults to non-territorial adults 10–20 years old. Statistically significant results are shown in bold type.

Table 3. Coefficient values of the response surface (number of breeding territories) and significance levels of the variables and interactions that were tested using mean demographic parameter values (Table 1).

	Estimate	SE	*t* value	*P*
Intercept	174.947	0.865	202.194	**0.0001**
Clutches	-3.792	0.499	-7.590	**0.0001**
Juveniles	-10.947	0.499	-21.915	**0.0001**
Adults	-3.138	0.499	-6.282	**0.0001**
Years	-16.181	0.499	-32.391	**0.0001**
Clutches x juveniles	-0.657	0.865	-0.760	0.461
Clutches x adults	0.585	0.865	0.676	0.511
Clutches x years	-5.452	0.865	-6.302	**0.0001**
Juveniles x adults	-0.735	0.865	-0.849	0.410
Juveniles x years	-9.025	0.865	-10.431	**0.0001**
Adults x years	-3.110	0.865	-3.594	**0.003**
Clutches ^{2}	0.299	0.706	0.423	0.679
Juveniles^2	0.640	0.706	0.906	0.381
Adults^2	0.156	0.706	0.221	0.828
Years^2	-1.250	0.706	-1.769	0.100

3.1. What would happen if the demographic parameters change?

Bearing in mind that productivity and survival rates may vary, another Box-Behnken design was constructed, evaluating the importance and effect of variations in these demographic parameters in combination with several types of extractions.

The results show that all four factors (productivity, juvenile survival, subadult survival and adult survival) had a significant effect on the number of breeding territories, with the most significant effects resulting from variations in productivity and adult survival rates (Fig. 4; Table 5). The duration of the intervention had no statistically significant effect due to the overwhelming effects of the other factors. According to our results, an increase in juvenile mortality (up to 0.086) resulted in a breeding population loss of seven breeding territories after 30 years. If an increase in mortality occurred in the subadult age-class (up to 0.055), the impact would be a loss of 20 breeding territories. Finally, in the case of adults (up to 0.055) 66 breeding territories would be lost. On the contrary, if the survival rates of all age-classes are maintained at the current levels (Table 1), but productivity falls (to 0.25 chicks/pair/year), 57 breeding territories would be lost after 30 years.

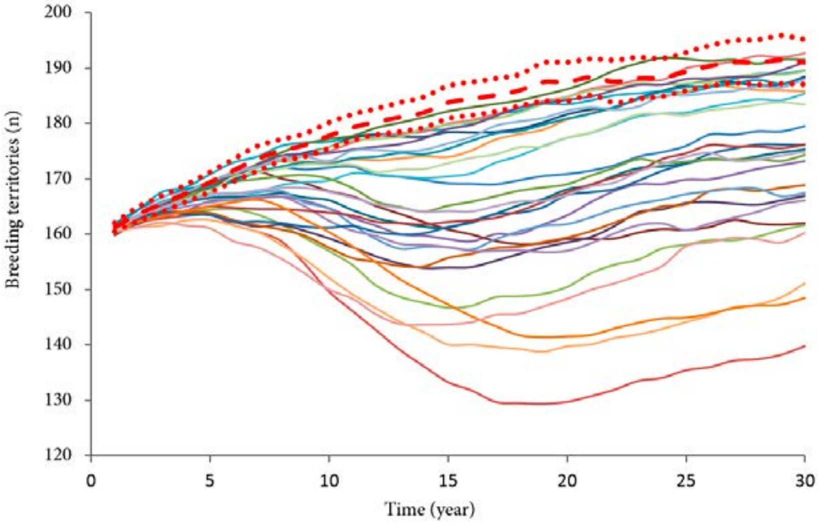

Figure 3. Projections of the number of bearded vulture territories in the Pyrenees over time as predicted under different combinations of extraction scenarios, using the currently estimated (mean values) for demographic parameters (Table 1). The red line shows the population trend without any intervention. The dashed line is the 95% CI. [Colour figure can be viewed at zslpublications.onlinelibrary. wiley.com.]

Table 4. Extraction scenarios that do not have a significant impact (95% CI) on the Pyrenean bearded vulture source population trend over a 30-year scenario.

Removal scenario			
Clutches	**Juveniles**	**Adults**	**Years**
5	0	5	1
0	5	5	1
10	5	5	1
5	5	0	1
5	5	10	1
0	0	5	6[a]
5	10	5	1
5	0	0	6[a]
0	5	5	1

[a] The five removals happen annually.

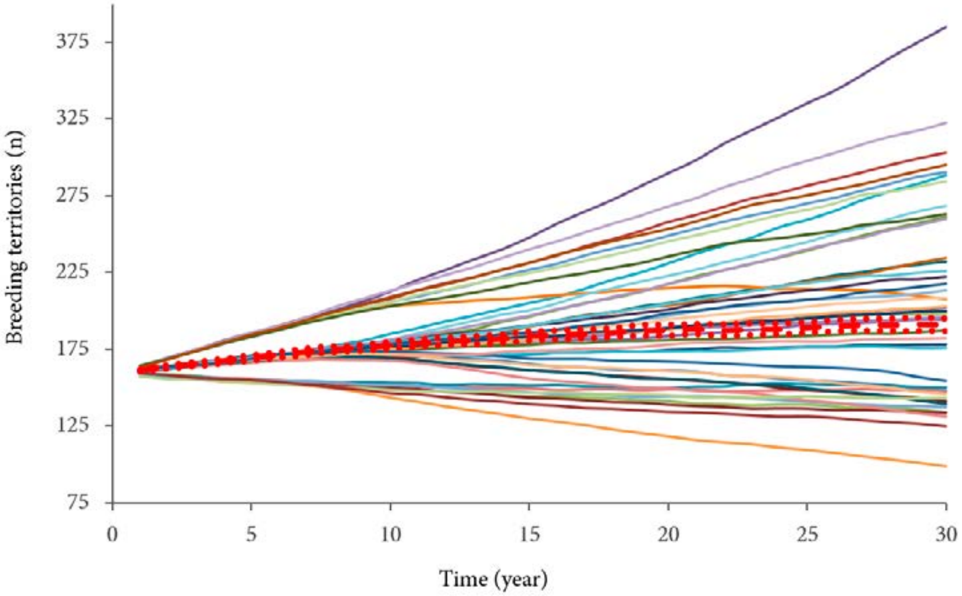

Figure 4. Projections of the number of bearded vulture territories in the Pyrenees over time as predicted under different combinations of demographic parameters and extraction scenarios, using the range (minimum–maximum) of demographic parameter values and extraction timeframes (Table 2). The red line shows the population trend without any intervention. The dashed line is the 95% CI. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com.]

4. Discussion

For threatened species, removal of individuals from the wild for reintroduction projects or to create a captive population could compromise the source population both due to the loss of the individuals extracted and because the population becomes more vulnerable to stochastic events (Margalida et al., 2015; Heinrichs et al., 2018, 2019). The simulations presented here offer sufficient evidence to assist managers and policy-makers to adopt the most rigorous and effective management measures for the restoration of European bearded vulture populations, without undue harm to the source population. These models, linked to various removal scenarios, could enable managers and policymakers to develop objective-based conservation trade-offs in current conservation projects to reinforce or reintroduce subpopulations of the species.

Until now, the Pyrenean bearded vulture population has been managed using two types of extractions: (1) the regular removal of clutches, started in 2008 and still ongoing (about five clutches are currently removed annually); and (2) the extraction of non-territorial adults, started in 2018 (when two adults were removed). To explore a broader spectrum of possible extraction scenarios we added a third option as an alternative: the extraction of trapped 1-year-old juveniles. This new simulated scenario could provide an alternative to reintroductions using fledglings derived from captive rearing projects, which are then hacked out into the wild.

With 164 breeding territories, the Pyrenees harbour the most important population of wild bearded vultures in Europe. This population is characterized by a progressive increase in breeding territories and, in parallel, a density-dependent decrease in productivity (Carrete et al., 2006). In addition, there has been an increase in the fraction of nonterritorial but potential breeders (i.e., mature floaters > 6 years of age), from 39% in 2006 to 68% in 2015 (Antor et al., 2007; Margalida et al., 2015). This situation prompted a technical-scientific debate regarding the need and opportunity to remove clutches and floating individuals for use in reintroduction programs (Ferrer et al., 2014; Margalida et al., 2015; Margalida et al., 2017b).

Table 5. Coefficient values of the response surface (number of breeding territories) and significance levels of the variables and interactions that were tested using a range (minimum–maximum) of demographic parameter values and extraction timeframes (Table 2).

	Estimate	**SE**	***t* value**	***P***
Intercept	200.170	2.479	80.731	**0.0001**
Productivity	56.946	1.240	45.934	**0.0001**
Juvenile mortality	-7.144	1.240	-5.762	**0.0001**
Subadult mortality	-19.869	1.240	-16.027	**0.0001**
Adult mortality	-80.053	1.240	-64.573	**0.0001**
Years	-2.071	1.240	-1.670	0.108
Productivity x juvenile mortality	-6.962	2.479	-2.808	**0.010**
Productivity x subadult mortality	-7.617	2.479	-3.072	**0.005**
Productivity x adult mortality	-32.075	2.479	-12.936	**0.0001**
Productivity x years	1.477	2.479	0.596	0.557
Juvenile mortality x subadult mortality	1.622	2.479	0.654	0.519
Juvenile mortality x adult mortality	2.315	2.479	0.934	0.360
Juvenile mortality x years	0.925	2.479	0.373	0.712
Subadult mortality x adult mortality	8.437	2.479	3.403	**0.002**
Subadult mortality x years	-1.030	2.479	-0.415	0.682
Adult mortality x years	0.340	2.479	0.137	0.892
Productivity^2	-1.203	1.825	-0.659	0.516
Juvenile mortality^2	-0.590	1.825	-0.323	0.749
Subadult mortality^2	1.202	1.825	0.659	0.516
Adult mortality^2	13.627	1.825	7.468	**0.0001**
Years^2	1.184	1.825	0.649	0.523

Statistically significant results are shown in bold type.

Our results show that of the three scenarios modeled, juvenile removal has twice the impact on the source population compared with the extraction of clutches or non-territorial adults. This is due to the fact that juveniles preserve intact their probabilities of beginning reproduction (from 6 years old onwards) whereas non-territorial adults (≥10 years), have lost part of their potential reproductive probabilities. However, reintroduction using juveniles is the principle management strategy used to reintroduce the species in Europe (i.e., hacking fledgling individuals from captive breeding) and is the most successful in many different European projects (see Schaub et al., 2009), although our results show that, in the wild, its impact on the source population is substantial. Therefore, from a conservation point of view, the extraction of clutches and non-territorial adults is preferable to the removal of juveniles. In the case of clutch removal, the extraction should be focused on low quality territories with low breeding success to reduce the demographic impact on the source population. In this sense, in future work it would be necessary to estimate the number of removals that could occur (Margalida et al., 2017b) without a negative impact if the removals were to be from these low quality territories (i.e., with low breeding success). This could potentially be an optimal management approach because releasing juveniles into the reintroduction site may be of benefit to the reintroduced population (i.e., potentially higher release site fidelity and high reproductive potential) with relatively low impact on the source population.

The next question is how many clutches and/or non-territorial adults could be removed from the Pyrenean population for use in reintroduction projects elsewhere in Europe without affecting the source population. There are several ways to view this question. First, from a conservation biology perspective, there is an argument that the Pyrenean population should be conserved through management programs and not subject to the removal of individuals. As such, reintroduction programs should focus on species recovery in existing reintroduction areas, but do not justify extractions from source nuclei. Expanding the species' range should prioritize alternative reintroduction methods such as captive breeding, management of supplementary feeding sites and so on and the removal of wild birds from existing populations should not be the first option. Second, we need to be cautious regarding the projections forecast here because some scenarios show no impact at the 95% CI (Table 4), indicating the pronounced influence of stochastic variations. The demographic parameters that we used in the models (survival and productivity) may be considered optimistic and their true values may well be lower (e.g., the falls in productivity resulting from density-dependent factors) and mortality values could increase as a result of non-natural factors such as illegal poisoning (Margalida, 2012). In this regard we can confirm that the observed mean values of subadult and adult survival rates (c. 0.96) are the most accurate so far obtained for this species in our study area and are higher than the 0.88 reported by Oro et al., (2008) and comparable to that of reintroduced populations in the Alps (0.96, Schaub et al., 2009). The current mean values of productivity in our density-dependent model are c. 0.38 chicks/pair/year; higher than those recorded during the previous 5 years (0.32 chicks/pair/year) and on the southern side of the Pyrenees (0.29; Margalida et al., 2014). Therefore, all of the model projections based on rates of survival and productivity higher than the current observations (Table 2) can be considered relatively unlikely (Fig. 4). To assess the effects of parameter variations we constructed a second Box-Behnken design which showed that population size is very sensitive to productivity rate. A decrease in productivity to 0.25 chicks/pair/year suggests that 57 pairs would be lost from the breeding population after 30 years. An increase in subadult and adult mortality predicts a substantial slowing of population growth. For example, an increase in subadult mortality (up to 0.055), predicts a loss of 20 breeding territories and similarly in adults, a loss of 66 breeding territories. Our results therefore suggest that even in a long-lived species such as the bearded vulture, population size and viability are very sensitive to changes in adult mortality (Saether and Bakke, 2000) but also productivity. Productivity also seems to exert a very strong effect on population viability in other species (see Genovart et al., 2018). In addition, since population growth is very sensitive to adult survival rate, the extraction of floating adults should also have substantial demographic effects. It is therefore important to know the true effect of extractions in order to avoid levels which would leave a population vulnerable to damage from stochastic increases in mortality or reductions in productivity. It should be noted that our models assume that extractions of floating adults are homogenous across all age classes between 10 and 20 years. Therefore, some of the individuals extracted from the Pyrenean population could be potential breeders (mainly those between 10 and 14 years old), while others (from 15 to 20 years old) could be extracted with less demographic impact. However, because the priority of reintroductions is to establish a viable or self-sustaining population at the new site, it seems imperative to evaluate trade-offs between objectives, and the benefits through a structured decision-making framework when faced with such complex decisions (Seddon et al., 2007; Converse et al., 2013). For example, the experimental reintroduction of non-territorial 15- to 20-year-old individuals would have less demographic impact on the source population but these individuals would have low probability of breeding at the release site. The post-release dispersal behaviour of individuals released as adults is unknown, and there is a possibility they could return immediately to the natal site. As such, there could be benefit in reintroducing 15–20 year old birds to improve knowledge about management options available to maximise site fidelity.

4.1. How many clutches and non-territorial adults could be removed?

Focusing on management by the removal of clutches and non-territorial adults, our results suggest that there are only three scenarios inside the 95% CI (i.e., those that do not affect the source population size after 30 years): (1) the removal of five clutches and five non-territorial adults during a single year; (2) the annual removal of five non-territorial adults during a 6–year period; and (3) the annual removal of five clutches during a 6–year period. We identify a limiting temporal removal scenario of 6 years in which two removal actions are combined (i.e., clutches and non-territorial adults) rather than extractions concentrated into a single year. All the rest of the combinations modelled will have detrimental effects on the dynamics of the source population, although some of the forecast trajectories showed population growth. This is because selective harvesting can indirectly increase recruitment, thereby potentially impacting population growth rate (Milner et al., 2007). Furthermore, our simulations show that the effects of extractions will not be detectable until 10–15 years after the start of such interventions. Therefore, applying the precautionary principle, interventions in the Pyrenean bearded vulture source population should be performed with caution. As an experimental project, the extractions could focus on floating individuals >15 years, which should have a lower demographic impact and allow an increase in the number of extractions without undue population effects. A next step would be to use GPS transmitters to monitor the behaviour of translocated individuals and their success in settling into their new locations in order to fine-tune future management procedures. With respect to clutch extractions, we suggest there would be value in future work to evaluate the impact of removing clutches from high-quality versus low-quality habitat. For example, removal of clutches from low quality habitat potentially has a lower demographic impact (Margalida et al., 2017b) and would allow an increase in the number of extractions without undue population effects.

Concluding remarks

Our simulation approach provides a vital tool for planning the management and conservation of a threatened long-lived population and for making objective decisions regarding trade-offs in future reintroduction projects (Converse et al., 2013; Lloyd et al., 2019; Panfylova et al., 2019). Quantitative modeling provides explicit predictions about future population trends and the uncertainty surrounding forecast population trajectories under different extraction regimes and allows the pros and cons of different removal alternatives to be assessed. Our results will be of value to decision-makers, allowing them to anticipate and avoid detrimental impacts on source populations. However, due to the inevitable stochastic changes in demographic parameters and the potential limitations of computational models, it is essential to constantly update future model replications with current observed parameter values when using theoretical models to implement management actions for threatened species, in order to increase credibility, efficiency and objectivity (Thiele and Grimm, 2015; Margalida et al., 2018).

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GENERAL DISCUSSION

Cinereous vultures *Aegypius monachus* in the Lleida Pre-Pyrenees, Catalonia (Spain)
Photo: Pilar Oliva-Vidal

Humans have brought about unprecedented changes to the Earth's many environments and have had a profound impact on biodiversity conservation and ecosystem service maintenance (Johnson et al., 2017). In terms of the ecosystem services they provide, vultures are a highly functional group that is suffering from global population declines at a critically extreme rate (Ogada et al., 2012a; McClure et al., 2018; IUCN 2022). The crucial role of vultures in delivering regulation and maintenance services (e.g., disease control and carcass removal) due to their capacity to efficiently consume organic matter (i.e., carrion) from ecosystems is widely recognized (Morales-Reyes et al., 2015); furthermore, their non-material contribution (i.e., cultural services such as ecotourism and sky burials) is today also receiving growing attention (Aguilera-Alcalá et al., 2020; García-Jiménez et al., 2021, 2022; Bhattacharjee et al., 2022; Carucci et al., 2022). However, a myriad of non-natural anthropogenic factors (e.g., poisoning, environmental contaminants, changes in landscape configuration, the proliferation of anthropic infrastructures and certain policy decisions; Donázar et al., 2009; Margalida and Oliva-Vidal, 2017; Arrondo et al., 2020; Monclús et al., 2020; Serrano et al., 2020; Moreno-Opo et al., 2021) are threatening vulture populations and their functional roles (Margalida and Ogada, 2018). Given this multi-faceted human-mediated scenario, vulture conservation has become a challenge and policy makers are therefore now being forced to implement multidimensional approaches and establish conservation actions from an evidence-based perspective. This thesis, which draws on a variety of fields of study, aims to provide a multidisciplinary framework and increase the scientific understanding of many highly significant emerging threats. Similarly, it also hopes to contribute to improving and optimizing conservation management and policies targeting avian scavengers in Europe and elsewhere.

Vultures and humans have coexisted and derived mutual benefits for millennia (Moleón et al., 2014). Yet, this reciprocity is disappearing in our currently changing rural landscapes (Gangoso et al., 2013), largely due to widespread rural abandonment, the loss of traditional agropastoral practices and an increasing negative view of vultures as predators (Chapters 1 and 2). Extensive global rural abandonment and subsequent landscape restoration (i.e., 'passive rewilding', see Pereira and Navarro, 2015; Corlett, 2016; Perino et al., 2019) by natural vegetation regeneration (e.g., woody encroachment) is generating concern in the scientific community and amongst policy-makers due to its consequences for biodiversity and ecosystem services (Pereira and Navarro, 2015; Tsunoda and Enari, 2020). Within a scavenging context, woody encroachment and consequent 'landscape closure' could have significant ecological consequences for the functioning of scavenger assemblages and the dynamics of carrion consumption. In rewilding agroecosystems, landscape type is the main factor governing scavenging dynamics through its influence on the composition of scavenger assemblages and their scavenging efficiency (Chapter 1). The progressive woody encroachment into traditionally maintained open areas in mountain agroecosystems could negatively affect species including avian scavengers that rely on sight to forage (Ruxton and Houston, 2004; Potier et al., 2016) while favouring those including mammals that rely on scent (DeVault and Rhodes, 2002; Enari and Enari, 2021).

Our findings in Chapter 1 demonstrate that while open landscapes favour carcass discovery and exploitation by avian scavengers (especially obligate scavengers), mammals are dominant at carcasses in landscapes with greater vegetation cover (e.g., shrublands and particularly forests) (Ogada et al., 2012b; Pardo-Barquín et al., 2019; Inagaki et al., 2020; Tobajas et al., 2022). In addition, from a perspective of scavenging efficiency, both birds and mammals take longer to locate carcasses in landscapes with greater vegetation cover. This highlights how changes in landscape configuration due to 'landscape closure' after farmland abandonment can reduce the efficiency of vertebrate scavenging assemblages (Wilson and Wolkovich, 2011; Beasley et al., 2019) by affecting, in general, a hugely important functional group (i.e., obligate avian scavengers) and, in particular, the most efficient species (i.e., the griffon vulture) (Arrondo et al., 2019). This vulture plays an important role in driving trophic facilitatory processes for other scavenger species (Cortés-Avizanda et al., 2014; Sebastián-González et al., 2016, 2021) and in terms of scavenging efficiency. Indeed, we found that carcasses consumed by griffon vultures persist much less (~3 days) than those that are not consumed by this species (~15 days), and that consumption rates increase up to 125 times when griffons are among the consumers, evidence of the key role, mediated by landscape characteristics, they play in maintaining healthy ecosystems (Chapter 1). Despite this, the growing belief that the griffon vulture is a species that attacks living livestock could jeopardize the coexistence of vultures and livestock in agropastoral landscapes (Chapter 2). From a trophic facilitation perspective, our results show that carcasses consumed by griffon vultures are exploited by a richer avian scavenger assemblage, and are discovered more rapidly by both avian and mammal scavengers, particularly by avian species. Griffon vultures therefore have three major functions: 1) they signal the location of carcasses, ii) facilitate access to the interior of carcasses, thereby offering feeding opportunities to less powerful species, and iii) provide resources for more specialist species such as bearded and cinereous vultures (Moreno-Opo et al., 2016). However, woody encroachment could reduce the scavenging function of this species and favour mammalian facultative scavengers (Chapter 1).

Despite the importance of vultures in rural regions, an unexpected human-wildlife conflict (HWC) between griffon vultures and livestock has emerged in Europe over the past two decades (Chapter 2). Certain policy decisions and a biased social perception, fuelled by public and media misrepresentation, help explain this emerging conflict. On the one hand, changes in sanitary legislation banned the abandoning of livestock carcasses in the countryside in the period 2002–2011, thereby provoking general alarm among farmers and generating a perceived causal relationship between food shortages and presumed vulture attacks on live livestock (Donázar et al., 2009; Margalida et al., 2014a; Duriez et al., 2019). On the other hand, the approval in Catalonia of a regulation including the griffon vulture among potential livestock predators (i.e., by listing them as a species like the European brown bear *Ursus arctos* and wolf *Canis lupus* whose attacks are financially compensated) aggravated this situation and led to government pay-outs for most farmers' complaints, above all in 2008 and 2009. However, compensatory measures will not necessarily alleviate this problem because they rarely cover the total costs, are open to abuse, and often involve complex bureaucracy (Sillero-Zubiri et al., 2004). Thus, broader-based assessments of environmental and social risk factors affecting the frequency of interactions are required if we are to fully understand the nature of this conflict and how it can be addressed (Dickman, 2010). In addition, improved livestock welfare is essential for reducing the vulnerability of livestock (e.g., injured, sick and weak animals, or those suffering parturition problems), especially during the birthing season. This is critical in areas with high densities of extensive livestock, above all where conflicts are recurrent. In 2011, more flexible regulations were approved allowing livestock carcasses to be left in the field to feed scavengers. However, in decentralized Spain these regulations are interpreted differently according to the specific policies of each region. For example, in Catalonia, health regulations only allow the carcasses of extensive livestock to be left in the field at high altitudes (i.e., > 1,400 m). These political decisions show how important it is to balance out the needs of public health protection and the long-term conservation of biodiversity (Margalida et al., 2010).

From a social perspective, it is vital to develop awareness campaigns and broader interdisciplinary approaches that can bring managers, scientists and farmers together to explain the importance of the ecosystem services vultures provide to society, and to persuade farmers to move away from a conflict mentality towards one of coexistence with vultures. Our findings illustrate how farmers declaring attacks on their livestock have a more negative attitude towards the coexistence of vultures and livestock and tend to consider vultures to be more harmful than beneficial (Gigante et al., 2021; Chapter 2). This may be due to their lack of awareness of vulture behaviour and the pivotal ecosystem services they provide, particularly in areas where vultures are scarce, since most conflicts occur in areas where vultures do not breed. The increasing amount of 'fake news' on social media has aggravated this conflict and paved the way for a growing negative perception of vultures as livestock predators that affects both Old and New World vultures (Margalida and Donázar, 2020; Ballejo et al 2021; Lambertucci et al 2021). This scenario has potentially undesirable consequences including the deliberate poisoning of severely threatened vulture species worldwide (Margalida et al., 2011).

Large avian scavengers perform long-distance movements and have vast foraging ranges covering a wide diversity of habitats (Margalida et al., 2013; Alarcón and Lambertucci, 2018). Such habitats include highly anthropic environments such as landfill sites and intensive livestock farms, where vultures (including breeders) commonly congregate to feed (Donázar et al., 2010; Plaza and Lambertucci, 2017, 2018; Tauler-Ametller et al., 2017; Arévalo-Ayala et al., 2022; Delgado-Gonzálet et al., 2022; Fernández-Gómez et al., 2022), resulting a greater potential for health risks (Chapter 3). In fact, large-scale foraging movements of both breeding and non-breeding griffon vulture populations probably play a larger role in determining the occurrence of vulture-livestock conflicts – above all, those occurring close to landfill sites or to supplementary feeding stations 'SFS' – than the actual number of griffon vulture breeding pairs present in the areas in which conflicts occur (Chapter 2). Within the context of these typical large foraging movements, griffon vultures may find significant amounts of trophic resources in areas far from their breeding sites. This has a number of implications not only for the growing number of conflicts with livestock that arise but also for the need for transboundary conservation approaches and to model accurately trophic availability (Chapter 4).

We explore in this thesis as a case study three obligate avian scavenger (griffon, bearded and Egyptian vultures) populations inhabiting a region with the highest density of scavenging birds in Spain (Navarre), where traditional farming practices are still practiced. The findings reported in Chapter 4 highlight the importance of transhumance in increasing food availability in mountain landscapes, above all of sheep, which provide > 50% of the trophic biomass available for scavengers. Hence, this practice plays a large role for the conservation of avian scavengers (Olea and Mateo-Tomás, 2009; Bernués et al., 2011; Aguilera-Alcalá et al., 2022) in terms of food biomass provisioning and preserving open landscapes (Chapters 1 and 4). However, because of the seasonal character of this livestock movement (which takes place in summer or the non-breeding period), it is vital to consider the temporal and spatial availability of food resources when attempting to objectively forecast population trends and improve management tools such as the creation of supplementary feeding stations. Although the overall annual availability of food for all scavenger populations is enough to cover their energetic requirements, important asymmetries in food availability exist, being substantially higher in summer due to the presence of transhumance herds and lower in winter when it is unable to satisfy the energetic requirements of the most abundant species (i.e., the griffon vulture) (Chapter 4). There is a seasonal reduction of trophic resources available for this species during the breeding period and so it is important to estimate food availability over the whole annual cycle (Marra et al., 2015; Zupo et al., 2017). Specialized and less abundant species such as the bearded and Egyptian vultures do not seem to be so limited by food resources, which suggests that trophic availability is not a limiting factor for the establishment of new territories or their geographic expansion in this study area (e.g., Margalida et al., 2017).

Two factors help explain how griffon vultures overcome potential food shortages during the breeding period. First, their large foraging movements (Delgado-Gonzálet et al., 2022) and use of other potential food resources not considered in the model (e.g., landfills and intensive livestock farms, see above) suggest that this species may find alternative food resources in neighbouring or more distant areas, thereby making accurate theoretical modelling estimations highly complex (Chapters 2 and

4). As has been shown for other large birds of prey, home ranges vary according to prey density and individual reproductive status, with habitat quality serving to regulate their use of space (Fernández et al., 2009; Pérez-García et al., 2013). Second, the network of SFSs recently established by the Spanish administrations (Morales-Reyes et al., 2017) are important for the breeding species inhabiting regions with limited food resources or which are affected by sanitary regulations (Donázar et al., 2009). From a management perspective, the location of SFSs should be related and regulated according to the spatio-temporal distribution of natural resources as well as avian scavenger populations. Only if these issues are heeded will we be able to optimize the value of SFSs — providing that the controversial pros and cons of this widespread conservation management tool are taken into account (Moreno-Opo et al., 2015; Cortés-Avizanda et al., 2016).

Modelling studies estimating the availability of trophic resources are useful for assessing the precise carrying capacity of an environment and for forecasting population trends based on different food availability scenarios (Chapter 4). The projected population estimated by our modelling approach suggests that changes in the spatial dynamics and distribution of avian scavengers are governed by: i) the maximum carrying capacity of the habitat (i.e., density); ii) the availability of suitable nesting sites; iii) the availability of food; and, to a lesser extent, iv) long-distance movements of birds. This information will help managers and policy-makers take decisions regarding the quality of a territory, assess the suitability of a particular region for reintroduction projects, and assess the impacts of policy decisions regarding health and sanitary regulations on population dynamics (Sarrazin and Legendre, 2000; Hirzel et al., 2004; Margalida and Colomer, 2012). These studies should consider large spatial areas, the proportion of accessible carcasses (e.g., of both domestic and wild ungulates) bearing in mind the landscape in which they occur (Chapter 1), the dietary habits of avian scavengers (Moreno-Opo et al., 2015, 2016), and all alternative food sources (e.g., livestock farms and landfill sites). However, it is crucial to obtain precise datasets since the sensitivity of some demographic parameters can have a significant impact on the results (Chapters 4 and 5). For instance, even a 1–2% change in livestock mortality can substantially modify estimates of carrion availability and, consequently, its effect on scavenger population dynamics. Thus, even small errors in livestock mortality estimates could lead to serious mistakes in management measures, which can have important repercussions for conservation (Chapter 4). From a broader perspective, it is essential to consider large spatial scales if we are to generate effective conservation measures based on transboundary approaches (Lambertucci et al., 2014; Bischof et al., 2015). This is crucial for correctly assessing the availability of trophic resources and for understanding the relevance of transboundary agreements between regional administrations as a means of developing, coordinating and applying conservation measures for species that move over long distances (Arrondo et al., 2018). Given that large-scale ecosystem management is needed for biodiversity conservation, the political or administrative boundaries that divide ecosystems and apply different rules and guidelines can pose serious problems for ecological processes and conservation efforts (Zbicz 1999; Papadopoulou and Sitsoni, 2012). This is particularly critical in decentralized countries such as Spain where each region can take independent decisions on management and policy regulations (López-Bao and Margalida, 2018; Chapter 2).

The computational modelling of biological systems is becoming increasingly important as part of efforts to better understand complex biological behaviour (Fisher and Henzinger, 2007). PDPs are bioinspired computational models that aim to mimic ecological phenomena, making them a highly useful tool in ecological research (Colomer et al., 2011; Margalida and Colomer, 2012). These flexible models enable the heterogeneity of populations and environments to be taken into account and allow demographic parameters to be modelled – for example, with regard to food resources – and provide an effective tool in conjunction with other approaches when classical modelling approaches fail (Colomer et al., 2013). Bioinspired PDP models can help in conservation planning for species of concern where available trophic spectra can be assessed objectively (e.g., biomass provided by domestic livestock) and can be used to combine trophic resource estimations with demographic parameters to improve the effectiveness of conservation management (Chapter 4). However, as mentioned above, it is imperative to have good datasets given that the sensitivity of certain parameters can have a significant impact on estimates and therefore on future conservation measures that often involve the active management of threatened species (Chapter 5).

The intentional movement of animals to restore populations (i.e., translocation) to formerly occupied habitats can play a key role in responses to biodiversity loss (Seddon et al., 2007, 2014). However, the population size of the donor population can be a limiting factor in threatened species. Thus, it is essential to evaluate the likely trade-offs between the *ex situ* (e.g., captive breeding) and *in situ* (e.g., threat management) options to improve the outcomes of reintroduction projects (Dolman et al., 2015) and optimize conservation efforts in terms of both economic and temporal costs (Smith et al., 2011; McCarthy et al., 2012). There is growing evidence of the success of translocations in reintroduction projects (Sarrazin and Legendre, 2000; Armstrong and Seddon, 2008; Le Gouar et al., 2008; Mihoub et al, 2013; Robert et al., 2015; Bertolero et al., 2018) but somewhat less information exists about their impact on source/donor populations (Chapter 5). The bearded vulture is the most threatened of the species that conform the avian scavenger guild in Europe. Consequently, several reintroduction projects have been carried out in Europe since the mid-1980s to counteract population declines and local extinctions of this species (Schaub et al., 2009). With 184 breeding territories, the Pyrenees harbour the most important population of wild bearded vultures in Europe. A recent study applying integrated population models (IPM) estimated a total Pyrenean population of 1026 individuals (range: 937–1119) (Margalida et al., 2020). This population is characterized by a progressive increase in breeding territories and, at the same time, a density-dependent decrease in productivity (Carrete et al., 2006; Margalida et al., 2014b). Moreover, there has been an increase in the fraction of non-territorial but potential breeding individuals (i.e., mature floaters > 6 years of age) (Antor et al., 2007; Margalida et al., 2015), which has prompted a technical-scientific debate regarding the aptness of removing clutches and floaters for reintroduction programs (Ferrer et al., 2014; Margalida et al., 2015, 2017). If a continuous extraction program for reintroduction or population reinforcement is to be contemplated, it is imperative to forecast the demographic impact of a translocation on the source population to ensure its viability and to establish guidelines to be followed as part of an adaptive management framework.

The results presented in Chapter 5 offer evidence that will assist policy-makers adopt strict and effective translocation protocols for the restoration of European bearded vulture populations without negatively affecting the source population. However, changes in demographic parameters, mainly in productivity and subadult/adult survival, could substantially change the forecasted results. On the other hand, the removal of juveniles has twice the impact on the source population compared to the extraction of clutches or non-territorial adults. Juveniles preserve their breeding potential intact (e.g., from 6 years old onwards), unlike non-territorial adults (≥ 10 years) that have lost part of this potential. Reintroduction using juveniles is the main strategy employed to re-establish this species in parts of Europe (i.e., hacking fledglings from captive breeding) and is successful in many European projects (Schaub et al., 2009), although the effect on the source population of removing juveniles from the wild is substantial. Thus, from a conservation point of view, the extraction of clutches and non-territorial adults is preferable to the removal of juveniles. Clutch removal should take place above all in low quality territories with low breeding success to reduce the demographic impact.

From a more conservationist perspective, the Pyrenean bearded vulture population should be conserved via management programs and not be subject to the removal of individuals. Work to expand this species' range should prioritize alternative reintroduction methods (e.g., captive breeding and the management of SFSs) and the removal of wild birds should never be the first option. Forecasts must be made with care due to the pronounced impact of stochastic variations. The modelled parameters of survival and productivity should generally be viewed as optimistic and values may well be lower due to a fall in productivity resulting from density-dependent factors or an increase in mortality due to non-natural threats (Margalida et al., 2012). Population size and viability are very sensitive to variations not only in subadult/adult mortality (Saether and Bakke, 2000; Margalida et al., 2020) but also in productivity, as has been shown in other species (Genovart et al., 2018). Moreover, since population growth is very sensitive to adult survival rates, the extraction of floating adults will also have substantial demographic effects (Chapter 5). As an experimental project and within an adaptive management context (IUCN/SSC, 2013), extractions should focus on floating individuals of >15 years, which will have less demographic impact, and translocated individuals should be closely monitored (i.e., using GPS transmitters). It must be remembered that demographic effects will not be detectable until 10–15 years after the start of any intervention.

Quantitative modelling using bioinspired PDP models provides explicit predictions about future population trends (Chapters 4 and 5) and this type of ecological modelling studies are important for decision-makers as they allow them to anticipate and avoid any detrimental impact on threatened species. However, due to inevitable stochastic changes (or small errors in parameter estimates) and the potential limitations of computational models, it is essential to constantly update modelled replications with currently observed parameter values when using theoretical models to implement management actions designed to increase credibility, efficiency and objectivity (Thiele and Grimm, 2015; Margalida et al., 2018).

Finally, the exploitation of trophic resources in anthropic environments such as open-air landfills and livestock farms could increase scavengers' exposure to several environmental pollutants and pharmaceutical residues, resulting in significant impact on their health (Plaza and Lambertucci, 2017, 2018; Tauler-Ametller et al., 2018; Blanco et al., 2019; Ortiz-Santaliestra et al., 2019; García-Alfonso et al., 2020; Moreno-Opo et al., 2021). The exposure (either intentional or unintentional) to different pesticides has been widely recognized as one of the most serious threats currently affecting vultures worldwide (Plaza et al., 2019). However, the current exposure of avian scavengers – and, in particular, both Old and New World vultures – to ARs remains poorly investigated despite being highlighted by the Convention on the Conservation of Migratory Species of Wild Animals as one of the most important toxicological hazards for migratory birds (CMS, 2014). This thesis is the first study to assess the exposure of the four European vultures and certain facultative avian scavengers to SGARs (Chapter 3), which is, in turn, a first step towards the recognition of the impact of these toxic compounds on living scavenging birds.

The current widespread and increasing use of SGARs and their high persistence in animal tissues make these compounds ubiquitous in rodent-predator-scavenger food webs (López-Perea and Mateo, 2018; Pay et al., 2021; Cooke et al., 2022; Elliott et al., 2022). Wildlife exposure to SGARs, especially raptors and avian scavengers, has to date usually been determined by passive monitoring (i.e., using dead individuals). However, these studies potentially introduce an important bias by underestimating observed concentrations and prevalence values compared to those obtained through active monitoring, that is, the sampling of free-living individuals (Chapter 3). Most birds found dead probably died from other non-natural causes (e.g., trauma or electrocution due to collision with energy infrastructures; Pérez-García et al., 2022) and their corpses are more easily detected at the site of death (González et al., 2008) than clinically poisoned birds, which tend to die in more remote sites (Peshev et al., 2022). Although the use of ARs is necessary for rodent control, the well-recognized risks of primary and secondary poisoning of non-target species obliges debate on the measures that can mitigate the risks of AR use (Buckle and Prescott, 2018; Eisemann et al., 2018; Witmer, 2018; Thornton et al., 2022). For example, the well-known greater persistence in animal tissues (i.e., bioaccumulative capacity) of some forms of diastereomer underlines the need for developing SGAR formulations with a lower impact on non-target wildlife (Damin-Pemink et al., 2016; Fourel et al., 2017, 2021; Chapter 3). Current secondary or even tertiary exposure routes to SGAR can harm not only facultative (e.g., kites and large eagles) but also obligate avian scavengers – as well as carnivores – that frequently exploit rodents and medium-size carrion (Chapter 1) or that forage in anthropic environments, including Egyptian and bearded vultures (Chapter 3).

Our findings illustrate that, although vulture populations in the Iberian Peninsula are currently characterized by favourable positive trends, changes in landscape configuration (Chapter 1) and other non-natural threats (e.g., Chapters 2 and 3) could curtail this encouraging scenario and threaten the largest vulture reservoir in Europe and the important ecosystem services that this avian scavenger guild provides. Future conservation actions must consider both local and large-scale perspectives and plan conservation measures based on transboundary approaches (Chapter 4); as well, policy-makers should prioritize scientific evidence-based sustainable management measures (Chapter 5). The global negative trend in obligate and some facultative avian scavenger populations due to anthropic disturbance poses an urgent need for the development of multidisciplinary approaches linking different conservation fields to help preserve and ensure the future viability of avian scavenger populations throughout our ever-changing world.

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CONCLUSIONS

Egyptian vulture *Neophron percnopterus* in the Lleida Pre-Pyrenees, Catalonia (Spain)
Photo: Pilar Oliva-Vidal

CONCLUSIONS

This thesis combines different disciplines and research fields to provide a multidisciplinary conservation framework and improve scientific understanding of emerging threats challenging avian scavengers in our ever-changing human-dominated world. Safeguarding the coexistence of avian scavengers and humans and improving conservation efforts requires integrative research linking aspects of disciplines such as conservation biology, behavioural ecology, ecotoxicology and social sciences. The development of multidisciplinary approaches can help establish priority research lines and provide policy-makers with useful insights for the design and optimization of future conservation management actions. The main conclusions thus obtained are as follows:

1. The effects of rural abandonment and subsequent passive rewilding due to natural revegetation on scavenger communities remains largely unexplored. This thesis throws light on how woody encroachment in mountain agroecosystems affects the functioning of scavengers by assessing differences in the scavenger assemblage composition and scavenging efficiency at community and species levels. The monitoring of large numbers of carcasses of different types (herbivores and carnivores) and sizes in landscapes with differing vegetation cover (open areas, shrublands and forests) provides an appropriate approach for assessing how ‘landscape closure’ after farmland abandonment shapes scavenging dynamics.

2. Landscape type is the main factor governing scavenging dynamics in rewilding situations. Unlike mammals, avian scavenging frequency decreases as vegetation cover increases, especially in the case of obligate scavengers. Open landscapes attract greater scavenger abundances and carrion is discovered and consumed more rapidly, largely due to its exploitation by griffon vultures. This species plays an important function in reducing carcass persistence given their high carcass consumptions rates and in driving trophic facilitation processes, particularly for other endangered avian scavengers such as cinereous and bearded vultures. Woody encroachment after farmland abandonment jeopardizes the scavenging roles of obligate avian scavengers and reduces the scavenging efficiency of the assemblage while favouring facultative mammalian scavengers.

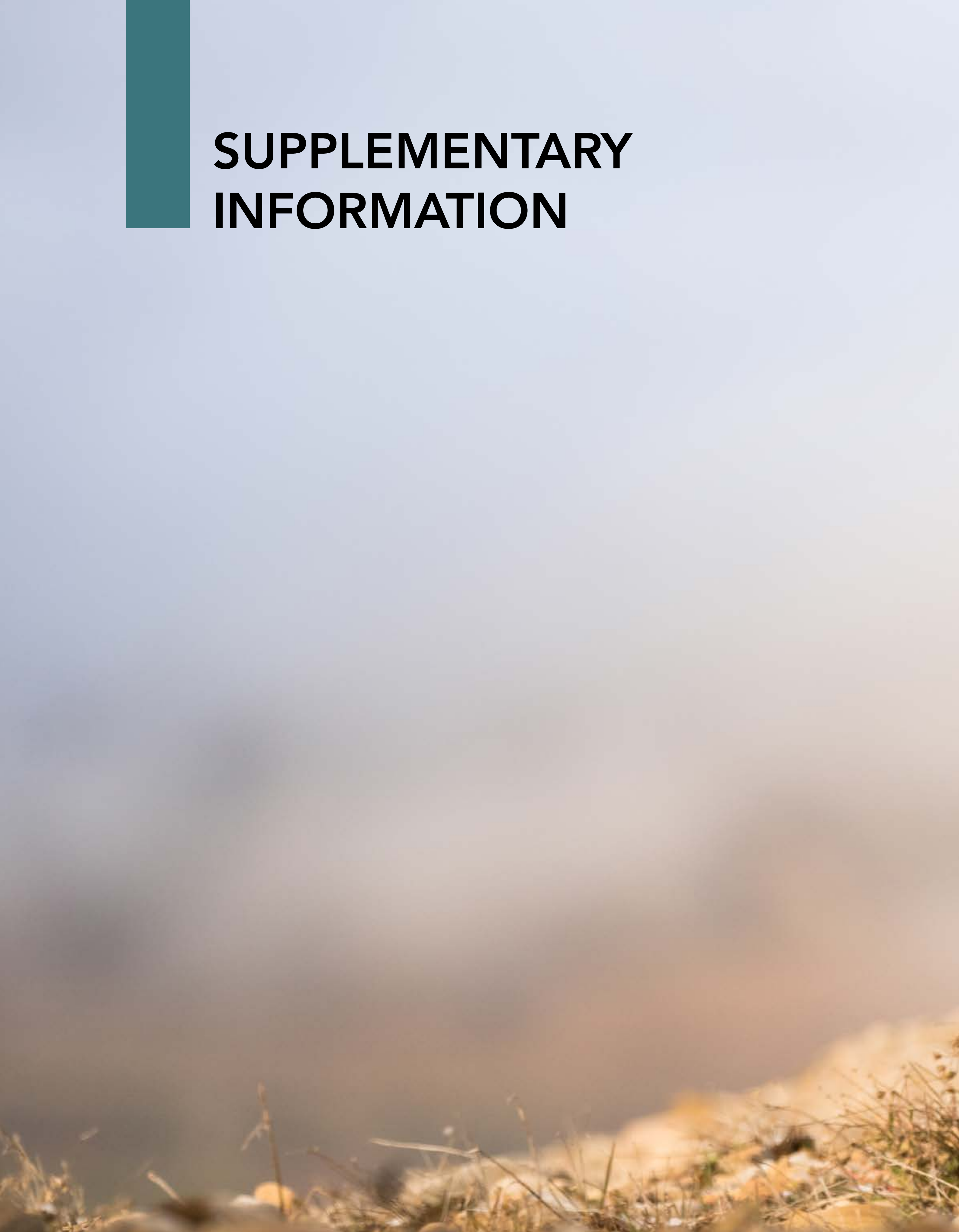
3 Agri-environmental actions encouraging traditional extensive farming practices have direct conservation benefits for avian scavengers due to the role played by extensive livestock farming in increasing carrion availability in mountain areas and in maintaining landscape heterogeneity. The preservation of extensive stockbreeding and transhumant herds should be a priority in biodiversity conservation programs to not only reverse the current decline of extensive grazing livestock but also to safeguard the high ecological value of traditional cultural mountain landscapes.

4 The vulture-livestock conflict is a complex issue that emerged after changes in sanitary regulations. It has been aggravated by growing media misrepresentation, thereby jeopardizing the coexistence of vultures and livestock in rural areas and threatening the ecosystem services vultures provide. The long-range foraging movements of vultures play a greater part in determining the appearance of conflicts than the number of vulture breeders occurring in an area. Interactions are more frequent during the birthing season in areas with higher extensive livestock numbers, especially those close to landfill sites and, to a lesser extent, to supplementary feeding stations. Addressing social perceptions and encouraging farmers to move away from a conflict mentality towards one of coexistence with vultures is vital for reconciling biodiversity conservation and agropastoral practices.

5 Computational bioinspired modelling using Population Dynamic P systems is an appropriate tool for generating explicit predictions of population trends, which can help policy-makers plan and develop conservation strategies such as the translocation of endangered species for reintroduction projects. However, the inevitable stochastic nature of certain demographic parameters (e.g., productivity and survival) or even small errors in parameter estimates (e.g., livestock mortality rates) can substantially alter forecasted results, and lead to significant mistakes with important conservation repercussions. Thus, conservation actions based on ecological modelling approaches should be performed with care due to uncertainties arising from stochastic changes in demographic parameters. Large foraging movements of avian scavengers not only make ecological modelling highly complex but also highlight the need for transboundary cooperation when managing their populations.

6 Medium-sized avian scavengers (black and red kites and Egyptian vultures) are the most exposed and vulnerable species to SGARs. The prevalence and concentrations of these compounds was lower in large avian scavengers, of which bearded vultures had the highest prevalence. The exposure we detected through active monitoring demonstrate the potential threat posed by SGARs to the most endangered European avian scavengers, including red kites and Egyptian vultures. The effects of these pesticides at population level thus require further research due to the lack of knowledge of exposure levels in free-living individuals. Active monitoring studies are also needed to improve knowledge of the true exposure to anthropogenic compounds. Sampling individuals tagged with GPS transmitters can provide crucial information for identifying areas of conflict that need to be closely monitored by managers and policy-makers.

SUPPLEMENTARY INFORMATION



Red fox *Vulpes vulpes* in the Lleida Pre-Pyrenees, Catalonia (Spain)

Photo: Pilar Oliva-Vidal

Supplementary information to Chapter 1

Scavenging in changing environments: woody encroachment shapes rural scavenger assemblages in Europe

Oliva-Vidal, P., Sebastián-González, E., Margalida, A. 2022. Scavenging in changing environments: woody encroachment shapes rural scavenger assemblages in Europe. *Oikos*.

Table 1. Vertebrate scavengers recorded consuming carcasses monitored in the three landscape types studied: open 'O' (n=70), shrubland 'S' (n=71) and forest 'F' (n=37). We present the scavenger taxon and group, the percentage of scavenged carcasses, the maximum abundance at a single carcass (abundance) and the conservation status (EN: Endangered, VU: Vulnerable, NT: Near Threatened, LC: Least Concern; IUCN 2020) for each species. Uppercase letters indicate the conservation status at national level (RD 139/2011). *Migratory species.

Scavenger species	Taxon	Group	Scavenged carcasses			Abundance			IUCN
			O	S	F	O	S	F	
Accipiter gentilis	Avian	Predator	1.43	0	0	1	0	0	LC
Aegypius monachus^{VU}	Avian	Obligate	25.71	8.45	0	3	4	0	NT
Aquila chrysaetos	Avian	Apex predator	24.29	22.54	2.70	2	2	2	LC
Buteo buteo	Avian	Facultative	1.43	1.41	0	1	1	0	LC
Corvus corax	Avian	Facultative	57.14	45.07	18.92	7	7	2	LC
Corvus corone	Avian	Facultative	2.86	0	0	7	0	0	LC
Garrulus glandarius	Avian	Facultative	7.14	7.04	10.81	1	1	1	LC
Gypaetus barbatus^{EN}	Avian	Obligate	41.43	22.54	0	2	3	0	NT
Gyps fulvus	Avian	Obligate	80.00	61.97	2.70	112	72	10	LC
Milvus migrans*	Avian	Facultative	2.86	4.23	0	1	1	0	LC
Milvus milvus$^{EN\,*}$	Avian	Facultative	11.43	1.41	0	1	1	0	LC
Neophron percnopterus^{VU*}	Avian	Obligate	31.25	25.64	0	6	1	0	EN
Pica pica	Avian	Facultative	0	1.41	0	0	1	0	LC
Canis familiaris	Mammal	Facultative	24.29	18.31	13.51	5	5	2	
Genetta genetta	Mammal	Facultative	2.86	1.41	0	1	0	0	LC
Martes spp.	Mammal	Facultative	21.43	19.72	40.54	1	2	2	LC
Meles meles	Mammal	Facultative	5.71	5.63	8.11	1	1	1	LC
Sus scrofa	Mammal	Facultative	54.29	73.24	72.97	13	15	7	LC
Vulpes vulpes	Mammal	Facultative	82.86	90.14	94.59	3	4	3	LC

Table 2. Models (GLM) at species level used to assess the effect of landscape type ('open', 'shrubland' and 'forest'), carcass weight (kg), carcass type (herbivore and carnivore) and placement time (morning and afternoon) on carcass dynamics consumption. We present the model-averaged coefficients and standard error (SE) for models with AICc < 2. Significant p-values (< 0.05) are highlighted in bold. The percentage of explained deviance of the model (i.e., percentage of the variability explained by each model 'D^2') is shown.

Scavenger species	Model	Coefficient	SE	p-value	D^2
Griffon vulture	Open	1.212	0.483	**0.012**	**50.1%**
	Forest	-4.931	1.184	**< 0.001**	
	Carcass weight	1.793	0.342	**< 0.001**	
	Carcass type (herbivore)	0.474	0.610	0.440	
Cinereous vulture	Open	1.286	0.523	**0.015**	**13.7%**
	Carcass weight	0.726	0.256	**0.005**	
	Carcass type (herbivore)	-0.344	0.926	0.713	
	Placement (afternoon)	0.383	0.689	0.582	
Egyptian vulture	Open	0.287	0.484	0.558	**2.6%**
	Carcass weight	0.360	0.237	0.134	
	Carcass type (herbivore)	0.546	0.694	0.438	
Bearded vulture	Open	0.921	0.409	**0.025**	**18.3%**
	Carcass weight	0.815	0.242	**< 0.001**	
	Carcass type (herbivore)	1.553	1.109	0.165	
	Placement (afternoon)	0.820	0.567	0.151	
Common raven	Open	0.458	0.355	0.201	**12.2%**
	Forest	-1.101	0.498	**0.028**	
	Carcass weight	0.607	0.176	**< 0.001**	
	Carcass type (herbivore)	0.188	0.547	0.733	
	Placement (afternoon)	-0.387	0.400	0.337	
Golden Eagle	Open	-0.095	0.435	0.829	**19.3%**
	Forest	-2.067	1.073	**0.055**	
	Carcass weight	1.097	0.291	**< 0.001**	
	Carcass type (herbivore)	-1.527	0.776	**0.050**	
	Placement (afternoon)	0.227	0.574	0.695	

Scavenger species	Model	Coefficient	SE	p-value	D^2
Red kite	Open	2.179	1.090	**0.047**	**19.5%**
	Carcass weight	0.545	0.490	0.270	
	Carcass type (herbivore)	-2.032	0.987	**0.041**	
	Placement (afternoon)	0.502	1.123	0.657	
Black kite	Open	-0.330	0.954	0.731	**21.9%**
	Carcass weight	-2.029	1.249	0.107	
	Carcass type (herbivore)	-1.633	1.119	0.147	
	Placement (afternoon)	0.639	1.171	0.589	
Eurasian jay	Carcass weight	-0.091	0.285	0.751	**1%**
	Placement (afternoon)	0.593	0.786	0.454	
Domestic dog	Carcass weight	0.747	0.202	**< 0.001**	**8.7%**
	Placement (afternoon)	0.150	0.513	0.772	
Badger	Carcass weight	0.321	0.391	0.415	**3.4%**
	Carcass type (herbivore)	-1.241	0.826	0.135	
Martes spp.	Open	0.100	0.418	0.812	**4.2%**
	Forest	1.019	0.451	**0.025**	
	Carcass weight	-0.164	0.187	0.383	
	Carcass type (herbivore)	-0.563	0.484	0.248	
	Placement (afternoon)	0.417	0.451	0.359	
Wild boar	Open	-1.093	0.394	**0.006**	**12.5%**
	Forest	0.209	0.491	0.672	
	Carcass weight	0.501	0.194	**0.010**	
	Carcass type (herbivore)	-0.453	0.537	0.401	
	Placement (afternoon)	1.313	0.403	**0.001**	
Red fox	Open	-0.770	0.531	0.150	**12.6%**
	Forest	0.921	0.842	0.278	
	Carcass weight	0.962	0.323	**0.003**	

Table 3. Univariate GLMs to test for differences between detection times (h) of the first carcass detector according to the 'taxon' (avian or mammalian) at each landscape type. We present the coefficient, standard error (SE) and the p-value of each model.

Landscape type	Model	Coefficient	SE	p-value
Open	Detection time ~ taxon (mammal)	1.349	0.490	**0.007**
Shrubland	Detection time ~ taxon (mammal)	1.416	0.366	**< 0.001**
Forest	Detection time ~ taxon (mammal)	2.494	0.707	**0.001**

Table 4. Univariate GLMs to assess the effect of the presence of griffon vultures on the variables relating to scavenger assemblage composition and scavenging efficiency. We also considered the arrival time (h) of the first bird and mammal scavengers after griffon vulture appearance. We present the model coefficients and the standard error (SE) of each variable. Significant p-values are highlighted in bold.

Explanatory variable	Coefficient	SE	p-value
Scavenger richness	0.661	0.083	**< 0.001**
Avian richness	1.779	0.170	**< 0.001**
Mammalian richness	-0.048	0.105	0.646
Scavenger abundance	2.349	0.102	**< 0.001**
Detection time	-1.347	0.267	**< 0.001**
First bird arrival	-1.222	0.44	**0.002**
First mammal arrival	-0.405	0.19	**0.036**
Consumption time	-2.23	0.251	**< 0.001**
Consumption rate	3.627	0.254	**< 0.001**

Figure 1. Images of the three landscape types (open, shrubland and forest) considered in this study. Landscapes were selected according to their vegetation cover characteristics within a 25 m radius around experimentally placed carcasses. Open landscapes were mainly large open areas (e.g., grasslands) without shrub and tree cover (i.e., < 5%); shrublands were sites with abundant shrub and/or tree vegetation cover (i.e., ranging 50–70%); and forests were mainly of mature pines and beech with a moderate canopy closure (i.e., tree cover > 90%).

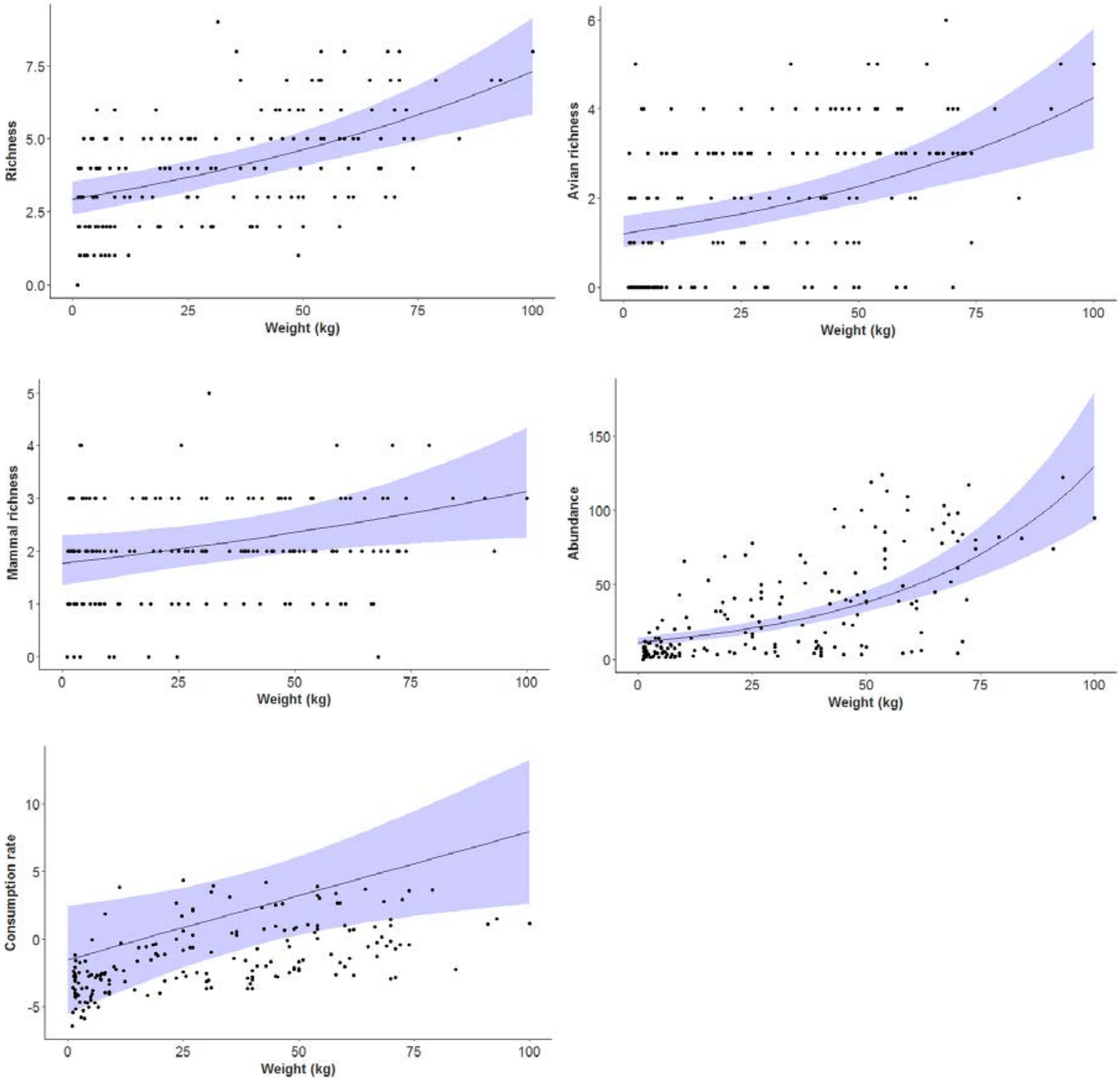


Figure 2. Significant relationships between carcass weight (kg) and scavenger richness, avian and mammal richness, scavenger abundance and consumption rates. The plots show the relationship predicted by the averaged models (black line) and the 95% confidence interval for each variable (blue shade). Dots represent real data

Supplementary information to Chapter 2

Griffon vultures, livestock and farmers: unraveling a complex socio-economic ecological conflict from a conservation perspective

Oliva-Vidal, P., Hernández-Matías, A., García, D., Colomer M. À., Real, J., Margalida, A. 2022. Griffon vultures, livestock and farmers: unraveling a complex socio-economic ecological conflict from a conservation perspective. *Biological Conservation* 272, 109664.

Appendix S1

Questionnaire used in this study

FARMER'S PERCEPTIONS OF VULTURE ATTACKS ON LIVESTOCK

A) LIVESTOCK TYPE

1. Livestock type (cattle, sheep/goats, horses):

B) LINKING SCAVENGING BIRDS TO LIVESTOCK FARMS

2. Has your livestock been attacked by vultures?

a) Yes
b) No

3. If you have suffered attacks, which livestock were affected?

4. In which circumstances were the livestock involved?

a) Female during parturition
b) New-born during parturition
c) New-born
d) Healthy adult
e) Healthy young
f) Sick or weak adult
g) Sick or weak young
h) Old
i) Dying
j) Dead
k) Don't know

5. Where was the livestock at the time of the attack?

a) Closed farm
b) Semi-closed farm
c) Field protected with an electric shepherd
d) Open field
e) Others: ___________________________

6. Do you know which species started the attack? If so, how do you know it?

a) Griffon vulture
b) Cinereous vulture
c) Common raven
d) Egyptian vulture
e) Bearded vulture
f) Golden eagle
g) Red fox
h) Bear
i) Wolf
j) Other
k) Don't kno
Reason: ____

7. How did you detect the attack?

a) I was in the area and I observed vultures
b) I was told by a known person
c) I was told by an unknown person
d) When I supervised the livestock on the same day
e) When I supervised the livestock after _ days (specify how many days)
f) Others: ___________________________

8. Do you think vultures can attack live cattle?

a) Yes
b) No
c) I don't know

9. Do you think that farmer's supervision of livestock, especially during the birthing season, is sufficient?

a) Yes
b) No
c) I don't know

10. Do you think that vulture attacks have increased in recent years?

a) Yes
b) No
c) I don't know

10.1 To what do you attribute this increase?

a) To the lack of available food (i.e., dead livestock) due to the prohibition on leaving carcasses in the countryside
b) Because there is an increase in the vulture population
c) Because vultures have changed their habits and have learned to attack live animals
d) Because sick or weakened animals are an easy resource
e) Others: ____

C) FARMERS' PERCEPTION OF THE ADMINISTRATION'S RESPONSE

11. If you have suffered an attack, have you reported it to the Administration?

a) Yes
b) No

If Yes:

a) **Have you received any financial compensation?** a)Yes b) No
b) **Do you think this compensation is sufficient?** a)Yes b) No

12. Do you think the response of the Administration was positive?

a) Yes
b) No
c) I don't know

13. Do you think that the Administration's compensation satisfies the farmers who have suffered an attack?

a) Yes
b) No
c) I don't know

14. Do you think that current levels of compensation are sufficient?

a) Yes
b) No
c) I don't know

15. Do you think it is necessary to apply management measures to reduce the conflicts between vultures and livestock?

a) Yes
b) No
c) I don't know

15.1 If Yes, what measures do you consider the most effective?

a) Create more SFSs
b) Increase the presence of livestock protection dogs
c) Provide more sites to deposit dead livestock on farms
d) Reduce the current population of vultures
e) Current sanitary regulations to facilitate the abandonment of dead livestock in the mountains
f) Others: ______________________________

16. If measures are not applied, do you think that the social conflict between vultures and livestock will increase?

a) Yes
b) No
c) I don't know

D) FARMERS' PERCEPTION OF THE POSSIBIITY OF VULTURE/LIVESTOCK COEXISTENCE AND THE ECOLOGICAL RELATIONSHIPS BETWEEN THEM

17. Do you think domestic cattle can coexist with vultures?

a) Yes
b) No
c) I don't know

18. In your opinion, what is the relationship between vultures and livestock? Why?

a) Beneficial
b) Harmful
c) Indifferent
d) Don't know

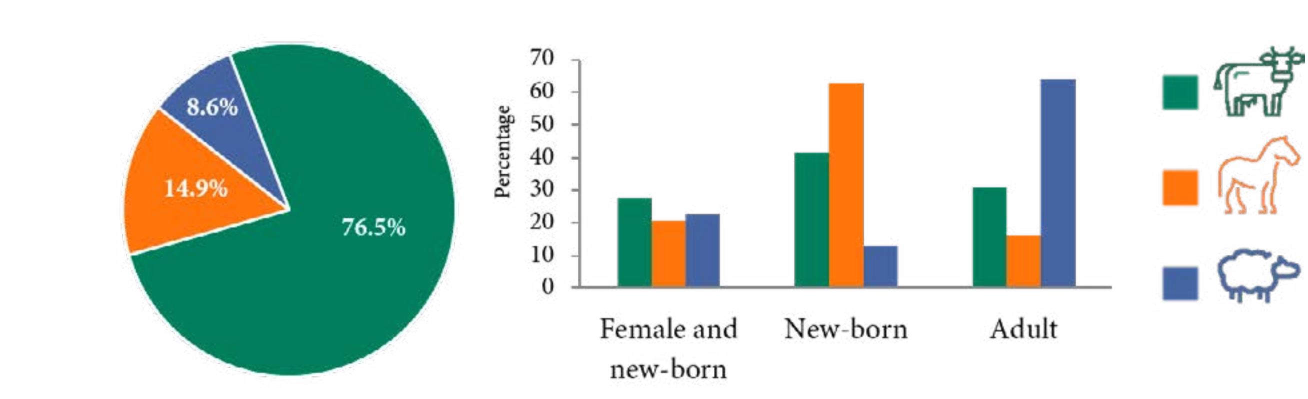
Appendix S2

Number of farmers who have responded each question and number of responses obtained.

Question	Respondents	Total responses	Closed responses	Others	Type of response			
					Yes	No	Don’t know	Not available
1	127	166	-	-	-	-	-	-
2	127	127	-	-	67	60	-	-
3	67	71	-	-	-	-	-	-
4	67	104	104	-	-	-	-	-
5	67	72	72		-	-	-	-
6	67	76	76	-	-	-	8	-
6.1	46	46	-		-	-	-	12
7	67	84	84	-	-	-	-	-
8	127	127	-	-	112	11	4	-
9	124	124	-	-	79	45	-	3
10	127	127	-	-	98	23	6	-
10.1	97	241	235	6	-	-	-	1
11	67	67	-	-	46	21	-	-
11a	46	46	-	-	14	32	-	-
11b	14	14	-	-	7	7	-	-
12	108	108	-	-	4	93	11	19
13	109	109	-	-	1	90	18	18
14	110	110	-	-	6	73	31	17
15	127	127			116	10	1	-
15.1	116	216	207	9	-	-	-	-
16	127	126	-	-	93	14	19	1
17	127	122	-	-	88	34	-	5
18	127	135	-	-	-	-	6	-

Appendix S3

Livestock type and characteristics (percentages) involved in the 616 complaints reported from 2008–2020: cattle (n = 471), horses (n = 92) and sheep/goats (n = 53)



Appendix S4

Number of farms for each livestock type managed by the 133 farmers interviewed, and number of farms reporting attacks and characteristics (%) of the livestock involved. Some farmers provided multiple responses regarding the characteristics of the livestock involved in the attacks.

	Cattle	Horses	Sheep/Goat
Farms (n)	89	27	50
Farms reporting attacks (n)	51	5	15
Livestock involved in the attacks			
Number of cases (n)	72	11	21
Female parturition (%)	23.6	18.2	9.5
Female and new-born (%)	37.5	9.1	9.5
New-born (%)	30.6	27.3	14.3
Juvenile (%)	6.9	18.2	14.3
Adult (%)	1.4	27.3	52.4

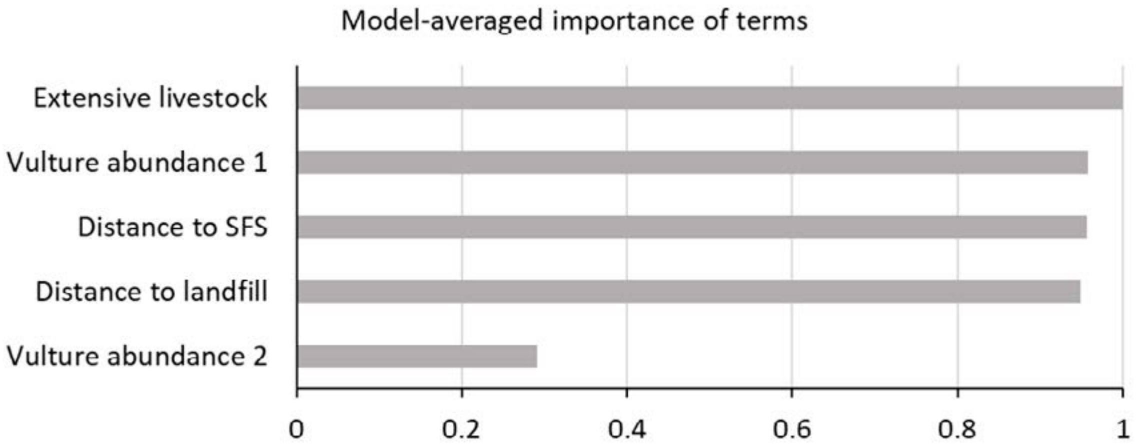
Appendix S5

Models (GLMs) determining the number of complaints for vulture attacks on livestock. We present the variables included in the set of models (model description); the number of estimated parameters (K); the Akaike Information Criteria corrected for small sample sizes (AICc); and the Akaike weight (wi) by the models evaluated (32 models; n = 110). Abbreviations relating to variables correspond to: 'extensive livestock density' (Ext); 'distance to nearest landfill site' (Dland); 'number of griffon vulture pairs' (Gpair); 'griffon vulture abundance' (Gabun); and 'distance to nearest supplementary feeding station' (DSFS). Selected models are shown in bold.

Model description	K	AIC_c	w_i
$Y \sim 1 + G_{pair} + G_{abun} + D_{land} + Ext$	**5**	**686.4231**	**0.6179**
$Y \sim 1 + G_{pair} + G_{abun} + D_{SFS} + D_{land} + Ext$	**6**	**688.1909**	**0.2553**
$Y \sim 1 + G_{pair} + D_{SFS} + D_{land} + Ext$	5	689.8276	0.1126
$Y \sim 1 + G_{pair} + D_{land} + Ext$	4	694.1791	0.0128
$Y \sim 1 + D_{SFS} + D_{land} + Ext$	4	700.7503	0.0005
$Y \sim 1 + D_{land} + Ext$	3	700.9221	0.0004
$Y \sim 1 + G_{abun} + D_{land} + Ext$	4	702.0973	0.0002
$Y \sim 1 + G_{abun} + D_{SFS} + D_{land} + Ext$	5	702.9417	0.0002
$Y \sim 1 + G_{pair} + D_{SFS} + Ext$	4	708.6972	<0.0001
$Y \sim 1 + G_{pair} + G_{abun} + D_{SFS} + Ext$	5	710.8094	<0.0001
$Y \sim 1 + G_{pair} + Ext$	3	710.8982	<0.0001
$Y \sim 1 + G_{pair} + G_{abun} + Ext$	4	711.878	<0.0001
$Y \sim 1 + G_{abun} + D_{SFS} + Ext$	4	721.3636	<0.0001
$Y \sim 1 + Ext$	2	722.6632	<0.0001
$Y \sim 1 + G_{abun} + Ext$	3	723.5644	<0.0001
$Y \sim 1 + D_{SFS} + Ext$	3	724.1304	<0.0001
$Y \sim 1 + G_{pair} + G_{abun} + D_{land}$	4	785.9484	<0.0001
$Y \sim 1 + G_{pair} + G_{abun} + D_{SFS} + D_{land}$	5	788.0146	<0.0001
$Y \sim 1 + G_{pair} + D_{SFS} + D_{land}$	4	791.6788	<0.0001
$Y \sim 1 + G_{abun} + D_{land}$	3	792.146	<0.0001
$Y \sim 1 + D_{SFS} + D_{land}$	3	793.1007	<0.0001
$Y \sim 1 + G_{abun} + D_{SFS} + D_{land}$	4	793.7733	<0.0001
$Y \sim 1 + D_{land}$	2	795.7488	<0.0001
$Y \sim 1 + G_{pair} + D_{land}$	3	796.5054	<0.0001
$Y \sim 1 + G_{pair} + D_{SFS}$	3	803.7333	<0.0001
$Y \sim 1 + G_{pair} + G_{abun}$	3	804.3183	<0.0001
$Y \sim 1 + G_{pair} + G_{abun} + D_{SFS}$	4	805.0536	<0.0001
$Y \sim 1 + D_{SFS}$	2	806.8502	<0.0001
$Y \sim 1 + G_{pair}$	2	807.1305	<0.0001
$Y \sim 1$	1	807.7727	<0.0001
$Y \sim 1 + G_{abun}$	2	808.8544	<0.0001
$Y \sim 1 + G_{abun} + D_{SFS}$	3	808.8951	<0.0001

Appendix S6

Relative importance of the variables estimated from the Akaike weight of the 32 models evaluated in the analysis of the determinants of the number of complaints for vulture attacks on livestock. Values close to 1 indicate variables that appear in most models and that show the highest likelihood given the data, i.e., those that show the highest Akaike weight values.



Supplementary information to Chapter 3

Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers

Oliva-Vidal, P., Martínez J. M., Sánchez-Barbudo, I., Pablor, R. Camarero., Colomer, M. À., Margalida, A., Mateo, R. 2022. Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers. *Environmental Pollution* 315, 120385.

Table S1. Number of individuals sampled according to species, age and sex.

Species	Age class					Sex	
	Nestling	Juvenile	Subadult	Adult	Total	Females	Males
Griffon vulture	7	-	11	47	65	20	45
Cinereous vulture	16	-	-	-	16	9	7
Bearded vulture	7	7	19	34	67	44	23
Egyptian vulture	33	-	7	27	67	36	31
Red kite	20	8	-	8	36	17	19
Black kite	-	1	-	7	8	6	2
Golden eagle	-	-	-	1	1	1	-
Bonelli's eagle	1	-	-	-	1	1	-

Table S2. Capture systems used for sampling each species included in our active monitoring study to assess avian scavenger exposure to anticoagulant rodenticides in NE Spain.

Species	Method
Griffon vulture	Pull and cannon net Walk-in trap (portable box) Noose carpet Specialized climbers
Cinereous vulture	Pull and cannon net Specialized climbers
Bearded vulture	Pull and cannon net Specialized climbers
Egyptian vulture	Pull and cannon net Noose carpet Specialized climbers
Red kite	Japanese raptor nets with artificial decoy Noose carpet Specialized climbers
Black kite	Pull and cannon net Noose carpet
Golden eagle	Pull net
Bonelli’s eagle	Specialized climbers

Table S3. MS/MS dMRM transition parameters, recovery and limits of quantification (LOQ) for the analysed SGARs and the internal standard (IS).

Compound	**Fragmentation voltage (V)**	**Parent ions**	**Quantifier collision energy (V)**	**Quantifier ions**	**Qualifier collision energy (V)**	**Qualifier ions**	**Average recovery (%)**	**RSD (%)**	**LOQ in blood (ng/ml)**
Bromadiolone	65	525.1	51	79	47	263	94.4	3.3	0.5
					43	250.1			
					47	93.1			
Brodifacoum	30	521.1	47	79	59	143.1	89.0	9.9	0.5
					43	135			
					59	93			
Difenacoum	65	443.2	35	293.1	43	219.1	95.8	4.9	0.07
					39	135			
					60	93.1			
Flocoumafen	65	541.2	27	382.1	39	289.1	91.6	9.0	0.04
					35	174			
					43	161			
Brodifacoum-d_4 (IS)	191	525.1 (526.1)	35	526.1	35	375.1	-	-	-
					35	187			
					35	135			
					35	79			

Table S4. Prevalence (%) and concentrations (ng/ml) [1] of the ΣSGARs concentration > LOQ (i.e., considering only individuals with SGAR levels > LOQ) in the blood of free-living obligate and facultative avian scavengers from the northwest Iberian Peninsula according to age class (nestling, juvenile, subadult and adult).

Species	**Nestling**				**Juvenile**				**Subadult**				**Adult**			
	N	**N+**	**%**	**Mean ± SE [min-max]**	**N**	**N+**	**%**	**Mean ± SE [min-max]**	**N**	**N+**	**%**	**Mean ± SE [min-max]**	**N**	**N+**	**%**	**Mean ± SE [min-max]**
Egyptian v.	33	15	45.45	8.82 ± 2.27 [0.12-28.02]		-	-	-	7	3	42.86	10.51 ± 9.19 [0.52-28.87]	27	25	92.59	5.03 ± 0.85 [1.05-17.71]
Bearded v.	7	3	42.86	2.53 ± 1.10 [0.54-4.35]	7	2	28.57	1.98 ± 1.81 [0.17-3.78]	19	2	10.53	3.44 ± 3.10 [0.37-6.53]	34	7	20.59	1.95 ± 0.57 [0.09-4.47]
Griffon v.	7	ND	-	ND	-	ND	-	-	11	ND	-	ND	47	11	23.40	2.23 ± 1.85 [0.04-20.73]
Cinereous v.	16	1	6.25	0.17	-	-	-	-	-	-	-	-	-	-	-	-
Black kite	-	-	-	-	1	1	100	16.71	-	-	-	-	7	7	100	6.31 ± 2.05 [0.20-16.71]
Red kite	20	11	55.0	7.61 ± 2.04 [0.49-18.44]	8	6	75.0	18.16 ± 6.14 [0.58-45.95]	-	-	-	-	8	7	87.50	16.18 ± 4.22 [1.86-32.30]

[1] Concentrations in birds with values > LOQ.

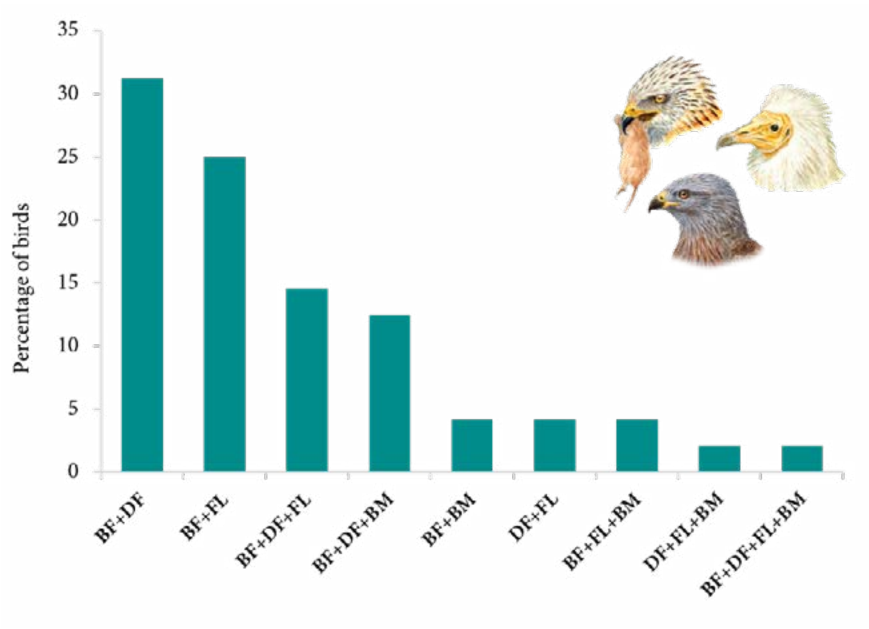


Fig. S1. Percentage of birds containing residues of more than one SGARs in the blood. Regarding SGAR-positive birds (n = 102), we found more than one compound in 48 (47.1%) of the birds. Of these, 31 birds (64.6%) presented with two different compounds; 16 (33.3%) presented with three compounds and one bird (2.1%) presented with four different SGARs. Of all SGAR-positive birds showing more than one SGAR in the blood, 81.2% were Egyptian vultures (n = 25; 58.14% of all), red kites (n = 10; 40%) and black kites (n = 4; 50%). BF (Brodifacoum), DF (Difenacoum), FL (Flocoumafen), BM (Bromadiolone).

Supplementary information to Chapter 4

Bioinspired models for assessing the importance of transhumance and transboundary management in the conservation of European avian scavengers

Margalida, A., Oliva-Vidal, P., Llamas, A., Colomer, M. À. 2018. Bioinspired models for assessing the importance of transhumance and transboundary management in the conservation of European avian scavengers. *Biological Conservation* 228, 321–330.

The study was carried out in northern Spain, where breeding sites for three European vulture species are located,—Eurasian griffon vulture (*Gyps fulvus*), Egyptian vulture (*Neophron percnopterus*), and bearded vulture (*Gypaetus barbatus*). This area is characterized by an abundance of grazing livestock, mainly sheep (*Ovis aries)*, cattle (*Bos taurus*), and horses (*Equus caballus*). Four wild ungulate species are also present, namely red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), southern chamois (*Rupicapra pyrenaica*), and wild boar (*Sus scrofa*).

The study area was divided into five subareas for which detailed data censuses of avian scavengers and wild and domestic ungulates are available. Wild and domestic ungulates are herbivores (except for the omnivorous wild boar) and their carcass remains form the basic food source for the avian scavenger guild (>80% of the diet is based on these species) (Donázar, 1993; Margalida et al., 2009; Margalida et al., 2012).

The study area contains 10 supplementary feeding sites (SFS) in which food is provided principally during the breeding season (winter). These SFS are provided with carcasses and meat remains delivered by farmers and regional administrations, as well as sheep extremities for bearded vultures (Table S1).

The year was divided into two periods: *'winter or reproductive period'* (hereafter *breeding*), which includes the months from October to May, and *'summer or non-reproductive period'* (hereafter *summer*), from June to September.

Table S1. Official Supplementary Feeding Stations (SFS) present in Navarra distributed according to the zone of the study area, food characteristics (bone and meat remains), the temporal distribution (reproductive [breeding] or non-reproductive [summer] period) and amount of biomass (kg of bones and meat) provided. *B_s: bones summer; B_b: bones breeding; M_s: meat summer; M_b: meat breeding.*

Zones	B_s	B_b	M_s	M_b
Northwest (NW)				
Gorramendi	2060	2904	39134	34534
Zudaire	1537	1382	29202	26254
Midwest (MW)				
Zurucuain	1792	1504	34044	28569
Pyrenees (P)				
Napal	88	1618	1663	1406
Garralda	1028	1093	19528	20775
Zandueta	580	371	11026	7042
Middle East (ME)				
Lumbier	4213	4440	74896	68891
Sangüesa	2080	2378	39515	45186
Izagaondoa	645	591	12250	11235
Ribera (R)				
Carcastillo	3850	3200	73148	60797

Since individuals can move from one area to another depending on the resources available, the ecosystem appears to function as a single unit (overall ecosystem) composed of a network of five subunits according to specific data obtained in each area. In this way, whenever there is a lack of food in one of the subareas or the carrying capacity reaches its peak, the individuals move to the nearest suitable area (Fig. 2). For example, if there is an avian scavenger who lacks sufficient resources after carrying out the rules of feeding and controlling for the maximum density of each species, it will move to another environment, running the feeding process and/or density previously unrealized for this animal. If space is not a limiting factor, they will return to the source environment or otherwise colonize a new area. The avian scavengers move to search for food in the peripheral areas (F, E, A and R-CL) when feeding resources in the regular home ranges are insufficient. For the foraging areas, we consider the maximum linear foraging movement from the nest for the griffon vulture to be 90 km (although the model consider that this species can exploit all the study area and peripheral zones), 40 km for the bearded vulture and 15 km for the Egyptian vulture (A.M. unpubl. data). The Table S2 refers to a matrix table that shows the probabilities the species have to visit one zone or another.

Table S2. Matrix table with the probabilities of the possible movements that scavenger birds can make in the different zones of the study area. 1: can access; 0: cannot access.

Species	Zones	NW	MW	Pyrenees	ME	Ribera
Gypaetus barbatus	NW	1	0	1	1	0
	MW	0	1	0	0	0
	Pyrenees	1	0	1	1	0
	ME	1	0	1	1	0
	Ribera	0	0	0	0	1
Neophron percnopterus	NW	1	1	1	1	0
	MW	1	1	0	0	1
	Pyrenees	1	0	1	1	0
	ME	0	0	1	1	0
	Ribera	0	0	0	0	1
Gyps fulvus	NW	1	1	1	1	1
	MW	1	1	1	1	1
	Pyrenees	1	1	1	1	1
	ME	1	1	1	1	1
	Ribera	1	1	1	1	1
Wild ungulates	NW	1	0	0	0	0
	MW	0	1	0	0	0
	Pyrenees	0	0	1	0	0
	ME	0	0	0	1	0
	Ribera	0	0	0	0	1

In the peripheral areas outside of the study area, individuals can obtain the same amount of resources in relation to the values estimated on the same border of the study area (Table S3).

Table S3. Peripheral subareas to which the scavenger birds can have access (1 can access; 0 cannot access). We consider that the amount of food available in each subarea is the same as that offered on the border of the study area. *A*: Aragon; *F*: France; *R-CL*: Rioja-Castilla León; E: Basque Country.

	PHERIPHERAL AREAS							
Zones	**Wild ungulates**				**Domestic ungulates**			
	A	**F**	**R-CL**	**E**	**A**	**F**	**R-CL**	**E**
NW	0	1	0	1	0	1	0	1
MW	0	0	0	1	0	0	0	1
Pyrenees	1	1	0	0	1	1	0	0
ME	1	0	0	0	1	0	0	0
Ribera	1	0	1	0	1	0	1	0

The maximum carrying capacity for wild ungulates and avian scavengers (Table S4) was established according to the data provided by the literature. Estimates are based on the population growth observed during the previous 20 years and are used to adjust the variables of the model (for details see Margalida et al., 2011; Margalida and Colomer, 2012).

The subarea in which the nest is located is considered the core area and the neighboring subareas the potential home range from which each species will obtain their energetic requirements. Thus, in our model, the individuals can move from one area to the other and the ecosystem's local carrying capacity is limited to the appropriate areas and habitats for the different species according to the estimates of Table S4.

Table S4. Maximum carrying capacity (breeding pairs) for scavenger birds and domestic and wild ungulates (individuals) in the different zones of the study area.

Species	MAXIMUM CARRYING CAPACITY				
	NW	MW	Pyrenees	ME	Ribera
Gypaetus barbatus	2	2	10	4	2
Neophron percnopterus	28	34	63	31	45
Gyps fulvus	1330	1202	2088	592	388
Rupicapra pyrenaica	0	0	500	0	0
Cervus elaphus	1913	0	5391	1034	1762
Capreolus capreolus	9567	4898	8984	9305	10575
Sus scrofa	3827	3810	7187	8271	10575
Ovis aries	No limit	No limit	No limit	No limit	No limit
Bos taurus	No limit	No limit	No limit	No limit	No limit
Equus caballus	No limit	No limit	No limit	No limit	No limit

Censuses, dietary habits and demographic parameters

Data on the avian scavenger and wild ungulate populations were obtained through censuses carried out by technicians from the Navarra Government, from the literature and from personal observations from 1994-2011.

During the breeding period, the reproductive period of avian scavengers takes place and egg-laying occurs from December to February (except for the Egyptian vulture, with egg-laying in April). Fledging occurs from June to August (in the case of Egyptian vultures, coinciding with the start of migration), coinciding with the presence of transhumant livestock in mountain areas. During the summer period, the number of livestock in the mountain areas increases significantly as a consequence of transhumant livestock. Thus, food availability differs among seasons being greater during the summer, whereas the energetic requirements of avian scavengers are higher during the breeding season (winter and spring). In addition, to population fluctuations there are also some demographic (i.e., mortality, see Table S5, S6) and biological (i.e., energetic requirements) parameters that vary between the summer and the breeding periods (Table S7).

Table S5. Demographic parameters used to build the model. ARE: average reproductive age; AAFE: average age at which fertility ends; ALE: average life expectancy; FR: female ratio; P: productivity; MAP: maximum annual productivity.

Species	ARE	AAFE	ALE	FR	P	MAP
Gypaetus barbatus	8	20	21	0.65	0.45	1
Neophron percnopterus	5	24	25	0.8	0.75	1
Gyps fulvus	5	24	25	0.7	0.7	1
Rupicapra pyrenaica	2	18	18	0.55	0.75	1
Cervus elaphus	2	20	20	0.5	0.75	1
Capreolus capreolus	1	10	10	0.67	1	1
Sus scrofa	1	4	6	0.5	0.55	4
Ovis aries	2	8	8	0.96	0.75	1
Bos taurus	2	9	9	0.9	0.9	1
Equus caballus	3	9	20	0.97	0.9	1

Table S6. Pre-adult and adult mortality (expressed in %) of the species considered in the study area according to the two phenological periods established. P_s: *pre-adult summer;* P_b: *pre-adult breeding;* A_s: *adult summer;* A_b: *adult breeding. M indicates males.*

Species	P_s	P_b	A_s	A_b
Gypaetus barbatus	4	2	1	3
Neophron percnopterus	4	2	5	2
Gyps fulvus	4	2	5	2
Rupicapra pyrenaica	40	20	4	2
Cervus elaphus (M)	23	11	4	2
Capreolus capreolus	39	19	4	2
Sus scrofa	9	5	7	3
Ovis aries	10	5	2	1
Bos taurus	4	2	3	2
Equus caballus	2	1	1	0

Table S7. Annual energy requirements per breeding pair (expressed in kg) of the three species of obligate scavenger birds present in the study area (for details see Material and Methods). B_s: bones summer, Ms: meat summer, Bb: bones breeding, Mb: meat breeding.

Species	**B_s**	**M_s**	**B_b**	**M_b**
Gypaetus barbatus	70	24	160	54
Neophron percnopterus	0	60	0	40
Gyps fulvus	0	132	0	272

With respect to interspecific differences in dietary habits, the diet of the bearded vulture is based on bone remains, whereas the diet of griffon and Egyptian vultures is based on meat provided by wild and domestic ungulates and to a lesser degree, small animal carcasses. However, because some cadavers cannot be used by vultures as a consequence of landscape (i.e., forestry areas), we considered only a proportion of the carrion to be available (Table S8).

Table S8. Proportion of biomass from the carcasses of domestic ungulates that birds can access. *O: Ovis aries, B: Bos taurus, E: Equus caballus.*

	NW			**PYRENNEES**			**RIBERA**			**NW**			**ME**		
	O	**B**	**E**	**O**	**B**	**E**	**O**	**B**	**E**	**O**	**B**	**E**	**O**	**B**	**E**
Summer	1	0.75	1	1	0.75	1	0.5	0	0	1	0.75	1	0.5	0.75	1
Breeding	0.75	0.5	0.75	0	0	0	0.5	0	0	0.5	0.5	1	0.5	0.5	1

In the case of bearded vultures, remains provided by larger species are not selected, reducing the opportunity for using the remains from adult horses and cows. The model considers that only young individuals of these species are selected. In the case of meat and bone remains, we also reduced the true exploitation of the carrion available as a consequence of accessibility to forest areas (Tables S8, S9). For example, we assume that for forestry species as wild boar or roe deer, the percentage of available biomass is 25% whereas in species inhabiting open areas (e.g., Pyrenean chamois, red deer) it increase until 50-60%.

Table S9. Use of the biomass remains (expressed in kg) that the carcasses of the species present in the study area provide according to their category (meat vs. bones). Data obtained from Margalida et al. (2011); Margalida and Colomer (2012); and unpubl. data. M indicates male.

Species	Bone pre-adults	Meat pre-adults	Bone adults	Meat adults	% bones useful	% meat useful
Rupicapra pyrenaica	3	4	6	24	50	50
Cervus elaphus M	12	15	24	96	60	60
Capreolus capreolus	1	4	1	19	25	25
Sus scrofa	4	6	12	60	25	25
Ovis aries	3	4	7	38	35	35
Bos taurus	10	60	6	518	2.5	30
Equus caballus	10	60	9	891	2.5	40

In addition, for bearded and Egyptian vultures, small mammals are important in the diet (Donázar, 1993; Margalida et al., 2009; Margalida et al., 2012). Because the population trends in these species have been increasing or stable, we consider the ecosystem to provide enough resources to cover their energetic requirements. Thus, we established a minimum of available biomass provided by small animal carcasses (i.e., rabbits, reptiles, small birds) as a supplement to the diet of all avian scavengers (4,500 kg during the breeding season and 2,000 kg in summer, for details see Margalida and Colomer, 2012). The remaining biomass is obtained from the food provided by the feeding stations.

Population Dynamic P System Model

Population Dynamic P System models (PDPs) are a variant of P systems, inspired by the functioning of cells and capable of performing different processes simultaneously in a synchronized way. PDP are a simplification of cells in which the cell is represented by a skin membrane that separates it from the environment in which it is located and with which it can communicate. Inside, there are different spaces in which different activities are carried out. Membranes internal to the skin membrane bound these spaces. The organelles that the cells contain are represented by objects that are the input of the models. These objects evolve through rules that describe the actual processes either sequentially or in parallel. Most of these processes are random, so the rules have associated probabilities. The definition of the parameters used appear in Table S10.

Table S10. Definition of the parameters used for the population dynamics model applied in the study area.

Parameters	Definition
g(i,3)	Age at which fertility begins in species *i*.
g(i,4)	Age at which fertility ends in species *i*.
g(i,5)	Average life expectancy of the species *i*.
k(i,1)	Proportion of females (sex-ratio) of the species *i*.
k(i,2)	Fertility ratio of the species *i*.
k(i,3)	Number of descendants (individuals) of the species *i*.
m(i,1,c)	Pre-adult mortality of the species *i* and period *c*.
m(i,2,c)	Adult mortality of the species *i* and period *c*.
h(i,1)	% males hunted of the species *i*
h(i,2)	% females hunted of the species *i*
hp(i)	Carcasses of the species *i* that remains in the field.
f(i,1)	Bones of young animals left by the species *i*.
f(i,2)	Meat of young animals left by the species *i*.
f(i,3)	Bones of adult animals left by the species *i*.
f(i,4)	Meat of adult animals left by the species *i*.
f(i,5)	% of usable bones left by the species *i*.
f(i,6)	% of useable meat left by the species *i*.
f(i,7)	Bones summer consumed by the species *i*.
f(i,8)	Meat summer consumed by the species *i*.
f(i,9)	Bones breeding consumed by the species *i*.
f(i,10)	Meat breeding consumed by the species *i*.
TM(i)	Type of movement of the species *i* (See Table S2).
p(TM(i), e,s)	Probability that species *i* move from the *e* area to *s*.
ff(e,k,j)	Amount of biomass from domestic or wild ungulates to which the scavengers from the environment *e* from the peripheral zone *k* have access. *j*= 1: wild, 2: domestic.
m1(i,1)	Bones summer SFS *i*.
m1(i,2)	Bones breeding SFS *i*.
m1(i,3)	Meat summer SFS *i*.
m1(i,4)	Meat breeding SFS *i*.
m1(i,5)	Biomass from small animals summer SFS *i*.
m1(i,6)	Biomass from small animals breeding SFS *i*.
q(e,i,j)	Total animals (not included those that realize movements in breeding and summer periods), zone *e*, species *i*, age *j*.
qa(e,i,j,k)	Total animals zone *e*, species *i*, age *j*; *k*=1 present in summer, *k*=2 leaves in breeding.

Modeling processes

In the ecosystem, three obligate avian scavenger species, four species of wild ungulates, three species of domestic ungulates and the five zones in which the study area was divided interact. As in many other areas with extensive livestock, there is an important transhumance and this movement of domestic animals involves significant fluctuations in the amount of energetic resources available to scavenger birds. Apart from the traditional transhumance, the Navarre community has a forest regime ("facerías") that affects an important area of the territory and that increases the annual movement of domestic animals between the five zones and the other peripheral areas.

The processes to be taken into account in the model are:

Dynamics of the three species of scavenger birds, namely the processes of reproduction, feeding, mortality, carrying capacity and the response to the lack of resources. In the case of a lack of resources (space or food), the scavengers can move (Fig. 2) to other areas or can look for food in areas peripheral to the study area. Individuals can move beyond an area in search of food or space.

Dynamics of the four species of wild ungulates, including the reproduction process, mortality, hunting and carrying capacity that the area can support. The feeding will not be modeled since it is not a limiting resource. It is necessary to assess the biomass available to the scavenger birds provided by dead wild and domestic ungulates. Their accessibility varies depending on the location of death.

Dynamics of the three species of domestic ungulates: the same processes are modeled as in the case of wild ungulates, except hunting, and the process of transhumance is added.

It is necessary to record the external supply to the 10 SFS distributed among the five zones. The food characteristics will depend on the type of SFS and time of year. Since the parameters that affect the majority of the processes vary according to the time of the year, the summer period will be differentiated from winter (breeding period).

Input of model and parameters to be taken into account

The inputs of the model are the populations of the different species in each of the areas, the biological parameters of each species, the external input of biomass by humans at the SFS and the network of the possible foraging movements among zones.

The output of the model includes predictions of the population size, and trends for each species and provided over each area and year.

Sequencing and parallelization of the processes

The scheme proposed for the model is reproduced in the Figure 2.

Designing of the model

The four components of the PDP model must be defined:

1. Environments

Six environments have been defined, one for each of the zones in which the ecosystem has been divided, and a virtual one that is used to simplify the movement of scavengers between zones when there is a lack of resources.

2. Structure of membranes

$$\mu = [[[\quad]_3]_1[[\quad]_4]_2]_0$$

The structure consists of five membranes; within the skin membrane there is a repeated structure consisting of two membranes, 1 and 2, inside of which there is another membrane labeled 3 and 4, respectively. The repeated internal structure serves to model the summer and breeding period separately.

3. Initial objects

The skin membrane, labeled with 0, contains the following objects:

$$\begin{aligned}
\mu_0 = &\left\{XA_{ij}^{q_{kij}}, AC_{ik}^{q_{kij}}, 8 \leq i \leq N, 1 \leq k \leq E, 1 \leq j \leq g_{i,5}\right\} \\
&\cup \left\{X_{ij}^{q_{kij}}, 4 \leq i \leq 7, 1 \leq k \leq E, 1 \leq j \leq g_{i,5}\right\} \\
&\cup \left\{X_{ij}^{q_{kij}}, 1 \leq i \leq 3, 1 \leq k \leq E, g_{i,3} \leq j \leq g_{i,5}\right\} \\
&\cup \left\{XJ_{ij}^{q_{kij}}, 1 \leq i \leq 3, 1 \leq k \leq E, 1 \leq j \leq g_{i,53}\right\} \\
&\cup \left\{XS_{ijc}^{qa_{kijc}}, AC_{ik}^{q_{kijc}}, 8 \leq i \leq N, 1 \leq k \leq E, 1 \leq j \leq g_{i,5}, 1 \leq c \leq 2\right\} \\
&\cup \{d_i, , 1 \leq i \leq 7, 1 \leq k \leq E\} \cup \{co_1, 1 \leq k \leq E + 1\}
\end{aligned}$$

The $X_{i,j}$, $XJ_{i,j}$, $XA_{i,j}$, and $XS_{i,j,c}$ objects are associated with wild animals, floating scavengers, domestic ungulates that remain year-round in the same area and domestic ungulates that move seasonally, respectively. The first index indicates the species that it represents and the second, the age. In the case of the objects associated with the transhumant animals, the third index indicates the time of year.

Access to dead domestic ungulates depends on the characteristics of the area where the carcass is located and the time of year. In order to establish this difference the object used is AC.

The objects d and a control the maximum densities of the animals and the object co activates the execution of the processes.

Objects on the membrane labeled 1 and 2: $\mu_1 = \{R\}$, $\mu_2 = \{R\}$, refer only to an object (counter) that allows synchronization of the rules.

The following objects are in the membranes labeled 3 and 4: $\mu_3 = \{F0_k, 1 \leq k \leq E\}$, $\mu_4 = \{F0_k, 1 \leq k \leq E\}$. The object F0 allows the generation of objects associated with resources external to the ecosystem.

4. Evolution rules

The model consists of a loop (Fig. 2), which runs twice annually. Running the loop at once involves 21 steps. The number of rule types that are applied are 216, which are split by 37417 rules.

The model starts executing the process of reproduction (egg laying). The objects associated with the animals $X_{i,j}$ enter membrane 1 where they evolve to objects of type $Z_{i,j}$ and generate new objects of the type $Z_{i,0}$ corresponding to animals that are born.

In the next step the mortality process is executed. Some of the objects $Z_{i,j}$ when entering the membrane labeled 3 dissolve in the case of corresponding scavenger birds while in the case of corresponding ungulates evolve to objects M and B (meat and bones that leave when they die).

Within the membrane 3, there are objects associated with food and objects associated with the scavengers. Therefore, the process that is executed in this step is related with feeding. Objects associated with animals that have sufficient resources evolve to objects of type $W_{i,j}$. The process of feeding is based on a hierarchy with the griffon vulture the last to eat.

The trophic resources that remain as well as the objects associated with animals that have not eaten leave the environment corresponding to their physical area and enter the virtual environment labeled 6. In the internal membrane of this virtual environment, the feeding process is carried out as a function of the existence of resources and possibilities of displacement of the birds (Fig. 2). After this second feeding process, most animals return to the starting environment provided there are no space limitations in the environment. The loop ends by restoring the initial configuration, i.e., objects associated with unconsumed biomass are removed and objects associated with surviving animals evolve to $X_{i,j}$. The second round of the loop corresponds to the non-reproductive period (summer). After this second loop, the simulation of the second year starts and so on.

Rules with counters that allow the synchronization of the model.

$r_1 \equiv [R]_c^+ \rightarrow [R_0]_c^0, 1 \leq c \leq 2.$

$r_2 \equiv [R_i \rightarrow R_{i+1}]_c^0, 0 \leq i \leq 14, i \neq 3,4,5,12,13, 1 \leq c \leq 2.$

$r_3 \equiv R_3[\quad]_{c+2}^{+} \rightarrow R_4[b]_{c+2}^{0}, 1 \leq c \leq 2.$

$r_4 \equiv [R_4]_c^0 \rightarrow [R_5]_c^-, 1 \leq c \leq 2.$

$r_5 \equiv [R_5]_c^- \rightarrow [R_6]_c^0, 1 \leq c \leq 2.$

$r_6 \equiv [R_{12}]_c^0 \rightarrow [R_{13}]_c^-, 1 \leq c \leq 2.$

$r_7 \equiv [R_{13}]_c^- \rightarrow [R_{14}]_c^0, 1 \leq c \leq 2.$

$r_8 \equiv R_{15}[\quad]_{c+2}^{0} \rightarrow R\ [b]_{c+2}^{-}, 1 \leq c \leq 2.$

Rules that allow the control of the maximum carrying capacity of each species.

$r_{e1} \equiv \left(d_i \rightarrow d'_i, a_i^{d(i,k)\cdot 0.9}, e_i^{d(i,k)\cdot 0.2}\right)_k, 1 \leq k \leq E, 1 \leq i \leq N.$

$r_9 \equiv d'_i[\ \ ]_0^0 \rightarrow [d_i]_0^0, 1 \leq i \leq N.$

$r_{10} \equiv a_i[\ \ ]_0^0 \rightarrow [a_i]_0^0, 1 \leq i \leq N.$

$r_{11} \equiv e_i[\ \ ]_0^0 \xrightarrow{0.5} [a_i]_0^0, 1 \leq i \leq N.$

$r_{12} \equiv e_i[\ \ ]_0^0 \xrightarrow{0.5} [\ \ ]_0^0, 1 \leq i \leq N.$

$r_{13} \equiv d_i[\ \ ]_c^+ \rightarrow [d'_i]_c^0, 1 \leq i \leq N, 1 \leq c \leq 2.$

$r_{14} \equiv [d'_i]_c^- \rightarrow [d_i]_c^0, 1 \leq i \leq N, 1 \leq c \leq 2.$

$r_{15} \equiv [d_i]_c^- \rightarrow d'_i[\ \ ]_c^0, 1 \leq i \leq N, 1 \leq c \leq 2.$

$r_{16} \equiv [d'_i]_0^0 \rightarrow d_i[\ \ ]_0^0, 1 \leq i \leq N.$

Step 1. The object *co* activates the execution of the processes; when it takes the value 1, those corresponding to the non-reproductive period begin and when it takes value 2 it corresponds to the reproductive period.

$r_{17} \equiv co_c[\ \ ]_c^0 \rightarrow [co_c]_c^+, 1 \leq c \leq 2.$

Step 2. The processes corresponding to the non-reproductive period are executed in membranes 1 and 3 while those corresponding to the reproductive period are in membranes 2 and 4.

$$r_{18} \equiv co_c[\ \ ]_{c+2}^0 \rightarrow [co_c]_{c+2}^+, 1 \leq c \leq 2.$$

The objects associated with animals and those that will allow the control of the maximum carrying capacity of each species enter the membrane 1 or 2, depending on the period being executed.

$r_{19} \equiv a_i[\ \ ]_c^+ \rightarrow [a_i]_c^0, 1 \leq i \leq N, 1 \leq c \leq 2.$

$r_{20} \equiv X_{i,j}[\ \ ]_c^+ \rightarrow \left[X_{i,j}\right]_c^0, 0 \leq j \leq g_{i,5}, 1 \leq i \leq N, 1 \leq c \leq 2.$

$r_{21} \equiv XJ_{i,j}[\ \ ]_c^+ \rightarrow \left[X_{i,j}\right]_c^0, 0 \leq j \leq g_{i,3}, 1 \leq i \leq 4, 1 \leq c \leq 2.$

$r_{22} \equiv XA_{i,j}[\ \ ]_c^+ \rightarrow XA_{i,j}\left[XA_{i,j}\right]_c^0, 0 \leq j \leq g_{i,5}, 1 \leq i \leq N, 1 \leq c \leq 2.$

$r_{23} \equiv XS_{i,j,c}[\ \ ]_c^+ \rightarrow XS_{i,j,c}\left[XA_{i,j}\right]_c^0, 0 \leq j \leq g_{i,5}, 1 \leq i \leq N, 1 \leq c \leq 2.$

Each domestic ungulate is associated with one object AC.

$r_{24} \equiv AC_{i,k}[\ \ ]_c^+ \rightarrow AC_{i,k}\left[AC_{i,k}\right]_c^0, 8 \leq i \leq N, 1 \leq c \leq 2, 1 \leq k \leq E.$

Step 3. In this step, the objects associated with the biomass provided by humans in the supplementary feeding stations are generated, as well as those corresponding to small animals that can be consumed.

$$r_{25} \equiv \left[F0_k \rightarrow C_0^{\beta(k,c)}, H_0^{\alpha(k,c)}, M^{\alpha(k,c)}, B^{\beta(k,c)}, S^{\lambda(k,c)}, F_k\right]_c^+, 1 \le c \le 2, 1 \le k \le E.$$

$$r_{26} \equiv \ a_i[\quad]_{c+2}^+ \rightarrow [a_i]_{c+2}^+, 1 \le c \le 2, 1 \le i \le N.$$

Rules of reproduction

Non-reproductive period

$$r_{27} \equiv \left[X_{i,j} \xrightarrow{1-\varphi(i,j)} Z_{i,j}\right]_1^0, g_{i,3} \le j < \ g_{i,4}, 1 \le i \le N.$$

$$\varphi(i,j) = \frac{k_{i,1}(1-ht_{i,2})^j}{k_{i,1} \cdot (1-ht_{i,2})^j + (1-k_{i,1} \cdot (1-ht_{i,1}))^j}$$

$$r_{28} \equiv \left[XA_{i,j} \xrightarrow{1-k_{i,2} \cdot k_{i,1}} ZS_{i,j}\right]_1^0, g_{i,3} \le j < \ g_{i,4}, 1 \le i \le N.$$

$$r_{29} \equiv \left[X_{ij} \xrightarrow{\varphi(i,j)} Z_{ij}, Z_{i0}^{k_{i,3}}\right]_1^0, g_{i,3} \le j < \ g_{i,4}, 1 \le i \le N.$$

$$r_{30} \equiv \left[XA_{i,j} \xrightarrow{k_{i,2} \cdot k_{i,1}} ZS_{i,j} Z_{i0}^{k_{i,3}}\right]_1^0, g_{i,3} \le j < \ g_{i,4}, 1 \le i \le N.$$

$$r_{31} \equiv \left[X_{i,j} \xrightarrow{(1-k_{i,2}) \cdot \varphi(i,j)} Z_{i,j}\right]_1^0, g_{i,3} \le j < \ g_{i,4}, 1 \le i \le N.$$

$$r_{32} \equiv \left[X_{i,j} \rightarrow Z_{i,j}\right]_1^0, g_{i,4} \le j \le \ g_{i,5}, 1 \le i \le N.$$

$$r_{33} \equiv \left[XA_{i,j} \rightarrow ZS_{i,j}\right]_1^0, g_{i,4} \le j \le \ g_{i,5}, 1 \le i \le N.$$

$$r_{34} \equiv \left[X_{i,j} \rightarrow Z_{i,j}\right]_1^0, 0 \le j < \ g_{i,3}, 1 \le i \le N.$$

$$r_{35} \equiv \left[XA_{i,j} \rightarrow ZS_{i,j}\right]_1^0, 0 \le j < \ g_{i,3}, 1 \le i \le N.$$

Reproductive period:

$$r_{36} \equiv \left[X_{i,j} \rightarrow Z_{i,j}\right]_2^0, 0 \le j \le \ g_{i,5}, 1 \le i \le N.$$

$$r_{37} \equiv \left[XA_{i,j} \rightarrow ZS_{i,j}\right]_2^0, 0 \le j \le \ g_{i,5}, 1 \le i \le N.$$

Step 4. Mortality process: natural and related to hunting activity.

$$r_{38} \equiv Z_{ij,}\,[\quad]_{c+2}^+ \xrightarrow{1-m_{i,1,c}} \left[Z_{i,j}\right]_{c+2}^+, 0 \le j < \ g_{i,3}, 1 \le i \le 4, 1 \le c \le 2.$$

$r_{39} \equiv Z_{i,j}\ [\quad]^{+}_{c+2} \xrightarrow{m_{i,1,c}} [\quad]^{+}_{c+2}, 0 \leq j < \ g_{i,3}, 1 \leq i \leq 4, 1 \leq c \leq 2.$

$r_{40} \equiv Z_{i,j}\ [\quad]^{+}_{c+2} \xrightarrow{1-m_{i,1,c}} \left[Z_{i,j}\right]^{+}_{c+2}, 0 \leq j < \ g_{i,2}, 5 \leq i \leq 7, 1 \leq c \leq 2.$

$r_{41} \equiv ZS_{i,j}AC_{i,k}\ [\quad]^{+}_{c+2} \xrightarrow{1-m_{i,1,c}} [\quad]^{+}_{c+2}, 1 \leq j < \ g_{i,2}, 8 \leq i \leq N, 1 \leq c \leq 2, 1 \leq k \leq E.$

$r_{42} \equiv ZS_{i,0}\ [\quad]^{+}_{c+2} \xrightarrow{1-m_{i,1,c}} [\quad]^{+}_{c+2}, 8 \leq i \leq N, 1 \leq c \leq 2.$

$r_{43} \equiv Z_{i,j}\ [\quad]^{+}_{c+2} \xrightarrow{m_{i,1,c}} \left[H_i^{f_{i,1}\cdot f_{i,5}} C_i^{f_{i,2}\cdot f_{i,6}} B_i^{f_{i,1}\cdot f_{i,5}} M_i^{f_{i,2}\cdot f_{i,6}}\right]^{+}_{c+2}, 0 \leq j < \ g_{i2},\ 5 \leq i \leq 7, 1 \leq c \leq 2.$

$r_{44} \equiv ZS_{i,j}\ AC_{i,k}[\quad]^{+}_{c+2} \xrightarrow{m_{i,1,c}} \left[H_i^{f_{i,1}\cdot fa_{i,c,k}} C_i^{f_{i,2}\cdot fa_{i,c,k}} B_i^{f_{i,1}\cdot fa_{i,c,k}} M_i^{f_{i,2}\cdot fa_{i,c,k}}\right]^{+}_{c+2}, 1 \leq j < \ g_{i,2}, 8 \leq i \leq N, 1 \leq c \leq 2, 1 \leq k \leq E.$

$r_{45} \equiv ZS_{i,0}\ [\quad]^{+}_{c+2} \xrightarrow{m_{i,1,c}} \left[H_i^{f_{i,1}} C_i^{f_{i,2}} B_i^{f_{i,1}} M_i^{f_{i,2}}\right]^{+}_{c+2},\ 8 \leq i \leq N, 1 \leq c \leq 2.$

$r_{46} \equiv Z_{i,j}\ [\quad]^{+}_{c+2} \xrightarrow{1-m_{i,2,c}} \left[Z_{ij}\right]^{+}_{c+2}, g_{i,3} \leq j < g_{i,5}, 1 \leq i \leq 4, 1 \leq c \leq 2.$

$r_{47} \equiv Z_{i,j}\ [\quad]^{+}_{c+2} \xrightarrow{m_{i,2,c}} [\quad]^{+}_{c+2}, g_{i,3} \leq j < g_{i,5}, 1 \leq i \leq 4,1 \leq c \leq 2.$

$r_{48} \equiv Z_{i,j}\ [\quad]^{+}_{c+2} \xrightarrow{1-m_{i,2,c}-(\varphi_{i,j}\cdot ht_{i,2}+(1-\varphi_{i,j)}\cdot ht_{i,1})} \left[Z_{i,j}\right]^{+}_{c+2}, g_{i,2} \leq j < g_{i,5},\ 5 \leq i \leq 7, 1 \leq c \leq 2.$

$r_{49} \equiv ZS_{i,j}AC_{i,k}\ [\quad]^{+}_{c+2} \xrightarrow{1-m_{i,2,c}} [\quad]^{+}_{c+2},\ g_{i,2} \leq j \leq g_{i,5}, 8 \leq i \leq N, 1 \leq c \leq 2, 1 \leq k \leq E.$

$r_{50} \equiv Z_{i,j}\ [\quad]^{+}_{c+2} \xrightarrow{m_{i,2,c}} \left[H_i^{f_{i,3}\cdot f_{i,5}} C_i^{f_{i,4}\cdot f_{i,6}} B_i^{f_{i,3}\cdot f_{i,5}} M_i^{f_{i,4}\cdot f_{i,6})}\right]^{+}_{c+2}, 5 \leq i \leq 7, g_{i2} \leq j < g_{i5}, 1 \leq c \leq 2.$

$r_{51} \equiv ZS_{ij}AC_{ik}\ [\quad]^{+}_{c+2} \xrightarrow{m_{i,2,c}} \left[H_i^{f_{i,3}\cdot fa_{i,c,k}} C_i^{f_{i,4}\cdot fa_{i,c,k}} B_i^{f_{i,3}\cdot fa_{i,c,k}} M_i^{f_{i,4}\cdot fa_{i,c,k}}\right]^{+}_{c+2},\ g_{i,2} \leq j \leq g_{i,5}, 8 \leq i \leq N, 1 \leq c \leq 2, 1 \leq k \leq E.$

$r_{52} \equiv \ Z_{ij}\ [\quad]^{+}_{c+2} \xrightarrow{\varphi_{i,j}\cdot ht_{i,2}+(1-\varphi_{i,j)}\cdot ht_{i,1}} \left[H_i^{f(i,3)\cdot f(i,5)\cdot h'_i} C_i^{f(i,4)\cdot f(i,6)\cdot h'_i} B_i^{f(i,3)\cdot f(i,5)\cdot h'_i} M_i^{f(i,4)\cdot f(i,6)\cdot h'_i}\right]^{+}_{c+2}, 5 \leq i \leq 7, g_{i2} \leq j < g_{i5}, 1 \leq c \leq 2.$

Senescence: mortality when the estimated maximum life expectancy is reached.

$r_{53} \equiv Z_{i,g_{i,5}}\ [\quad]^{+}_{3} \xrightarrow{0.333} \left[H_i^{f(i,3)\cdot f(i,5)} C_i^{f(i,4)\cdot f(i,6)} B_i^{f(i,3)\cdot f(i,5)} M_i^{f(i,4)\cdot f(i,6)}\right]^{+}_{c+2}, 1 \leq i \leq N.$

$r_{54} \equiv Z_{i,g_{i,5}}\ [\quad]^{+}_{3} \xrightarrow{0.667} \left[Z_{i,g_{i,5}}\right]^{+}_{3}, 1 \leq i \leq N.$

$r_{55} \equiv Z_{i,g_{i,5}}\ [\quad]^{+}_{4} \rightarrow \left[H_i^{f(i,3)\cdot f(i,5)} C_i^{f(i,4)\cdot f(i,6)} B_i^{f(i,3)\cdot f(i,5)} M_i^{f(i,4)\cdot f(i,6)}\right]^{+}_{4}, 1 \leq i \leq N.$

Step 5. Rules related to the feeding process

Non-adult scavengers:

$$r_{56} \equiv \left[Z_{i,j} B^{\frac{f_{i,7}}{2}} M^{\frac{f_{i,8}}{2}} \rightarrow W_{i,j} \right]_3^+ , 0 \le j < g_{1,3} - 1, 1 \le i \le 2;$$

$$r_{57} \equiv \left[Z_{i,j} B^{\frac{f_{i,9}}{2}} M^{\frac{f_{i,10}}{2}} \rightarrow W_{i,j} \right]_4^+ , 0 \le j < g_{1,3} - 1, 1 \le i \le 2;$$

$$r_{58} \equiv \left[Z_{i,j} B^{\frac{f_{i,7}}{2}} S^{\frac{f_{i,8}}{2}} \rightarrow W_{i,j} \right]_3^+ , 0 \le j < g_{1,3} - 1, 1 \le i \le 2;$$

$$r_{59} \equiv \left[Z_{i,j} B^{\frac{f_{i,9}}{2}} S^{\frac{f_{i,10}}{2}} \rightarrow W_{i,j} \right]_4^+ , 0 \le j < g_{1,3} - 1, 1 \le i \le 2;$$

Non-adult scavengers evolve to adults:

$$r_{60} \equiv \left[Z_{i,g_{1,3}-1} a_i B^{\frac{f_{i,7}}{2}} M^{\frac{f_{i,8}}{2}} \rightarrow W_{i,g_{1,3}-1} \right]_3^+ , 0 \le j < g_{1,3} - 1, 1 \le i \le 2;$$

$$r_{61} \equiv \left[Z_{i,g_{1,3}-1} a_i \; B^{\frac{f_{i,9}}{2}} M^{\frac{f_{i,10}}{2}} \rightarrow W_{i,g_{1,3}-1} \right]_4^+ , 1 \le i \le 2;$$

$$r_{62} \equiv \left[Z_{i,g_{1,3}-1} \; a_i \, B^{\frac{f_{i,7}}{2}} S^{\frac{f_{i,8}}{2}} \rightarrow W_{i,g_{1,3}-1} \right]_3^+ , 1 \le i \le 2;$$

$$r_{63} \equiv \left[Z_{i,g_{1,3}-1} a_i B^{\frac{f_{i,9}}{2}} S^{\frac{f_{i,10}}{2}} \rightarrow W_{i,g_{1,3}-1} \right]_4^+ , 1 \le i \le 2;$$

Adult scavengers:

$$r_{64} \equiv \left[Z_{i,j} a_i B^{f_{i,7}} M^{f_{i,8}} \rightarrow W_{i,j} \right]_3^+ , g_{1,3} \le j \le g_{1,5}, 1 \le i \le 2;$$

$$r_{65} \equiv \left[Z_{i,j} a_i B^{f_{i,9}} M^{f_{i,10}} \rightarrow W_{i,j} \right]_4^+ , g_{1,3} \le j \le g_{1,5}, 1 \le i \le 2;$$

$$r_{66} \equiv \left[Z_{i,j} a_i B^{f_{i,7}} S^{f_{i,8}} \rightarrow W_{i,j} \right]_3^+ , g_{1,3} \le j \le g_{1,5}, 1 \le i \le 2;$$

$$r_{67} \equiv \left[Z_{i,j} a_i B^{f_{i,9}} S^{f_{i,10}} \rightarrow W_{i,j} \right]_4^+ , g_{1,3} \le j \le g_{1,5}, 1 \le i \le 2;$$

When the bearded vulture has no access to meat, it can only feed on bones.

$$r_{68} \equiv \left[Z_{1,j} B^{\frac{f_{i,7}}{2} + \frac{f_{i,8}}{2}} \rightarrow W_{1,j} \right]_3^+ , 0 \le j < g_{1,3} - 1;$$

$$r_{69} \equiv \left[Z_{1,g_{1,3}-1} a_1 B^{\frac{f_{i,7}}{2} + \frac{f_{i,8}}{2}} \rightarrow W_{1,g_{1,3}-1} \right]_3^+ ;$$

$$r_{70} \equiv \left[Z_{1,j} a_1 B^{f_{i,7}+f_{i,8}} \rightarrow W_{1,j}\right]_3^+, g_{1,3} \leq j \leq g_{1,5};$$

$$r_{71} \equiv \left[Z_{1,j} B^{\frac{f_{i,9}}{2}+\frac{f_{i,10}}{2}} \rightarrow W_{1,j}\right]_4^+, 0 \leq j < g_{1,3} - 1;$$

$$r_{72} \equiv \left[Z_{1,g_{1,3}-1} a_1 B^{\frac{f_{i,9}}{2}+\frac{f_{i,10}}{2}} \rightarrow W_{1,g_{1,3}-1}\right]_4^+;$$

$$r_{73} \equiv \left[Z_{1,j} a_1 B^{f_{i,9}+f_{i,10}} \rightarrow W_{1,j}\right]_4^+, g_{1,3} \leq j \leq g_{1,5};$$

The bearded and Egyptian vultures are the first to arrive at the carrion and/or to feed with respect to the griffon vulture.

$$r_{74} \equiv \left[Z_{3,j} \rightarrow Z''_{3,j}\right]_{c+2}^+, 0 \leq j \leq g_{3,5}, 1 \leq c \leq 2;$$

There is no food limitation for ungulates.

$$r_{75} \equiv \left[Z_{i,j} a_i \xrightarrow{g_{i,1}} W_{i,j}\right]_{c+2}^+, 0 \leq j \leq g_{i,5}, 4 \leq i \leq N, 1 \leq c \leq 2;$$

Step 6. The griffon vulture eats

$$r_{76} \equiv \left[Z''_{3,j} B^{\frac{f_{3,7}}{2}} M^{\frac{f_{3,8}}{2}}\right]_3^+ \rightarrow \left[W_{3,j}\right]_3^0, 0 \leq j < g_{3,3} - 1;$$

$$r_{77} \equiv \left[Z''_{3,j} B^{\frac{f_{3,9}}{2}} M^{\frac{f_{3,10}}{2}}\right]_4^+ \rightarrow \left[W_{3,j}\right]_4^0, 0 \leq j < g_{3,3} - 1;$$

$$r_{78} \equiv \left[Z''_{3,g_{3,3}-1} a_3\, B^{\frac{f_{3,7}}{2}} M^{\frac{f_{3,8}}{2}}\right]_3^+ \rightarrow \left[W_{3,g_{3,3}-1}\right]_3^0;$$

$$r_{79} \equiv \left[Z''_{3,g_{3,3}-1} a_3\, B^{\frac{f_{3,9}}{2}} M^{\frac{f_{3,10}}{2}}\right]_4^+ \rightarrow \left[W_{3,g_{3,3}-1}\right]_4^0;$$

$$r_{80} \equiv \left[Z''_{3,j}\; a_3\; B^{f_{3,7}} M^{f_{3,8}}\right]_3^+ \rightarrow \left[W_{3,j}\right]_3^0, g_{3,3} \leq j \leq g_{3,5};$$

$$r_{81} \equiv \left[Z''_{3,j}\; a_3\; B^{f_{3,9}} M^{f_{3,10}}\right]_4^+ \rightarrow \left[W_{3,j}\right]_4^0, g_{3,3} \leq j \leq g_{3,5};$$

Step 7. Objects associated with animals that did not have sufficient resources change environments (go to other areas) in search of resources that have been scarce as well as objects associated with surplus resources.

$$r_{82} \equiv \left[Z_{i,j}\right]_{c+2}^0 \xrightarrow{g_{i,1}} B'^{\,f_{i,3} \cdot f_{i,5}}_{c+2} M^{f_{i,4} \cdot f_{i,6}}\, H''^{\,f_{i,3} \cdot f_{i,5}}_{i,c+2} C'^{\,f_{i,4} \cdot f_{i,6}}_{i} \left[H'^{\,f_{i,3} \cdot f_{i,5}}_{i} C'^{\,f_{i,4} \cdot f_{i,6}}_{i}\right]_{c+2}^0, g_{i,2} \leq j \leq g_{i,5}, 4 \leq i \leq N, 1 \leq c \leq 2;$$

$$r_{83} \equiv \left[Z_{i,j}\right]_{c+2}^{0} \xrightarrow{g_{i,1}} B'^{f_{i,1} \cdot f_{i,5}}_{c+2} M^{f_{i,2} \cdot f_{i,6}} H''^{f_{i,1} \cdot f_{i,5}}_{i,c+2} C'^{f_{i,2} \cdot f_{i,6}}_{i} \left[H'^{f_{i,1} \cdot f_{i,5}}_{i} C'^{f_{i,2} \cdot f_{i,6}}_{i}\right]_{c+2}^{0}, 0 \leq j < g_{i,2}, 4 \leq i \leq N, 1 \leq c \leq 2;$$

$$r_{84} \equiv \left[Z_{i,j}\right]_{c+2}^{0} \rightarrow Z'_{i,j,c+2}[\quad]_{c+2}^{0}, 0 \leq j \leq g_{i,5}, 1 \leq i \leq 2, 1 \leq c \leq 2;$$

$$r_{85} \equiv \left[Z''_{3,j}\right]_{c+2}^{0} \rightarrow Z'_{3,j,c+2}[\quad]_{c+2}^{0}, 0 \leq j \leq g_{3,5}, 1 \leq c \leq 2;$$

$$r_{86} \equiv [H_i]_{c+2}^{0} \rightarrow H''_{i,c+2}[H'_i]_{c+2}^{0}, 4 \leq i \leq N, 1 \leq c \leq 2;$$

$$r_{87} \equiv [H_0]_{c+2}^{0} \rightarrow [H'_0]_{c+2}^{0}, 1 \leq c \leq 2;$$

$$r_{88} \equiv [C_i]_{c+2}^{0} \rightarrow C'_i[C'_i]_{c+2}^{0}, 4 \leq i \leq N, 1 \leq c \leq 2;$$

$$r_{89} \equiv [C_0]_{c+2}^{0} \rightarrow [C'_0]_{c+2}^{0}, 1 \leq c \leq 2;$$

$$r_{90} \equiv [B]_{c+2}^{0} \rightarrow B'_{c+2}[\quad]_{c+2}^{0}, 1 \leq c \leq 2;$$

$$r_{91} \equiv [M]_{c+2}^{0} \rightarrow M[\quad]_{c+2}^{0}, 1 \leq c \leq 2;$$

$$r_{92} \equiv [S]_{c+2}^{0} \rightarrow S[\quad]_{c+2}^{0}, 1 \leq c \leq 2;$$

$$r_{93} \equiv [a_i]_{c+2}^{0} \rightarrow a'_i[\quad]_{c+2}^{0}, 1 \leq i \leq N, 1 \leq c \leq 2;$$

Step 8. When in the previous space resources are generated, they can be consumed at this time. Non-adult individuals:

$$r_{94} \equiv \left[Z'_{i,j,3} B'^{\frac{f_{i,7}}{2}} M^{\frac{f_{i,8}}{2}}\right]_{1}^{-} \rightarrow \left[W''_{i,j}\right]_{1}^{0}, 0 \leq j < g_{i,3} - 1, 1 \leq i \leq 3;$$

$$r_{95} \equiv \left[Z'_{i,j,4} B'^{\frac{f_{i,9}}{2}} M^{\frac{f_{i,10}}{2}}\right]_{2}^{-} \rightarrow \left[W''_{i,j}\right]_{2}^{0}, 0 \leq j < g_{i,3} - 1, 1 \leq i \leq 3;$$

$$r_{96} \equiv \left[Z'_{i,j,3} B'^{\frac{f_{i,7}}{2}} S^{\frac{f_{i,8}}{2}}\right]_{1}^{-} \rightarrow \left[W''_{i,j}\right]_{1}^{0}, 0 \leq j < g_{i,3} - 1, 1 \leq i \leq 3;$$

$$r_{97} \equiv \left[Z'_{i,j,4} B'^{\frac{f_{i,9}}{2}} S^{\frac{f_{i,10}}{2}}\right]_{2}^{-} \rightarrow \left[W''_{i,j}\right]_{2}^{0}, 0 \leq j < g_{i,3} - 1, 1 \leq i \leq 3;$$

$$r_{98} \equiv \left[Z'_{i,g_{i,3}-1,3} a'_i B'^{\frac{f_{i,7}}{2}} M^{\frac{f_{i,8}}{2}}\right]_{1}^{-} \rightarrow \left[W''_{i,g_{i,3}-1}\right]_{1}^{0}, 0 \leq j < g_{i,3} - 1, 1 \leq i \leq 3;$$

$$r_{99} \equiv \left[Z'_{i,g_{i,3}-1,4} a'_i B'^{\frac{f_{i,9}}{2}} M^{\frac{f_{i,10}}{2}}\right]_{2}^{-} \rightarrow \left[W''_{i,g_{i,3}-1}\right]_{2}^{0}, 1 \leq i \leq 3;$$

$$r_{100} \equiv \left[Z'_{i,g_{i,3}-1,3} a'_i B'^{\frac{f_{i,7}}{2}} S^{\frac{f_{i,8}}{2}}\right]_{1}^{-} \rightarrow \left[W''_{i,g_{i,3}-1}\right]_{1}^{0}, 1 \leq i \leq 3;$$

$$r_{101} \equiv \left[Z'_{i,g_{i,3}-1,4} a'_i B'^{\frac{f_{i,9}}{2}} S^{\frac{f_{i,10}}{2}}\right]_{2}^{-} \rightarrow \left[W''_{i,g_{i,3}-1}\right]_{2}^{0}, 1 \leq i \leq 3;$$

Adult individuals:

$r_{102} \equiv \left[Z'_{i,j,3}a'_iB'^{f_{i,7}}M^{f_{i,8}}\right]_1^- \rightarrow \left[W''_{i,j}\right]_1^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

$r_{103} \equiv \left[Z'_{i,j,4}a'_iB'^{f_{i,9}}M^{f_{i,10}}\right]_2^- \rightarrow \left[W''_{i,j}\right]_2^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

$r_{104} \equiv \left[Z'_{i,j,3}a'_iB'^{f_{i,7}}S^{f_{i,8}}\right]_1^- \rightarrow \left[W''_{i,j}\right]_1^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

$r_{105} \equiv \left[Z'_{i,j,4}a'_iB'^{f_{i,9}}S^{f_{i,10}}\right]_2^- \rightarrow \left[W''_{i,j}\right]_2^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

$r_{106} \equiv \left[Z'_{i,j,c+2}a'_i\right]_c^- \rightarrow \left[Za'_{i,j,c+2}\right]_c^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3, 1 \leq c \leq 2;$

$r_{107} \equiv \left[Z'_{i,j,3}B'^{f_{i,7}}M^{f_{i,8}}\right]_1^- \rightarrow \left[Zm'_{i,j,3}\right]_1^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

$r_{108} \equiv \left[Z'_{i,j,4}B'^{f_{i,9}}M^{f_{i,10}}\right]_2^- \rightarrow \left[Zm'_{i,j,4}\right]_2^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

$r_{109} \equiv \left[Z'_{i,j,3}B'^{f_{i,7}}S^{f_{i,8}}\right]_1^- \rightarrow \left[Zm'_{i,j,3}\right]_1^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

$r_{110} \equiv \left[Z'_{i,j,4}B'^{f_{i,9}}S^{f_{i,10}}\right]_2^- \rightarrow \left[Zm'_{i,j,4}\right]_2^0, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3;$

If the bearded vulture does not have access to meat, it will feed on bones.

$r_{111} \equiv \left[Z'_{1,j,3}B'^{f_{i,7}+f_{i,8}}\right]_1^- \rightarrow \left[Zm'_{1,j,3}\right]_1^0, g_{1,3} \leq j \leq g_{1,5};$

$r_{112} \equiv \left[Z'_{1,j,4}B'^{f_{i,9}+f_{i,10}}\right]_2^- \rightarrow \left[Zm'_{1,j,4}\right]_2^0, g_{1,3} \leq j \leq g_{1,5};$

$r_{113} \equiv \left[Z'_{1,j,3}B'^{\frac{f_{i,7}+f_{i,8}}{2}}\right]_1^- \rightarrow \left[Zm'_{1,j,3}\right]_1^0, 0 \leq j < g_{1,3};$

$$r_{114} \equiv \left[Z'_{1,j,4}B'^{\frac{f_{i,9}+f_{i,10}}{2}}\right]_2^- \rightarrow \left[Zm'_{1,j,4}\right]_2^0, 0 \leq j < g_{1,3};$$

Step 9. The objects associated with the animals that had resources in the previous step enter the innermost membrane.

$r_{115} \equiv W''_{i,j}[\quad]_{c+2}^0 \rightarrow \left[W_{i,j}\right]_{c+2}^0, 0 \leq j \leq g_{1,5}, 1 \leq i \leq 3,1 \leq c \leq 2;$

The rest of the objects change environments, leaving the skin membrane.

$r_{116} \equiv \left[Z'_{i,j,c+2}\right]_c^0 \rightarrow Z'_{i,j,c+2}[\quad]_c^0, 0 \leq j \leq g_{1,5}, 1 \leq i \leq 3,1 \leq c \leq 2;$

$r_{117} \equiv \left[Za'_{i,j,c+2}\right]_c^0 \rightarrow Za'_{i,j,c+2}[\quad]_c^0, 0 \leq j \leq g_{1,5}, 1 \leq i \leq 3,1 \leq c \leq 2;$

$r_{118} \equiv \left[Zm'_{i,j,c+2}\right]_c^0 \rightarrow Zm'_{i,j,c+2}[\quad]_c^0, 0 \leq j \leq g_{,5}, 1 \leq i \leq 3,1 \leq c \leq 2;$

$r_{119} \equiv \left[H''_{i,c+2}\right]_c^0 \rightarrow H''_{i,c+2}[\quad]_c^0, 1 \leq i \leq N, 1 \leq c \leq 2;$

$$r_{120} \equiv [C'_i]_c^0 \rightarrow C'_i[\quad]_c^0, 1 \le i \le N, 1 \le c \le 2;$$

$$r_{121} \equiv [B'_{c+2}]_c^0 \rightarrow B'_{c+2}[\quad]_c^0, 1 \le c \le 2;$$

$$r_{122} \equiv [M]_c^0 \rightarrow M[\quad]_c^0, 1 \le c \le 2;$$

$$r_{123} \equiv [S]_c^0 \rightarrow S[\quad]_c^0, 1 \le c \le 2;$$

$$r_{124} \equiv [a'_i]_c^0 \rightarrow a'_i[\quad]_c^0, 1 \le i \le N, 1 \le c \le 2;$$

Step 10. Objects that did not evolve due to a lack of resources as well as those associated with surplus resources, leave the environment.

$$r_{125} \equiv \left[Z'_{i,j,c+2}\right]_0^0 \rightarrow Z'_{i,j,c+2}[\quad]_0^0, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2;$$

$$r_{126} \equiv \left[Za'_{i,j,c+2}\right]_0^0 \rightarrow Za'_{i,j,c+2}[\quad]_0^0, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2;$$

$$r_{127} \equiv \left[Zm'_{i,j,c+2}\right]_0^0 \rightarrow Zm'_{i,j,c+2}[\quad]_0^0, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2;$$

$$r_{128} \equiv \left[H''_{i,c+2}\right]_0^0 \rightarrow H''_{i,c+2}[\quad]_0^0, 1 \le i \le N, 1 \le c \le 2;$$

$$r_{129} \equiv \left[C'_i\ \right]_0^0 \rightarrow C'_i\ [\quad]_0^0, 1 \le i \le N;$$

$$r_{130} \equiv [B'_{c+2}]_0^0 \rightarrow B'_{c+2}[\quad]_0^0, 1 \le c \le 2;$$

$$r_{131} \equiv [M]_0^0 \rightarrow M[\quad]_0^0;$$

$$r_{132} \equiv [S]_0^0 \rightarrow S[\quad]_0^0;$$

$$r_{133} \equiv [a'_i]_0^0 \rightarrow a'_i[\quad]_0^0;$$

Step 11. Change of environment of the objects that have left.

$$r_{e2} \equiv \left(Z'_{i,j,c+2} \rightarrow ZE_{i,j,c+2,k}\right)_k, 1 \le k \le E, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2;$$

$$r_{e3} \equiv \left(Za'_{i,j,c+2} \rightarrow ZEa_{i,j,c+2,k}\right)_k, 1 \le k \le E, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2;$$

$$r_{e4} \equiv \left(Zm'_{i,j,c+2} \rightarrow ZEm_{i,j,c+2,k}\right)_k, 1 \le k \le E, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2;$$

Objects associated with food belonging to border/peripheral areas that the scavengers can access are generated.

$$r_{e5} \equiv \left(H''_{i,c+2} \rightarrow BE_{c+2,k}^{\omega(i,k)}\right)_k, \quad 1 \le i \le N, 1 \le c \le 2, 1 \le k \le E;$$

$$\omega(i,k) = g_{i,1} \cdot (ff(k,1,1) + ff(k,2,1) + ff(k,3,1) + ff(k,4,1) + (1 - g_{i1}) \cdot (ff(k,1,2) + ff(k,2,2) + ff(k,3,2) + ff(k,4,2)$$

$$r_{e6} \equiv \left(C'_i \rightarrow ME_{c+2,k}^{\omega(i,k)}\right)_k, \quad 1 \le i \le N, 1 \le k \le E;$$

$r_{e7} \equiv \left(B'_{c+2} \rightarrow BE_{c+2,k}\right)_k, \;\; 1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{e8} \equiv (M \rightarrow ME_k)_k, \;\; 1 \leq k \leq E;$

$r_{e9} \equiv (S \rightarrow SE_k)_k, \;\; 1 \leq k \leq E;$

$r_{e10} \equiv \left(a'_i \rightarrow aE_{i,k}\right)_k, \;\; 1 \leq i \leq N, 1 \leq k \leq E;$

Step 12. All objects from the five environments associated with geographic areas are moved to the virtual environment 6.

$r_{e11} \equiv \left(ZE_{i,j,c+2,k}\right)_k(\;\;)_6 \rightarrow (\;\;)_k\left(ZE'_{i,j,c+2,k}\right)_6, \;\; 0 \leq j \leq g_{i,5}, 1 \leq i \leq 3{,}1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{e12} \equiv \left(ZEa_{i,j,c+2,k}\right)_k(\;\;)_6 \rightarrow (\;\;)_k\left(ZEa'_{i,j,c+2,k}\right)_6, \;\; 0 \leq j \leq g_{i,5}, 1 \leq i \leq 3{,}1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{e13} \equiv \left(ZEm_{i,j,c+2,k}\right)_k(\;\;)_6 \rightarrow (\;\;)_k\left(ZEm'_{i,j,c+2,k}\right)_6, \;\; 0 \leq j \leq g_{i,5}, 1 \leq i \leq 3{,}1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{e14} \equiv \left(BE_{c+2,k}\right)_k(\;\;)_6 \rightarrow (\;\;)_k\left(BE'_{c+2,k}\right)_6, \;\; 1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{e15} \equiv (ME_k)_k(\;\;)_6 \rightarrow (\;\;)_k(ME'_k)_6, \;\; 1 \leq k \leq E;$

$r_{e16} \equiv (SE_k)_k(\;\;)_6 \rightarrow (\;\;)_k(SE'_k)_6, \;\; 1 \leq k \leq E;$

$r_{e17} \equiv \left(aE_{i,k}\right)_k(\;\;)_6 \rightarrow (\;\;)_k\left(aE'_{i,k}\right)_6, 1 \leq i \leq N, 1 \leq k \leq E;$

Step 13. When the objects are in environment 6, they enter the skin membrane.

$r_{134} \equiv ZE'_{i,j,c+2,k}[\;\;]_0^0 \rightarrow \left[ZE_{i,j,c+2,k}\right]_0^0, 0 \leq j \leq g_{i,5}\,, 1 \leq i \leq 3, 1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{135} \equiv ZEa'_{i,j,c+2,k}[\;\;]_0^0 \rightarrow \left[ZEa_{i,j,c+2,k}\right]_0^0, 0 \leq j \leq g_{i,5}\,, 1 \leq i \leq 3, 1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{136} \equiv ZEm'_{i,j,c+2,k}[\;\;]_0^0 \rightarrow \left[ZEm_{i,j,c+2,k}\right]_0^0, 0 \leq j \leq g_{i,5}\,, 1 \leq i \leq 3, 1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{137} \equiv BE'_{c+2,k}[\;\;]_0^0 \rightarrow \left[BE_{c+2,k}\right]_0^0, 1 \leq c \leq 2{,}1 \leq k \leq E;$

$r_{138} \equiv ME'_k[\;\;]_0^0 \rightarrow [ME_k]_0^0, 1 \leq k \leq E;$

$r_{139} \equiv SE'_k[\;\;]_0^0 \rightarrow [SE_k]_0^0, 1 \leq k \leq E;$

$r_{140} \equiv aE'_{i,k}[\;\;]_0^0 \rightarrow \left[aE_{i,k}\right]_0^0, 1 \leq i \leq N, 1 \leq k \leq E;$

Step 14. The objects enter membrane 1 where the feeding process will be executed if there are sufficient resources.

$r_{141} \equiv ZE_{i,j,c+2,k}[\;\;]_1^0 \rightarrow \left[ZE_{i,j,c+2,k}\right]_1^0, 0 \leq j \leq g_{i,5}\,, 1 \leq i \leq 3, 1 \leq c \leq 2{,}1 \leq k \leq E;$

$$r_{142} \equiv ZEa_{i,j,c+2,k}[\ \ ]_1^0 \rightarrow \left[ZEa_{i,j,c+2,k}\right]_1^0, 0 \le j \le g_{i,5}\ , 1 \le i \le 3, 1 \le c \le 2,1 \le k \le E;$$

$$r_{143} \equiv ZEm_{i,j,c+2,k}[\ \ ]_1^0 \rightarrow \left[ZEm_{i,j,c+2,k}\right]_1^0, 0 \le j \le g_{i,5}\ , 1 \le i \le 3, 1 \le c \le 2,1 \le k \le E;$$

$$r_{144} \equiv BE_{c+2,k}[\ \ ]_1^0 \rightarrow \left[BE_{c+2,k}\right]_1^0, 1 \le c \le 2,1 \le k \le E;$$

$$r_{145} \equiv ME_k[\ \ ]_1^0 \rightarrow [ME_k]_1^0, 1 \le k \le E;$$

$$r_{146} \equiv SE_k[\ \ ]_1^0 \rightarrow [SE_k]_1^0, 1 \le k \le E;$$

$$r_{147} \equiv aE_{i,k}[\ \ ]_1^0 \rightarrow \left[aE_{i,k}\right]_1^0, 1 \le i \le N, 1 \le k \le E;$$

Step 15. Feeding process

$$r_{148} \equiv \left[ZE_{i,j,3,k}BE_{3,v}^{\frac{f_{i,7}}{2}}, ME_v^{\frac{f_{i,8}}{2}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,3,k}[\ \ ]_1^-, 0 \le j < g_{i,3}, 1 \le i \le 3,1 \le v \le E, 1 \le k \le E;$$

$$r_{149} \equiv \left[ZE_{i,j,4,k}BE_{4,v}^{\frac{f_{i,9}}{2}}, ME_v^{\frac{f_{i,10}}{2}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,4,k}[\ \ ]_1^-, 0 \le j < g_{i,3}, 1 \le i \le 3,1 \le v \le E, 1 \le k \le E;$$

$$r_{150} \equiv \left[ZE_{i,j,3,k}BE_{3,v}^{\frac{f_{i,7}}{2}} SE_v^{\frac{f_{i,8}}{2}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,3,k}[\ \ ]_1^-, 0 \le j < g_{i,3}, 1 \le i \le 2,1 \le v \le E, 1 \le k \le E;$$

$$r_{151} \equiv \left[ZE_{i,j,4,k}BE_{4,v}^{\frac{f_{i,9}}{2}} SE_v^{\frac{f_{i,10}}{2}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,4,k}[\ \ ]_1^-, 0 \le j < g_{i,3}, 1 \le i \le 2,1 \le v \le E, 1 \le k \le E;$$

$$r_{152} \equiv \left[ZE_{i,j,3,k}aE_{i,s}BE_{3,v}^{f_{i,7}} ME_v^{f_{i,8}}\right]_1^0 \xrightarrow{p(TM(i),k,v)\cdot p(TM(i),k,s)} W'_{i,j,3,s}[\ \ ]_1^-, g_{i,3} \le j \le g_{i,5}, 1 \le i \le 3,1 \le v \le E, 1 \le k \le E, 1 \le s \le E;$$

$$r_{153} \equiv \left[ZE_{i,j,4,k}aE_{i,s}BE_{4,v}^{f_{i,9}} ME_v^{f_{i,10}}\right]_1^0 \xrightarrow{p(TM(i),k,v)\cdot p(TM(i),k,s)} W'_{i,j,4,s}[\ \ ]_1^-, g_{i,3} \le j \le g_{i,5}, 1 \le i \le 3,1 \le v \le E, 1 \le k \le E, 1 \le s \le E;$$

$$r_{154} \equiv \left[ZE_{i,j,3,k}aE_{i,s}BE_{3,v}^{f_{i,7}} SE_v^{f_{i,8}}\right]_1^0 \xrightarrow{p(TM(i),k,v)\cdot p(TM(i),k,s)} W'_{i,j,3,s}[\ \ ]_1^-, g_{i,3} \le j \le g_{i,5}, 1 \le i \le 2,1 \le v \le E, 1 \le k \le E, 1 \le s \le E;$$

$$r_{155} \equiv \left[ZE_{i,j,4,k}aE_{i,s}BE_{4,v}^{f_{i,9}} SE_v^{f_{i,10}}\right]_1^0 \xrightarrow{p(TM(i),k,v)\cdot p(TM(i),k,s)} W'_{i,j,4,s}[\ \ ]_1^-, g_{i,3} \le j \le g_{i,5}, 1 \le i \le 2,1 \le v \le E, 1 \le k \le E;$$

$$r_{156} \equiv \left[ZEm_{i,j,c+2,k}aE_{i,v}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,c+2,v}[\ \ ]_1^-, g_{i,3} \le j \le g_{i,5}, 1 \le i \le 3,1 \le v \le E, 1 \le c \le 2,1 \le k \le E;$$

$$r_{157} \equiv \left[ZEa_{i,j,3,k}BE_{3,v}^{f_{i,7}}ME_v^{f_{i,8}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,3,k}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3, 1 \leq v \leq E, 1 \leq k \leq E;$$

$$r_{158} \equiv \left[ZEa_{i,j,4,k}BE_{4,v}^{f_{i,9}}ME_v^{f_{i,10}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,4,k}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 3, 1 \leq v \leq E, 1 \leq k \leq E;$$

$$r_{159} \equiv \left[ZEa_{i,j,3,k}BE_{3,v}^{f_{i,7}}SE_v^{f_{i,8}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,3,k}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 2, 1 \leq v \leq E, 1 \leq k \leq E;$$

$$r_{160} \equiv \left[ZEa_{i,j,4,k}BE_{4,v}^{f_{i,9}}SE_v^{f_{i,10}}\right]_1^0 \xrightarrow{p(TM(i),k,v)} W'_{i,j,4,k}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5}, 1 \leq i \leq 2, 1 \leq v \leq E, 1 \leq k \leq E;$$

$$r_{161} \equiv \left[ZE_{1,j,3,k}BE_{3,v}^{\frac{f_{i,7}+f_{i,8}}{2}}\right]_1^0 \xrightarrow{p(TM(1),k,v)} W'_{1,j,3,k}[\quad]_1^-, 0 \leq j < g_{i,3}, 1 \leq v \leq E, 1 \leq k \leq E;$$

$$r_{162} \equiv \left[ZE_{1,j,4,k}BE_{4,v}^{\frac{f_{i,9}+f_{i,10}}{2}}\right]_1^0 \xrightarrow{p(TM(1),k,v)} W'_{1,j,4,k}[\quad]_1^-, 0 \leq j < g_{i,3}, 1 \leq v \leq E, 1 \leq k \leq E;$$

$$r_{163} \equiv \left[ZE_{1,j,3,k}aE_{1,s}\ BE_{3,v}^{f_{i,7}+f_{i,8}}\right]_1^0 \xrightarrow{p(TM(1),k,v)\cdot p(TM(i),k,s)} W'_{1,j,3,s}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5},, 1 \leq v \leq E, 1 \leq k \leq E, 1 \leq s \leq E;$$

$$r_{164} \equiv \left[ZE_{1,j,4,k}aE_{1,s}\ BE_{4,v}^{f_{i,9}+f_{i,10}}\right]_1^0 \xrightarrow{p(TM1,k,v)\cdot p(TM(i),k,s)} W'_{1,j,4,s}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5}, 1 \leq v \leq E, 1 \leq k \leq E, 1 \leq s \leq E;$$

$$r_{165} \equiv \left[ZEa_{1,j,3,k}\ BE_{3,v}^{f_{i,7}+f_{i,8}}\right]_1^0 \xrightarrow{p(TM(1),k,v)} W'_{1,j,3,k}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5}, 1 \leq v \leq E, 1 \leq k \leq E;$$

$$r_{166} \equiv \left[ZEa_{1,j,4,k}BE_{4,v}^{f_{i,9}+f_{i,10}}\right]_1^0 \xrightarrow{p(TM1,k,v)} W'_{1,j,4,k}[\quad]_1^-, g_{i,3} \leq j \leq g_{i,5}, 1 \leq v \leq E, 1 \leq k \leq E;$$

Step 16. Dissolution of the objects that have not been able to execute the process, elimination of surplus resources and movement of the objects that have finished the process to a physical environment.

$$r_{167} \equiv \left[ZE_{i,j,c+2,k}\right]_1^- \rightarrow [\quad]_1^0, 0 \leq j \leq g_{i,5}, 1 \leq i \leq 3, 1 \leq c \leq 2, 1 \leq k \leq E;$$

$$r_{168} \equiv \left[ZEa_{i,j,c+2,k}\right]_1^- \rightarrow [\quad]_1^0, 0 \leq j \leq g_{i,5}, 1 \leq i \leq 3, 1 \leq c \leq 2, 1 \leq k \leq E;$$

$$r_{169} \equiv \left[ZEm_{i,j,c+2,k}\right]_1^- \rightarrow [\quad]_1^0, 0 \leq j \leq g_{i,5}, 1 \leq i \leq 3, 1 \leq c \leq 2, 1 \leq k \leq E;$$

The 20% of bones left during the breeding period can be eaten the next spring. Those that are left in the summer are not used.

$$r_{170} \equiv \left[BE_{3,k}\right]_1^- \xrightarrow{0.2} BE_{3,k}[\quad]_1^0, 1 \le k \le E;$$

$$r_{171} \equiv \left[BE_{3,k}\right]_1^- \xrightarrow{0.8} [\quad]_1^0, 1 \le k \le E;$$

$$r_{172} \equiv \left[BE_{4,k}\right]_1^- \rightarrow [\quad]_1^0, 1 \le k \le E;$$

The meat surplus is not used.

$$r_{173} \equiv [ME_k]_1^- \rightarrow [\quad]_1^0, 1 \le k \le E;$$

$$r_{174} \equiv [SE_k]_1^- \rightarrow [\quad]_1^0, 1 \le k \le E;$$

$$r_{175} \equiv \left[aE_{i,k}\right]_1^- \rightarrow aa_{ik}[\quad]_1^0, 1 \le i \le N, 1 \le k \le E;$$

$$r_{176} \equiv \left[W'_{i,j,c+2,v}\right]_0^0 \rightarrow W'_{i,j,c+2,v}[\quad]_0^0, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2, 1 \le v \le E;$$

Step 17. Change the virtual environment to a physical environment.

$$r_{e18} \equiv \left(W'_{i,j,c+2,v}\right)_6 (\quad)_v \rightarrow (\quad)_6 \left(X'_{i,j+c-1,c}\right)_v, 0 \le j \le g_{i,5}, 1 \le i \le 3, 1 \le c \le 2, 1 \le v \le E;$$

$$r_{177} \equiv \left[BM_{3,k}\right]_0^0 \rightarrow BM_{3,k}[\quad]_0^0, 1 \le k \le E;$$

Step 18. Objects in the physical environment enter the skin membrane.

$$r_{178} \equiv X'_{i,j,1}[\quad]_0^0 \rightarrow \left[X_{i,j}\right]_0^0, g_{i,3} \le j \le g_{i,5}, 1 \le i \le 3;$$

$$r_{179} \equiv X'_{i,j,1}[\quad]_0^0 \rightarrow \left[XJ_{i,j}\right]_0^0, 0 \le j < g_{i,3}, 1 \le i \le 3;$$

$$r_{180} \equiv X'_{i,j,2}[\quad]_0^0 \rightarrow \left[X_{i,j}\right]_0^0, g_{i,3} < j \le g_{i,5}, 1 \le i \le 3;$$

$$r_{181} \equiv X'_{i,j,2}[\quad]_0^0 \rightarrow \left[XJ_{i,j}\right]_0^0, 0 \le j < g_{i,3}, 1 \le i \le 3;$$

$$r_{182} \equiv X'^2_{i,g_{i,3},2}[\quad]_0^0 \rightarrow \left[X''_{i,g_{i,3}}\right]_0^0, 1 \le i \le 3;$$

$$r_{e19} \equiv \left(aa_{i,k}\right)_6 (\quad)_k \rightarrow (\quad)_6 (sa_i)_k, 1 \le i \le 3, 1 \le k \le E;$$

$$r_{e20} \equiv \left(BM_{3,k}\right)_6 (\quad)_k \rightarrow (\quad)_6 \left(BM_{3,k}\right)_k, 1 \le k \le E;$$

Step 19. The objects associated with food that are not used in the following period are eliminated and the counters and activators of the processes evolve.

$$r_{183} \equiv BM_{3,k}[\quad]_0^0 \rightarrow [BB]_0^0, 1 \le k \le E;$$

$$r_{184} \equiv sa_i[\quad]_0^0 \rightarrow sa_i[\quad]_0^0, 1 \le i \le 3;$$

$$r_{185} \equiv \left[W_{i,j}\right]_{c+2}^- \rightarrow W_{i,j+c-1}[\quad]_{c+2}^0, g_{i,3} \le j \le g_{i,5}, 1 \le i \le N, 1 \le c \le 2;$$

$r_{186} \equiv [H'_i]^-_{c+2} \rightarrow H_i[\quad]^0_{c+2}, 1 \leq i \leq N, 1 \leq c \leq 2;$

$r_{187} \equiv [C'_i]^-_{c+2} \rightarrow C_i[\quad]^0_{c+2}, 1 \leq i \leq N, 1 \leq c \leq 2;$

$r_{188} \equiv [co_1]^-_3 \rightarrow co'_2[\quad]^0_3;$

$r_{189} \equiv [co_2]^-_4 \rightarrow co'_1[\quad]^0_4;$

$r_{190} \equiv [F_k]^-_{c+2} \rightarrow [F0_k]^0_{c+2}, 1 \leq c \leq 2, 1 \leq k \leq E;$

$r_{191} \equiv [b]^-_{c+2} \rightarrow [\quad]^0_{c+2}, 1 \leq c \leq 2;$

$r_{192} \equiv AC_{i,k}[\quad]^-_{c+2} \rightarrow [\quad]^0_{c+2}, 8 \leq i \leq N, 1 \leq c \leq 2, 1 \leq k \leq E;$

Step 20. The initial configuration is restored to restart the model loop.

$r_{193} \equiv \left[X''_{i,g_{i,3}} sa_i \rightarrow X_{i,g_{i,3}} i\right]^0_0, 1 \leq i \leq 3;$

$r_{194} \equiv \left[W_{i,j}\right]^0_c \rightarrow X_{i,j}[\quad]^0_c, 4 \leq i \leq N, 0 \leq j \leq g_{i,5}, 1 \leq c \leq 2;$

$r_{195} \equiv \left[W_{i,j}\right]^0_1 \rightarrow X_{i,j}[\quad]^0_1, 1 \leq i \leq 3, g_{i,3} \leq j \leq g_{i,5};$

$r_{196} \equiv \left[W_{i,j}\right]^0_1 \rightarrow XJ_{i,j}[\quad]^0_1, 1 \leq i \leq 3{,}0 \leq j < g_{i,3};$

$r_{197} \equiv \left[W_{i,j}\right]^0_2 \rightarrow X_{i,j}[\quad]^0_1, 1 \leq i \leq 3, g_{i,3} \leq j \leq g_{i,5};$

$r_{198} \equiv \left[W^2_{i,g_{i,3}}\right]^0_2 \rightarrow X''_{i,g_{i,3}}[\quad]^0_2, 1 \leq i \leq 3;$

$r_{199} \equiv \left[W_{i,j}\right]^0_2 \rightarrow XJ_{i,j}[\quad]^0_2, 1 \leq i \leq 3{,}0 \leq j < g_{i,3};$

$r_{200} \equiv [co'_i]^0_c \rightarrow co_i[\quad]^0_c, 1 \leq i \leq 2{,}1 \leq c \leq 2;$

$r_{201} \equiv [H_i]^0_c \rightarrow H_i[\quad]^0_c, 0 \leq i \leq N, 1 \leq c \leq 2;$

$r_{202} \equiv [C_i]^0_c \rightarrow C_i[\quad]^0_c, 0 \leq i \leq N, 1 \leq c \leq 2;$

$r_{203} \equiv BB[\quad]^0_1 \rightarrow [B]^0_1;$

Step 21 + Step 0. The loop begins again and the objects that had been allowed to save the generated biomass information are dissolved.

$r_{204} \equiv [H_i \rightarrow \#]^0_0, 0 \leq i \leq N;$

$r_{205} \equiv [C_i \rightarrow \#]^0_0, 0 \leq i \leq N;$

$r_{206} \equiv sa_i[\quad]^+_1 \rightarrow [\#]^0_1, 1 \leq i \leq 3;$

$r_{207} \equiv X''_{i,g_{i,3}}[\quad]^+_1 \rightarrow [\#]^0_1, 1 \leq i \leq 3;$

$r_{208} \equiv B[\quad]^+_3 \rightarrow [B]^+_3\ , 0 \leq i \leq N;$

Supplementary information to Chapter 5

Prioritizing among removal scenarios for the reintroduction of endangered species: insights from bearded vulture simulation modeling

Colomer, M. À., Oliva-Vidal, P., Jiménez, J., Martínez, J. M., Margalida, A. 2020. Prioritizing among removal scenarios for the reintroduction of endangered species: insights from bearded vulture simulation modeling. *Animal Conservation* 23, 396–406.

Table S1. Definition of the parameters used in the PDP model.

Parameters	Definition
qs_j	Number of floating individuals of age *j*
qp_j	Number of territorial individuals that form pairs of age *j*
qt_j	Number of territorial individuals that form trios of age *j*
Pcp_j	Probability of mating an animal of age *j*
g_1	Juvenile age ends (years)
g_2	Subadult age ends (years)
g_3	Average life expectancy
g_4	Age at which fertility begins
g_5	Age at which fertility ends
k	Number of annual descendants (individuals)
SB	Pairs that start the reproduction process
p	Productivity
Pb	Eggs fertility (%)
pf	Ratio between productivity (*p*) and the number of fertile eggs (*pb*) *p/pb*
$Maxp$	Productivity at low density
$Minp$	Productivity at high density
m_j	Mortality probability of an animal of age *j*

APPENDIX

Red kite *Milvus milvus* in the Lleida Pre-Pyrenees, Catalonia (Spain)
Photo: Pilar Oliva-Vidal

ORIGINAL PUBLICATIONS

This PhD thesis includes five published original articles. Additionally, a published short note (correspondence) complementary to the thesis topics (Box 2) can be found at the end of this section.

Chapter 1: Oliva-Vidal, P., Sebastian-Gonzalez, E., Margalida, A. 2022. Scavenging in changing environments: woody encroachment shapes rural scavenger assemblages in Europe. *Oikos*. https://doi.org/10.1111/oik.09310.

Chapter 2: Oliva-Vidal, P., Hernández-Matías, A., García, D., Colomer M. À., Real, J., Margalida, A. 2022. Griffon vultures, livestock and farmers: unraveling a complex socio-economic ecological conflict from a conservation perspective. *Biological Conservation* 272, 109664.

Chapter 3: Oliva-Vidal, P., Martinez J. M., Sanchez-Barbudo, I., Pablor, R. Camarero., Colomer, M. A., Margalida, A., Mateo, R. 2022. Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers. *Environmental Pollution* 315, 120385. https://doi.org/10.1016/j.envpol.2022.120385.

Chapter 4: Margalida, A., Oliva-Vidal, P., Llamas, A., Colomer, M. À. 2018. Bioinspired models for assessing the importance of transhumance and transboundary management in the conservation of European avian scavengers. *Biological Conservation* 228, 321–330.

Chapter 5: Colomer, M. À., Oliva-Vidal, P., Jiménez, J., Martínez, J. M., Margalida, A. 2020. Prioritizing among removal scenarios for the reintroduction of endangered species: insights from bearded vulture simulation modeling. *Animal Conservation* 23, 396–406.

OIKOS

Research

Scavenging in changing environments: woody encroachment shapes rural scavenger assemblages in Europe

Pilar Oliva-Vidal, Esther Sebastián-González and Antoni Margalida

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Rural abandonment and subsequent vegetation regeneration (‘passive rewilding’) are expected to increase worldwide, producing cascades of dynamic socioeconomic, landscape and biological changes. Although landscape characteristics strongly influence the structure and functioning of scavengers, little is known about the ecological consequences of passive rewilding due to woody encroachment (i.e.‘landscape closure’) on scavenging assemblages. We investigated differences in ‘scavenger assemblage composition’ (species richness and abundances) and ‘scavenging efficiency’ (scavenging frequency, detection and consumption times and consumption rates) in a mountain agroecosystem (Pyrenees) undergoing passive rewilding. We monitored 178 carcasses in three landscapes: ‘open’, ‘shrubland’ and ‘forest’, and evaluated the effects of landscape type on ‘scavenger assemblage composition’ and ‘scavenging efficiency’ at the community and species levels, while accounting for the influences of carcass size, type and placement time. We also examined whether the locally most abundant and efficient scavenger (i.e. the griffon vulture *Gyps fulvus*) affects scavenging patterns. We found that landscape type was the main factor governing scavenging dynamics. Overall and average scavenger richness were similar in open and shrubland landscapes, while forests contained the lowest number of scavengers, mainly comprising mammals. Unlike mammals, avian scavenging frequency decreased as vegetation cover increased, especially for obligate scavengers (i.e. vultures). Scavenger abundances were highest in open landscapes, and carcasses were detected and consumed more rapidly in these landscapes. Carcass size did not influence detection and consumption times, although it did affect average scavenger richness, abundances and consumption rates. Consumption rates were higher in open landscapes and were strongly associated with the presence of griffon vultures. Interestingly, we found that griffon vultures influenced scavenging dynamics via facilitation processes. However, woody encroachment could reduce the scavenging role of this species, while favoring mammalian facultative scavengers. Finally, our findings highlight the pivotal role of griffon vultures, mediated by landscape characteristics, in reducing carcass persistence.

Keywords: agropastoral landscapes, carrion, farmland abandonment, passive rewilding, Pyrenees, rural abandonment, scavengers, vultures

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Introduction

Rural Europe has been shaped by traditional land-use and encompasses a wide diversity of cultural landscapes, especially in mountain regions (Tieskens et al. 2017). European mountain landscapes are the result of several thousand years of interactions between people and agroecosystems and deliver valuable ecosystem services (Bernués et al. 2014), often with exceptional conservation value (Fisher et al. 2012). Over the centuries, agro-livestock practices have promoted biodiversity by preserving high spatial heterogeneity within forest–agricultural mosaic landscapes (Fahrig et al. 2011). However, socioeconomic changes during the 19th and 20th centuries have led to widespread abandonment of rural areas and the loss of landscape heterogeneity, posing major scientific and policy challenges due to the crucial role of traditional farming for biodiversity and ecosystem service maintenance (MacDonald et al. 2000, Plieninger 2006, Cramer et al. 2008, Lasanta et al. 2017, Ustaoglu and Collier 2018). Understanding the ecological consequences of rural abandonment for ecosystem dynamics is key to the design of biodiversity conservation strategies in such areas.

As in most European mountain regions, important landscape changes occurred in the Spanish Pyrenees during the mid-20th century due to rural depopulation and agricultural modernization (Lasanta-Martínez et al. 2005). Traditional livestock farming systems intensified during this period, resulting in a severe decline in sheep numbers and an increase in cattle. Some valleys in this region saw sheep numbers decline by more than 80%, drastically reducing grazing pressure (Lasanta-Martínez et al. 2005) and livestock biomass over large areas (Margalida et al. 2018). This trend is widespread across Europe, where extensive livestock numbers declined by 25% between 1990 and 2010 (Navarro and Pereira 2015). Furthermore, the ‘transhumance’, a seasonal livestock (mainly sheep) migration – usually across long distances – between winter and summer pastures, is in strong decline (García-Ruiz et al. 2020a). Nowadays, livestock are kept in the valleys during the cold season and moved to adjacent pastures during the summer (i.e. ‘transtermitance’). This drastic decline in sheep numbers has caused a significant reduction of livestock pressure, accelerating the processes of vegetation succession, especially in marginal areas (García-Ruiz et al. 2020a). These changes have been characterized by generalized farmland abandonment and subsequent vegetation encroachment, modifying the long-standing forest-agricultural mosaic, reducing the extent of open spaces and increasing the extent of woody landscapes (Poyatos et al. 2003, Lasanta-Martínez et al. 2005, Roura-Pascual et al. 2005, Améztegui et al. 2010). The result is passive landscape restoration, or ‘passive rewilding’, by natural shrub and tree colonization (‘landscape closure’), driving important changes in habitat structure and landscape configuration (Pereira and Navarro 2015, Corlett 2016, Perino et al. 2019).

Although active rewilding has recently been proposed as a conservation strategy to restore the loss of biodiversity and ecosystem functions (Pereira and Navarro 2015), there is ongoing debate regarding the associated impacts on species diversity and community dynamics (Queiroz et al. 2014, Nogués-Bravo et al. 2016, García-Ruiz et al. 2020b). For example, depopulation and agricultural abandonment in rural areas of Japan have posed major conservation challenges owing to changes in species distribution, resulting in increased human–wildlife conflicts (Tsunoda and Enari 2020) and plant encroachment has been associated with losses of mesocarnivore scavenging efficiency in tropical-grassy African savannas (Lima et al. 2021). In Europe, much attention has focused on how natural revegetation leads to an expansion of forest species (Falcucci et al. 2006, Acevedo et al. 2011, Martínez-Abraín et al. 2020), but the effects on species adapted to open-habitats or mountain agropastoral landscapes remain poorly understood (Laiolo et al. 2004, Regós et al. 2014). There is therefore a need to identify the ecological impacts of rewilding, and the resulting changes in landscape configuration, on functional assemblages linked to traditional mountain landscapes. Scavengers (i.e. carrion-eating species), form one such important assemblages. Understanding how scavenger assemblages function in different landscape configurations differing in vegetation structure is critical to inform management priorities dealing with passive rewilding.

Despite the essential role of scavengers in providing regulatory services by efficiently removing carrion from ecosystems (Moleón et al. 2014, DeVault et al. 2016), the effects of rewilding or other changes in habitat configuration on scavenging dynamics have not been extensively investigated (but see Cortés-Avizanda et al. 2015, García-Barón et al. 2018, van Klink et al. 2020). For example, Arrondo et al. (2019) found that in mountainous areas of south-east Spain, sheep carcasses in open pasturelands were detected and consumed faster than wild ungulate carcasses in more heterogeneous areas. Thus, partially abandoned mountain agroecosystems inhabited by both facultative scavengers (species that scavenge opportunistically, e.g. mammalian carnivores, raptors and corvids) and obligate scavengers (species that depend totally on carrion, i.e. vultures) present an ideal opportunity to improve our understanding of how vegetation encroachment affects scavenging dynamics.

Carrion occurs unpredictably in time and space, and provides an ephemeral, valuable food resource. It has high nutritional value and is generally rapidly consumed following the multiple complex interactions that structure scavenger communities and stabilize food webs (Wilson and Wolkovich 2011, Moreno-Opo et al. 2016, Sebastián-González et al. 2020). However, vegetation cover and structure may influence the ability of scavengers to locate and access carcasses, affecting consumption patterns and efficiency (Selva et al. 2005, Moleón et al. 2019, Pardo-Barquín et al. 2019). An increase in shrub and forest cover may favor carcass detection by scavengers that mainly use odor cues to locate food, such as mammals (DeVault and Rhodes 2002, Enari and Enari 2021), to the detriment of avian scavengers that mainly rely on visual cues, such as Old World vultures (Ruxton and Houston 2004).

To better understand these dynamics, we monitored a large number of carcasses (> 170) of several types and sizes, representing the widest carrion diversity appearing in a mountain agroecosystem in which the four European vulture species coexist with important facultative scavenger populations. Our main goal was to describe the 'scavenger assemblage composition' (species richness and abundances) and 'scavenging efficiency' (scavenging frequency, detection and consumption times and consumption rates) at both the community and species levels in three landscape types ('open'; semi-closed or 'shrubland'; and closed or 'forest') where passive rewilding processes occur. We also evaluated whether carcass size, type (herbivore/carnivore) and placement time (morning/afternoon) influenced carcass consumption patterns.

Our general hypothesis was that landscape type, carcass size and type, and placement time influence both scavenger assemblage composition and scavenging efficiency. We predicted: 1) that carcasses placed in open areas would be detected and exploited more rapidly, mainly by avian scavengers (i.e. species relying on sight for carcass detection), than those placed in shrublands and forests, which would mainly be consumed by mammals (i.e. species relying on scent for carcass detection); 2) that herbivore carcasses would show higher scavenger species richness, abundance and consumption rates, as carnivore carcasses are known to be avoided by carnivores (Moleón et al. 2017); 3) that carcasses deployed in the morning would be exploited faster, since the more efficient vultures are inactive at night; 4) that larger carcasses would be consumed at a faster rate and by a richer and more abundant scavenger assemblage than small ones, as found by Moleón et al. (2015) in African assemblages; and 5) that consumption by griffon vultures would influence the composition and efficiency of the scavenger assemblage, as this species is known to have higher consumption rates and to facilitate carrion location (Cortés-Avizanda et al. 2014). Our research provides a novel approach to the study of the effects of passive rewilding and 'landscape closure' on scavenging dynamics in mountain landscapes.

Material and methods

Study area

The Pyrenees is a mountain range extending over more than 50 000 km^2 in the north-east of the Iberian Peninsula. The study area is on the southern slope and covers around 170 000 ha. This area is characterized by a wide range of altitudes (from ~500 to > 3000 m a.s.l.) and shows significant climatic variation, which offers contrasting landscapes and great vegetational diversity. In the cultivated lands of the lower valleys (< 700 m a.s.l.), Mediterranean shrubland and mixed woodlands of oak *Quercus ilex*, *Q. faginea* and Scots pine *Pinus sylvestris* predominate. Montane forest zones occur at the middle altitudes (700–1600 m a.s.l.), mainly dominated by Scots pine, European beech *Fagus sylvatica* and a wide variety of scrublands, open grasslands and pasturelands. In the upper mountain areas (1600–1800 m a.s.l. upwards), mountain pines *Pinus uncinata* are abundant, and above the treeline (2300 m a.s.l.), a mosaic of different types of alpine pastures dominate (Ninot et al. 2007). Historically, these landscapes have been shaped by human activity (e.g. long-term use for crops, livestock and forestry created an increase in open areas at the expense of forest, Roura et al. 2005) but more recent rural abandonment and shepherding decline have led to an increase in natural vegetation cover in previously open areas .

Extensive and semi-extensively reared livestock (cattle, sheep and horses) provide most of the biomass for the scavenger guild (Colomer et al. 2011). Extensive livestock are kept outdoors from April to October, depending on the weather conditions, and some transhumant herds migrate to high summer pastures. Important populations of wild herbivorous ungulates, mainly red deer *Cervus elaphus*, roe deer *Capreolus capreolus*, Pyrenean chamois *Rupicapra pyrenaica* and fallow deer *Dama dama* are present. Of the obligate scavengers within a 30 km radius of the central study area, almost 900 pairs of griffon, 16 pairs of cinereous *Aegypius monachus*, 21 pairs of bearded *Gypaetus barbatus* and 50 pairs of Egyptian vultures *Neophron percnopterus* breed in the area. Regarding facultative scavengers, the study area holds a rich community of avian (e.g. corvids and raptors) and mammal species, while shepherd and free-ranging hunting dogs are also frequent (Supporting information).

Carcass monitoring

From October 2017 to August 2019, we monitored 178 carcasses (herbivores, n = 154; carnivores, n = 24) of different species ranging in size from 1 to 100 kg, placed across three landscape types (open, n = 70; shrubland, n = 71; and forest, n = 37). Herbivore carcasses consisted of sheep/goat *Ovis aries*/*Capra hircus* (n = 60, mean ± SD = 47.65 ± 12.53 kg, including lambs/kids: n = 31, 9.34 ± 6.33 kg); wild boar *Sus scrofa* (n = 40, 53.20 ± 22.47 kg); roe deer (n = 8, 22.04 ± 3.60 kg); and European rabbit *Oryctolagus cuniculus* (n = 15, 1.48 ± 0.29 kg). Carnivore carcasses (mean weight ± SD = 5.71 ± 3.14 kg) consisted of red fox *Vulpes vulpes* (n = 6), European badger *Meles meles* (n = 9), stone marten *Martes foina* (n = 2) and domestic cat *Felis catus* (n = 7). Carcasses were placed between 600 and 1750 m a.s.l. (i.e. the mountain sectors where passive rewilding is widespread, García-Ruiz and Lasanta 1990), and were randomly distributed among the three landscape types over all altitudes. We selected each landscape type according to the vegetation cover within a 25 m radius from the center of the carcass (Supporting information). For open landscapes, we chose large open areas (e.g. grasslands) without shrub/tree cover (i.e. shrub and tree cover < 5%) (Fig. 1A–D). For shrublands, we selected sites with abundant vegetation cover (i.e. shrub and tree cover ranging 50–70%), mainly large (e.g. *Buxus sempervirens* and *Juniperus* sp.) and medium-size shrub species (e.g. *Genista scorpius* and *Rosa* sp.), or dispersed trees (mainly oak species) (Fig. 1E–G). Regarding forest landscapes, we placed carcasses in mature pine and beech forests with moderate canopy closure (i.e.

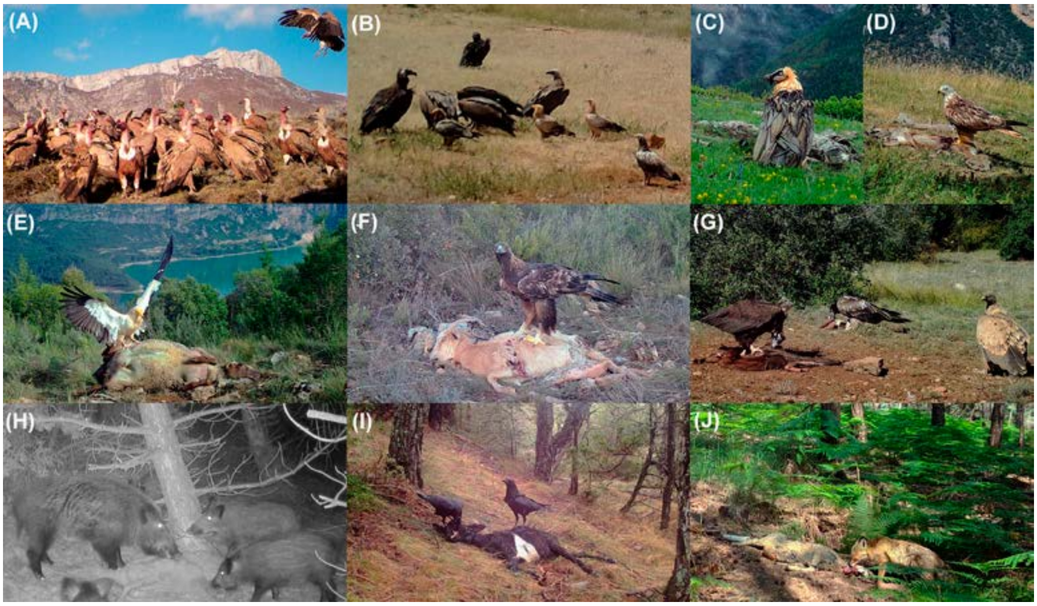

Figure 1. Images of some of the most frequent scavengers recorded in the three landscape types studied: 'open' (A–D), 'shrubland' (E–G) and 'forest' (H–J). (A) Griffon vultures *Gyps fulvus*, (B) cinereous *Aegypius monachus*, Egyptian *Neophron percnopterus* and griffon vultures, (C) bearded vulture *Gypaetus barbatus*, (D) red kite *Milvus milvus*, (E) Egyptian vulture, (F) golden eagle *Aquila chrysaetos*, (G) cinereous, bearded and griffon vultures, (H) wild boars *Sus scrofa*, (I) common ravens *Corvus corax* and (J) red fox *Vulpes vulpes*.

tree cover > 90%), allowing some visibility of the forest floor (Fig. 1H–J). Most carcasses in shrublands and forests were placed in areas where there was historical evidence of rewilding, e.g. from open landscapes to shrublands, or from shrublands to forests. Scavenging activity was recorded at all carcasses except for one stone marten placed in a shrubland. Carcasses were obtained from legal farms and slaughterhouses or collected as road kills.

Carcasses were monitored using Moultrie motion-triggered remote cameras (M-990i 10MP, M-999i 20MP and S-50i 20MP). Cameras were attached to a tree close to the carcasses (5–10 m away) and were programmed to take three consecutive photos when movement was detected, with a one-second delay between photos, and a delay of 15 s before the next set of photos. Carcasses were weighed and fixed to the ground using inconspicuous iron stakes to prevent scavengers moving them away from the camera focus. Carcasses were placed at random between dawn and dusk and separated by at least 1 km to maximize independence (Morales-Reyes et al. 2017). Once a carcass was consumed, we waited at least one month before placing another carcass closer than 1 km from the previous placement. A species was considered a 'consumer' when it was clearly recorded feeding on a carcass. When consumption was suspected, but not clearly recorded, we assumed consumption if that species had already been detected consuming another carcass (Sebastián-González et al. 2019). Carcasses were monitored continuously (24 h day^{-1}) until scavenging ended (i.e. only bones and/or skin remained) or the carcass was removed by a scavenger.

Scavenging measures

For each landscape type, we calculated the following variables relating to scavenger assemblage composition: 1) 'total richness' (number of vertebrate scavenger species recorded at all carcasses) and 2) 'richness' (number of vertebrate scavenger species per carcass). Richness was calculated individually for each species and also for birds and mammals separately. For some analyses we grouped scavengers by 'taxon' (avian or mammalian) and as 'obligate' or 'facultative', or at the species level. We also recorded 3) the 'abundance' (maximum number of unequivocally different individuals of each species per carcass). This was calculated by counting the highest number of individuals appearing simultaneously in a picture, and by differentiating individuals of different age, sex or individual features (Sebastián-González et al. 2019). Regarding scavenging efficiency (i.e. carcass location and consumption ability), we first calculated the scavenging frequency (i.e. percentage of consumed carcasses) for each species in each landscape type. Then, for each carcass and landscape type we estimated: 1) 'detection time' (time elapsed in hours between carcass placement and the arrival of the first scavenger), and separately for birds and mammals; 2) 'total consumption time' (time elapsed in hours between carcass placement and its complete consumption); 3) 'active consumption time'

(time elapsed in hours between start of carcass consumption and its complete consumption); and 4) ‘consumption rate’ (kilograms of carrion biomass consumed divided by carcass consumption time). To calculate consumption rates, we excluded the carcass stomach contents and the unconsumed parts from the carcass weight, so that the consumed biomass was calculated as: (initial weight of the carcass) – (weight of unconsumed remains) – (weight of stomach contents). Unconsumed remains were weighed at the end of each trial (Moleón et al. 2015) and stomach contents were estimated as 10% of the animal weight (Selva et al. 2004).

Statistical analyses

We investigated factors affecting scavenger assemblage composition and scavenging efficiency by fitting generalized linear models (GLMs), where ‘richness’, ‘avian/mammalian richness’, ‘abundance’, ‘detection time’, ‘total and active consumption time’ and ‘consumption rate’ were the response variables; and ‘landscape type’ (‘open’, ‘shrubland’, ‘forest’), ‘carcass type’ (herbivore or carnivore), ‘placement time’ (morning, from dawn to midday; afternoon, from midday to dusk) and ‘carcass weight’ (kg) were predictors. We also used the ‘taxon’ (avian or mammalian) of the first detector as a categorical predictor to test for differences in carcass detection time using univariate (i.e. single-predictor variable) GLMs, where ‘detection time’ (hours) was the response variable. We used Poisson error distributions and log link functions to model ‘richness’; negative binomial error distributions and log link functions for ‘abundance’; and Gaussian error distributions and identity link functions for ‘detection time’, ‘total and active consumption times’ and ‘consumption rate’, in all analyses. ‘Detection time’, ‘consumption times’ and ‘consumption rate’ were log-transformed to improve normality assumptions. We compared the unconsumed remains (i.e. skin and skeletal remains, in kg) of medium-size ungulates among landscape types using the Kruskal–Wallis test.

We also performed GLMs at the species level to investigate factors influencing scavenging frequency according to the same predictors: ‘landscape type’, ‘carcass type’, ‘placement time’ and ‘carcass weight’, using a binomial error distribution. For each species, we only used the presence/absence matrix of those landscapes in which the consumption of at least one carcass was detected. We fitted models with all possible combinations and subsets of the predictor variables. We selected the model with the lowest AICc, but when there was more than one model with a ΔAICc < 2 relative to the best model, we used a model-averaging function in the ‘MuMIn’ package (Barton 2013). This function averages parameter estimates across all considered models for each dependent variable where the respective parameter appeared, weighted by the relative importance of each model. We also calculated the percentage of deviance explained (i.e. the amount of variability explained) of each model. Finally, because one of our goals was to evaluate whether the presence of griffon vultures at carcasses could affect scavenging dynamics, we evaluated the relationship between all the composition and efficiency variables and the presence of griffon vultures using single-predictor GLMs with a binomial error distribution. We checked the model assumptions using statistical tests for normality and homogeneity of variance, and the fit of all models was visually inspected using residual versus fitted values and Q–Q plots. All analyses were performed using R ver. 3.6.1 (<www.r-project.org>).

Results

Differences in scavenger assemblage composition

We analyzed 286 572 photos and detected 19 vertebrate scavenger species (Supporting information). Total species richness in open and shrubland landscapes was similar (18 and 17 species, respectively), including the four European vultures, while forests showed the lowest scavenger richness (nine species) (Table 1). Mean scavenger richness was dependent on landscape type (open > shrubland > forest, Table 1) although GLM analyses revealed that it was only significantly lower in forests and that it increased with carcass size (Table 2, Supporting information). Mean avian species richness was slightly higher in open landscapes compared with shrublands, and up to eight times higher compared with forests, and also increased with carcass size. In contrast, mean mammalian richness only increased with carcass size (Table 1, 2, Supporting information). Placement time and carcass type did not influence scavenger richness (Table 2).

Table 1. Measures of scavenger assemblage composition recorded in each landscape type: total scavenger richness, scavenger richness, avian and mammalian scavenger richness and scavenger abundance per carcass. Values represent the mean ± standard deviation and range. The number of obligate and facultative avian and mammalian species and the number of carcasses monitored are shown.

	Open	Shrubland	Forest	Total
Total richness	18	17	9	19
Richness	4.70 ± 1.75 [1–9]	3.97 ± 1.88 [0–8]	2.65 ± 1.25 [1–7]	
Avian richness	2.77 ± 1.36 [0–5]	1.90 ± 1.47 [0–6]	0.35 ± 0.67 [0–3]	
Mammal richness	1.93 ± 1.04 [0–5]	2.07 ± 0.85 [0–4]	2.30 ± 0.94 [1–4]	
Scavenger abundance	51.5 ± 37.7 [2–124]	28.2 ± 24.8 [0–89]	4.3 ± 2.7 [1–12]	
Avian species	12	11	4	13
Mammal species	6	6	5	6
Obligate scavengers	4	4	1	4
Facultative scavengers	14	13	8	15
Number of carcasses	70	71	37	178

Table 2. Models (GLMs) used to assess the effect of landscape type ('open', 'shrubland' and 'forest'), carcass weight (kg), carcass type (herbivore and carnivore) and placement time (morning and afternoon) on the composition of scavenger assemblages (scavenger richness, avian and mammalian species richness, scavenger abundance) and scavenging efficiency (carcass detection time, consumption times and consumption rate). We present the model-averaged coefficients and standard error (SE) for models with AICc < 2. Significant p-values (< 0.05) are highlighted in bold. The percentage of explained deviance of the model (i.e. percentage of the variability explained by each model 'D^2') is shown.

Response variable	Model	Coefficient	SE	p-value	D^2
Scavenger richness	Open	0.133	0.082	0.105	**40%**
	Forest	−0.311	0.118	**0.009**	
	Carcass weight	0.224	0.039	**< 0.001**	
	Carcass type (herbivore)	−0.109	0.133	0.417	
	Placement (afternoon)	0.128	0.098	0.195	
Avian richness	Open	0.325	0.113	**0.003**	**43.1%**
	Forest	−1.562	0.291	**< 0.001**	
	Carcass weight	0.308	0.054	**< 0.001**	
	Carcass type (herbivore)	−0.076	0.202	0.707	
Mammalian richness	Open	−0.105	0.120	0.383	**13.1%**
	Forest	0.159	0.138	0.251	
	Carcass weight	0.121	0.053	**0.024**	
	Placement (afternoon)	0.220	0.137	0.110	
Scavenger abundance	Open	0.489	0.115	**< 0.001**	**65.1%**
	Forest	−1.461	0.158	**< 0.001**	
	Carcass weight	0.636	0.060	**< 0.001**	
	Carcass type (herbivore)	0.259	0.179	0.150	
	Placement (afternoon)	0.184	0.131	0.162	
Detection time	Open	−0.852	0.297	**0.004**	**15.4%**
	Forest	0.725	0.358	**0.04**	
	Carcass weight	−0.183	0.136	0.182	
	Carcass type (herbivore)	−0.356	0.407	0.385	
	Placement (afternoon)	0.989	0.321	**0.002**	
Total consumption time	Open	−1.306	0.281	**< 0.001**	**31.3%**
	Forest	1.221	0.338	**< 0.001**	
	Carcass type (herbivore)	−1.190	0.366	**0.001**	
	Placement (afternoon)	1.176	0.300	**< 0.001**	
Active consumption time	Open	−1.761	0.396	**< 0.001**	**31.7%**
	Forest	1.772	0.479	**< 0.001**	
	Carcass weight	0.206	0.202	0.311	
	Carcass type (herbivore)	−2.023	0.552	**< 0.001**	
	Placement (afternoon)	1.570	0.427	**< 0.001**	
Consumption rate	Open	1.270	0.299	**< 0.001**	**49.7%**
	Forest	−1.178	0.363	**0.001**	
	Carcass weight	1.112	0.152	**< 0.001**	
	Carcass type (herbivore)	1.350	0.433	**0.002**	
	Placement (afternoon)	−1.020	0.330	**0.002**	

Mean scavenger abundance was almost double in open compared with shrubland landscapes, and up to 12 times higher compared with forests (Table 1). It increased with carcass size but was not affected by placement time or carcass type (Table 2, Supporting information). The most abundant avian scavengers were griffon vultures, followed by *Corvus* spp. and Egyptian vultures. Wild boars, domestic dogs and red foxes were the most abundant mammals (Supporting information).

Differences in scavenger efficiency

Avian and mammalian scavenging frequency showed clear differences according to landscape type and carcass size (Fig. 2). Birds scavenged at 91.4, 73.1 and 27% whereas mammals scavenged at 90, 97.2 and 100% of the carcasses in open, shrubland and forest landscapes, respectively. Obligate scavengers, except for the Egyptian vulture, consumed carrion significantly more frequently in open landscapes than in shrublands (Fig. 2, Supporting information). Griffon vultures were the most frequent scavenger, followed by bearded, Egyptian and cinereous vultures (Fig. 2). Facultative avian scavenging frequency did not differ between open and shrubland landscapes, except for the red kite *Milvus milvus* (Supporting information). Ravens *Corvus corax* and golden eagles *Aquila chrysaetos* were the most frequent facultative avian scavengers (Fig. 2). Only ravens, Eurasian jays *Garrulus glandarius*, golden eagles and griffon vultures consumed carcasses within forests (Fig. 2). Mammals scavenged carcasses in all landscapes to a similar extent, except for the wild boar and *Martes* spp. (Fig. 2, Supporting information). Foxes and wild boars were the most frequent mammalian scavengers. GLMs confirmed that, except for the Egyptian vulture, kites

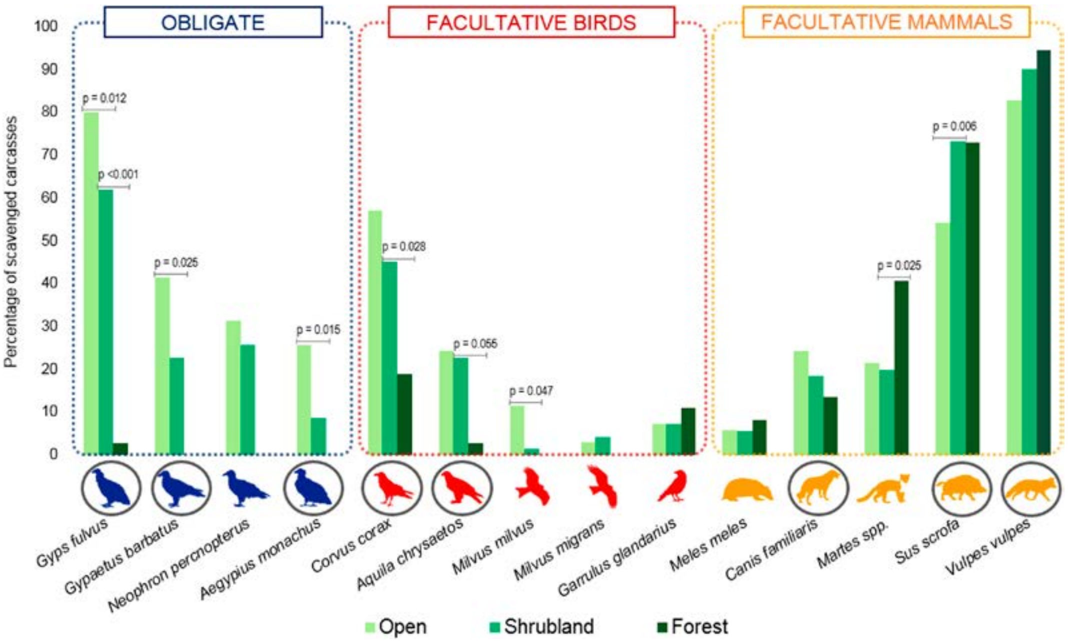

Figure 2. Scavenging frequency (percentage of consumed carcasses) of the main vertebrate scavengers recorded in each landscape type. Significant p-values among landscape types and significant effect (circles) of carcass size (i.e. preference for large carcasses, Supporting information) are shown for each species. Obligate (blue square), avian facultative (red square) and mammalian facultative (orange square) scavengers are shown.

Milvus spp., badger and *Martes* spp., species visited carcasses more frequently as carcass size increased (Fig. 2, Supporting information).

Landscape type influenced both the main carcass detector species (Fig. 3) and detection time (open < shrubland < forest, Table 2, 3). Carcasses were detected faster in open landscapes, while detection times increased significantly in shrublands and forests. Carcasses placed in the morning were detected more rapidly, while carcass size and type did not influence detection time (Table 2). In all landscapes, birds detected carcasses significantly faster than mammals (Fig. 4, Supporting information), although birds discovered only four carcasses within forests. Mean total consumption time was 2.5 times faster in open than shrubland landscapes, and up to five times faster than in forests (Table 3). Regarding carcass type, herbivore carcasses were consumed more rapidly than carnivore carcasses (Table 2). In addition, carcasses placed during the morning were exploited faster, while carcass size did not influence consumption times (Table 2). Active consumption time showed a similar pattern to that of total consumption time (Table 2 and 3). Consumption rate was three times higher in open than in shrubland landscapes, and up to 12 times higher than in forests (Table 3). It increased with carcass size and was higher for herbivore than for carnivore carcasses, decreasing when carcasses were placed in the afternoon (Table 2, Supporting information). The percentage of unconsumed parts of medium-sized ungulates (n = 82, mean ± SD = 51.03 ± 17.8 kg) was less in open (8.1%) than shrubland (10.6%) and forest (14.6%) landscapes (Kruskal–Wallis test, $\chi^2_2 = 10.9$, $p < 0.05$).

The influence of griffon vultures on scavenging dynamics

GLMs revealed that griffon vultures influenced the scavenger assemblage composition and scavenging efficiency (Fig. 5, Supporting information). Scavenger richness, avian richness and abundance per carcass were higher when griffon vultures consumed them, although this did not influence mammalian richness. Both birds and mammals arrived faster at carcasses when griffon vultures exploited them. Consumption times decreased when griffon vultures were among the consumers of a carcass (80 versus 362 h with and without the participation of griffon vultures, respectively) and consumption rates were notably higher (7.6 versus 0.06 kg h^{-1} with and without the participation of griffon vultures, respectively).

Discussion

Allowing passive rewilding has been recognized as a novel conservation strategy in abandoned European agricultural

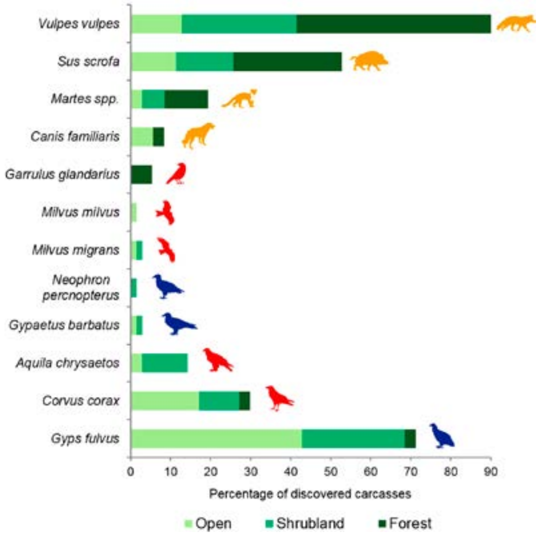

Figure 3. Main carcass detector species (%) in each landscape type. Avian scavengers were the main detectors in open landscapes (44.3% by obligate and 22.9% by facultative species). Both birds (51.4%) and mammals (48.6%) discovered carcasses in shrublands to a similar extent, while mammals were the main detectors (89.2%) in forest landscapes. Griffon vultures *Gyps fulvus* and red foxes *Vulpes vulpes* were the main detectors in open and forest landscapes, respectively. The percentages were calculated on the basis of carcasses monitored in each landscape type.

landscapes, where it is estimated that between 2000 and 2030, up to 20 million ha may have been released from agricultural use (Pereira and Navarro 2015). Rewilding processes may increase carrion availability due to the expansion and increased abundance of wild ungulates (Acevedo et al. 2011), but little is known about the effects of passive rewilding on vertebrate scavenging communities (Mateo-Tomás et al. 2015). Previous studies have highlighted habitat type as an important factor influencing carcass utilization (Arrondo et al. 2019, Pardo-Barquín et al. 2019, Stiegler et al. 2020). However, this study is the first to assess the effects of woody encroachment or 'landscape closure' due to farmland abandonment and undergrazing on the functioning of scavenging guilds in mountain agroecosystems.

Our findings indicate that landscape type is a major factor influencing scavenger assemblage composition and scavenging efficiency. However, carcass size and the presence of griffon vultures also modulated the functioning of scavenging assemblages. Carcasses were more frequently visited by avian scavengers in open landscapes, while in shrub and forest landscapes mammals dominated at carcasses. In addition, open landscapes were associated with higher scavenger abundances, faster carcass detection and consumption times and higher consumption rates, and favored rapid carcass location and more efficient consumption by griffon vultures. We found that carcass size influenced the composition of scavenger assemblages (i.e. higher scavenger richness and abundance at larger carcasses). However, regarding scavenging efficiency, carcass size only affected carcass consumption rates (i.e. carcasses with larger biomass were consumed more rapidly), suggesting that landscape type is the main factor determining carrion discovery and consumption times in agroecosystems inhabited by both obligate and facultative scavengers .

Contrary to our expectations, we detected a similar number of obligate and facultative scavenger species in open and shrubland landscapes. Furthermore, the mean number of species per carcass was similar in both. These results differ from those of Arrondo et al. (2019), who found that sheep carcasses in open areas were visited by fewer species but showed higher mean scavenger richness than wild ungulates placed in more heterogeneous sites. This discrepancy could be related to the wide diversity of carcass types we monitored. The provision of more carcass sizes/types may result in greater carrion exploitation by scavenger assemblages. For example, we detected higher scavenger species in both open and shrubland landscapes compared with those recorded in previous studies (Arrondo et al. 2019). We also found higher average avian richness in open landscapes, which could be related to the high scavenging frequency of griffon vultures and ravens at these carcasses, since these species could indicate the location of food to other scavenging birds (Cortés-Avizanda et al. 2012, Orr et al. 2019). On the contrary, our results demonstrate that carcasses within forests were consumed by fewer species, mainly mammals as has been reported in other temperate forests (Inagaki et al. 2019, Pardo-Barquín et al. 2019, Tobajas et al. 2022).

The adaptive abilities of scavengers to locate and exploit carrion (i.e. visual/olfactory abilities, capacity to break into carcasses and foraging behavior), determine carcass detection and utilization (Selva et al. 2005). Our findings revealed that birds found carcasses faster than mammals across all landscape types studied. This is probably because birds are generally better adapted to locating food because they can fly and

Table 3. Scavenging efficiency measures recorded in each landscape type: overall detection time (h), avian and mammalian detection time (h), total and active consumption time (h) and consumption rate (kg h^{-1}) per carcass. Values represent the mean ± standard deviation and range.

Landscape	Detect. time	Avian detect. time	Mammalian detect. time	Total consum. time	Active consum. time	Consum. rate
Open	21.90 ± 29.54	15.4 ± 19.17	34.91 ± 40.99	80.91 ± 152.55	59.0 ± 143.94	7.89 ± 16.27
	(0.02–182.13)	(0.02–70.80)	(0.16–182.13)	(0.22–945.56)	(0.10–945.27)	(0.004–82.12)
Shrubland	43.20 ± 63.25	24.56 ± 37.34	53.93 ± 52.70	207.12 ± 274.40	163.93 ± 244.0	2.70 ± 7.82
	(0.10–394.46)	(0.1–189.18)	(1.87–241.67)	(0.90–1234.16)	(0.13–1044.98)	(0.0005–48.11)
Forest	65.32 ± 67.03	22.65 ± 33.93	70.50 ± 68.50	411.12 ± 336.91	345.79 ± 51.30	0.66 ± 3.66
	(0.32–297.05)	(0.32–72.0)	(5.50–297.05)	(1.25–1261.98)	(0.03–1008.82)	(0.002–22.32)

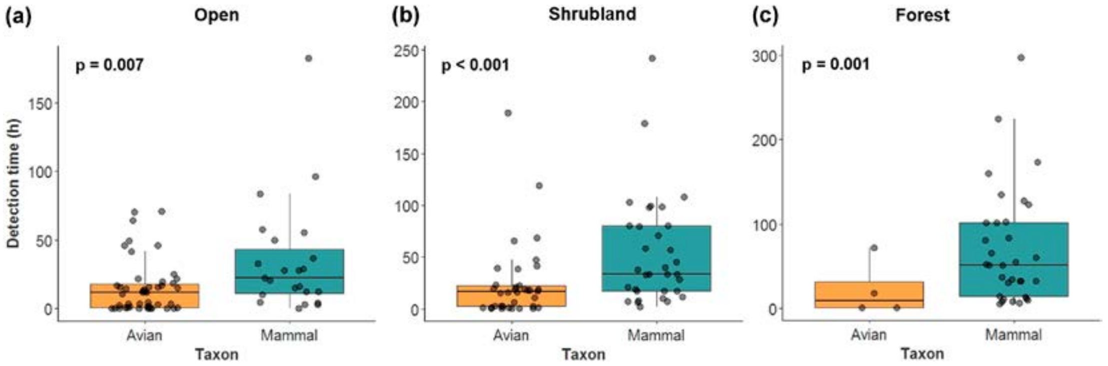

Figure 4. Boxplots and carcass detection times values (hours) at each carcass for birds and mammals in the three landscape types studied. Significant values of the univariate generalized linear models (Supporting information) are shown.

have acute eyesight (Ruxton and Houston 2004, Potier et al. 2016). However, vegetation cover affects the rate and speed at which carrion is discovered by a species (Ogada et al. 2012). We found that avian scavengers were the main detectors in open landscapes whereas both birds and mammals discovered carcasses in shrublands equally easily. Conversely, mammals detected almost 90% of the carcasses within forests. On the other hand, our results showed that increased vegetation cover led to higher detection times, since scavengers found carcasses in open landscapes up to three times faster than in forests. For example, birds found carcasses more rapidly in open landscapes (~15 h) than shrublands and forests (~25 h). Also, similar to the findings of Lima et al. (2021), mammals discovered carrion more rapidly in open landscapes than forests (~35 h versus ~70 h).

Unlike mammals, avian scavenging frequency decreased as vegetation cover increased. Overall, obligate scavengers consumed carrion more frequently in open landscapes, while this preference was not observed for the most frequent but less efficient biomass consumers (i.e. facultative avian and mammalian scavengers, Morales-Reyes et al. 2017, Arrondo et al. 2019). Birds, which rely mainly on visual cues to detect food, found carcasses easily in open landscapes and rarely detected them underneath dense forest cover (Moleón et al. 2019). Conversely, the well-developed olfactory sense of mammals plays a larger role in food discovery, making them better adapted to foraging in closed landscapes (Enari and Enari 2021). Despite this, some birds, such as corvids and eagles, are extremely efficient in locating carcasses, which could explain our observed scavenging frequency of these species within forests (Selva et al. 2005).

In accordance with our predictions, we found that carcass size is an important factor driving scavenging patterns in terms of scavenger assemblage composition (Moleón et al. 2015, Moreno-Opo et al. 2015, Turner et al. 2017, Stiegler et al. 2020). The greater biomass provided by large carcasses (above ~25 kg) helped explain the higher scavenger richness and abundances we observed at these carcasses (Moleón et al. 2015, Turner et al. 2017). However, contrary to our expectations, neither detection time nor total and active consumption times were related to carcass size (Moleón et al. 2015, Turner et al. 2017), suggesting that, in rewilding situations, landscape type is the major factor influencing carcass detection and the time until complete consumption. Despite this, larger carcasses showed higher consumption rates, which could be attributed to exploitation by highly efficient griffon vultures (Sebastián-González et al. 2016). Indeed, our results suggest that griffon vultures play a similar role in terms of consumption rates as that observed for large facultative scavengers in African scavenging assemblages (Moleón et al. 2015), since consumption rates were ~125 times higher when carcasses were consumed by this species.

As expected, carrion removal was faster in open landscapes than within forests, because of the absence of vultures and dominance of mammals in forested areas (Morales-Reyes et al. 2017). In fact, our findings showed that griffon vultures play a crucial role in reducing carcass persistence. Carcasses that were consumed by griffon vultures persisted much less (~3 days) than those that were not consumed by this species (~15 days), highlighting their crucial regulatory service in maintaining healthy ecosystems. Both total and active consumption times increased when carcasses were deployed during the afternoon, since scavenging birds are generally inactive at night. Otherwise, our results support the view that vegetation encroachment leads to longer carcass persistence times. Increased carcass persistence in a landscape has been shown to increase carcass decomposition rates and the likelihood of disease transmission (Markandya et al. 2008, Ogada et al. 2012, Buechley and Şekercioğlu 2016), while both obligate and facultative scavengers can recycle carrion more efficiently than decomposers (DeVault et al. 2003, Ray et al. 2014).

We found that carnivore carcasses persisted longer and showed lower consumption rates than herbivore carcasses (Oliva-Vidal et al. 2021, Peers et al. 2021). However, contrary to our predictions, we detected no differences in species richness, abundance and detection time between herbivore and carnivore carcasses. Indeed, some avian scavengers such

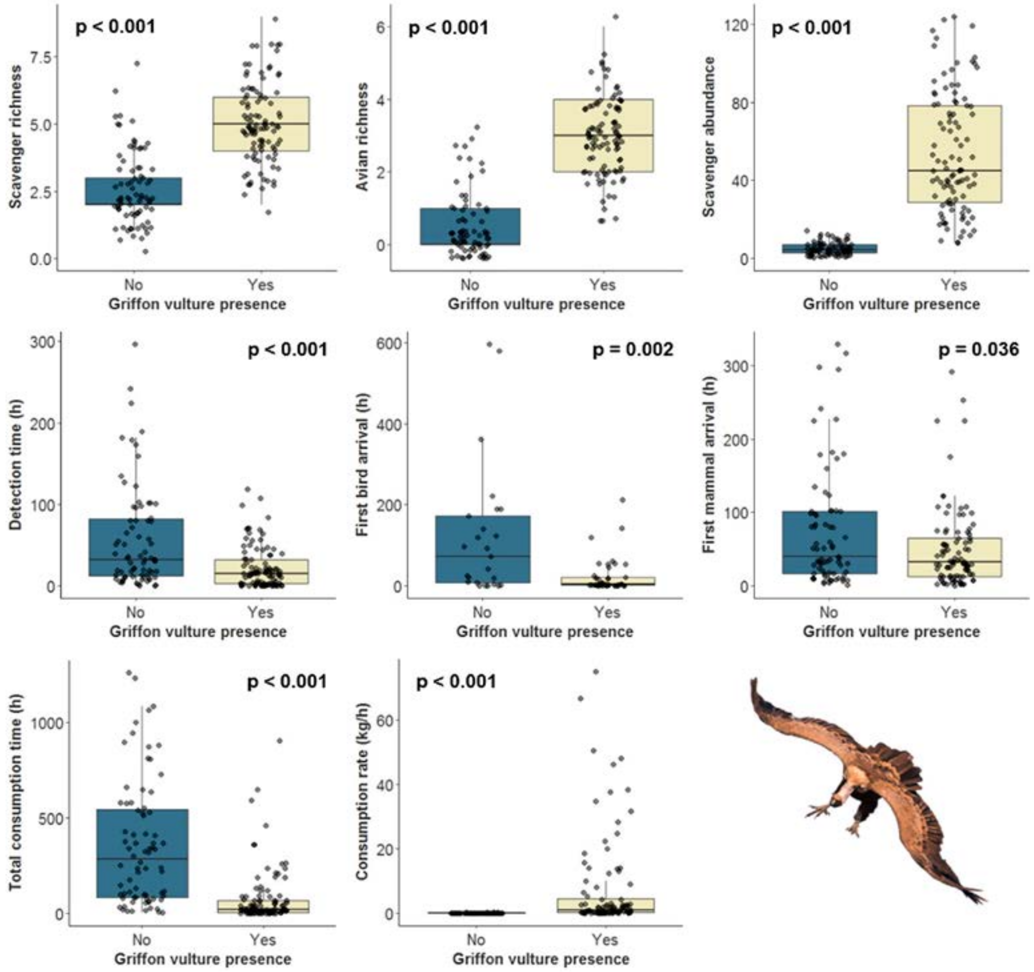

Figure 5. Boxplots showing the significant univariate relationships between the presence of griffon vultures at carcasses and the scavenger assemblage composition, scavenging efficiency and arrival time (h) of the first bird and mammal after griffon vulture appearance (Supporting information). Dots represent real data.

as golden eagles and red kites were more frequent at carnivore carrion. This could be attributed to the smaller size of carnivore carcasses, which may make them harder for griffon vultures to detect. Further, we recorded conspecific necrophagy at all red fox and at 57.5% of wild boar carcasses, where wild boars consumed mainly bone remains from conspecifics. The conspecific necrophagy we observed in red foxes contrasts with the findings of Moleón et al. (2017). They evidenced avoidance of carnivore and conspecific consumption by other carnivores, and attributed this behavior to a strategy to reduce the probability of disease transmission. However, our observations suggest that conspecific carcasses can provide an alternative resource for red foxes and other scavengers in certain areas or circumstances (Oliva-Vidal et al. 2021).

Overall, griffon vultures consumed 80% of the carcasses in open landscapes and 62% in shrublands. These findings support the idea that their functional traits (e.g. effective foraging strategies and conspecific visual information transfer, Cortés-Avizanda et al. 2014, Gutiérrrez-Canovas et al. 2020) make griffon vultures the most frequent, abundant and efficient scavengers in our study area. However, our results also show that landscape rewilding may jeopardize their high scavenging efficiency because increasing shrub and, especially, tree cover, hinder their ability to locate carrion and reduce both their scavenging frequency and, ultimately, their abundance in more closed landscapes (Ogada et al. 2012, Martin-Díaz et al. 2020). The higher griffon vulture abundances in open areas could also relate to landscape characteristics in other ways, since high vegetation cover may leave insufficient clear space for them to take-off easily (Bamford et al. 2009).

We found strong evidence for the dominant role of griffon vultures in shaping scavenging through conspecific and heterospecific trophic facilitatory processes (Sebastián-González et al. 2016, 2021). First, more avian scavenger species consumed carcasses when griffon vultures exploited them. Mammalian richness was not influenced, perhaps because of the low number of diurnal mammal species in our study area. Second, because of their effective use of social information from conspecifics in their foraging strategy, a greater number of griffon vultures will gather to feed on a carcass (e.g. more than a hundred individuals can be attracted to a carcass once it is discovered). Third, both birds and mammals (mainly foxes, which can forage at the same time as griffon vultures) arrived faster at carcasses discovered by griffon vultures. These findings are consistent with Kane et al. (2017), who found that mammals might find carrion twice as fast when following vultures. Although griffon vultures consumed almost all of the meat biomass, we observed that bone remains provide an essential resource for some species (e.g. bearded vultures) and are valuable resources for mammals (especially red fox and wild boar), because bones are as energetically rich as meat over the long term (Blasco et al. 2019). Thus, in our study area griffon vultures may provide three major trophic facilitatory functions: 1) signaling carcass location; 2) facilitating access to the interior of carcasses offering feeding opportunities to less powerful species (i.e. access to small meat pieces, tendons, skin and skeletal remains); and 3) providing resources to more specialist species such as bearded or cinereous vultures (Moreno-Opo et al. 2015).

Concluding remarks

Scavengers are subject to large-scale habitat changes, such as farmland abandonment, which is projected to increase worldwide (Pereira and Navarro 2015, Tsunoda and Enari 2020, Lima et al. 2021). The abandonment of rural grazing and farmland practices often results in drastic landscape changes through general processes of vegetation encroachment (passive rewilding) and consequent 'landscape closure' (García-Ruiz et al. 2020b). The effects of habitat type on the functioning of scavenging assemblages have been previously recognized, and understanding the ecological consequences of passive rewilding is necessary to harmonize the coexistence of humans and scavengers. Our findings show that, in rewilding agroecosystems, landscape type is the main factor governing scavenging dynamics through its influence on the composition of scavenger assemblages and their scavenging efficiency. We demonstrate that progressive vegetation encroachment could jeopardize a hugely important functional group (i.e. vultures, which provide crucial ecosystem and scavenging services), reducing the scavenging efficiency of the assemblage (Morales-Reyes et al. 2017). While open landscapes promote carcass removal by the most efficient avian scavengers, in more forested landscapes mammals dominated at carcasses. Moreover, woody encroachment increases carcass persistence, which is known to result in negative ecological, economic and human health consequences (Markandya et al. 2008). We highlight the high consumption rates of griffon vultures as well as their role in driving trophic facilitatory processes, especially to endangered avian scavengers. Our results suggest that future studies should explore the necessity of maintaining open areas (e.g. by supporting traditional pastoral practices) to mitigate the ecological impact of rural abandonment on the functioning of scavenger assemblages.

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Author contributions

Pilar Oliva-Vidal: Conceptualization (equal); Investigation (lead); Formal analysis (equal); Methodology (equal); Resources (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Esther Sebastián-González**: Conceptualization (equal); Formal analysis (equal); Methodology (equal); Writing – review and editing (equal). **Antoni Margalida**: Conceptualization (equal); Project administration (lead); Funding acquisition (supporting); Resources (equal); Supervision (lead); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.9zw3r22hn> (Oliva-Vidal et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

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Griffon vultures, livestock and farmers: Unraveling a complex socio-economic ecological conflict from a conservation perspective

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ABSTRACT

An unexpected human-wildlife conflict between vultures and livestock has emerged in Europe during the last two decades. Farmers attributed changes in vulture behavior, due to food shortages caused by sanitary regulations, to increasing livestock interactions ('vulture attacks'). To disentangle this conflict, we analyzed 683 farmer complaints between 1996 and 2020 in Catalonia (northeastern Spain) and investigated the eco-anthropological factors driving their frequency. We also assessed farmers' perception through 127 interviews. Most complaints (80 %) occurred during the birthing season, mainly involving cattle (76.5 %), followed by horses (14.9 %) and sheep/goats (8.6 %). From 2008 to 2020, vulture-livestock conflicts cost the government €192,000 (~22 % of claims compensated). The frequency of complaints was positively associated with extensive livestock density, griffon vulture *Gyps fulvus* abundance (breeding and non-breeding), shorter distances to landfill sites and, to a lesser extent, to supplementary feeding stations. In contrast, there was a negative relationship between complaints and the number of griffon vulture breeding pairs, suggesting that long-distance foraging movements by both breeding and non-breeding individuals may play a major role in determining the occurrence of conflicts. Farmers (88 %) said that vultures attack livestock and that attacks had increased in recent years because of significant vulture population increases and food shortages due to sanitary regulations. They considered government policies and compensation ineffective. We highlight the critical need for mitigation in areas with high extensive livestock numbers, particularly during birthing times. Scientific assessments and interdisciplinary awareness campaigns on the coexistence of vultures and livestock are necessary to harmonize biodiversity conservation and agro-pastoral practices in rural economies.

1. Introduction

Human-wildlife conflicts (HWCs) are becoming more common and are of increasing concern to ecologists and managers because of their impact on biodiversity loss (Woodroffe et al., 2005; Treves et al., 2009; Nyhus, 2016). Wildlife attacks on livestock and damage to crops are major causes of HWCs worldwide (Torres et al., 2018) and are projected to increase due to climate change, increasing economic insecurity and retaliations against wildlife in agro-pastoral communities (Abrahms, 2021). Economic losses produced by HWCs, mainly due to endangered predators (carnivores and birds of prey) killing livestock, are considered the main driver of this conflict (Graham et al., 2005; Dickman, 2010; Redpath et al., 2013; Araneda et al., 2021). The resulting direct persecution of predator species can jeopardize conservation efforts (Peterson et al., 2010; Zimmermann et al., 2010). Torres et al. (2018) identified 263 terrestrial vertebrate species involved in HWCs worldwide, of which 53 were listed as threatened, such as vultures. Policy-makers therefore need evidence-based information to design sustainable management actions and policy tools to address this problem (Baynham-Herd et al., 2020).

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Old World vulture populations are declining throughout their ranges, mainly due to anthropogenic pressures (Green et al., 2004; Ogada et al., 2012). However, in contrast, European vulture populations are stable, or even increasing. Nonetheless, over the past two decades critical threats to the conservation of European vultures have emerged (Safford et al., 2019). The main current threat, in the shape of new sanitary legislation (EC 1774/2002), appeared after the outbreak of bovine spongiform encephalopathy (BSE) at the beginning of 21st century. This controversial regulation prohibited the abandonment of dead extensively reared livestock in the countryside from 2006 to 2011 (Donázar et al., 2009). A collateral effect subsequently emerged; a widespread view, fueled by public and media misrepresentation, claimed a causal relationship between food shortages for scavengers and presumed attacks on livestock, leading to increasingly negative perceptions of vultures (Margalida et al., 2011).

Negative perceptions of wildlife can promote anthropogenic pressures such as lethal control. Indeed deliberate poisoning is considered one of the major causes of death in wildlife species in Europe (Guitart et al., 2010). Avian scavengers in general, and vultures in particular, are major victims of illegal poisoning, either intentionally or unintentionally (Pfeiffer et al., 2015; Ogada et al., 2016; Santangeli et al., 2016; Plaza et al., 2019; Safford et al., 2019). Laying the blame for livestock killing on Old and New World vultures has recently aggravated this situation (Margalida et al., 2011, 2014; Duriez et al., 2019; Plaza and Lambertucci, 2021). Generalized perceptions of vultures attacking livestock have increased in recent years, associated with the viral spread of partial and biased information through social media, despite the limited empirical support for these assertions (Margalida and Donázar, 2020; Lambertucci et al., 2021). In spite of the long-established mutual relationship between humans and vultures (Moleón et al., 2014), negative perceptions may drive discontent and incite vengeance from some farmers, creating a source of tension and the debate on vulture/livestock conflicts is increasing and remains open and controversial.

The study of public perceptions, "*the way an individual observes, understands, interprets, and evaluates a referent object, action, experience, policy or outcome*" (Bennett, 2016) has received growing attention in conservation biology because of increasing awareness of the critical role of the human dimension (i.e., perceptions, values, beliefs and attitudes) in improving conservation efforts (Bennett et al., 2017) and effectively resolving HWCs (Dickman, 2010). However, perceptions are not always objective since they are mediated by multiple contextual factors, including past experience, personal motivation or the reports of individuals, communities or social media (Naughton-Treves and Treves, 2005; Bennett, 2016; Ballejo et al., 2021). A person's perceptions can also be driven by contradictory governmental policies (e.g., when a species, such as vultures, is listed both as a livestock-predator and a protected species) requiring different mitigation and conservation measures. Therefore, making objective decisions can be challenging because human realities and perceptions may vary widely between and within the stakeholder groups involved (König et al., 2020).

A recent example of differing perceptions regarding the relationship between scavengers and livestock relates to farmers. Their perceptions, attitudes and values may change along a species geographical distribution (Ballejo et al., 2019), due to different local legislation (Gigante et al., 2021) or paradoxically, the same species and species within the same guild can be perceived as either beneficial or harmful, or a mixture of both (Morales-Reyes et al., 2018). Moreover, although farmers usually attribute severe damage to livestock as being due to scavenging birds, when the interaction is assessed it is often found to be minimal or even nonexistent (Margalida et al., 2014; Duriez et al., 2019). Indeed livestock losses to bird predation are often found to be less than those produced by other causes, such as disease, injury or poor nutrition (Graham et al., 2005). Recent research on the behavioral conflict between New and Old world vultures and livestock has focused on the human dimension (Ballejo et al., 2019; Gigante et al., 2021; Salom et al., 2021) and to a lesser extent on field observations (Ballejo et al., 2020). However, a complete examination of this phenomenon would require integrating the management/responses of local authorities, complaint assessments, farmer perceptions and broader assessments by technical experts and veterinarians.

Here, we try to disentangle the factors associated with increasing conflicts between vultures and livestock in Catalonia, a region of northern Spain inhabited by the four European vultures. We first assessed the complaints reported by farmers to the authorities and explored their temporal trend. Then, we investigated the influence of ecological and anthropic factors (e.g., global griffon vulture *Gyps fulvus* abundance, number of griffon vulture breeding pairs, extensive livestock density and the distances to the nearest landfill site and supplementary feeding stations 'SFSs') on the frequency of complaints. Finally, we interviewed farmers to analyze their perceptions and concerns regarding this emergent conflict, so as to assess the vulture/livestock interface from an ecological-economic-social perspective. Based on our results, we propose solutions and guidelines to manage this unexpected conflict and to harmonize vulture conservation with agro-pastoral practices.

2. Material and methods

2.1. Study area

Catalonia covers over 32,000 km^2, with altitudes ranging from sea level to >3000 m. The landscape is heterogeneous, dominated by forests (34.7 % of the total area), crops (30.1 %), shrublands (22.4 %) and grasslands (4.8 %), while urbanized areas cover 5.3 % of the surface (González-Guerrero and Pons, 2020). The region is characterized by a high population density (241.8 inhabitants/km^2) and powerful industrial and service sectors, while agriculture is less important (1 % of the regional GDP). Intensive farming of pork (54.9 %) and poultry (17.9 %) are the most important agricultural systems. Cattle (13.0 %), sheep/goat (1.8 %) and horse (0.4 %) rearing are economically less important (DARPA, 2018). Catalonia has at least 5457 extensive or semi-extensively farms of cattle (37.03 %), sheep/goats (34.9 %) and horses (28.07 %) (http://agricultura.gencat.cat/), which are present throughout the region, but especially in mountainous areas. Extensive livestock in mountainous zones graze on high summer pastures from June to October and remain stabled in the valley bottoms exploiting the surroundings of small hamlets for the rest of the year (García-Ruiz et al., 2020). Rural areas hold important populations of facultative and obligate scavenger species. At least 1628 breeding pairs of griffon, 20 of cinereous *Aegypius monachus*, 88 of Egyptian *Neophron percnopterus* and 49 of bearded vultures *Gypaetus barbatus* occur in the region (Del Moral, 2017; Del Moral and Molina, 2018a, 2018b; Margalida and Martínez, 2020).

2.2. Farmer complaints

We used an official database containing all complaints (n = 683) made by farmers from 1996 to 2020 relating to reported vulture 'attacks' on livestock. It is important to note that while farmers reported incidents as 'attacks', our study took the view that the precise nature of the interaction was still to be proven, so we additionally used the term 'interaction' to refer these conflicts. We used the complete database to assess the temporal trend, and the complaints reported from 2008 to 2020, for which more detailed information was available, to characterize the interactions (n = 616, 90.2 % of the total). A subset of this data (2008–2018, n = 573 claims, 83.9 % of the total) was considered for modelling the determinants of conflicts, as updated information of livestock density was not available for the latest period. For each complaint, the official data included: i) date of the interaction; ii) date of the assessment; iii) municipality and county; iv) livestock type (cattle, horses or sheep/goats) and category ('adult', 'female and new-born' or 'new-born'); v) the number of individuals affected; vi) validation (i.e., 'accepted' when the assessment considered that the animal was still alive when vultures started to consume it, or 'rejected' when no evidence was found or when consumption by vultures was considered to be post-mortem); vii) financial compensation (€); and viii) UTM coordinates. To characterize the interactions, we recorded for each complaint: i) type of livestock involved; ii) livestock category; iii) seasonality (i.e., the month the claim was reported); iv) number of complaints 'accepted' (i.e., financially compensated) or 'rejected'; and v) total economic compensation by the authorities.

2.3. *Frequency of complaints*

We first recorded the number of complaints reported by farmers in a grid of 10 × 10 km UTM cells during 2008–2018, since each cell was considered as one single statistical observation in the analyses ($n = 110$ cells accounting for 573 complaints). Next, we calculated the following variables for each cell: i) 'number of griffon vulture breeding pairs'; ii) 'global griffon vulture abundance'; iii) 'extensive livestock density'; iv) 'distance to nearest SFS'; and v) 'distance to nearest landfill site'.

We estimated the 'number of griffon vulture breeding pairs' as the average of two censuses carried out in 2008 and 2018 throughout Catalonia (Del Moral and Molina, 2018a); and the 'global griffon vulture abundance', according to the values obtained by García-Ferré et al. (2011) following the methods described in Herrando et al. (2011). In brief, sampling was carried out through surveys performed in three consecutive winters (2006–2009) over the whole study area, and then was standardized by sampling effort. After that, all the data was processed statistically to achieve a value of abundance for each 1 × 1 km cell. Here, we averaged the 100 1 × 1 km cells to obtain a value of abundance for each 10 × 10 km cell. An advantage of this measure is that it accounts for the abundance of both breeding and non-breeding individuals. It is known that griffon vultures routinely perform movements at large distances from breeding sites (Delgado-González et al., 2022), so they can be abundant in areas located far from breeding sites. Moreover, non-breeding vultures may represent an important fraction of the population, which frequently exploit food resources far from the breeding grounds. Similar information on abundance does not exist for later years, although based on our personal observations we do not expect the spatial pattern of vulture abundances to have changed significantly during the period considered in the analyses.

The 'extensive livestock density' (e.g., extensive, mixed or transhumant farming systems) was estimated considering the potential capacity of the farms (i.e., maximum number of animals that a farm can raise) for each livestock type (cattle, sheep/goats and horses). We assumed that farm capacities are a good proxy of the number of livestock animals (information that was not yearly available) because farmers pay fees on this basis. To estimate the density of extensive livestock at the cell level, we first calculated for each year an average density of livestock heads for each municipality. Then, we calculated a weighted average for each cell according to the municipalities' surface within each cell. Lastly, we averaged the yearly estimates in each cell for the entire period. Farming information was extracted from the Livestock Information System (www.sir.gencat.cat). Geoprocessing analyses were performed using the ArcMap 10.5 program (ESRI, 2016). The distances (km) to the nearest landfill site and SFS from the nearest limit of each cell were estimated assigning a distance equal to zero for those cells containing a landfill site or SFS. Because some SFSs were not active in every year, an average distance value was obtained for the whole study period.

2.4. *Farmer interviews*

We assessed farmers' perception of vulture conflicts with livestock through face-to-face and telephone interviews from 2018 to 2021 (see Appendix S1 for the full list of questions). We surveyed 127 farmers from Pyrenean and adjacent regions, obtaining information on 166 farms with cattle (53.6 %), sheep/goats (30.1 %) and horses (16.3 %), since thirty-four respondents had mixed farms. We first recorded information regarding the farm characteristics (e.g., livestock type) and then asked both closed and open questions about conflicts between vultures and livestock. Some of the closed questions were in multiple-choice format (Appendix S2). Further, we asked if they had experienced vulture interactions on their livestock and if so, we collected information about the livestock involved (e.g., where and under what circumstances the dead livestock was found) as well as if they had recognized the species starting the interaction and how they detected it. Respondents were also asked a range of questions regarding their views on whether vultures could attack live livestock, the temporal trend of such conflicts, the type of livestock supervision during birthing times and the response of the local authorities and any financial compensations paid. Finally, we recorded farmers' views on the coexistence of vultures and livestock, the ecological relationships between them, and on measures that could be implemented to mitigate conflicts.

2.5. *Statistical analysis*

We investigated ecological and anthropic factors influencing the frequency of vulture/livestock interactions (i.e., number of complaints) using generalized linear models (GLM), where 'number of complaints' (per 10 × 10 km cell) was the response variable and 'number of griffon vulture breeding pairs', 'global griffon vulture abundance', 'extensive livestock density', 'distance to nearest landfill site', and 'distance to nearest SFS' were the explanatory variables. We used Poisson error distributions and logarithmic link functions. Models considering the main effects of the explanatory variables were evaluated. We used Akaike's information criterion for small sample sizes (AICc) to identify the most parsimonious model (i.e., the one with the lowest AICc) and to rank the remaining models. When there was more than one model competing with the best AICc model (those with $\Delta\text{AICc} < 2$), we performed conditional model-averaging across the candidate models (Burnham and Anderson, 2002) using the *MuMIn* package (Barton, 2013). This procedure averages parameter estimates across the set of selected models in which the respective parameter appeared, weighted by the relative importance of each model. The analyses were performed using the R Software for Statistical Computing program (R Development Core Team, 2020). We used Chi-squared analyses to look for differences in farmers' perceptions of the coexistence and ecological relationships between vultures and livestock, based on whether they had suffered attacks.

3. Results

3.1. *Complaints assessment*

We found three temporal trends in the 683 complaints reported from 1996 to 2020. Until 2006, the number of complaints was low and with little variability between years (mean ± SD = 1.8 ± 1.6 claims/year). From 2007 to 2011 complaints increased dramatically reaching a maximum in 2010 (86 complaints). After 2011 the trend diminished progressively until 2020 (Fig. 1). Complaints reported during 2008–2020 mainly involved cattle ($n = 471$, 76.5 %), followed by horses ($n = 92$, 14.9 %) and sheep/goats ($n = 53$, 8.6 %) (Appendix S3). Of these, 78.2 % were rejected. The proportion of accepted complaints ($n = 134$, 21.8 %) was similar: 77.6 %, 14.2 % and 8.2 % for cattle, horses and sheep/goats, respectively, and did not differ between species ($\chi^2_2 = 0.13$, $P = 0.94$). We identified a seasonal pattern (e.g., 80 % of complaints between February and July) associated with the birthing season: 76.4 %, 96.7 % and 83 % for cattle, horses and sheep/goats, respectively. Further, at least 68.3 % of the complaints occurred during birthing times (i.e., involving the female and new-born, or the new-born): 69 % for cattle, 83.7 % for horses and 35.8 % for sheep/goats, respectively (Appendix S3). The period March–June included 69.6 % of the total complaints. During this period, on average 8.3 claims were reported per

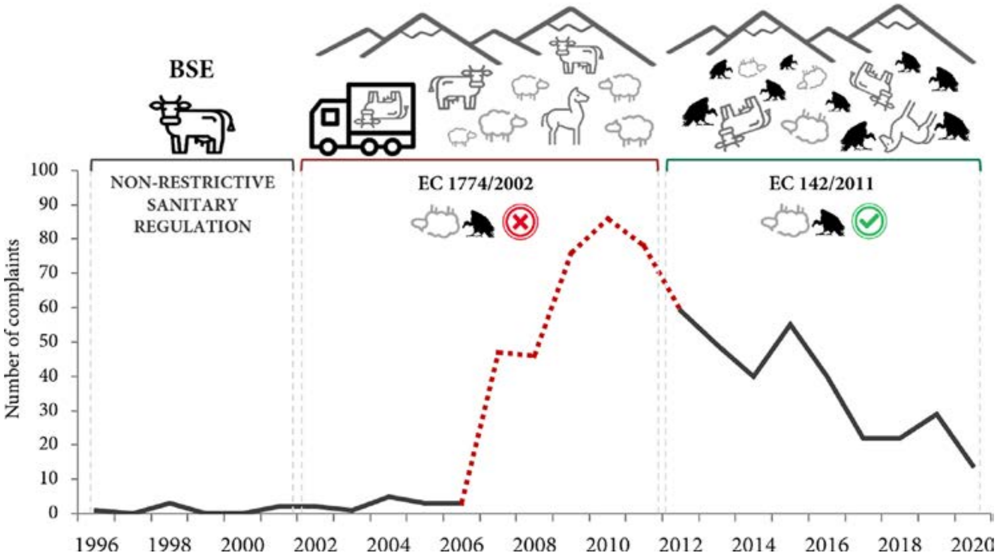

Fig. 1. Complaints regarding griffon vulture attacks on livestock from 1996 to 2020 in Catalonia. Three periods are highlighted: a) appearance of bovine spongiform encephalopathy (BSE) in Europe (1996) when no sanitary regulations were in force (e.g., livestock carcasses could be left in the countryside and available for scavengers); b) the approval of restrictive sanitary regulations (EC 1774/2002) prohibiting the abandonment of dead livestock in the countryside (2002–2011); and c) the approval of new regulation (EC 142/2011) allowing farmers to leave livestock carcasses in the countryside.
*The slow adoption of European environmental policies helps to explain the delay between regulation approval EC 1774/2002 and the increase in complaints from 2006 (López-Bao and Margalida, 2018).

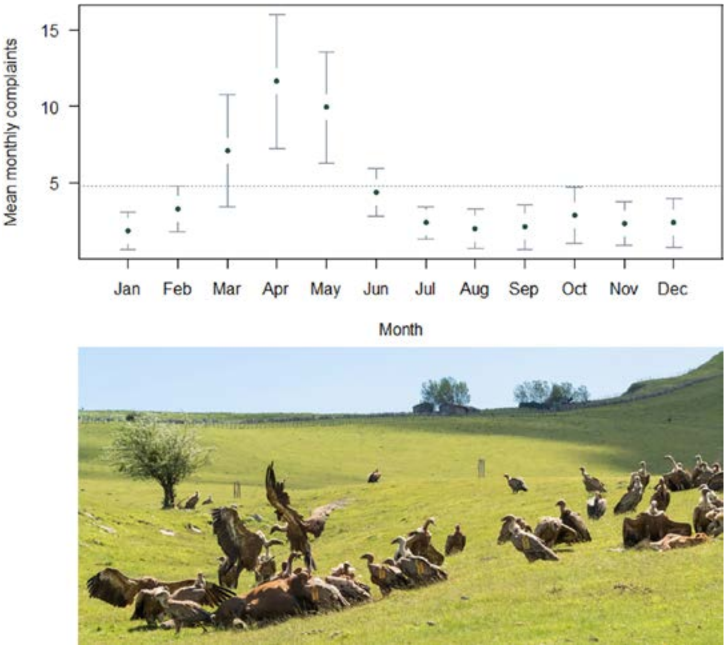

Fig. 2. Average number of monthly complaints (n = 616) from 2008 to 2020. The dashed line shows the average number of complaints reported during the entire period. The photograph shows a group of griffon vultures feeding on a dead mare and her new-born foal just after a problematic calving, which could be construed as an 'attack' (photo: Pilar Oliva-Vidal).

month, whereas for the rest of the year the mean number of monthly complaints was ≤3 (Fig. 2). Most accepted complaints (75.4 %) were made during the birthing season (65.7 % from March to June). The highest percentage of accepted complaints was in 2008 (56.6 %) and 2009 (61.8 %) and decreased in 2010 (39.5 %) and remained below 10 % until 2018. The estimated economic cost during 2008–2020 of the complaints assessed positively was €192,000.

3.2. Factors driving the frequency of complaints

We evaluated 32 models to investigate the factors influencing the frequency of complaints (n = 110 cells; Appendix S5). The averaged model included all of the predictors, of which four showed values of relative importance (i.e., > 0.8, Appendix S6). ‘Extensive livestock density’, ‘distance to nearest landfill site’, ‘number of griffon vulture breeding pairs’ and ‘global griffon vulture abundance’ were the predictors with the highest weights, although the averaged model also included the effect of ‘distance to nearest SFS’. We found that higher densities of extensive livestock and shorter distances to both the nearest landfill site and SFS were associated with higher numbers of complaints (Table 1), although the effect of the distance to the nearest SFS was weak. In addition, our results showed that a higher abundance of griffon vultures (including both the breeding and non-breeding populations) was associated with higher numbers of complaints, but conversely, the number of griffon vulture breeding pairs was negatively associated with the number of complaints (Fig. 3).

3.3. Farmers' perceptions

Of 127 farmers interviewed, 67 (52.8 %) reported having suffered vulture attacks on their livestock, cattle being the most affected (71.9 %), followed by sheep/goats (21.1 %) and horses (7.0 %) (Appendix S4). Most interactions (75.9 %) described occurred during the birthing season. However, while most cases involving cattle (91.7 %) and horses (54.6 %) were associated with calving, interactions with juveniles and adults unrelated to parturition were more important for sheep (66.7 %) (Appendix S4). Farmers associated 72.2 % of the interactions on adult livestock with old, sick or dying individuals. The attacks described mainly occurred while livestock remained in open fields (48.6 %) or fields protected with electric shepherds (44.4 %), compared with cases in semi-open (5.6 %) or closed (1.4 %) farms. Most respondents (76.5 %) thought that griffon vultures had initiated the attack, followed by ‘don't know’ (10.5 %), ravens *Corvus corax*/foxes *Vulpes vulpes* (3.9 %) and cinereous vultures/bears *Ursus arctos* (2.6 %). However, none claimed to have seen the actual start of an attack. Farmers detected attacks because they were in the area and observed griffon vultures (42.9 %) while supervising livestock at the time (32.1 %), by their noticing a known (21.4 %) or unknown (1.2 %) person, or while supervising livestock on the following day (2.4 %).

Overall, farmers believed that vultures can attack live livestock (88.2 %) and most of them (77.2 %) said that attacks had increased in recent years, attributing them mainly to the population increase of griffon vultures and to the lack of food in the field due to sanitary regulations (Fig. 4A). Regarding farmers declaring attacks, 68.7 % (n = 46) had reported it to the authorities. Of these, 30.4 % were financially compensated and half had considered the economic compensation to be sufficient. However, considering all respondents, 86.1 % thought that the response of the authorities had not been favorable and 82.6 % believed that financial compensation did not satisfy farmers suffering attacks. Few respondents (5.4 %) considered that current economic compensations were sufficient. Farmers reporting attacks had a more negative perception of the coexistence of vultures and livestock (Fig. 5A) and the ecological relationships between them (Fig. 5B) than those who had not reported attacks. Further, 91.3 % of respondents said that it was necessary to apply management measures to mitigate the conflict (Fig. 4B) and 73.8 % believed that interactions would increase further otherwise.

Table 1

Generalized linear model (GLM) relating the frequency of vulture attack complaints to ecological and anthropic variables. The conditional model-averaged estimates and standard errors (SE) of models with ΔAICc < 2 are shown.

Variables	Estimate	SE
Intercept	1.050	0.137
Extensive livestock density (animals/km^2)	0.011	0.001
Distance to nearest landfill site (km)	−0.027	0.005
Number of griffon v. breeding pairs	−0.010	0.003
Global griffon v. abundance	0.872	0.334
Distance to nearest SFS[a] (km)	−0.004	0.005

[a] SFS: supplementary feeding station.

4. Discussion

Livestock and wildlife conflicts have occurred for millennia, mainly involving carnivores and birds of prey (Torres et al., 2018). However, recent widespread perceptions that vultures attack livestock threaten Old and New World vulture populations (Lambertucci et al., 2021). In southern Europe, claims that vultures attack livestock began in the mid-1990s but have increased since the mid-2000s, notably in Spain and southern France (Margalida et al., 2014; Duriez et al., 2019) and an unprecedented and complex socio-economic ecological conflict has emerged.

We identified three temporal trends in reported vulture attacks on livestock. From 1996 to 2006 the number of annual complaints was low but rose significantly from 2007 to 2011, followed by a progressive decline from 2012 to 2020. Certain policy decisions could explain these trends. First, changes in sanitary legislation (Regulation EC 1774/2002) prohibited the abandonment of livestock carcasses in the countryside, provoking general alarm among farmers and establishing a perceived causal relationship between food shortages and presumed vulture attacks (Donázar et al., 2009; Margalida et al., 2010, 2011). Second, the approval in Catalonia of a regulation (Decret 176/2007) including griffon vultures among potential livestock predators (listing them as compensating species with wolves *Canis lupus* and bears). This regulation coincided with a period of perceived vulture food shortage and growing alarm among farmers, leading to government compensation for most complaints (e.g., ~60 % of those reported in 2008 and 2009). In 2011, coinciding with the highest frequency of complaints, more flexible regulations were approved (EC 142/2011) allowing livestock carcasses to be left in the field to feed scavengers (Margalida et al., 2012). However, to date few farmers have requested permission to leave livestock carcasses in areas designated for this purpose. In fact, only 9.4 % of the farmers interviewed reported leaving carcasses in the field. Official collection and transport of dead livestock from farms to authorized processing plants is still the main means of dealing with carcasses following the BSE outbreak. Our results highlight a paradoxical lack of awareness since ‘being able to leave carcasses in the countryside’ was the second most frequent measure proposed to mitigate the conflict by the farmers interviewed. The steady fall in farmer complaints since 2011 may result from more rigorous assessments (e.g., accurate necropsies) verifying that livestock deaths were due to other causes (Dalmasso et al., 2012), increasing the percentage of rejected complaints and suggesting that the conflict had been overestimated.

Presumed vulture attacks were highest during the period of severest food shortage. However, Spanish griffon vulture populations increased 20.5 % during 2008–2018 (Del Moral and Molina, 2018a), suggesting that the severest food shortage had no demographic effects. Despite this, farmers continue to believe that vultures attack livestock when food is in short supply. Our findings showed that almost 90 % of farmers believe that vultures attack livestock and most of them thought that attacks have increased in recent years, mainly because of the drastic increase in the vulture population and a lack of food due to sanitary regulations.

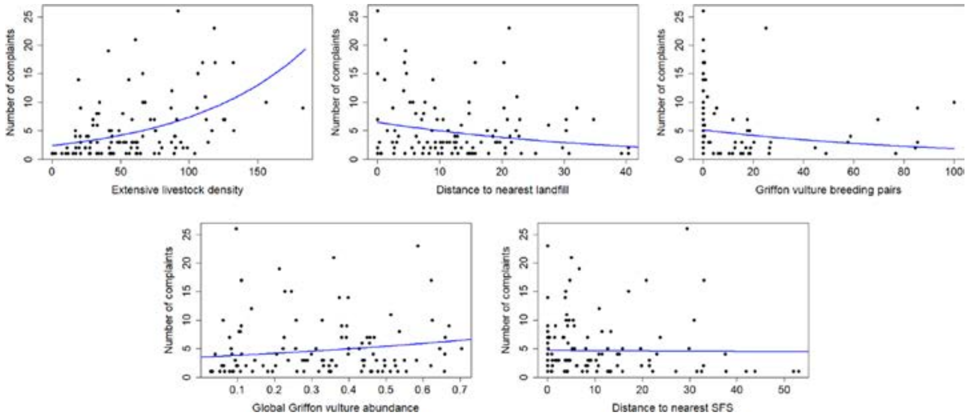

Fig. 3. Observed (black spots) and predicted (blue lines) values of the explanatory variables: 'extensive livestock density' (animals/km^2), 'distances to the nearest landfill site and SFS' (km), 'number of griffon vulture breeding pairs' and 'global griffon vulture abundance' considered in the generalized linear model determining the frequency of complaints of vulture/livestock interactions during the period 2008–2018. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

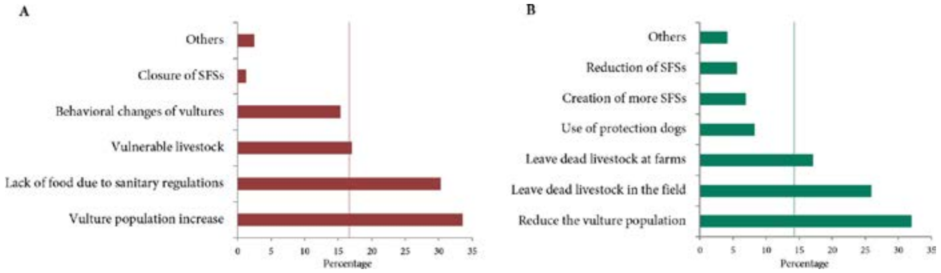

Fig. 4. A) Main causes of the increase in vulture/livestock interactions reported by the farmers interviewed (n = 241), and B) main management measures proposed to reduce this conflict (n = 216). Some farmers proposed multiple responses (Appendixes S1 and S2). The vertical line indicates the percentage of responses expected if the responses occurred equally frequently.

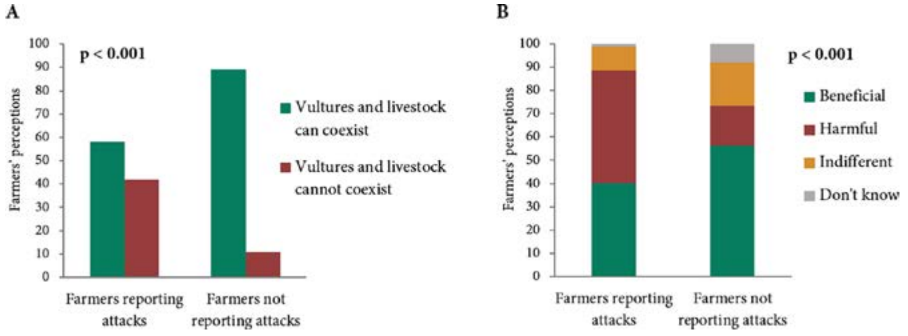

Fig. 5. Farmers' perceptions (in %) regarding the coexistence of vultures and livestock (A); and the ecological relationships between them (B). The significance of the Chi-squared tests are shown.

Nevertheless, no spatial relationship was found between the food availability and the frequency of complaints (Margalida and Campión, 2009). Further, 70 % of complaints in Spain and France were associated with post-mortem consumption and French shepherds were not present at 95 % of livestock deaths, so could not confidently attribute the death to vulture attack, suggesting that farmers' biased opinions led to perceived conflicts rather than any change in vulture behavior (Margalida et al., 2014; Duriez et al., 2019).

Our results showed that complaints were strongly associated with high extensive livestock densities and were influenced by shorter distances to landfills and, to a lesser extent, to SFSs. Griffon vultures and other scavenging birds commonly congregate at landfill sites to forage

(Donázar et al., 2010; Tauler-Ametller et al., 2017). The weaker effect of the distance to SFSs could be related to the type and management of the SFSs in our study area, most being specifically designed for target species such as the bearded vulture. The specialized resources provided at these SFSs may be less attractive to griffon vultures (Moreno-Opo et al., 2015). Although griffon vultures may visit SFSs, they seem to be less attractive than landfills, where both breeding and floating non-breeding individuals often gather (Arévalo-Ayala et al., 2022; Fernández-Gómez et al., 2022). Griffon vulture abundance was positively associated with the number of complaints while the number of breeding pairs showed the opposite trend. However, while most cells with higher complaint numbers harbored few or no breeding pairs, some cells with greater numbers of breeding pairs were associated with a high number of complaints. This suggests that the effect of vulture abundance on the number of complaints may be complex. In this sense, reports of conflict could vary greatly in time and space due to vultures' long distance foraging habits (Spiegel et al., 2015; Gutiérrez-Canovas et al., 2020). The foraging strategies of vultures (Ruxton and Houston, 2004) may explain why griffon vultures find it easier to detect vulnerable livestock (e.g., injured, sick, weak, or those suffering parturition problems) in rural and anthropogenic (e.g., close to landfills) landscapes, resulting in increased conflicts compared to areas less attractive to vultures. Conversely, the negative relationship between complaints and the number of vulture breeding pairs may be because a significant number of complaints were reported in areas far from their breeding sites, suggesting that breeding birds may perform longer foraging movements (Delgado-González et al., 2022), or that floating populations may play a major role in determining the appearance of conflicts.

We identified a strong seasonality in complaints associated with the birthing season, particularly from March to June (~70 % of the claims). However, while most complaints involving cattle and horses were associated with calving, most interactions involving adult sheep were not related to parturition. Similarly, farmers reporting attacks (52.7 %), declared that cattle were most affected and that attacks were mainly associated with calving. Further, farmers reported that attacks on sheep mainly concerned adult individuals. In our study area, cattle were most often reported as affected and interactions were more frequent in spring, demonstrating a seasonal, birthing time, pattern of vulture/livestock encounters. However, because of the patchy nature of the official data, cases involving calving cattle were probably underestimated. Therefore, it is imperative to establish a standardized protocol, supervised by qualified personnel (e.g., veterinarians and biologists), to assess and decide on official complaints.

Livestock management could explain the seasonal pattern we found regarding both official complaints and attacks described by farmers. In spring during birthing times, livestock are kept extensively in grazing pasturelands, often with minimal supervision. For instance, almost 37 % of the farmers interviewed recognized that livestock supervision during the birthing season is insufficient, regardless of whether they had suffered attacks. On the other hand, while sheep usually graze accompanied by shepherds and/or sheepdogs, and most herds spend the night at farms or remain protected by electric shepherds, cattle and horses usually remain at large for longer periods with minimal supervision. This could help explain why most interactions involving sheep were unrelated to parturition, since lambing difficulties are much less frequent compared to cattle (Jacobson et al., 2020). In fact, >90 % of vulture/cattle interactions described by farmers were associated with calving.

Although scavenging birds may cause some harm, especially during parturition, evidence of actual predation is exceptional (Ballejo et al., 2020). However, farmer's perceptions are different, and they usually attribute livestock death to vulture attack, often prompted by increasing numbers of false reports, even if they have never experienced this conflict (Margalida and Donázar, 2020; Ballejo et al., 2021). Nevertheless, media reports just suggest attacks showing vultures feeding on a carcass (Fig. 2) or people claiming livestock losses due to vultures without actual evidence (Lambertucci et al., 2021). Highly visible and potentially dangerous species are especially likely to generate disproportionate antagonism, being perceived as innately evil or harmful, so that even low levels of damage can still elicit harsh responses and even where conflicts are entirely mitigated, negative perceptions can perpetuate (Dickman, 2010). Some people consider vultures 'disgusting birds', associated with death and decay due to their obligate scavenging habits (Hla et al., 2011), despite their having cultural significance in some religions and the valuable contributions they make to public health and ecosystem services (DeVault et al., 2016). Education can reduce these negative perceptions, but such deep-seated preconceptions are difficult to overcome and must be considered in HWC assessments (Dickman, 2010).

We identified certain social factors that may contribute to the conflict. First, most farmers declaring attacks believed that vultures initiated the attack, but only because they had observed vultures or their signs (e.g., feathers). However, none confirmed observing the start of an attack. Second, 68.7 % of farmers reported interactions, and half of those compensated thought that the economic compensation was enough (Bauer et al., 2017). However, compensatory measures may not alleviate the problem because they rarely cover the total costs, are open to corruption, and are often expensive to administer (Sillero-Zubiri et al., 2004). Third, we found that sheep farmers complained much less often (8.6 %) than cattle (76.5 %) or horse (14.9 %) farmers. Complex bureaucratic procedures and livestock values can help explain these differences (e.g., in Catalonia, financial compensation for vulture interactions were ~€1200 for cattle, ~€1300 for horses and ~€150 for sheep; Decret 176/2007). Furthermore, 86 % of all farmers thought that the authorities' response was ineffective; 83 % said that financial compensations did not satisfy farmers suffering attacks; and only 5 % believed that compensations were enough – even though the compensation was considered fair by some farmers actually receiving it. Addressing these perceptions is crucial to alleviating this conflict. It is vital to improve administrative processes, and develop awareness campaigns and broader interdisciplinary approaches (e.g., to bring managers, scientists and farmers together) in order to move farmers away from a conflict mentality towards one of coexistence with vultures. The fact that we were able to interview 127 farmers on this topic highlights the opportunities to work together in future, and effectively resolve this complex conflict in the long term.

Farmers declaring attacks on their livestock had a more negative attitude towards the coexistence of vultures and livestock and considered vultures more often harmful than beneficial. This could be due to their lack of knowledge of vultures' behavior and the pivotal ecosystem services they provide, particularly in areas where vultures are scarce. In fact, we found that many interactions were reported in areas without breeding vulture pairs, suggesting that people's negative perceptions could be influenced by reports of others or the media (Margalida and Donázar, 2020). Finally, our findings illustrate that most farmers considered that reducing griffon vulture populations was necessary to mitigate the conflict. We argue that it is necessary to promote awareness campaigns to rural people that explain that if vulture numbers are reduced, may result in an increase in mesopredators and the spread of pathogens among livestock, wildlife and humans (Markandya et al., 2008; O'Bryan et al., 2019).

4.1. Management implications

To mitigate 'vulture attack' conflicts, ~22 % of claims were compensated between 2008 and 2020. However, financial compensation is not always the best way to minimize HWCs (Sillero-Zubiri et al., 2004). Instead, broader assessments of environmental and social risk factors affecting the frequency of interactions are critical to better understand the nature of this conflict and how it can be addressed (Dickman, 2010). Damage verification by veterinarians is crucial to clarify the real cause of death, avoid waste of public resources and obtain rigorous data to identify the real magnitude of the conflict (Dalmasso et al., 2012). Further, improving livestock welfare is essential. This is critical in areas with high extensive livestock density, especially those with recurrent conflicts. Increased livestock protection is crucial to reduce livestock vulnerability, especially during the birthing season. As recognized by some farmers "*livestock calving has to be at home and not in the mountains, as was traditionally done*". Governments should enact regulations integrating livestock welfare and hold livestock owners accountable for providing adequate protection (López-Bao and Mateo-Tomás, 2021). The European Directive 98/58/EC on the protection of animals kept for farming purposes, states that "*all animals kept in husbandry systems in which their welfare depends on frequent human attention shall be inspected at least once a day*". Some farmers stated that livestock supervision was insufficient, and that regular inspections are essential, enabling farmers to recognize vulnerable or dead livestock, thus reducing the likelihood of an 'attack' or the uncertainty in verifying suspected attacks (López-Bao et al., 2017). Many complaints occurred in areas where no vultures breed, due to the large numbers of vultures congregating at nearby predictable food sources such as landfills (Arévalo-Ayala et al., 2022). Clearly, proper management of landfills is essential, particularly taking into account the expected drastic decrease of food available to wildlife at these sites over the coming years (Landfill Waste Directive 2008/98/EC; Circular Economy Action Plan). In addition, it is necessary to allow carcasses to be disposed of more widely than in the currently permitted high altitude (i.e., >1400 m) areas. Finally, because farmers perceive the arrival of vultures in newly colonized areas as a novel threat, it is crucial to implement educational campaigns to explain the importance of the ecosystem services they provide to society. Our findings demonstrate that scientific assessments and interdisciplinary awareness campaigns are urgently required to reconcile biodiversity conservation with rural economies.

CRediT authorship contribution statement

Conceptualization: A.M., P.O.-V., M.A.C., A.H.M., J.R.; Data curation: A.M., P.O.-V., D.G.; Formal analysis: A.H.M., J.R., P.O.-V., M.A.C.; Funding acquisition: A.M., M.A.C.; Project administration: A.M., M.A.C.; Writing original draft: P.O.-V., A.M., A.H.M., J.R.; Writing - review & editing: All authors.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2022.109664.

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Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers☆

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ABSTRACT

The widespread use of second-generation anticoagulant rodenticides (SGARs) and their high persistence in animal tissues has led to these compounds becoming ubiquitous in rodent-predator-scavenger food webs. Exposure to SGARs has usually been investigated in wildlife species found dead, and despite growing evidence of the potential risk of secondary poisoning of predators and scavengers, the current worldwide exposure of free-living scavenging birds to SGARs remains scarcely investigated. We present the first active monitoring of blood SGAR concentrations and prevalence in the four European obligate (i.e., vultures) and facultative (red and black kites) avian scavengers in NE Spain. We analysed 261 free-living birds and detected SGARs in 39.1% (n = 102) of individuals. Both SGAR prevalence and concentrations (ΣSGARs) were related to the age and foraging behaviour of the species studied. Black kites showed the highest prevalence (100%), followed by red kites (66.7%), Egyptian (64.2%), bearded (20.9%), griffon (16.9%) and cinereous (6.3%) vultures. Overall, both the prevalence and average ΣSGARs were higher in non-nestlings than nestlings, and in species such as kites and Egyptian vultures foraging in anthropic landscapes (e.g., landfill sites and livestock farms) and exploiting small/medium-sized carrions. Brodifacoum was most prevalent (28.8%), followed by difenacoum (16.1%), flocoumafen (12.3%) and bromadiolone (7.3%). In SGAR-positive birds, the ΣSGAR (mean ± SE) was 7.52 ± 0.95 ng mL^{-1}; the highest level detected being 53.50 ng mL^{-1}. The most abundant diastereomer forms were *trans*-bromadiolone and flocoumafen, and *cis*-brodifacoum and difenacoum, showing that lower impact formulations could reduce secondary exposures of non-target species. Our findings suggest that SGARs can bioaccumulate in scavenging birds, showing the potential risk to avian scavenging guilds in Europe and elsewhere. We highlight the need for further studies on the potential adverse effects associated with concentrations of SGARSs in the blood to better interpret active monitoring studies of free-living birds.

1. Introduction

The contamination of food webs with anticoagulant rodenticides (ARs) is currently of major concern to environmental toxicologists and wildlife ecotoxicologists (Rattner et al., 2014; Lohr and Davis, 2018; van den Brink et al., 2018; Ravindran et al., 2022). Rodents comprise the largest mammalian order with >2500 species (Kay and Hoekstra, 2008) and human-rodent conflicts occur worldwide (e.g., by consuming and spoiling crops and stored grain, damaging infrastructure, predating endemic species, and spreading human and livestock diseases; van den Brink et al., 2018) and cost several billion Euros each year (Jacob and Buckle, 2018). The multi-faceted nature of these conflicts requires increasing continuous anthropogenic controls, and the use of ARs has been the most frequent lethal method since the 1950s. However, the rapid development of rodent resistance to the early forms of rodenticides (i.e., first generation ARs or FGARs) has led to the development of more toxic and bioaccumulative second generation ARs (SGARs), also so-called "super-warfarins" (Thomas et al., 2011). The bioaccumulation

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of SGARs after repeated secondary exposure negatively affects the vitamin K-dependent coagulation pathway in birds of prey, leading to bleeding in or around critical organs (e.g., brain, heart, lungs) and causing sublethal impairments or death (Murray, 2018).

Although the use of ARs may be necessary for rodent control, the well-known risks of primary and secondary poisoning of non-target species necessitates debate on the need to require measures to mitigate the risks of ARs use (Buckle and Prescott, 2018; Eisemann et al., 2018; Witmer, 2018; Thornton et al., 2022). Indeed, the Convention on the Conservation of Migratory Species of Wild Animals (the CMS, or Bonn Convention) highlighted secondary exposure to ARs as one of the most important toxicological hazards for migratory birds (CMS, 2014). The high acute toxicity and persistence of ARs in animal tissues, especially of SGARs, has led to their becoming ubiquitous in rodent-predator-scavenger food webs (López-Perea and Mateo, 2018; Pay et al., 2021; Elliott et al., 2022; Cooke et al., 2022). Baits containing ARs may be consumed by a number of non-target primary consumers (e.g., invertebrates, fish, wild birds and mammals), increasing the risk of exposure across the entire food web (Shore and Coeurdassier, 2018; Regnery et al., 2020; Alabau et al., 2020).

Predator species that are highly specialized rodent-feeders, such as snakes, kestrels, owls and mustelids, are at a high risk of SGAR exposure (Lettoof et al., 2020; Roos et al., 2021; Elliott et al., 2022). However, generalist predators and scavengers may be exposed to secondary SGAR poisoning at similar or even higher levels than specialist predators (López-Perea and Mateo, 2018). This could be because clinically poisoned (e.g., sick) animals containing high SGAR concentrations can be easily caught and consumed by predators such as raptors (e.g., eagles and kites) and mammalian carnivores. They could also be eaten by obligate scavengers (i.e., species that depend entirely on carrion, such as vultures) and facultative scavengers (i.e., species that exploit carrion opportunistically, including raptors, corvids and mammalian carnivores) (Hindmarch and Elliott, 2018). In addition, avian scavengers frequently exploit food sources in urban landfill sites (Tauler-Ametller et al., 2017, 2019; Plaza and Lambertucci, 2018; Arévalo et al., 2022; Fernández-Gómez et al., 2022), where SGARs are constantly deployed (Coeurdassier et al., 2018). Despite these risk factors, few studies have investigated the exposure of avian scavengers to SGARs, particularly vulture species (Sánchez-Barbudo et al., 2012; Mateo et al., 2015; Plaza et al., 2019; Rial-Berriel et al., 2021; Moriceau et al., 2022).

The prevalence of wildlife exposure to SGARs has usually been assessed by passive monitoring of animals found dead in the field or admitted to wildlife rehabilitation centres. However, this method is probably biased towards higher prevalence values due to the inherent over-representation of poisoned animals in the samples. Active monitoring (i.e., by sampling live animals in the field) may therefore provide more accurate data on the prevalence of contaminant exposure than passive monitoring (Descalzo et al., 2021). However, active monitoring studies of wild bird exposure to SGARs are much less common. Regarding raptors, Martínez-Padilla et al. (2017) detected bromadiolone in the blood of 16.9% (n = 112) of common kestrel (*Falco tinnunculus*) fledglings with an average (±SE) concentration of 0.25 ± 0.02 ng mL^{-1}, in a region of central Spain during a common vole (*Microtus arvalis*) population outbreak. Badry et al. (2022) analysed ARs in the blood of nestling raptors from Germany and detected ARs in 22.6% (n = 53) of red kites (*Milvus*) and 8.6% (n = 35) of common buzzards (*Buteo*), with the highest median concentration (of brodifacoum at 13 ng mL^{-1}) observed in red kites, evidencing this species' risk of AR exposure. In France, Powolny et al. (2020) detected SGARs in the blood of 30% (n = 47) of red kite nestlings, with a median (and range) of 6.1 ng mL^{-1} (0.2–29.4). It should be noted that, as with other chemicals, blood samples may show lower SGAR concentrations than other tissues in which these compounds tend to bioaccumulate and persist for longer periods (i.e., the liver; see Horak et al., 2018), limiting their detectability in active monitoring studies relying on blood SGAR levels. For example, Murray (2020) detected SGARs in the liver of 100% (n = 43) of red-tailed hawks (*Buteo jamaicensis*) admitted to a wildlife clinic in the north-eastern United States, while only 32.6% showed SGARs in their serum. Another current limitation of blood SGAR levels is the lack of reference toxicity thresholds in blood, contrary, for example, to the established toxicity threshold in the liver (i.e., >0.1 μg g^{-1} in wet weight (Thomas et al., 2011; Rattner et al., 2014).

We carried out an active monitoring program in NE Spain to determine the prevalence and concentration of different SGARs (difenacoum, bromadiolone, brodifacoum and flocoumafen) in the blood of the four European obligate scavengers (griffon *Gyps fulvus*, cinereous *Aegypius monachus*, bearded *Gypaetus barbatus*, and Egyptian, *Neophron percnopterus* vultures) and facultative avian scavengers (black and red kites, *Milvus migrans* and *M. milvus*) with different dietary preferences to test five hypotheses: (1) that a higher proportion of rodents in the diet may increase the exposure to SGARs, because rodents are the target species for these biocides; (2) that non-nestlings bioaccumulate more SGAR residues than nestlings, because of their longer time at risk of exposure; (3) that certain diastereomer (*cis* vs *trans*) forms tend to bioaccumulate more in predator/scavenger species because of their different half-lives in animal tissues; (4) that species foraging in anthropic habitats would suffer greater exposure to these compounds because of the potential association between the exploitation of anthropogenic food resources found on landfill sites and livestock farms; and (5) that the SGAR prevalence observed in living individuals would be lower than that described in animals found dead in other studies in the same geographical area, because SGARs bioaccumulate and persist for longer periods in liver tissue.

2. Material and methods

2.1. Study area

The study was carried out in the Pyrenees (NE Spain) and adjacent regions (Fig. 1). This mountain range covers around 50,000 km^2 and runs from east-west forming a natural boundary between France and Spain (Ameztegui et al., 2010). It is characterized by a strong altitudinal gradient ranging from sea level to >3000 m a.s.l. and encompasses a great diversity of vegetation types (Ninot et al., 2007). The area is characterized by extensively and semi-extensively grazed livestock (cattle, sheep and horses) and holds important populations of wild herbivorous ungulates, which provide most of the biomass for the scavenger guild (Colomer et al., 2011; Margalida et al., 2018). The scavenger assemblage (mainly facultative avian species) also exploits carnivorous mammal carcasses (see Fig. 2). The Pyrenees holds breeding populations of the four obligate avian scavengers (Table 1) and a rich community of facultative avian (e.g., raptors and corvids) and mammalian scavenger species (e.g., red fox *Vulpes*, wild boar *Sus scrofa*, *Martes*spp. and badgers *Meles*) (Oliva-Vidal et al., 2022). A network of active supplementary feeding stations (SFS) for avian scavengers is present throughout the Pyrenees (Moreno-Opo et al., 2015) and wildlife, mainly avian species, frequently exploit anthropogenic food resources at the urban open-air landfills that occur throughout the study area (Arizaga et al., 2018; Tauler-Ametller et al., 2018, 2019).

2.2. Studied species

We collected blood samples from 261 free-living obligate and facultative avian scavengers, from 2017 to 2021. Our study included the four European obligate avian scavengers (griffon, cinereous, bearded and Egyptian vultures). As for the facultative avian scavengers, we sampled red and black kites, one golden eagle (*Aquila chrysaetos*) and one Bonelli's eagle (*Aquila fasciata*) (Table S1), the latter being the only one that scavenges only rarely. The Spanish breeding population sizes and overall population trends of these species, and their main biological traits (e.g., breeding behaviour and principle feeding habits) are detailed in Table 1.

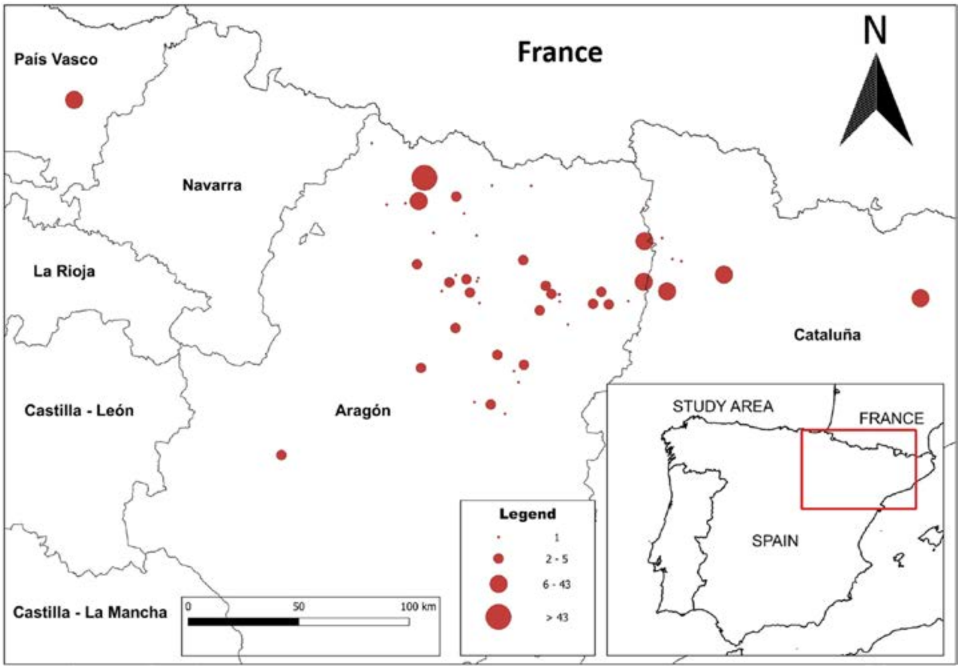

Fig. 1. Map of the study area showing the Pyrenees and adjacent regions (NE Spain) that were sampled from 2017 to 2021. The sampling points are shown according to the number of birds sampled at each location. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

Fig. 2. Main avian scavenger species included in this study consuming carcasses of different mammalian carnivores in the study area. The consumption of carnivore carrion could help explain tertiary SGAR exposure or poisoning routes to scavenging species. A) red kite consuming a red fox; B) black kite consuming a badger; C) Egyptian vulture, black kite and common raven consuming a domestic cat (*Felis catus*); D) golden eagle consuming a domestic cat; E) bearded vulture consuming a stone marten (*Martes foina*); and F) griffon vulture consuming a red fox (see Oliva-Vidal et al., 2022). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

2.3. Sampling

Blood samples were collected when birds were handled to be fitted with satellite transmitters or ringed and marked with patagial tags. Both nestlings and non-nestling individuals (juveniles, subadults and adults, Table S1) were sampled. Each individual's age class was identified according to its moult pattern and plumage characteristics (Forsman, 2016). All individuals were handled by trained and authorized personnel. Specialized climbers visited nests when nestlings were feathered but not yet ready to fly. Non-nestling individuals were captured using a variety of methods (e.g., baited traps) according to the species concerned (Table S2). Whole-blood samples (3–5 mL, and never exceeding 1% of the body weight) were collected from the brachial vein, placed into EDTA or heparinized tubes and stored at −80 °C until

Table 1

Number of breeding pairs, population trend, breeding behaviour, main feeding habits and migratory status in Spain of the four obligate avian scavengers (vultures) and the main facultative avian scavenger species included in this study. The percentage of the European population represented by the Spanish populations of the four European vulture species are shown.

Species	Scavenger group	Breeding pairs	Population trend	Breeding behaviour$^{(8)}$	Main feeding habits$^{(9,\ 10,\ 11)}$	Migratory status
Griffon vulture	Obligate	30,946$^{(1)}$ [90%]	+	Colonial	Medium-sized and large carrion, garbage	Sedentary (adults) Migrant (juveniles)
Cinereous vulture	Obligate	2,548$^{(2)}$ [> 90%]	+	Colonial and territorial	Medium-sized and large carrion remains (tendons, muscle), small carrion	Sedentary
Bearded vulture	Obligate	140$^{(3)}$ [63%]	+	Territorial	Bone remains of medium-sized and large carrion, small carrions	Sedentary
Egyptian vulture*	Obligate	1,490$^{(4)}$ [32.2%]	–	Territorial	Small pieces of medium-sized and large carrion, small carrions, garbage	Migrant
Red kite	Facultative	1,994$^{(5)}$	–	Territorial	Small carrion, garbage	Sedentary
Black kite*	Facultative	2,061$^{(6)}$	+	Territorial	Small carrion, garbage	Migrant
Golden eagle^{+}	Facultative	1,553(7)	+	Territorial	Small and medium-sized prey/carrion	Sedentary

Medium-sized and large carrion items mainly represent domestic and wild ungulate carcasses. Small carrion items represent rodents, birds, invertebrates and small/medium-sized mammals. Garbage refers to food sources found at open-air landfill sites.

(1) Del Moral and Molina (2018a); (2) Del Moral (2017); (3) Margalida and Martínez (2020); (4) Del Moral and Molina (2018b); (5) Cardiel (2006); (6) Palomino (2006); (7) Del Moral (2009); (8) van Overveld et al., (2020); (9) Moreno-Opo et al. (2015); (10) Tauler-Ametlller et al. (2019); (11) Ortiz-Santaliestra et al. (2019).

*Species with a landfill-based diet.

$^{+}$Apex predator.

analysis. Two drops of blood were used for sexing the individuals using molecular procedures.

2.4. SGAR analysis

SGAR extraction was performed following the method described by Martínez-Padilla et al. (2017) with some modifications. Briefly, whole blood samples were thawed, and 400 μl were placed in a 10 mL-glass tube with 3 mL of dichloromethane:acetone (70:30), 2 g of sodium sulphate and 50 μl of internal standard (brodifacoum-d4 at 0.1 ng μL^{-1}) in methanol. Then, the sample was vortexed (10 min), sonicated (5 min) and centrifuged (1048 rcf, 5 min). The organic phase was transferred to another glass tube. This extraction was repeated again with another 3 mL of dichloromethane:acetone (70:30) and the extract obtained was pooled with the first one. The extract was then evaporated to dryness under a stream of N_2, resuspended in 200 μL of methanol, filtered through a 0.2 μm mesh nylon filter, and collected in a chromatography vial for analysis using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). The analytical equipment consisted of a liquid chromatograph (Agilent UHPLC Series 1290 Infinity II) coupled to a triple quadrupole mass spectrometer (Agilent 6470 LC/TQ). The chromatographic separation was performed using a reverse-phase column (Agilent InfinityLab Poroshell 120 EC–C18, 2.1 × 150 mm, 2.7 μm) in an oven at 40 °C. The injection volume was 10 μL. The mobile phase comprised a gradient elution of two solvents (A: ammonium acetate 10 mM, pH: 6.03; B: methanol). The initial conditions were 50% A and 50% B, reaching 100% B at min 13. This was maintained until min 14, returned to the initial conditions at min 15, and then left to stabilize until min 20 before the next sample injection. The flow rate was 0.250 mL min^{-1}. Ionization was performed using an electrospray ionization source (ESI) in negative mode with an Agilent Jet Stream. The conditions were as follows: gas temperature 300 °C; gas flow 8 L min^{-1}; nebulizer at 40 psi; sheath gas temperature 300 °C; sheath gas flow 11 L min^{-1}; capillary at 4000 V; and nozzle voltage/charging 1750 V. Fragment ion spectra were obtained in a dynamic multiple reaction monitoring (dMRM) scan. The fragmentor voltage, collision energy and ion fragments for quantification or qualification were optimised for every compound (Table S3). Up to four dMRM transitions were performed for each SGAR and identifications were considered positive when all the ratios between qualifier and quantifier ions in the sample differed by less than 20% compared with the standard ratios. *Cis* and *trans* diastereomers of the SGARs (with indistinguishable mass spectra) were identified according to the elution order of each form given by Fourel et al. (2017a). Data collection and processing were performed using the Masshunter™ Work-station from Agilent Technologies.

Matrix-matched calibrations were performed using partridge whole-blood extracts at concentrations of 6.25–250 ng mL^{-1} in a final volume of 200 μL, including 25 ng mL^{-1} of internal standard. Procedural blanks were analysed for each sample batch. Precision and accuracy were calculated using partridge whole-blood fortified with 31.25, 62.5 and 125 ng mL^{-1} of SGAR standards. The recovery percentage ranged between 89.0 and 95.8% and the relative standard deviation (RSD) was between 3.3 and 9.9%. The limits of quantification based on the minimum detected concentrations followed the identification criteria described above and ranged between 0.04 and 0.5 ng mL^{-1} with a response to noise ratio greater than 10 (Table S3).

2.5. Statistical analysis

We first calculated the blood concentration (ng mL^{-1}) of each SGAR (brodifacoum, bromadiolone, difenacoum and flocoumafen) in SGAR-positive birds ($n = 102$ individuals). Then, to ascertain whether avian species, age class (nestling or non-nestling) or sex (female or male) affected the prevalence and concentrations of SGARs, we fitted generalized linear models (GLMs) where 'SGARs presence' (positive or negative), 'ΣSGARs concentration' (i.e., sum of the concentrations of all the compounds detected in each bird) and 'ΣSGARs concentration > LOQ' (limit of quantification) were the response variables; and 'avian species', 'age class' and 'sex' were categorical predictors. Given that we did not sample individuals of all age classes for all species (see Table S1), we lumped the age data into two categories, 'nestling' and 'non-nestling' in all models. We used binomial error distributions and log link functions for SGARs prevalence, and Gaussian error distributions and identity link functions for ΣSGARs concentration and ΣSGARs concentration > LOQ. To improve normality assumptions, both ΣSGARs concentration and ΣSGARs concentration > LOQ were log-transformed. When we found significant differences among avian species, we used a post hoc Tukey's HSD test for pairwise comparisons using the *multcomp* package with the Bonferroni correction. At the intraspecific level, we reported 'SGARs presence', 'ΣSGARs concentration' and 'ΣSGARs concentration > LOQ' for all age class categories (nestling, juvenile, subadult and adult). Given sample size limitations for some age class categories (see Table S1), we did not model SGARs prevalence and concentrations at the species level. We used the Kruskal-Wallis test and post hoc pairwise comparisons using the pairwise Wilcoxon rank sum test with Bonferroni adjustment to test for intraspecific differences in ΣSGAR concentrations among age classes for species with both sample sizes ≥ seven individuals and with individuals of at least three age classes (griffon vultures, bearded vultures, Egyptian vultures and red kites; Table S1). To explore differences in the proportions of the most abundant *cis* or *trans* diastereomers of each SGAR, we performed GLMs where 'proportion of *cis* forms' or 'proportion of *trans* forms' were the response variables, and 'avian species', 'age class' and 'sex' were categorical predictors. We used Gaussian error distributions and identity link functions, and only birds with residues of each SGAR were considered. Analyses were performed using R version 3.6.1 (R Development Core Team, 2019).

3. Results

SGARs were detected in 102 (39.1%) of the 261 avian scavengers analysed, including every species studied except Bonelli's eagle (Table 2). The highest prevalence of SGARs was observed in black kites (100%), followed by red kites (66.7%), and Egyptian (64.2%), bearded (20.9%), griffon (16.9%) and cinereous vultures (6.3%) (Table 2). SGARs prevalence differed among species with sample sizes ≥7 ($p <$ 0.001), with higher values in black kites, red kites and Egyptian vultures than in the other species (Tables 2 and 3). SGARs prevalence was lower in nestlings compared with individuals of other age classes ($p = 0.003$), whereas sex had no influence (Table 3). Regarding the specific SGARs, brodifacoum showed the highest overall prevalence in the birds studied (28.8%), followed by difenacoum (16.1%), flocoumafen (12.3%) and bromadiolone (7.3%). We found that 18.4% (n = 48) of all birds analysed contained residues of multiple (>1) SGARs, representing 47.1% (48/102) of the SGAR-positive birds. Of all SGAR-positive birds, 30.4% contained residues of two SGARs, 15.7% of three SGARs, and four different SGARs were detected in one individual (Fig. S1).

Considering concentrations in SGAR-positive birds (n = 102) only, ΣSGARs showed a mean ± SE value of 7.52 ± 0.95 ng mL^{-1}, with a maximum level of 53.50 ng mL^{-1}. Considering all individuals analysed (n = 261), the mean total ΣSGARs was 2.94 ± 7.03 ng mL^{-1}. Brodifacoum was the compound found with the highest levels in birds with any SGARs present (5.95 ng mL^{-1}), followed by bromadiolone (0.72 ng mL^{-1}), difenacoum (0.72 ng mL^{-1}) and flocoumafen (0.13 ng mL^{-1}) (Fig. 3). The maximum blood concentration of brodifacoum (51.66 ng mL^{-1}) was found in a golden eagle without evident signs of toxicosis; the 27-year-old female eagle had been equipped with a GPS transmitter since the time of sampling and died by electrocution two years later without showing any uncharacteristic behaviours.

In all of the species studied with sample sizes ≥7 individuals, the mean individual concentration of ΣSGARs considering all individuals (n = 259) was significantly higher in black kites, red kites and Egyptian vultures than in bearded, cinereous and griffon vultures ($p < 0.001$; Fig. 4, Table 3). ΣSGARs concentration was lower in nestlings than in non-nestlings ($p < 0.001$; Table 3), particularly in Egyptian vultures and red kites (Fig. 5). The mean concentration of ΣSGARs differed between species ($p < 0.001$) in individuals with SGARs concentration > LOQ, with higher concentrations in red kite, black kite and Egyptian vultures than in bearded and griffon vultures, and showed no significant differences related to age class (nestling vs. non-nestling) or sex (Tables 2 and 3).

The proportions of SGAR diastereomers did not differ between species, sexes or age classes. The *trans* diastereomer was the most frequent form of bromadiolone and flocoumafen, whereas the *cis* diastereomer was the most frequent form of brodifacoum and difenacoum. The highest difference between diastereomer forms was found for bromadiolone, in which the *cis* form was almost absent (Fig. 6).

4. Discussion

Our active monitoring indicated that medium-sized facultative avian

Table 2
Prevalence (%) and concentration (ng mL^{-1})^{1} of second-generation anticoagulant rodenticides (SGARs) in the blood of free-living obligate and facultative avian scavengers sampled in the Pyrenees and adjacent regions (NE Spain).

		SGARs			Brodifacoum			Bromadiolone			Difenacoum			Flocoumafen		
	N	N+	%	Mean ± SE [min-max]	N+	%	Mean ± SE [min-max]	N+	%	Mean ± SE [min-max]	N+	%	Mean ± SE [min-max]	N+	%	Mean ± SE [min-max]
Obligate																
Egyptian v.	67	43	64.18^{A}	6.74 ± 1.1^{A} [0.13–28.87]	39	58.21	6.54 ± 1.11 [0.37–27.15]	8	11.94	1.48 ± 0.25 [0.85–2.95]	18	26.87	0.85 ± 0.18 [0.13–2.84]	16	23.88	0.47 ± 0.08 [0.12–1.39]
Bearded v.	67	14	20.90^{B}	2.29 ± 0.5^{B} [0.1–6.53]	12	17.91	2.48 ± 0.53 [0.17–6.53]	1	1.49	0.50	1	1.49	0.15	5	7.46	0.35 [0.04–1.31]
Griffon v.	65	11	16.92^{B}	2.23 ± 1.85^{B} [0.04–20.73]	3	4.62	7.15 ± 6.53 [0.53–20.21]	ND	–	–	4	6.15	0.19 ± 0.08 [0.1–0.43]	7	10.77	0.33 ± 0.13 [0.04–0.90]
Cinereous v.	16	1	6.25^{B}	0.17	ND	–	–	ND	–	–	1	6.25	–	ND	–	–
Facultative																
Black kite	8	8	100^{A}	7.61 ± 2.20^{A} [0.20–16.71]	7	87.50	7.60 ± 2.28 [0.14–16.71]	ND	–	–	5	62.50	1.44 ± 0.85 [0.07–4.77]	1	12.50	0.50
Red kite	36	24	66.67^{A}	12.75 ± 2.29^{A} [0.49–45.95]	13	36.11	15.08 ± 3.62 [0.49–45.95]	10	27.78	6.08 ± 2.20 [0.58–18.44]	12	33.33	4.02 ± 1.56 [0.23–17.38]	3	8.33	0.29 ± 0.02 [0.26–0.32]
Golden e.	1	1	100	53.50	1	–	51.66	ND	–	–	1	–	1.84	ND	–	–
Bonelli's e.	1	0	–	–	ND	–	–	ND	–	–	ND	–	–	ND	–	–

[1] Concentrations in birds with values > LOQ.

[2] Percentages with different letters are significantly different.

Table 3

Generalized linear models (GLMs) used to assess the effects of 'avian species' (red kite, black kite, Egyptian, bearded, griffon and cinereous vultures), 'age class' (nestling or non-nestling) and gender (female or male) on SGARs prevalence, ΣSGARs concentration (i.e., sum of concentrations of all compounds detected in each bird) and ΣSGARs concentration > LOQ (i.e., considering only individuals with SGARs levels > LOQ). The coefficients and standard errors (SE) of all models are shown. Significant p-values (<0.05) are highlighted in bold.

Response variable	Explanatory variable	Coefficient	SE	p-value
SGARs prevalence	Black kite	15.073	–	0.986
	Egyptian vulture	−0.206	0.453	0.649
	Bearded vulture	−0.716	0.542	**< 0.001**
	Griffon vulture	−2.867	0.552	**< 0.001**
	Cinereous vulture	−2.962	1.105	**0.007**
	Age class ~ non-nestling	1.1966	0.408	**0.003**
	Sex ~ male	−0.302	0.319	0.344
ΣSGARs concentration	Black kite	0.678	0.837	0.418
	Egyptian vulture	−0.596	0.430	0.167
	Bearded vulture	−3.645	0.459	**< 0.001**
	Griffon vulture	−3.905	0.458	**< 0.001**
	Cinereous vulture	−3.251	0.642	**< 0.001**
	Age class ~ non-nestling	1.220	0.337	**< 0.001**
	Sex ~ male	−0.274	0.268	0.308
ΣSGARs concentration > LOQ	Black kite	−0.482	0.558	0.390
	Egyptian vulture	−0.530	0.337	0.119
	Bearded vulture	−1.735	0.449	**< 0.001**
	Griffon vulture	−3.136	0.514	**< 0.001**
	Age class ~ non-nestling	0.095	0.309	0.758
	Sex ~ male	0.396	0.283	0.164

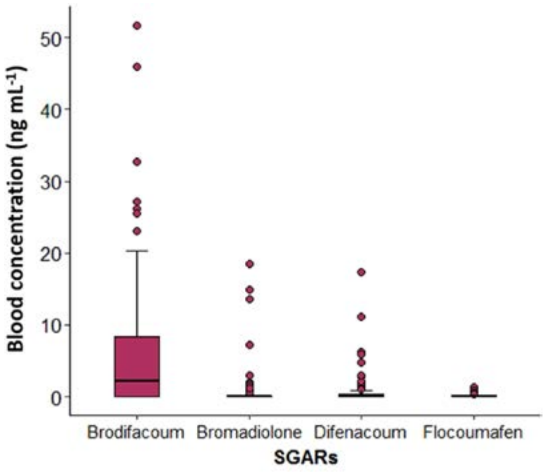

Fig. 3. Boxplots showing the blood concentration (ng mL^{-1}) of each second-generation anticoagulant rodenticide (SGAR) analysed in the avian scavenger species sampled in the Pyrenees and adjacent regions (NE Spain). Only SGAR-positive birds are shown. Boxes encompass the 25–75th quartiles from the median (thick line); vertical lines represent the maximum and minimum values, excluding outliers, shown as circles and defined as values further than 1.5 times the interquartile range.

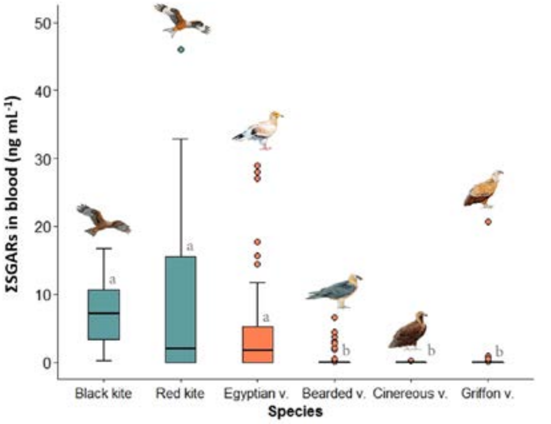

Fig. 4. Boxplots representing the blood concentration of the sum of second-generation anticoagulant rodenticides (ΣSGARs) in obligate and facultative avian scavengers with sample sizes ≥7 individuals sampled in the Pyrenees and adjacent regions (NE Spain). Boxes encompass the 25–75th quartiles from the median (thick line); vertical lines represent the maximum and minimum values, excluding outliers, shown as circles and defined as values further than 1.5 times the interquartile range. Different letters show significant differences between species as indicated by post hoc Tukey's tests after Bonferroni correction of ΣSGARs concentration generalized linear model (GLM; see Table 3). Facultative (green boxes) and obligate (orange boxes) avian scavenger species are shown separately. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

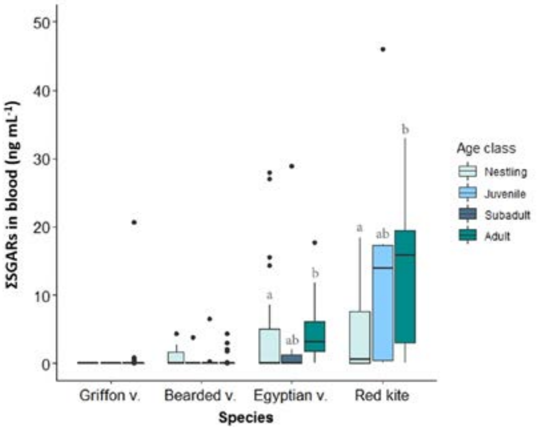

Fig. 5. Boxplots representing differences in blood concentrations of the sum of second-generation anticoagulant rodenticides (ΣSGARs) in the obligate and facultative avian scavengers studied in the Pyrenees and adjacent regions (NE Spain) according to age class (i.e., nestling, juvenile, subadult and adult; also see Table S4). Boxes encompass the 25–75th quartiles from the median (thick line); vertical lines represent the maximum and minimum values, excluding outliers, shown as circles and defined as values further than 1.5 times the interquartile range. Different letters show significant intraspecific differences between age classes as indicated by the Kruskal-Wallis test with Bonferroni adjustment (see Methods).

scavengers, such as red and black kites, and Egyptian vultures (an obligate scavenger) were the species most exposed to SGARs, with a prevalence >64% in red kites and Egyptian vultures, and up to 100% in black kites. Prevalence values were lower for the larger obligate avian scavengers (bearded, griffon and cinereous vultures) but still ranged between 6.25% and 20.9%. The average blood SGAR concentrations were similar in kites and Egyptian vultures (ranging between ~7 and 13 ng mL^{-1}) while among large vultures blood levels were lower (<2.5 ng mL^{-1}). The highest concentration was detected in the only golden eagle

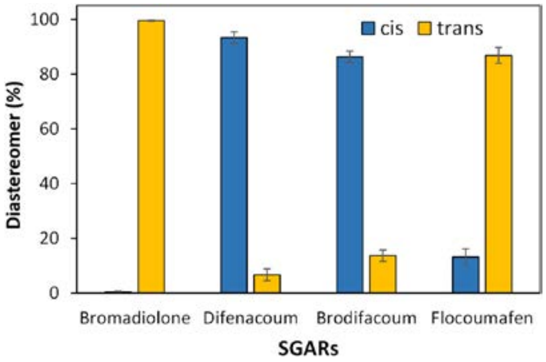

Fig. 6. Proportion (mean ± SE) of *cis* and *trans* diastereomer forms of each SGAR detected in the obligate and facultative avian scavengers studied in the Pyrenees and adjacent regions (NE Spain).

(i.e., an apex predator) we sampled, being 53.50 ng mL^{-1}. These results showed that bioaccumulation of SGARs presents a risk not only to top predators, but also to the European avian scavenger guild as a whole.

Exposure of avian scavengers to SGARs has previously been investigated mainly by analysing the livers of animals found dead, but such passive monitoring method may introduce significant bias in the observed concentration and prevalence values. For example, Sánchez-Barbudo et al. (2012) detected ARs in 13% of griffon vultures throughout Spain (n = 23) and López-Perea et al. (2019) found that 19% of griffon vultures were affected (n = 42) in NE Spain. In griffon vultures in France, Berny et al. (2015) detected ARs in 4.2% of the liver samples examined (n = 119) and Moriceau et al. (2022) in 32.2% of livers (n = 90). Regarding bearded vultures, López-Perea et al. (2019) detected ARs in 22.2% of the birds examined (n = 9) in NE Spain. In France, Berny et al. (2015) did not detect residues in bearded vultures (n = 8), but Moriceau et al. (2022) found AR residues in 33.3% of birds (n = 9). Concerning cinereous vultures, Moriceau et al. (2022) detected ARs in 76.5% of the birds examined (n = 17). For Egyptian vultures, Berny et al. (2015) detected ARs in 22.2% of the individuals examined (n = 9) while López-Perea et al. (2019) found residues in two out of three birds in NE Spain. In the Canary Islands (Spain), Rial-Berriel et al. (2021) detected ARs in 29.9% of the Egyptian vultures analysed (n = 67).

Considering the number of birds studied by Berny et al. (2015) and López-Perea et al. (2019), in areas which overlap or are close to our study area, we estimate an overall prevalence of AR occurrence of 8.1% (13/161) for griffon, 11.8% (2/17) for bearded and 33.3% (4/12) for Egyptian vultures. The values estimated from birds found dead were half of those we obtained by analysing living individuals (i.e., griffon vulture: 16.9%, n = 65; bearded vulture: 20.9%, n = 67; and Egyptian vulture: 64.2%, n = 67), highlighting the need for further studies on the relationship between AR levels in blood and liver tissues. Assuming similar limits of detection in the studies mentioned, we observed that, contrary to our expectations, the prevalence of ARs discovered in birds found dead may underestimate those we observed in living individuals. This fact could be due to significant bias in the sampling method. Most birds found dead could have died from other non-natural causes, such as those related to anthropogenic infrastructures (e.g., trauma or electrocution due to collision with energy infrastructures; Pérez-García et al., 2022) and their carcasses could be more easily detected at the site of death (González et al., 2008). In contrast, clinically poisoned birds tend to move less and remain in safer places (e.g., roosting sites) before dying in the field, making them much harder to find (Peshev et al., 2022). Indeed, there are significant differences in the reported causes of mortality of dead vultures discovered by chance (e.g., 75% relating to shootings and collisions with power lines) compared with dead radio-tagged vultures (e.g., 86% relating to intentional and unintentional poisoning), demonstrating a methodological sampling bias (Margalida et al., 2008). Thus, assessment of threats to avian scavengers by analysing untagged individuals found dead may lead to important biases in the accurate calculation of epidemiological parameters (Franson et al., 1996; González et al., 2008).

Regarding New World vultures, Hosea (2000) found AR residues in one of the two turkey vultures (*Cathartes aura*) examined between 1997 and 2010. McMillin (2012) reported death due to AR poisoning in four turkey vultures in California (USA). Stone et al. (2003) detected AR residues in the liver of both of two turkey vultures in New York state (USA), while Kelly et al. (2014) detected ARs in the liver of seven out of 23 (30.4%) turkey vultures in California (USA). Turkey vultures therefore show a prevalence of exposure to ARs similar to that of Egyptian vultures in Europe. The dietary habits of these opportunistic avian scavengers are similar, feeding on substantial quantities of garbage and the carrion of wild and domestic mammals, ranging from small rodents to small/medium-sized mammals, large ungulates, birds, reptiles, amphibians and invertebrates (Margalida et al., 2012; Hill et al., 2022).

Exposure to ARs in facultative avian scavengers such as kites or large eagles has been more frequently studied. Regarding kites, Badry et al. (2021) detected ARs in the liver of 80.5% of the birds (n = 41) in Germany. In England, SGAR poisoning was diagnosed as the cause of death in 17.3% of reintroduced red kites found dead (n = 110) (Molenaar et al., 2017). In Scotland, 70% of red kites found dead (n = 114) contained ARs in their liver and in 10% of the individuals AR poisoning was the cause of death (Hughes et al., 2013). In France, Coeurdassier et al. (2014) sampled in an area where bromadiolone has been intensively used to control water voles (*Arvicola terrestris*) and poisoning by this compound was confirmed (or highly suspected) in all red kites found dead (n = 28). More recently, Moriceau et al. (2022) detected AR residues in the liver of 100% of red kites (n = 16) and in one black kite (n = 1) found dead in France. In Spain, ARs have been detected by López-Perea et al. (2019) in the liver of red kites (77%, n = 13) and black kites (33%, n = 6) and Sánchez-Barbudo et al. (2012) found a similar situation (red kites, 88%, n = 8; black kites, 60%, n = 5). In Asia, Hong et al. (2018) detected SGARs in the liver of 42.9% of black kites (n = 7) in Taiwan.

Regarding large eagles, Badry et al. (2021) detected ARs in 38.3% of white-tailed sea eagles (*Haliaeetus albicilla*) (n = 60) in Germany. Sell et al. (2022) detected SGARs in 100% of white-tailed sea eagles found dead with suspected poisoning (n = 40) in Poland. In France, Moriceau et al. (2022) detected ARs in the liver of 100% of golden eagles found dead (n = 7). In Norway, Langford et al. (2013) detected SGARs in the liver of 68.8% of golden eagles (n = 16). In Spain, López-Perea et al. (2019) found no ARs in the liver of golden eagles (n = 5) but Sánchez-Barbudo et al. (2012) detected SGARs in one individual (25%, n = 4). In the USA, Viner et al. (2022) detected ARs in the liver of 38.7% of golden eagles (n = 62) found dead under power lines or wind turbines, with no significant differences in AR prevalence between these infrastructures, and Niedringhaus et al. (2021) detected ARs in the liver of 83% of bald eagles (*Haliaeetus leucocephalus*) (n = 96) and 77% of golden eagles (n = 17). AR poisoning was also previously detected in bald and golden eagles by Stone et al. (1999). In Australia, Pay et al. (2021) detected AR residues in 74% of Tasmanian wedge-tailed eagles (*Aquila audax fleayi*) found dead (n = 50). We detected SGARs in the only golden eagle we analysed, but every previous study of large eagles has indicated significant exposure levels varying from 25% to 100%, depending on the sampling method or location.

Our findings evidence that facultative avian scavengers (black and red kites) showed the highest prevalence of SGARs (100% and 66.7%, respectively) although Egyptian vultures (an obligate scavenger) also showed a high SGAR prevalence (64.2%). Moreover, we found that both kites and Egyptian vultures showed a high prevalence of individuals containing multiple different SGARs (four compounds were identified in a single Egyptian vulture). These exposures could relate to the foraging and dietary habits of these species, which comprise the carrion of small and medium-sized mammals, such as rodents, as well as carnivores (Oliva-Vidal et al., 2022) and large quantities of garbage and food items gleaned from landfill sites and livestock farms (Margalida et al., 2012; Tauler-Ametller et al., 2017, 2018; Arévalo et al., 2022; Fernández-Gómez et al., 2022). In the specific case of the red kite, a diet based on small rodents is more likely to result in bioaccumulation of ARs (Coeurdassier et al., 2014) although this species is well-known for frequently exploiting garbage dumps, slaughterhouses and agricultural areas (Seoane et al., 2003; García-Macía et al., 2022). The widespread use of ARs at landfills and farms could result in a high number of contaminated rodents around these areas, which could be consumed by predators and scavengers foraging in such anthropogenic landscapes. Thus, species which exploit anthropogenic habitats are very likely to suffer greater exposure to SGARs through secondary or even tertiary pathways due to their consumption of contaminated rodents or their predators (López-Perea and Mateo, 2018).

Large obligate avian scavengers (griffon, cinereous and bearded vultures) showed lower prevalence and concentration values than Egyptian vultures and kites. However, our results showed that the endangered bearded vulture suffered the highest prevalence (20.9%) among the large vultures. The consumption of small carrion items (e.g., birds, rodents and small/medium-sized mammals), which represent 14% of their diet (Margalida et al., 2009), could explain the vulnerability of this species to ARs and the prevalence that we found. Their greater dietary plasticity could also explain the high AR exposure observed in Egyptian vultures, since small to medium-sized vertebrate carrion items are frequent in their diet during the breeding season (Margalida et al., 2012). Direct and indirect poisoning is the most widespread non-natural mortality factor for Egyptian vultures and other avian scavengers in Spain (Hernández and Margalida, 2009; Mateo et al., 2015) and our findings demonstrate the critical vulnerability of these threatened species to ARs.

We found that both SGAR prevalence and average concentration were related to the age of an individual. Overall, our results showed that nestlings exhibited lower prevalence and concentration values than non-nestling individuals. Badry et al. (2022) detected ARs in the blood of 22.6% of red kite nestlings in Germany, and Powolny et al. (2020) in the blood of 30% of red kite nestlings in France, much lower prevalence values than we found in red kite nestlings (55.0%, 11/20), juveniles (75%, 6/8) and adults (87.5%, 7/8). Several factors may explain the age class differences we found. There may be dietary differences in the trophic spectrum between chicks and adults. In some species, variations in the diet during the breeding period are related to specific energetic requirements or food quality. However, the high bioaccumulative capacity of SGARs could help explain this age-related exposure, resulting in lower concentrations in nestlings than in adults because of the shorter time nestlings have spent at risk. SGARs have long half-lives in the liver of an exposed animal (e.g., 91.7–307.4 days), which could explain the higher concentrations we observed in adults compared with nestlings (Shore and Coeurdassier, 2018). As for differences in SGAR diastereomer concentrations, our results are in line with other studies in which the prevalence in birds of prey of the *trans* forms of bromadiolone and flocoumafen and the *cis* forms of brodifacoum and difenacoum were greatest because of their higher persistence in animal tissues (Fourel et al., 2017b, 2021). This is a relevant finding and highlights the need for developing SGAR formulations with a lower impact on non-target wildlife (Damin-Permink et al., 2016). Currently, commercial SGAR formulations have varying proportions of *cis*:*trans* forms, ranging between 35:65 and 55:45 (Alabau et al., 2020), and the proportions observed in free-living avian scavengers clearly show that the different diastereomer forms bioaccumulate differently across the food chain.

From a conservation and management point of view, our results suggest that scavenging birds foraging in anthropogenic landscapes (e.g., landfill sites and livestock farms) are more vulnerable to SGAR exposure, as reported in other raptor and scavenger species (Lopez-Perea et al., 2019; Badry et al., 2021) and for other pollutants in Egyptian vultures (Ortiz-Santaliestra et al., 2019). The exploitation of predictable food resources at landfills has increased coinciding with the changes in the Spanish sanitary regulations between 2006 and 2011, which have resulted in a sudden reduction in food availability by restricting the availability of livestock carcasses in the field, causing changes in the foraging behaviour and dietary habits of avian scavengers, particularly griffon vultures (Donázar et al., 2010; Fernandez-Gómez et al., 2022). As with other chemical pollutants, the risk of avian scavenger exposure to SGARs could be mitigated by securing food availability using managed SFSs where livestock and game animal carcasses can be provided regularly under strict controls to prevent wildlife exposure to toxic compounds (e.g., veterinary pharmaceutical residues or lead).

Our study highlights the problems associated with widespread use of ARs to control commensal rodents, which can affect both facultative avian scavengers, such as kites and eagles, and obligate avian scavengers that frequently include rodents and small/medium-sized carrion items in their diet (e.g., bearded and Egyptian vultures). Oliva-Vidal et al. (2022) found that some facultative avian scavengers, such as golden eagles and red kites, exploit carnivorous mammal carcasses more frequently than herbivore carcasses, although other avian and mammalian scavengers can also consume carnivore carrion (Fig. 2), so increasing the risk of secondary or tertiary AR exposure routes across the scavenger guild. The use of bromadiolone against field voles (*Arvicola terrestris*), coypu (*Myocastor coypu*) and musk rat (*Ondathra zibethicus*) in France has been associated with AR exposure and poisoning of both predators and scavengers (Berny et al., 1997; Fournier-Chambrillon et al., 2004; Coeurdassier et al., 2014). A similar scenario could occur in other countries and affect facultative and obligate avian scavengers due to the current underestimation of the potential problems. Future research to reduce biases in the study of SGAR exposure could focus on sampling individuals tagged with GPS transmitters in order to provide temporal and spatial information to identify conflict areas that should be monitored by managers and policy-makers to reduce the negative impact of SGARs on scavenger populations.

5. Conclusions

We performed the first ever active monitoring of SGAR concentrations and prevalence in the blood of the four European obligate (vultures) and facultative (red and black kites) avian scavengers in the Pyrenees and adjacent areas (NE Spain). SGARs were detected in 39.1% of the birds sampled, with variations in prevalence and concentrations among species that could be explained by differences in their foraging and trophic behaviours. Red and black kites and Egyptian vultures, which mainly feed on small/medium-sized carrion items (e.g., rodents and mammals, including carnivores) and frequently forage in anthropic areas (e.g., landfill sites and livestock farms), showed the highest prevalence of SGARs (64%–100%). In contrast, large vulture species mainly exploiting medium-sized and large domestic and wild ungulate carcasses, such as griffon, cinereous and bearded vultures, showed the lowest prevalence (<21%), the highest being found in bearded vultures. By analysing the blood of free-living vultures, we found prevalence values higher than those previously described from the analysis of liver samples from individuals found dead in the same area, which may point to methodological sampling biases. Both prevalence and ΣSGAR concentrations showed differences between age classes, being higher in adult individuals than in nestlings, which could be explained by their longer exposure to ARs leading to greater levels of bioaccumulation. The most abundant SGAR was brodifacoum, followed by difenacoum, flocoumafen and bromadiolone, and in all of these compounds one diastereomer form was clearly more bioaccumulated than the other. These results highlight the need to monitor and regulate the use of anthropogenic compounds such as SGARs. The current exposure levels in scavenging birds could have negative impacts on conservation efforts, particularly for the most endangered European avian scavengers. Further studies on the potential adverse effects associated with blood SGAR levels are necessary to better interpret the concentrations found in the blood of free-living birds and to better understand their potential population effects.

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Credit author statement

Pilar Oliva-Vidal: Fieldwork & Sample collection, Conceptualization, Formal analysis, Data curation, Investigation, Writing – original draft, Writing – review & editing, Visualization; **José Ma Martínez**: Fieldwork & Sample collection, Investigation; **Inés Sánchez- Barbudo**: Validation, Investigation; **Pablo R. Camarero**: Validation, Investigation; **Antoni Margalida**: Fieldwork & Sample collection, Conceptualization, Resources, Methodology, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition; **Ma Àngels Colomer**: Resources, Methodology, Writing – review & editing, Funding acquisition; **Rafael Mateo**: Conceptualization, Resources, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120385.

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Bioinspired models for assessing the importance of transhumance and transboundary management in the conservation of European avian scavengers

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ABSTRACT

The assessment of temporal and spatial availability of food resources is an important prerequisite in developing improved management tools for effective conservation action. It is especially useful in the conservation of avian scavengers inhabiting regions where livestock move on a regular basis (transhumance). Important management decisions can be taken on the basis of theoretical analyses that need to be regularly checked. In this case study, we consider models of Griffon vulture *Gyps fulvus*, Egyptian vulture *Neophron percnopterus* and bearded vulture *Gypaetus barbatus* populations in a part of Spain with one of the highest densities of scavenging birds, and where traditional farming practices remain. We applied bioinspired Population Dynamic P System models (PDP) to assess these species' population trends against the distribution, quantity and availability of carrion for food. We show asymmetries in the availability of food resources, which are substantially higher in summer due to transhumant movements. In the study area, a lack of food resources in winter leads to a seasonal reduction in food supplies to levels unable to meet the energetic requirements of the most abundant vulture species, the Griffon vulture. Our results suggest that regardless of active management (e.g. supplementary feeding sites) and the birds' use of other potential food resources not included in the model, Griffon vultures are able to find important alternative food resources in more remote areas. We show the importance of variations at spatio-temporal scales in the objective forecasting of population trends, and in the correct application of management actions. Because of the importance of robust assessments for management applications, we discuss the advantages and limitations of ecological modelling for avian scavengers, highlighting the importance of transhumance processes and transboundary approaches.

1. Introduction

Successful conservation action rests upon harmonizing the best available knowledge with management actions appropriate to the prevailing political and economic situation (Linnell et al., 2016). To this end, conservationists and managers must adapt their activities to administrative and regional scales, and be increasingly aware of the importance both of large scale ecological processes and transboundary cooperation (Rands et al., 2010; Wiens and Bachelet, 2010; Rüter et al., 2014, Lim, 2016; Linnell et al., 2016). The need for, and the benefits arising from, transboundary cooperation in managing wildlife populations beyond simple administrative and jurisdictional limits has led to the emergence of wider scale approaches as a major conservation paradigm, and these are being increasingly applied in many locations (Chapron et al., 2014; Fleurke and Trouwbors, 2014).

Because large avian scavengers have extensive foraging ranges, their management and conservation requires transboundary approaches (Margalida et al., 2013; Lambertucci et al., 2014; Arrondo et al., 2018). Because of their specialized diet based mainly on the carcasses of domestic and wild ungulates, the assessment of food resource availability for these species, and its spatio-temporal distribution, is key and provides an important management tool in improving their conservation status (Margalida and Colomer, 2012; Cortés-Avizanda et al., 2016; Kane et al., 2015). The health of vulture populations is good indicators

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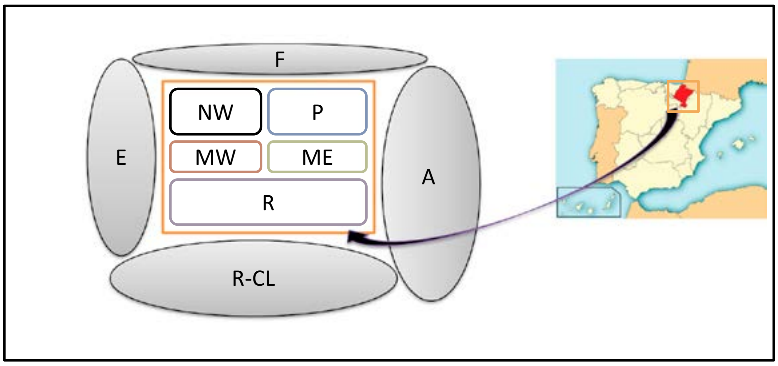

Fig. 1. Location of study area in northern Spain showing the five zones considered within the study area (surrounded by orange) and the peripheral zones (surrounded by grey) in which scavengers can obtain alternative resources. The regular foraging ranges were estimated based on the maximum distance that a bird will fly in a straight line from the nest in search of food (see Material and Methods).

of habitat modification and unsustainable land management at large spatial scales. Therefore, quantitative assessments of trophic availability, in conjunction with information regarding vulture food preferences and selection (Moreno-Opo et al., 2015, 2016) provide useful information about territory quality and can be used to estimate carrying capacity. Such information can also help to develop guidelines regarding the need for, and form of, supplementary feeding programs and the suitability of particular habitats for vulture reintroduction projects.

Spain is home to 90% of the European Union's avian scavenger population, and conservation management actions carried out there are fundamental for the Europe-wide scavenging bird metapopulation. In contrast with other scavenging bird populations worldwide (e.g. Ogada et al., 2016), Spanish vulture populations have shown moderate and/or important increases in recent years (Donázar et al., 2009a, 2009b). Transhumance is characteristic of many Spanish ecosystems. It is a traditional farming practice whereby livestock are regularly moved between winter and summer pastures, maximizing the exploitation of grazing resources (Ruiz and Ruiz, 1986; Fernández-Giménez and Fillat, 2012). This was a common practice in many European countries, but is now in decline elsewhere (Vicente-Serrano et al., 2004; Oteros-Rozas et al., 2013). Vultures make use of the food resources from transhumant livestock during at least a third of the year, and this is particularly useful for Griffon vulture management (Olea and Mateo-Tomás, 2009). Because larger scale ecosystem management is important for biodiversity conservation, political or administrative boundaries, which divide ecosystems and apply different rules and guidelines, pose special problems for ecological processes and conservation efforts (Zbicz, 1999; Papadopoulou and Sitsoni, 2012). These issues were highlighted in Spain during the outbreak of bovine spongiform encephalopathy in 2001, when changes in sanitary regulations suddenly reduced the food available to vultures provided by livestock carcasses (Tella, 2001, Donázar et al., 2009a, 2009b; Margalida et al., 2010). The various Spanish regions applied different sanitary policies, each affecting the distribution and availability of animal carrion biomass. After this, a network of protection areas for the feeding of scavengers in Spain was designated, but the criteria adopted to manage carrion resources differed among regions (Morales-Reyes et al., 2017).

Clearly, it is therefore important to determine whether the available food resources are sufficient to cover the energetic requirements of an avian scavenger assemblage and whether spatio-temporal variations in food availability may affect their population levels and trends. This information allows managers and policy-makers to anticipate and forecast the effects of food shortages, or changes in their spatio-temporal distribution, on scavenger populations, and enable management and conservation measures to minimize such effects (Margalida and Colomer, 2012). The use of bioinspired models (PDP Systems) allows the assessment of the influence of spatio-temporal changes in food availability on the population dynamics (e.g. Colomer et al., 2011; Kane et al., 2015; Cortés-Avizanda et al., 2016). These estimates, based on data collected in the field, allow the modeling of hypothetical scenarios that can enable managers to anticipate decision-points regarding conservation measures such as the provision of Supplementary Feeding Sites (SFS) or "vulture restaurants". However, the different scenarios provided by modeling approaches are subject to degrees of uncertainty, and to minimize bias in the results it is necessary to estimate the sensitivity of any model to changes in the parameters involved, and the degrees of causality between them.

Considering the important ecosystem services provided by vultures (Dupont et al., 2012; Moleón et al., 2014; Morales-Reyes et al., 2015) and the lack of empirical data on the influence of transhumance effects, we undertook a case study on three vulture species (Egyptian vulture *Neophron percnopterus*, Eurasian Griffon vulture *Gyps fulvus* and bearded vulture *Gypaetus barbatus*), all of which are obligate scavengers, in a part of Spain with one of the highest avian scavenger population densities (Navarra, N Spain). Our goals were: i) to estimate the carrying capacity of the ecosystem based on the availability of trophic resources; ii) to quantify the spatio-temporal distribution of these resources and their relationship to transhumance practices, in order to determine how the distribution of food impacts vulture population dynamics; iii) to examine the advantages and limitations of ecological modelling in the management of carrion and its effects on ecosystem services provided by vultures, to assess the usefulness of modelling as a decision making tool for managers and policy-makers.

2. Material and methods

2.1. Model building and assumptions

Using a Population Dynamic P System (see Supporting information) we built a model to study the ecosystem dynamics in an area subdivided into five zones and four peripheral zones surrounding the main study area (Fig. 1). PDP models are computational methods that are analogous to the machinery of cells (Colomer et al., 2013). The cells of the model correspond to the physical space of the environment. Animals (which along with things such as resources, are represented by model 'objects') will feed, reproduce, develop, etc. within an environment which is accounted for by a set of mathematical rules describing these behaviours in the model (Colomer et al., 2011). The application of PDP models constitute an effective computational tool to model a complex problem, because these bioinspired models are characterized by the ability to work in parallel (simultaneously interrelating different processes, for example combining demographic parameters with energetic requirements), being modular and with a high computational efficiency.

The subdivision of the study area has been based on climatic, topographic, landscape and ecological criteria (see Elósegui and Pérez

Table 1
Population of domestic and wild ungulates (individuals) in each zone in the study area. Non-transhumant animals are those that are not moved by farmers and remain in the same place year round. R.p. *Rupicapra pyrenaica*; C.e. *Cervus elaphus*; C.c. *Capreolus capreolus*; S.s. *Sus scrofa*; O.a. *Ovis aries*; B.t. *Bos taurus*; E.c. *Equus caballus*.

Zones	*R. p.*	*C. e.*	*C. c.*	*S. s.*	*O. a.*	*B. t.*	*E. c.*
NW							
No transhumants	0	153	4668	2564	130,429	14,893	7982
Summer	0	0	0	0	0	0	0
Breeding	0	0	0	0	10,800	700	975
MW							
No transhumants	0	0	1556	2177	38,651	1291	2662
Summer	0	0	0	0	15,800	1250	1520
Breeding	0	0	0	0	0	0	0
Pyrenees							
No transhumants	260	4132	3917	4456	71,099	8697	2660
Summer	0	0	0	0	28,000	0	0
Breeding	0	0	0	0	0	0	0
ME							
No transhumants	0	41	3908	5252	58,176	4005	1858
Summer	0	0	0	0	0	0	0
Breeding	0	0	0	0	3200	550	545
Ribera							
No transhumants	0	0	0	0	221,972	7588	5820
Summer	0	0	0	0	0	0	0
Breeding	0	0	0	0	20,800	0	0

Ollo, 1982). This area of 10 391 km^2 is inhabited by three avian scavenger species: seven bearded vulture pairs, 129 Egyptian vulture pairs, and 2798 Eurasian Griffon vulture pairs.

Regarding carrion provided by wild species, we considered the Pyrenean chamois (*Rupicapra pyrenaica*), the red deer (*Cervus elaphus*), the roe deer (*Capreolus capreolus*) and the wild boar (*Sus scrofa*). Carrion provided in the study area by domestic ungulates, mainly comprises sheep (*Ovis aries*), cows (*Bos taurus*) and horses (*Equus caballus*), occurring either naturally after death (*in situ*) or artificially at the network of supplementary feeding sites (SFS) (Table 1, Table S1). The study area contains 10 SFS, where farmers and administrators provide carcasses and bone remains (Table S1). In addition, we added the contribution of alternative carrion from other species such as birds, small mammals, rodents, and lagomorphs (see Supporting information). These constitute a very important part of the diet of the Egyptian vulture (see Donázar, 1993; Margalida et al., 2012) and complement the trophic spectrum of the bearded vulture (Margalida et al., 2005; Margalida et al., 2009).

The study area is characterized by husbandry related seasonal movements of livestock (transhumance). Two periods are defined annually according to the use of the grasslands and the variations in certain biological parameters throughout the year: 'summer or non-reproductive period' (hereafter *summer* - the months between June and September), and 'winter or reproductive period' (hereafter *breeding* - the period between October and May). Since livestock are man-managed, we considered their spatio-temporal distribution to be seasonally fixed and the model takes this effect of transhumance into account. Humans also partially manage the feeding of scavengers artificially by placing bones and meat at the SFS. Some of these SFS are specifically targeted at the bearded vulture (for which lamb carrion is the only food offered), while others are more generic, in which any type of carcass is provided and the entire scavenging vulture guild can feed at them. In addition, some wild ungulates are hunted (some selectively for trophies), so that humans manage the number of animals killed through decisions based on the quarry population sizes or through the issue of hunting permits.

2.2. Annual energetic requirements

To estimate regular foraging ranges, we constructed circular areas around the nesting site based on the maximum distance that a bird will fly in a straight line from the nest in search of food: Griffon vulture 90 km, bearded vulture 40 km, Egyptian vulture 15 km (for more details see Margalida and Colomer, 2012). We used an extension of the central place forager theory known as the foraging radius concept at which every individual is energetically constrained in terms of the spatial range they can cover while foraging (Sinclair and Norton-Griffiths, 1995). As central place foragers, breeding individuals must return to their breeding sites after they forage every day. The energetic requirements of the three avian scavengers according to food type (bones and meat) and period (summer vs breeding) were estimated following Donázar (1993). Eurasian Griffon vultures need 404 kg/pair/year, Egyptian vultures 100 kg and bearded vultures 308 kg (Table S7).

In addition to including the natural and non-natural mortality rates of ungulates, the model assumes that an animal dies of starvation when it exceeds the carrying capacity of the habitat. In defining the model, a directed network table of avian scavenger movement was specified following the rationale given in Table S2.The model predicts that as a mean foraging range (Table S2), a species will move to a nearby zone if food resources become insufficient at its current location, and that it returns to the starting point (nesting area) if there are food limitations but no space (density) limitations. In this sense, we do not consider the large metabolic cost that result from the requirement to move greater distances. Accordingly, an individual colonizes a new area if insufficient space is available at its current location. Scavengers can choose between more than one available destination if they need to move, and the model assumes that they select one at random. If the new area selected also lacks resources, this random sampling continues until resources are found. If an individual cannot find sufficient resources after the process of random sampling, it will move to another area subject to the maximum density of each species in each zone. If space is not a limiting factor, it will return to its original location, or otherwise colonize a new area.

When feeding resources are insufficient in an avian scavenger's usual home range, birds move to the peripheral zones (A, F, E, R-CL) in search of food, assuming they can obtain the same food resources found in the neighboring areas (Table S3). The model assumes that floater individuals can obtain a part of the resources available in the study area. However, the model does not take into account the use of the resources by neighboring individuals, including obligate and facultative scavengers.

The model takes into account the fact that each species uses the resources closest to their nesting area first, and then widens the radius of search as these deplete. The amount of meat and bones consumed by scavengers depends on the season. Excess meat disappears from the ecosystem at the end of each period (breeding or summer). The model assumes that 20% of the unconsumed bones remain available in the ecosystem in spring, as a consequence of bone preservation (Margalida and Villalba, 2017). In addition, because not all carrion remains available due to its location (i.e. it lies in forested areas), we reduced the food actually available for scavengers by applying a correction factor (Tables S8, S9).

The bone remains of large bovine ungulates and equines are rarely consumed by bearded vultures (Margalida et al., 2009) and simply counting them would therefore overstate their importance, so we reduced their quantification by applying a factor according the size of the various bones (Table S9).

Population growth is restricted due to limitations on physical space imposed by each species foraging range and the food available to cover their energetic requirements. This informs the habitat carrying capacity used in the model (Table S4). Running the model requires input of initial parameters (such as reproduction, mortality and feeding), which are entered before generating the output. The model is first run for each individual and then again simultaneously for all individuals. Therefore, the system operates in parallel, allowing for competition when birds of the same or different species share resources. In this regard, the bearded

Table 2
Annual mortality of domestic ungulates according to the different food availability scenarios, age classes and the two temporal periods. Scenario variation indicates the annual mortality variation in the low and high scenarios regarding the medium scenario.

Juveniles		Annual	Summer	Breeding	Scenario variation
Low	*O. aries*	0.100	0.067	0.033	-0.05
	B. taurus	0.050	0.033	0.017	-0.01
	E. caballus	0.020	0.013	0.007	-0.01
	O. aries	0.150	0.100	0.050	0
Medium	*B. taurus*	0.060	0.040	0.020	0
	E. caballus	0.030	0.020	0.010	0
	O. aries	0.200	0.133	0.067	0.05
High	*B. taurus*	0.070	0.042	0.028	0.01
	E. caballus	0.040	0.040	0	0.01

Adults		Annual	Summer	Breeding	Scenario variation
Low	*O. aries*	0.020	0.013	0.007	−0.01
	B. taurus	0.040	0.024	0.016	−0.01
	E. caballus	0.009	0.009	0.000	−0.01
	O. aries	0.030	0.02	0.01	0
Medium	*B. taurus*	0.050	0.03	0.02	0
	E. caballus	0.010	0.01	0	0
	O. aries	0.040	0.027	0.013	0.1
High	*B. taurus*	0.060	0.036	0.024	0.1
	E. caballus	0.020	0.020	0	0.1

and Egyptian vultures are the first to arrive at the carrion and/or to feed with respect to the griffon vulture (see Supporting information).

The values of the parameters used in the model were derived from published sources (for more details see Colomer et al., 2011; Margalida et al., 2011a, 2011b; Margalida and Colomer, 2012; see also Supporting information).

2.3. Food availability scenarios

Three possible scenarios were studied to test the impact of different food availability regimes based on different livestock mortality rates, to examine their potential effects on population projections over time. The *Medium food availability scenario* represents the estimated food available in a normal year based on average domestic ungulate mortality rates (Table 2). The *High* scenario models the situation where available food increases relative to an average year (optimistic scenario). The *Low* scenario simulates an ecosystem where the food available is less than average (conservative scenario). The scavenger population trends were simulated on the basis of the demographic parameters typical of each species (Table S5) and the availability of biomass provided by the different domestic ungulate mortality scenarios, plus the biomass provided by wild ungulates and feeding stations (see Supporting information).

2.4. PDP model

We used PDP models to build the ecosystem model. These are probabilistic computational models inspired by studies of cell function, and can perform a high number of simultaneous and perfectly-synchronized processes. These models resemble multi-agent models, although they have some special characteristics which enable them to model complex processes (see Colomer et al., 2013; Colomer et al., 2014). The integrated data on food availability, food requirements and population dynamics of the avian scavenging guild and the ungulate populations of the study area (Fig. 1 and Supporting Information) try to determine if carcass availability could meet the demands of the avian scavenger population over a 20-year period. The conceptual bases of the models are given in Fig. 2.

The parameters used are defined in Table S10 and the model is thoroughly described in the Supporting information. The model was executed using MeCoSim (a free software under license) developed by the Computation Group at the University of Sevilla (GNU GPL; http://www.p-lingua.org).

3. Results

3.1. Food availability

The availability of animal biomass (meat and bones) was estimated for the three scenarios considered (low, medium and high availability of food resources), during two periods (summer and breeding). In all scenarios, the meat and bone biomass available to scavenging birds was higher in summer than during breeding (low-meat: 787,823 vs 325,854 kg; low-bones: 144,448 vs 34,377 kg; medium-meat: 1,005,630 vs 416,021 kg; medium-bones: 195,563 vs 46,024 kg; high-meat: 1,339,084 vs 585,049 kg; high-bones: 247,220 vs 58,292 kg), with sheep providing the most domestic meat and bone biomass in all the scenarios considered (meat range: 34.80%–42.11%; bones range: 65.40%–77.39%). During the breeding period, the sheep is also the domestic ungulate that provides the most amount of meat and bone biomass (meat range: 34.57%–39.96%; bones range: 60.35%–74.05%) (Fig. 3).

Regarding the wild species, wild boar and red deer provided in a similar way the highest amount of meat biomass (summer meat range: 9.87%–5.80% and 7.76%–4.61% for wild boar and red deer respectively; breeding meat range: 6.09%–3.38% and 5.91%–3.27% for wild boar and deer respectively) while deer provided the most amount of bones biomass (summer bones range: 13.70%–8.01%; breeding bones range: 17.19%–10.14%) (Fig. 3).

3.2. Temporal availability of food resources to cover energetic requirements under different scenarios

Comparing the total meat trophic resources available (green bars) with the total energy requirements of the avian scavenger species (red bars), the food available is substantially greater than requirements in the summer, in the medium and high scenarios (Fig. 4). On the contrary, during breeding in all three scenarios, the food available is insufficient to cover the energy requirements of the scavenging species assemblage.

When the total bone trophic availability (green column) is compared with the energy requirement (red column), the availability of bones is clearly much higher than needed to cover the energy requirements of the breeding bearded vulture population, even in the low food availability scenario (Fig. 5).

3.3. Population trends in the Eurasian griffon, bearded and Egyptian vultures according to the carrying capacity

In the case of the Griffon vulture, the model forecasts significant differences among scenarios ($F_{2,\,60} = 19.45$, $P < 0.0001$). The differences were found between the low and the rest of scenarios. In the high and medium food availability scenario the population grows and stabilizes at 3900 pairs after 20 years, whereas in the low food availability scenarios the trend is different. In this scenario, the model predicts that the population stabilizes at 3500 pairs after 20 years (Figure 6).

Regarding the bearded vulture, the results suggest oscillations between the current seven pairs and a maximum of 8, stabilizing over the following 20 years, with no significant differences between years ($F_{2,\,60} = 1.126$, $P = 0.331$, Figure 6).

Considering the Egyptian vulture, the results show a positive population trend in all three scenarios with no significant differences between years ($F_{2,\,60} = 0.149$, $P = 0.862$, Figure 6). The model predicts an increase of c. 11 pairs in the first six years, before rising later and stabilizing at 143 pairs after 20 years, in all three scenarios with no significant differences between them.

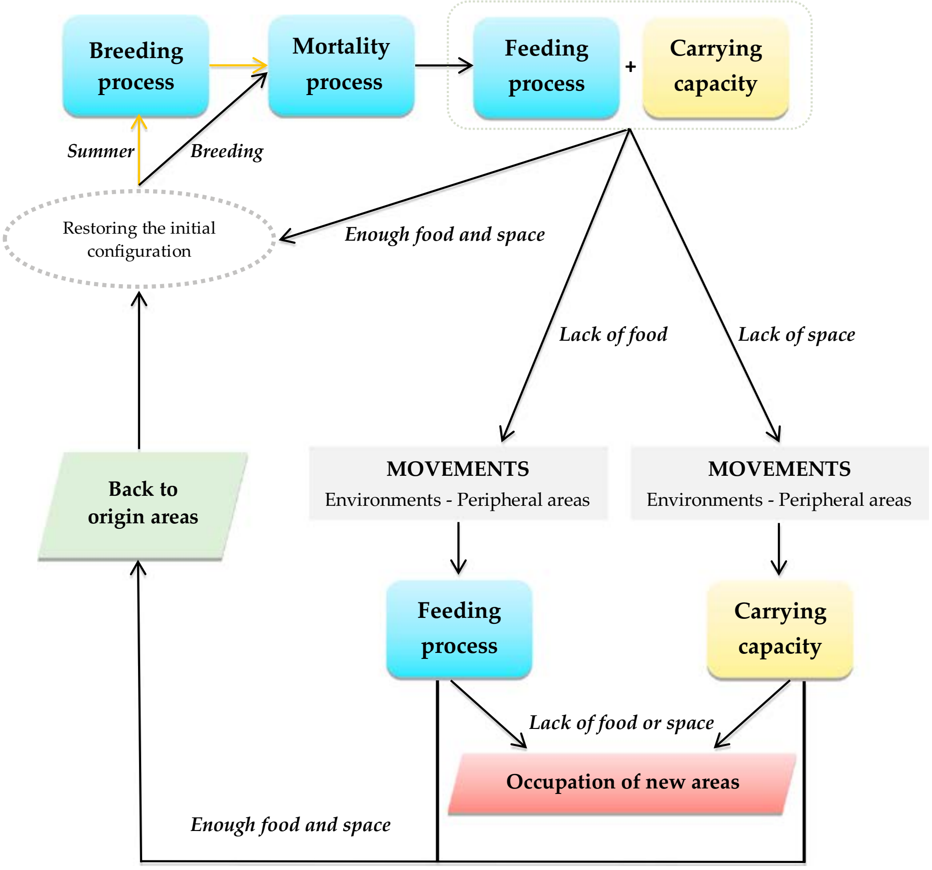

Fig. 2. Conceptual basis and sequencing of the processes considered in the PDP model. The model takes into account two periods (summer and breeding) and the processes of reproduction, mortality, feeding and the carrying capacity. When food is insufficient in the foraging area, the scavenger birds forage in peripheral areas. If they find food they return to their nesting site. On the contrary, when food is also insufficient in peripheral areas, the individual disappear from the study area. Two executions of a loop are equivalent to the passage of one year in the ecosystem.

4. Discussion

The availability of resources limits the population size of an animal species, and sets the carrying capacity of an area (Hanski et al., 1993; Turchin, 2001). In the case of avian scavengers, the availability of food provided by wild ungulate carcasses has gradually decreased as a result of their replacement by domestic ungulates (Lambertucci et al., 2009; Margalida et al., 2011a, 2011b; Ogada et al., 2012a, 2012b). Livestock is man-managed and this makes it easy to obtain accurate data on numbers of animals and their demographic parameters, as well as their spatio-temporal distribution and its effect on the amount of food that they provide for scavengers. This quality of information makes it possible to assess the precise carrying capacity of an environment and to forecast scavenger population trends based on estimates of food availability. Modeling this information can help managers and policy-makers to make decisions regarding reintroduction projects, conservation measures, and to assess the impact of policy decisions regarding health and sanitation regulations on scavenger population dynamics (Sarrazin and Legendre, 2000; Hirzel et al., 2004; Margalida and Colomer, 2012). As this study shows, it is imperative to have good datasets in order to model population trends or assess carrying capacity because the sensitivity of some demographic parameters can have a significant impact on the results obtained. As we show, even a 1–2% change in livestock mortality can substantially modify the assessment of carrion available and the effects on scavenger population dynamics. For example, with respect to the differences between the low vs medium food availability scenario, in the case of the griffon vulture the model forecasts a difference of 384 pairs after 20 years (Fig. 6). Therefore, even small errors in livestock mortality estimates could lead to serious mistakes in management measures, with important conservation repercussions. However, it is important to remark that predictions were only different significantly among the three scenarios for the griffon vulture but not for Egyptian and bearded vultures.

We show that asymmetries exist in the availability of food resources during the year, emphasizing the importance of estimating food availability over the full annual cycle (Marra et al., 2015; Zupo et al., 2017). According to our results, although the overall annual availability of carrion for the scavenger populations studied is enough to cover their energetic requirements, when we separate the breeding from the summer period (when transhumance occurs) the results suggest that seasonal food shortages do exist in our study area (Fig. 4), at least for the most abundant species, the Griffon vulture. An additional issue which suggests that results are conservative is that some facultative scavengers and breeding pairs inhabiting outside the study area can

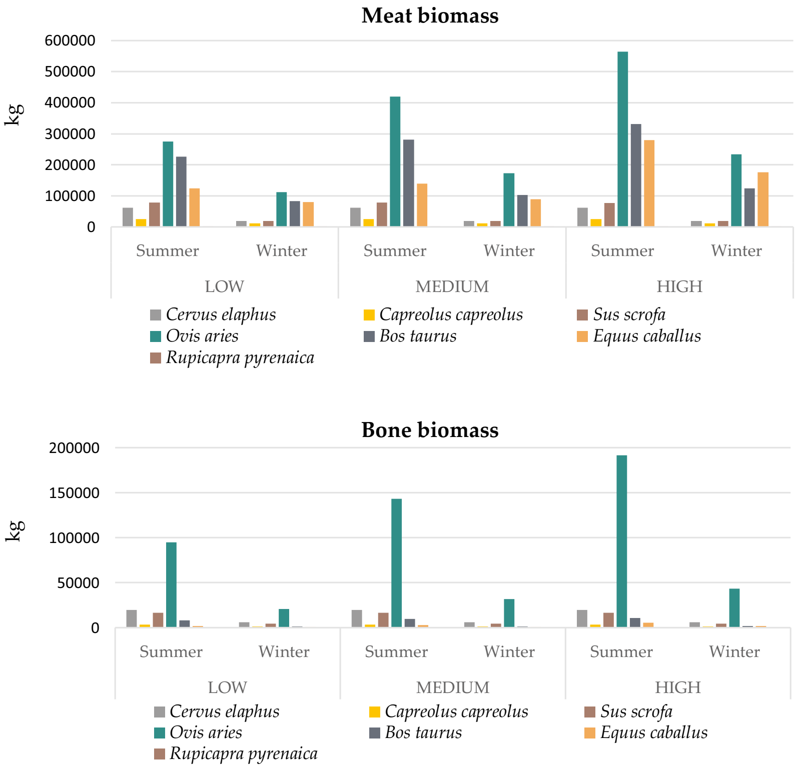

Fig. 3. Food availability (meat and bone biomass) provided by the different domestic and wild ungulates in the study area.

take advantage of the carrion present (Moreno-Opo et al., 2016). The quantification of the impact of biomass consumption by these facultative carrion eaters (birds and mammals) is difficult and will require future approximations to improve the models. However, the progressive increase in the griffon vulture population size (from 312 pairs in 1979 to 2783 in 2009, Del Moral, 2009), suggests that an important proportion of their food is obtained from zones peripheral to the core area, or even from more distant areas, possible because of the high mobility of these species (Monsarrat et al., 2013). Therefore, as has been shown for other large birds of prey, home ranges vary according to prey density and individual reproductive status, with habitat quality serving to regulate their use of space (Fernández et al., 2009; Pérez-García et al., 2013). Spatial scales are therefore important to assess correctly available feeding resources and to understand the relevance of trans-boundary agreements between regional administrations to develop, coordinate and apply conservation measures for species with extensive

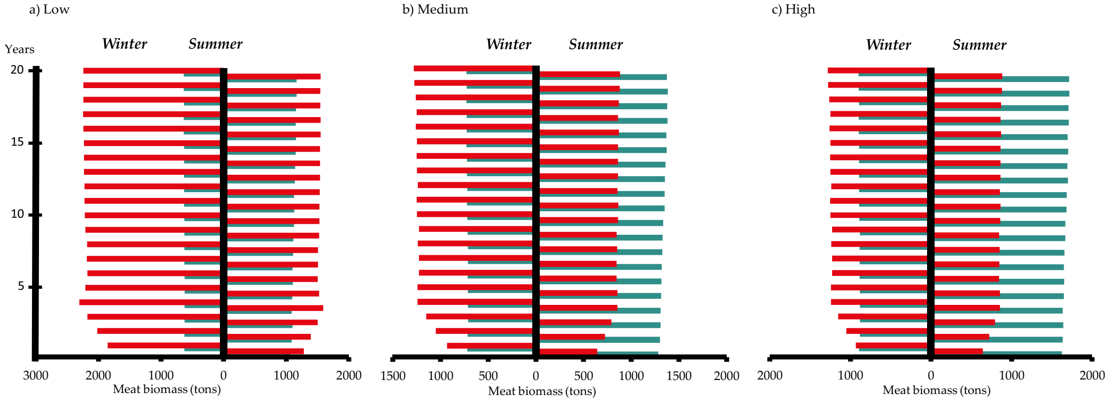

Fig. 4. Temporal availability of food resources (green column) compared with the energetic requirements of the scavenging species assemblage (red column) in the three trophic availability scenarios: a) low, b) medium and c) high.

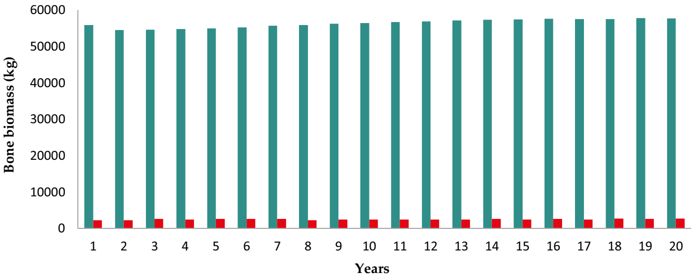

Fig. 5. Bone biomass available (green column) in the study area with respect to the energetic requirements necessary (red column) for the breeding bearded vulture population in a low trophic availability scenario.

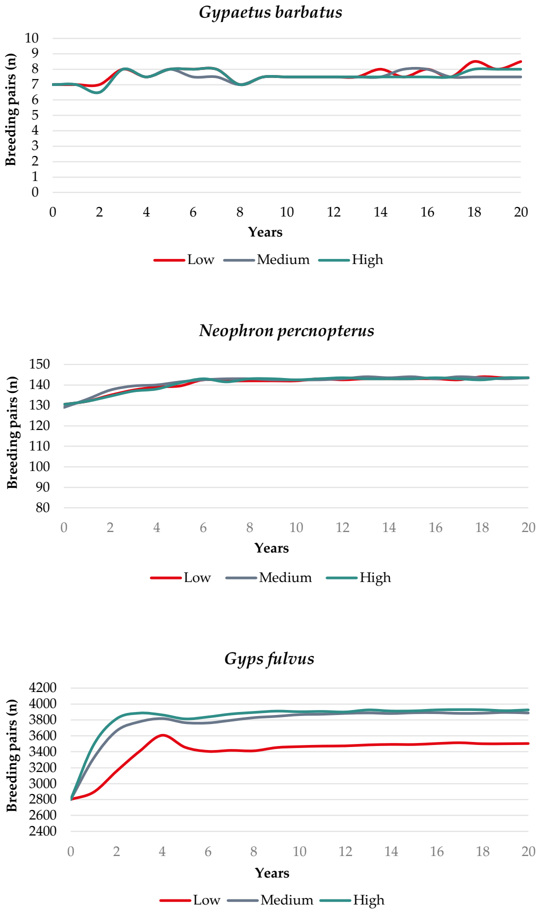

Fig. 6. Predicted population trends for the three avian scavengers in the study area, for each of the three scenarios tested (low, medium and high), expressed as the percentage of domestic and wild ungulate carcasses available in the ecosystem. Note the different y-axis scales.

foraging areas (Margalida et al., 2013; Lambertucci et al., 2014; Margalida et al., 2016; Morales-Reyes et al., 2017; Arrondo et al., 2018). This is the case of our study area that, as the results show, do not provides enough resources to cover the energetic requirements of the griffon vulture population being dependent of the availability and management of food in neighbouring areas. Accordingly, species with large foraging areas, like griffon vultures, are difficult to implement on computational models as a consequence of the use of alternative food resources from far-away areas. As a result, the coordination between different Spanish administrations and countries (France and Portugal) regulating health policies are necessary.

From a temporal perspective, we show that the availability of carrion is substantially higher in summer because of the increased numbers of livestock in mountain pastures. Mountain areas are the main zones which benefit from livestock seasonal movements, as occurs in the Pyrenean and MW regions. Sheep provide most of the carrion biomass, and constitute 50% of the food available. Therefore, transhumance, mainly of sheep, is important in increasing food availability in mountain ecosystems and this practice plays an important role in the conservation of avian scavengers and other wild species (Olea and Mateo-Tomás, 2009; Bernués et al., 2011; López-Santiago et al., 2014; Tyrrell et al., 2017). However, transhumance practice is suffering a progressive decline (Olea and Mateo-Tomás, 2009) that can have important consequences for biodiversity conservation (Mateo-Tomás and Olea, 2010, Carmona et al., 2013; Oteros-Rozas et al., 2014). Therefore, any conservation measure which facilitates extensive sheep husbandry should be a priority from a conservation point of view. However, this is compromised by the fact that the critical energetic shortfall for this avian scavenger guild occurs during the breeding period (winter-spring). At this time, the constraints of breeding limit the foraging movements at a time of reduced hours of daylight, adverse weather conditions, and the increased energetic requirements due to feeding chicks. We show that evident food shortages exist during the breeding period. The network of special feeding sites for avian scavengers (ZPAEN) recently established by Spanish administrations (Morales-Reyes et al., 2017) therefore play an important role for breeding birds inhabiting regions with limited food resources, or which are affected by sanitary regulations which remove carcasses from the landscape (Donázar et al., 2009a, 2009b). From a management perspective, the location of SFS should be related to the spatio-temporal distribution of natural resources and the avian scavenger population. Only by taking these into account can managers optimize the value of SFS, always considering the controversial pros and cons of this widespread conservation management tool (Moreno-Opo et al., 2015; Cortés-Avizanda et al., 2016).

Given that all the scenarios modelled show a deficit of food resources in the breeding season, our results suggest that a high proportion of the trophic resources available to the nesting population in the study area are obtained from: i) peripheral zones outside the study area; and/or ii) the exploitation of other sources of food not considered in this study (e.g. landfills, intensive farms, e.g. Plaza and Lambertucci, 2017, Tauler-Ametller et al., 2017). The first explanation can be confirmed by satellite tracking results obtained from several breeding individuals that exploited resources located far from nesting sites, such as certain areas in Extremadura, located 600 km from their breeding colony (C. Fernández, unpubl. rep.). Regarding the second explanation, Griffon vultures have been observed exploiting other resources such as garbage dumps following food shortages (Donázar et al., 2010; Plaza and Lambertucci, 2017). Therefore, models which assess food resource availability should consider larger spatial areas and every possible source of scavenger food. In contrast, specialized and less abundant species such as the bearded or Egyptian vulture do not seem limited by food resources. This is probably due to the small size of their breeding populations and the diet plasticity of both species, suggesting that trophic availability is not a limiting factor either for the establishment of new territories or the geographic expansion of these species. This agrees with previous studies (Margalida and Colomer, 2012; Margalida et al., 2017) suggesting that, quantitatively, food is not a limiting factor for Egyptian and bearded vultures. The results suggest that the available food is still substantially more than is needed by the breeding birds. For example, in the case of bearded vultures, while we included the smaller items of carrion from horses and cows, these are only rarely selected by this species (Margalida et al., 2009), and even considering only sheep remains, the potential food available each year in a medium food availability scenario is 175,000 kg, sufficient to sustain 568 bearded vulture breeding pairs. Therefore, the geographical expansion of bearded vultures does not seem to be limited by food resources, and limiting factors are more likely to be other aspects of habitat quality (e.g. disturbance, habitat modification), non-natural mortality factors (i.e. illegal poisoning) and the potential overcrowding with conspecifics attracted to the supplementary feeding sites established in the Pyrenees (Carrete et al., 2006; Margalida et al., 2009; Margalida et al., 2017).

Among the 23 Old World vulture species, 81% are globally threatened or near threatened and most of these species are declining, particularly in Africa and Asia, as a consequence of anthropogenic activities such as the illegal use of poisons, landscape transformation, health policies and ingestion of toxic veterinary drugs (Ogada et al., 2012a, 2012b). These threats persist and continue to increase, despite the fact that vultures provide important ecosystem services (Moleón et al., 2014). Regarding the projected population estimates, changes in spatial dynamics and distribution of the different species will be governed by factors such as: i) the maximum carrying capacity of the region (density); ii) the availability of suitable nesting sites; iii) the availability of food; and iv) to a lesser extent, longer distance movements of individual birds. Data regarding the increase in the breeding population of the Spanish Griffon vulture suggests that there is no density-dependent regulation of numbers, since the population grew more steadily in the more densely occupied provinces (Parra and Tellería, 2004). However, this large-scale result does not preclude the possibility of local regulatory processes in more densely occupied zones, where decreased breeding success has been observed (see Fernández et al., 1996). In the case of the bearded vulture, its population dynamics will depend fundamentally on management measures carried out in the rest of the Pyrenean range. During the last 20 years, there has been hardly any geographic expansion and this is attributable to the effect that supplementary feeding points have in attracting this species (Margalida et al., 2013). The large concentrations of individuals at these sites probably reduce geographic expansion of this species westwards, as evidenced by the lack of movement of pre-adult individuals beyond these areas (Margalida et al., 2013; Margalida et al., 2016). There is an abundant trophic supply and limiting factors could be the quality of available nesting sites and the population density (Donázar et al., 1993; Margalida et al., 2009). With respect to Griffon vultures, following an exponential growth in numbers during the last 30 years (Del Moral, 2009), the indications are of reduced growth leading to a possible stabilization of the population. Recent changes in sanitary policies have modified the behavior and diet of this species (Donázar et al., 2009a, 2009b, 2010; Margalida et al., 2011a, 2011b) and probably affected demographic parameters in similar ways to those noted for bearded vultures (Margalida et al., 2014). Finally, with respect to Egyptian vultures, the models suggest a population increase because its dietary plasticity allows it to utilize a wide spectrum of different prey. Consequently, the primary factor limiting the population viability of this species appears to be illegal poisoning (Hernández and Margalida, 2009; Ogada, 2014; Sanz-Aguilar et al., 2015).

Because each region/country may make independent decisions and work according to their own specific interests and conservation policies, approaches based on large spatial scales are essential to generate effective conservation measures based on transboundary approaches (Bischof et al., 2015). This approach is also required for avian scavengers where management centers on the provision of SFS or “vulture restaurants” at which surplus resources modify the quality of a habitat and provide predictable food resources that might affect spatial distribution and breeding density. Accordingly, the economic costs of providing SFS and their effects on ecological processes (see Donázar et al., 2009a, 2009b; Cortés-Avizanda et al., 2010; Dupont et al., 2012; Cortés-Avizanda et al., 2016) should force managers and policy-makers to assess the natural food provided by the ecosystem and to carefully evaluate the usefulness of supplementary feeding sites (Kane et al., 2015). The identification of optimal areas (i.e. those with abundant food resources and nesting-sites) could provide conservation tools to identify priority areas for reintroduction projects. It is important to consider large spatial scales in order to manage species with large foraging areas and to apply the correct management and conservation measures.

4.1. *Pros and cons of computational models in ecology*

Bioinspired models, such as PDP systems that work in parallel, are more flexible and enable the consideration of the heterogeneity of the population and the environment. These models allows to capture the randomness of the natural environmental processes using stochastic strategies based on Gillespie's kinetics (Gillespie, 1976) and the semantics defined by using probabilistic functions (Colomer et al., 2011; Colomer et al., 2013). However, modeling complex systems in which several environments and species interact competing for resources requires experienced researchers familiarized with these models. Although their complexity could limit the use of this tool, PDP systems allows modelling of demographic parameters with regard to food resources, and provides an effective tool in conjunction with other considerations (Colomer et al., 2013). PDP models are a complementary approach to be used when the classical modeling approaches fail (Colomer et al., 2011), and can aid in conservation planning for species of concern where available trophic spectra can be assessed objectively, and should be used to combine trophic resource measurements with demographic parameters to improve the effectiveness of conservation management. However, as a result of the limitations in computational models, replication of the models seems necessary to increase credibility and efficiency to facilitate theory development (Thiele and Grimm, 2015) and, as occurs in the case of threatened species, to optimize management and conservation actions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2018.11.004.

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Prioritizing among removal scenarios for the reintroduction of endangered species: insights from bearded vulture simulation modeling

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Abstract

Translocations are an increasing feature of threatened species conservation plans, but the impact of removal of individuals on the source population is seldom studied. Using computational Population Dynamics P System models and the Pyrenean Bearded Vulture population as a case study we looked at: the effect on the source population of alternative strategies for removal of individual birds for use in reintroduction projects; and the trade-offs between the various management options. According to our models (over a 30 year prediction horizon) the removal of one clutch, juvenile or non-territorial adult each year over an 11 year period, results in an annual loss of 1.57, 3.71 and 0.97 territories, respectively. We forecast the impact of a plausible removal scenario for the Pyrenees source population (the removal of five clutches and five non-territorial adults each year over 11 years), leading to a predicted loss of 16 breeding territories. Nevertheless, changes in demographic parameters, mainly in productivity and adult survival, could substantially affect these predicted results. With the current demographic parameters, the removal scenarios that were estimated to not affect population size after 30 years (95% CI) are limited to: (1) the removal of five clutches and five non-territorial adults during a single year; (2) the annual removal of five non-territorial adults during a 6 year period; and (3) the annual removal of five clutches during a 6 year period. Our results suggest that removals from the Pyrenean Bearded Vulture source population should be performed with caution due to uncertainties arising from stochastic changes in survival and productivity.

Introduction

The search for innovative and effective methods to improve and optimize species conservation programs is a key issue for managers, conservationists and policy-makers responding to biodiversity loss. The translocation of individuals to formerly occupied habitats is a widespread and increasingly used tool in conservation programs to restore wild populations of endangered species (Seddon, Armstrong & Maloney, 2007; Pérez *et al.*, 2012; Seddon *et al.*, 2014; Brichieri-Colombi & Moehrenschlager, 2016; Swan, Lloyd & Moehrenschlager, 2018). Because the economic and time costs of population replenishment and reintroduction projects can be limiting factors (Smith *et al.*, 2011), reliable assessments of the effectiveness of various management options are essential in choosing the best course of action (McCarthy, Armstrong & Runge, 2012). In the case of threatened species, population size of donor population can be an additional limiting factor. As a result, researchers and practitioners need to evaluate the likely trade-offs among the ex situ (e.g. captive breeding) and in situ (e.g. threat management) options available to improve reintroduction outcomes (Dolman *et al.*, 2015).

There are many studies of translocation projects and the success of reintroductions, including settlement, survival and reproduction of translocated individuals and their effects on the viability of the reintroduced population (e.g. Sarrazin & Legendre, 2000; Armstrong & Seddon, 2008; Le Gouar *et al.*, 2008; Mihoub *et al*, 2013; Robert *et al.*, 2015; Bertolero, Pretus & Oro, 2018). However, there is less information regarding the impacts of translocations on the source or donor population (see McCleery, Hostetler & Oli, 2014; Margalida *et al.*, 2015).

This is especially relevant when translocated individuals are extracted from endangered populations, as in the threatened Bearded Vulture *Gypaetus barbatus*.

Bearded Vulture reintroduction projects have been carried out in several European countries since the mid-1980s to counter the population declines and local extinctions of this species during the 19th and the beginning of the 20th centuries. The ambitious and successful reintroduction project conducted in the Alps is the most noteworthy (Schaub *et al.*, 2009). Birds have been bred in captivity and subsequently released not only in the Alps (France, Italy, Austria, Switzerland), but also in the Massif Central (France), Andalucia (Spain) and elsewhere, specifically in Corsica (5 pairs) and Crete (5 pairs) to reintroduce the species or to reinforce threatened subpopulations.

The Pyrenees (Spain, France and Andorre), with 164 breeding territories, holds the most important European population of Bearded Vultures. There are currently three active Bearded Vulture reintroduction projects in Spain: Andalusia (started in 2006), Asturias (Picos de Europa, started in 2012) and, most recently, Maestrazgo (Castellón, started in 2018). To date, captive bred individuals have been used in the Andalusia and Maestrazgo projects (European Endangered Species Programme) while the Asturias project has used birds raised from eggs taken from clutches removed from nests in the Aragonese Pyrenees. There is also a new proposal for an experimental reintroduction project involving the translocation and release of non-territorial adults taken from the Pyrenean population. To date, two non-territorial Pyrenean adults have been captured and released in November 2018 as part of the Maestrazgo project to reinforce the hacking of captive reared birds, but one returned to the Pyrenees after 6 days (authors unpubl. data).

Because the size of the Pyrenean Bearded Vulture population is regulated by a density-dependent effect on fecundity and its geographic expansion is slow (Carrete, Donázar & Margalida, 2006; Margalida *et al.*, 2008) managers and policy-makers have proposed that more individuals be extracted from the non-territorial population to strengthen and/or reintroduce new populations elsewhere. It is therefore imperative to assess the effects of translocation on the population dynamics of the source population to establish future guidelines for reintroduction or population reinforcement projects. So far, the only study to forecast the impact of different translocation management scenarios (removal of eggs, chicks or fledglings) on Spanish Bearded Vulture populations (Margalida *et al.*, 2015) was based on only a part of the Pyrenean population (Spanish Pyrenees) and used demographic records up to 2006 only (see Oro *et al.*, 2008). Any quantitative study of the entire Pyrenean chain (Spain, France and Andorra) on the effects of different extraction scenarios on the source population dynamics will ideally use computational Population Dynamics P System (PDP) models: an important advance in estimating the population dynamics of threatened species in relation to climatic, energetic (trophic availability) or anthropogenic variables that provides a more rigorous and objective alternative to traditional models of population viability (Colomer *et al.*, 2011; Margalida & Colomer, 2012; Margalida *et al.*, 2018).

We applied PDP models to estimate the effects of different management actions on the Bearded Vulture population trend using data from the long-term monitoring of the entire Pyrenean Bearded Vulture population (1987–2016) and updated demographic parameters for the whole Pyrenean chain (A. Margalida, J. Jiménez, J. M. Martínez, J. A.Sesé, D. García, A. Llamas, M. Razin, M. A. Colomer, B. Arroyo, in prep). Specifically, we modelled the population impact (number of breeding territories) over a 30-year prediction horizon of different extraction scenarios for reintroduction purposes: removal of clutches; first year juveniles; and non-territorial adults >10–20 years old. In addition we modelled the effects of changes in demographic parameters on the simulation forecasts. Our goal was to provide accurate information for managers and policy-makers in order to establish conservation guidelines for translocation management which would not compromise the source Pyrenean Bearded Vulture population.

Materials and methods

Study species

The Bearded Vulture is the most threatened European vulture species. They can breed from 6 years of age, although most start breeding at between 9 and 12 years old (Antor *et al.*, 2007; Lopez-Lopez *et al*. 2013; A. Margalida, J. Jiménez, J. M. Martínez, J. A. Sesé, D. García, A. Llamas, M. Razin, M. A. Colomer, B. Arroyo, unpubl. data). One or two eggs are laid per nest but only one chick ever fledges as a result of siblicide (Margalida *et al*., 2004). The chick-rearing period is about 121 days (Margalida *et al*., 2003) and after fledging the young enter a dispersal phase until they settle as territorial adults at between 5 and 7 years old (Antor *et al.*, 2007).

In the Pyrenean study area, breeding ocurs in about 60% of territories each year (each occupied either by a pair or a polyandrous trio) and the annual productivity ranges between 0.30 and 0.40 chicks/pair/year (Margalida, Colomer & Oro, 2014). However, this population is regulated according to the site-dependency hypothesis: as the population increases, average productivity decreases as progressively poorer quality territories are used. In addition, productivity is negatively correlated to the proximity to the nearest conspecific breeding pair (due to crowding) and to the proximity to the nearest supplementary feeding point where floaters congregate (Carrete *et al.*, 2006).

Population modeling

Our methodology first estimated the population parameters based on historical records. Next, a PDP model was constructed to perform 'virtual experiments' to evaluate the effect of removals of clutches and floating individuals of different ages. In order to study the effects of various life history parameters and extractions on the population trends, we used a surface response model (Box–Behnken). We combined the extreme range values of some demographic parameters (productivity and juvenile, subadult and adult survival) in a second Box–Behnken model to assess the potential effects of changes in these parameters on the population's dynamics.

Data collection and parameter estimates

The Pyrenean (Spain and France) Bearded Vulture population has been intensively monitored since the 1980s, including field surveys to monitor population trends, breeding parameters, and survival rates (including a specific capture-mark-resighting sub-programme) (Oro *et al.*, 2008; Margalida *et al.*, 2014, 2015). Between 1987 and 2016, all known breeding Pyrenean territories were visited several times each month to record reproductive parameters. Productivity (number of fledglings per pair/trio per year) and breeding success (number of fledglings per territory with egg-laying) were estimated (Margalida *et al.*, 2014). The ranges of these observed parameters were used to populate our models, assuming an even sex ratio at birth (Bretagnolle *et al.*, 2004). From 1987 to 2016, a total of 151 individuals of known age were identified using rings and wing-marks and were radio- or satellite-tracked in the study area. This has allowed the estimation of demographic parameters such as age of first reproduction, survival and age structure using Integrated Population models (IPM) which integrate capture and recapture methods and counts in a multistate Bayesian approach (Oro *et al.*, 2008; Margalida *et al.*, 2014, 2015; A. Margalida, J. Jiménez, J. M. Martínez, J. A.Sesé, D. García, A. Llamas, M. Razin, M. A. Colomer, B. Arroyo, in prep.). We distinguished three age classes according to the most parsimonious age-model obtained in previous analyses (Oro *et al.*, 2008; Margalida *et al.*, 2014): juveniles (1–2 year old), subadults (3–5 years old) and adults (>6 years old). Given that productivity and survival rates have decreased over the years (Margalida *et al.*, 2014), we used the range of values obtained during the last 5 years (2012–2016), gathered from various demographic studies (Table 1, Supporting Information Table S1). Maximum carrying capacity of the region has been estimated at 1000 breeding individuals (500 breeding territories) based on estimates of natural (i.e. without artificial feeding) food biomass availability, which is more than twice the current population size (Margalida & Colomer, 2012; Margalida, Pérez-García & Moreno-Opo, 2017*a*).

PDP model

Population Dynamic P Systems are computational models inspired by the functioning of cells that operate in parallel at both the individual and process levels, and are capable of modeling processes that interact with each other and animals that compete for resources of different types. The components of a PDP are: environments, membrane structure, initial work alphabet and evolution rules (Colomer, Margalida & Pérez-Jiménez, 2013). The number of environments is usually associated with the number of different zones in the ecosystem to be modelled (i.e. subpopulations inhabiting different regions). The initial alphabet is the input of the model (in our case the objects associated with each individual at the start of the model run, for example, population size, demographic parameters). The rules of evolution use some parameters (e.g. demographic parameters) and aim to describe the processes and process changes that each individual will undergo.

Table 1. Values of demographic parameters obtained from our own empirical data (for details, see Methods) used to calculate the viability of the Pyrenean Bearded Vulture population

Parameter	Value
End juvenile stage	2
End sub-adult stage	5
Life expectancy	30
Age first breeding attempt	6
Number of descendents	1
Productivity ratio low density[a]	0.45
Productivity ratio high density[a]	0.25
Maximum density (pairs)	500
Annual mortality age 1–2 year	0.066
Annual mortality age 3–5 year	0.035
Annual mortality age ≥ 6 year	0.036

[a] Values are taken from the high and low end of the productivity distribution that was estimated across all densities, and assigned to low- and high-density, respectively.

Here we use a PDP model related to the population's dynamics, the density-dependent reproductive process, and the possible effects of extraction of clutches, juveniles >1 year old (hereafter juveniles), and non-territorial adults of ≥10–20 years old (hereafter adults). The results of the model were compared with a baseline non-intervention scenario, based on the demographic rates considered. In the density-dependent model, we take into account the variations in fecundity according to the population size. Our model incorporated negative density-dependence effects on fecundity (Carrete *et al.*, 2006), applying the equation used in *Vortex*: $P(N) = \left(P(0) - (P(0) - P(K)) \cdot \left(\frac{N}{K}\right)^{B}\right) \cdot \frac{N}{N+A}$, where $P(N)$ is the percentage of females that breed at population size N, $P(K)$ is the percentage of females that breed when the population is at carrying capacity (K) and $P(0)$ is the percentage of females that breed when the population is close to zero. In the extractions model, we subdivided the fecundity variables into subcategories (the percentage of pairs that lay eggs and both the hatching and reproductive success) to explore the population effects of the different intervention scenarios (clutches, juveniles and adults) on productivity and population size (breeding fraction).

Description of the PDP model

The model takes into account all the items described previously such as: the population density and the processes of mortality; reproductive parameters; and the removal interventions (clutches or individuals of different age classes) to be performed (Fig. 1) each year. These items were sequenced in order to simplify the model, although this simplification does not affect the final results. The PDP defined is formed by three membranes with the following structure: $\mu = [[\,]_1[\,]_2]_0$

(see Colomer *et al.*, 2013). The main, or skin membrane (labelled 0), contains two inner membranes labelled 1 and 2, respectively. Most processes are carried out between the skin membrane and membrane 1. Membrane 2 serves to save the information on the removals that will be extracted from the ecosystem each year and to restore the initial configuration at the end of the cycle, to reset the model at beginning of the following year.

The objects that appear in the initial configuration in the membrane labeled by 0, that is, the model inputs, are as follows:

$$\mu_0=\left\{\mathrm{XS}_j^{\mathrm{qs}_j},1\leq j\leq g_3,\right\}\cup\left\{\mathrm{XP}_j^{\mathrm{qp}_j},g_4\leq i\leq g_3\right\}$$
$$\cup\left\{\mathrm{XT}_j^{\mathrm{qt}_j},g_4\leq i\leq g_3\right\}\cup\{\mathrm{NES}^{\mathrm{Eg}_1},\mathrm{CHI}^{\mathrm{Chi}_1},D,\mathrm{ANY}_1,$$
$$\mathrm{ANY}_1^{\prime g_3}\}\cup\{b_i,1\leq i\leq g_3\}.$$

A XS_j object is associated to each non-territorial animal of age *j*, while territorial animals are associated to a XP_j or XT_j object, depending on whether they form a pair or trio, respectively. qs_j is the number of objects XS_j (number of non-territorial individuals of age *j*), qp_j the number of objects XP_j (territorial individuals that form pairs of age *j*) and qt_j the number of objects XT_j (territorial individuals that form trios of age *j*). The model allows the removal of clutches, chicks and floating individuals of different age-classes. In this study, we only considered the removal of clutches and non-territorial individuals (juveniles and adults). The number and type of extraction depend on the year. For each year, the quantity of NES objects that are generated equals the number of clutches that are extracted from nests that year. The number of CHI objects is the number of chicks to be removed from nests (not used in the current model). D is an object that generates a counter (R_1) in the first step, which allows control of the model and avoids inconsistency in the application of the rules. In addition, D generates other objects that allow control of the maximum carrying capacity in the study area. The object ANY_i stores the year that is being simulated in its index. Finally, the objects ANY_i' and b_j allow the generation of FLY_j objects, which are used to extract i non-territorial individuals of age j in the simulation.

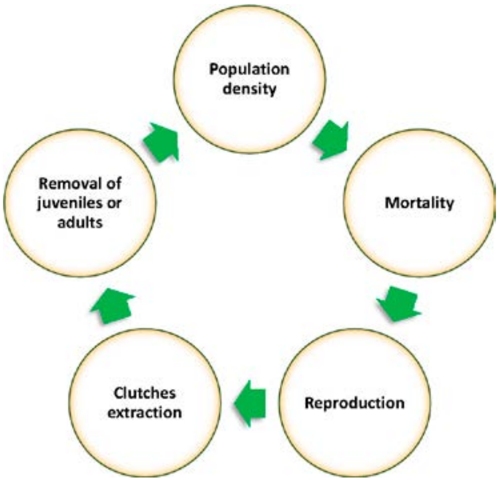

Figure 1 Representation of the modeling process carried out with the Population Dynamics P System. The input of the model is the population size at the start of the process (0) and the output is the population size in the year *t* using the parameters showed in Table 1.

The starting point is the census of animals of each age estimated through the IPM. The definitions of the parameters of the model are shown in Table 1. The model assumes that productivity varies according to the number of breeding territories as a result of density-dependent effects on fecundity (Carrete *et al.*, 2006; Oro *et al.*, 2008). The probability that an individual mates and reproduces depends on its age. The reproductive success is density-dependent and depends on the size of the population (Carrete *et al.*, 2006). The probability of mortality depends on the age of an individual, and the probablility of survival was estimated using observations during the last 5 years for the three age classes considered.

Box–Behnken design

We used a response surface (number of breeding territories) approach to estimate the size of the Bearded Vulture population in the Pyrenees over a 30-year period, depending on the mean values of the parameters considered (Table 1) as well as a range (maximum and minimum) of demographic parameter values and removal timeframes (Table 2). The response surface designs are a subset of the experimental designs used to model the relationship between the independent variables or factors (x_1, x_2, … x_n) and the response variable (Box & Behnken, 1960), using linear models and quadratic or higher order models.

To study the effect of removals on Bearded Vulture population dynamics we constructed a Box–Behnken design using four factors: clutch extractions (range 0–10); removal of 1 year old juveniles (range 0–10); removal of non-territorial adults ≥10–20 years old (range 0–10) and the time of extractions (range 1–11 years). This results in a total of 28 experiments examining 25 different scenarios and four repetitions.

Since some demographic parameters, such as productivity and survival are subject to progressive decreases (in productivity) or annual fluctuations (in survival), we subsequently created another Box–Behnken design to consider the possible

Table 2. Range (minimum–maximum values) of demographic parameters and extraction timeframes used to calculate the viability of the Pyrenean Bearded Vulture population

Factor	Low level	High level
Productivity	0.25	0.45
Juvenile mortality (age 1–2 year)	0.046	0.086
Subadult mortality (age 3–5 year)	0.015	0.055
Adult mortality (age ≥ 6 year)	0.015	0.055
Years extraction	5	11

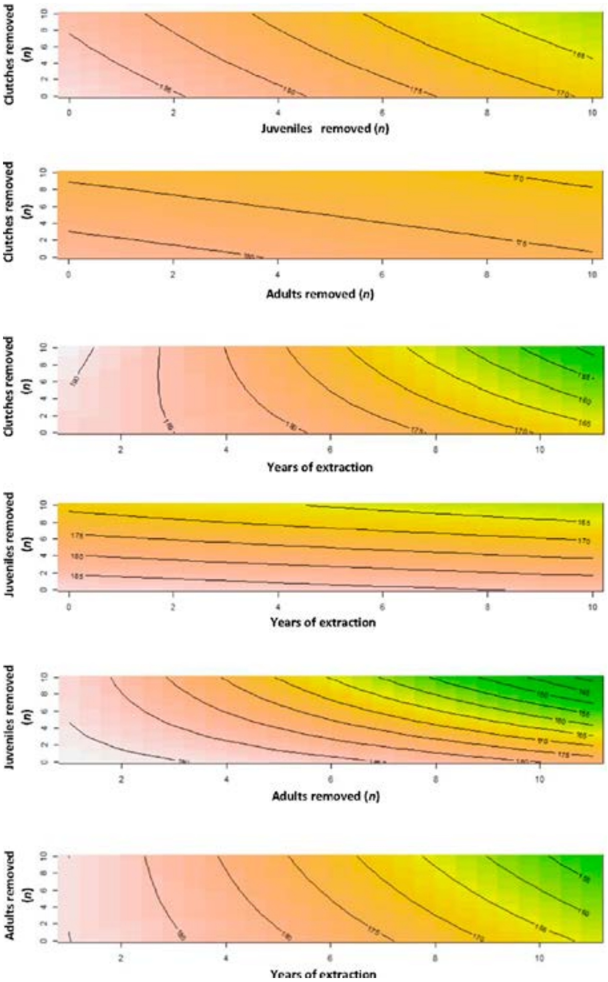

Figure 2 Response surface (number of breeding territories) obtained using the current estimates of the population demographic parameters. The colours represent the number of breeding territories: brown represents the higher values and green the lower ones.

variations (Table 2). In this case, the number of experiments was 44 with a total of 41 different scenarios with four repetitions.

For the design and the statistical analyses we used DoE.-base, a package in the R program (R 3.5.2.) R Core Team, 2018.

Results

All of the four factors considered (clutches, juveniles, non-territorial adults and years) had a significant effect on the number of breeding territories (Fig. 2; Table 3). In addition, the interactions among clutches, juveniles and adults and the timing of extractions were also significant. The removal of one clutch, juvenile or non-territorial adult each year over an 11 year period, resulted in an annual loss of 1.57, 3.71 and 0.97 territories, respectively. Therefore, an annual extraction of five clutches lead to the loss of 10 breeding territories over 11 years, compared with 20 territories lost due to an annual extraction of five juveniles and seven territories lost due to an annual extraction of five adults. The annual extraction of five clutches and five adults over an 11-year period is a plausible scenario in the Pyrenees and would lead to the loss of 16 breeding territories after 30 years.

As shown in Fig. 3 and Table 4, there were nine removal scenarios that did not result in a significant change (95% CI)

Table 3. Coefficient values of the response surface (number of breeding territories) and significance levels of the variables and interactions that were tested using mean demographic parameter values (Table 1)

	Estimate	SE	*t* value	*P*
Intercept	174.947	0.865	202.194	**0.0001**
Clutches	−3.792	0.499	−7.590	**0.0001**
Juveniles	−10.947	0.499	−21.915	**0.0001**
Adults	−3.138	0.499	−6.282	**0.0001**
Years	−16.181	0.499	−32.391	**0.0001**
Clutches × juveniles	−0.657	0.865	−0.760	0.461
Clutches × adults	0.585	0.865	0.676	0.511
Clutches × years	−5.452	0.865	−6.302	**0.0001**
Juveniles × adults	−0.735	0.865	−0.849	0.410
Juveniles × years	−9.025	0.865	−10.431	**0.0001**
Adults × years	−3.110	0.865	−3.594	**0.003**
Clutches^2	0.299	0.706	0.423	0.679
Juveniles^2	0.640	0.706	0.906	0.381
Adults^2	0.156	0.706	0.221	0.828
Years^2	−1.250	0.706	−1.769	0.100

Juvenile refers to individuals 1-year-old and adults to non-territorial adults 10–20 years old. Statistically significant results are shown in bold type.

in the population trend over 30 years compared to the non-intervention scenario (Fig. 3). The only combinations lying inside this confidence interval were extractions performed during 1 year (7 scenarios) and 6 years (2 scenarios) (Table 4).

What would happen if the demographic parameters change?

Bearing in mind that productivity and survival rates may vary, another Box–Behnken design was constructed, evaluating the importance and effect of variations in these demographic parameters in combination with several types of extractions.

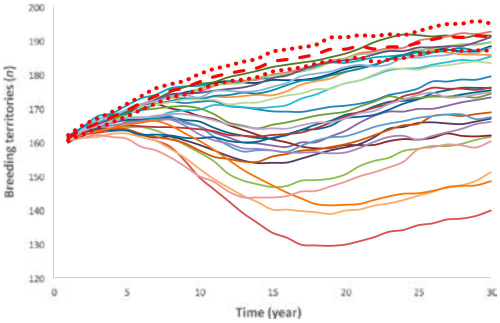

Figure 3 Projections of the number of Bearded Vulture territories in the Pyrenees over time as predicted under different combinations of extraction scenarios, using the currently estimated (mean values) for demographic parameters (Table 1). The red line shows the population trend without any intervention. The dashed line is the 95% CI.

The results show that all four factors (productivity, juvenile survival, subadult survival and adult survival) had a significant effect on the number of breeding territories, with the most significant effects resulting from variations in productivity and adult survival rates (Fig. 4; Table 5). The duration of the intervention had no statistically significant effect due to the overwhelming effects of the other factors. According to our results, an increase in juvenile mortality (up to 0.086) resulted in a breeding population loss of seven breeding territories after 30 years. If an increase in mortality occurred in the subadult age-class (up to 0.055), the impact would be a loss of 20 breeding territories. Finally, in the case of adults (up to 0.055) 66 breeding territories would be lost. On the contrary, if the survival rates of all age-classes are maintained at the current levels (Table 1), but productivity falls (to 0.25 chicks/pair/year), 57 breeding territories would be lost after 30 years.

Table 4. Extraction scenarios that do not have a significant impact (95% CI) on the Pyrenean Bearded Vulture source population trend over a 30-year scenario

Removal scenario			
Clutches	Juveniles	Adults	Years
5	0	5	1
0	5	5	1
10	5	5	1
5	5	0	1
5	5	10	1
0	0	5	6[a]
5	10	5	1
5	0	0	6[a]
0	5	5	1

[a]The five removals happen annually.

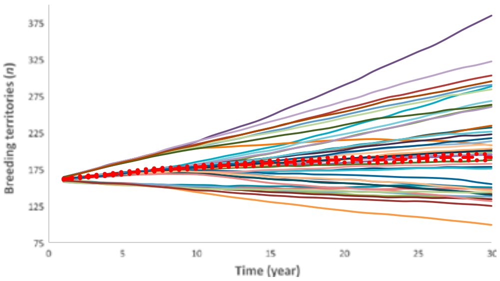

Figure 4 Projections of the number of Bearded Vulture territories in the Pyrenees over time as predicted under different combinations of demographic parameters and extraction scenarios, using the range (minimum–maximum) of demographic parameter values and extraction timeframes (Table 2). The red line shows the population trend without any intervention. The dashed line is the 95% CI.

Discussion

For threatened species, removal of individuals from the wild for reintroduction projects or to create a captive population could compromise the source population both due to the loss of the individuals extracted and because the population becomes more vulnerable to stochastic events (Margalida *et al.*, 2015; Heinrichs *et al.*, 2018; Heinrichs *et al.*, 2019). The simulations presented here offer sufficient evidence to assist managers and policy-makers to adopt the most rigorous and effective management measures for the restoration of European Bearded Vulture populations, without undue harm to the source population. These models, linked to various removal scenarios, could enable managers and policy-makers to develop objective-based conservation trade-offs in current conservation projects to reinforce or reintroduce subpopulations of the species.

Until now, the Pyrenean Bearded Vulture population has been managed using two types of extractions: (1) the regular removal of clutches, started in 2008 and still ongoing (about five clutches are currently removed annually); and (2) the extraction of non-territorial adults, started in 2018 (when two adults were removed). To explore a broader spectrum of possible extraction scenarios we added a third option as an alternative: the extraction of trapped 1 year old juveniles. This new simulated scenario could provide an alternative to reintroductions using fledglings derived from captive rearing projects which are then hacked out into the wild.

With 164 breeding territories, the Pyrenees harbour the most important population of wild Bearded Vultures in Europe. This population is characterized by a progressive increase in breeding territories and, in parallel, a density-dependent decrease in productivity (Carrete *et al.*, 2006). In addition, there has been an increase in the fraction of non-territorial but potential breeders (i.e. mature floaters > 6 years of age), from 39% in 2006 to 68% in 2015 (Antor *et al.*, 2007; Margalida *et al.*, 2015). This situation prompted a technical-scientific debate regarding the need and opportunity to remove clutches and floating individuals for use in reintroduction programs (Ferrer *et al.*, 2014; Margalida *et al.*, 2015; Margalida *et al.*, 2017*b*).

Table 5. Coefficient values of the response surface (number of breeding territories) and significance levels of the variables and interactions that were tested using a range (minimum–maximum) of demographic parameter values and extraction timeframes (Table 2)

	Estimate	SE	*t* value	*P*
Intercept	200.170	2.479	80.731	**0.0001**
Productivity	56.946	1.240	45.934	**0.0001**
Juvenile mortality	−7.144	1.240	−5.762	**0.0001**
Subadult mortality	−19.869	1.240	−16.027	**0.0001**
Adult mortality	−80.053	1.240	−64.573	**0.0001**
Years	−2.071	1.240	−1.670	0.108
Productivity × juvenile mortality	−6.962	2.479	−2.808	**0.010**
Productivity × subadult mortality	−7.617	2.479	−3.072	**0.005**
Productivity × adult mortality	−32.075	2.479	−12.936	**0.0001**
Productivity × years	1.477	2.479	0.596	0.557
Juvenile mortality × subadult mortality	1.622	2.479	0.654	0.519
Juvenile mortality × adult mortality	2.315	2.479	0.934	0.360
Juvenile mortality × years	0.925	2.479	0.373	0.712
Subadult mortality × adult mortality	8.437	2.479	3.403	**0.002**
Subadult mortality × years	−1.030	2.479	−0.415	0.682
Adult mortality × years	0.340	2.479	0.137	0.892
Productivity^2	−1.203	1.825	−0.659	0.516
Juvenile mortality^2	−0.590	1.825	−0.323	0.749
Subadult mortality^2	1.202	1.825	0.659	0.516
Adult mortality^2	13.627	1.825	7.468	**0.0001**
Years^2	1.184	1.825	0.649	0.523

Statistically significant results are shown in bold type.

Our results show that of the three scenarios modeled, juvenile removal has twice the impact on the source population compared with the extraction of clutches or non-territorial adults. This is due to the fact that juveniles preserve intact their probabilities of beginning reproduction (from 6 years old onwards) whereas non-territorial adults (≥10 years), have lost part of their potential reproductive probabilities. However, reintroduction using juveniles is the principle management strategy used to reintroduce the species in Europe (i.e. hacking fledgling individuals from captive breeding) and is the most successful in many different European projects (see Schaub *et al.*, 2009), although our results show that, in the wild, its impact on the source population is substantial. Therefore, from a conservation point of view, the extraction of clutches and non-territorial adults is preferable to the removal of juveniles. In the case of clutch removal, the extraction should be focused on low quality territories with low breeding success to reduce the demographic impact on the source population. In this sense, in future work it would be necessary to estimate the number of removals that could occur (Margalida *et al.*, 2017*b*) without a negative impact if the removals were to be from these low quality territories (i.e. with low breeding success). This could potentially be an optimal management approach because releasing juveniles into the reintroduction site may be of benefit to the reintroduced population (i.e. potentially higher release site fidelity and high reproductive potential) with relatively low impact on the source population.

The next question is how many clutches and/or non-territorial adults could be removed from the Pyrenean population for use in reintroduction projects elsewhere in Europe without affecting the source population. There are several ways to view this question. First, from a conservation biology perspective, there is an argument that the Pyrenean population should be conserved through management programs and not subject to the removal of individuals. As such, reintroduction programs should focus on species recovery in existing reintroduction areas, but do not justify extractions from source nuclei. Expanding the species' range should prioritize alternative reintroduction methods such as captive breeding, management of supplementary feeding sites and so on and the removal of wild birds from existing populations should not be the first option. Second, we need to be cautious regarding the projections forecast here because some scenarios show no impact at the 95% CI (Table 4), indicating the pronounced influence of stochastic variations. The demographic parameters that we used in the models (survival and productivity) may be considered optimistic and their true values may well be lower (e.g. the falls in productivity resulting from density-dependent factors) and mortality values could increase as a result of non-natural factors such as illegal poisoning (Margalida, 2012). In this regard we can confirm that the observed mean values of subadult and adult survival rates (*c.* 0.96) are the most accurate so far obtained for this species in our study area and are higher than the 0.88 reported by Oro *et al.,* (2008) and comparable to that of reintroduced populations in the Alps (0.96, Schaub *et al.*, 2009). The current mean values of productivity in our density-dependent model are c. 0.38 chicks/pair/year; higher than those recorded during the previous 5 years (0.32 chicks/pair/year) and on the southern side of the Pyrenees (0.29; Margalida *et al.*, 2014). Therefore, all of the model projections based on rates of survival and productivity higher than the current observations (Table 2) can be considered relatively unlikely (Fig. 4). To assess the effects of parameter variations we constructed a second Box–Behnken design which showed that population size is very sensitive to productivity rate. A decrease in productivity to 0.25 chicks/pair/year suggests that 57 pairs would be lost from the breeding population after 30 years. An increase in subadult and adult mortality predicts a substantial slowing of population growth. For example, an increase in subadult mortality (up to 0.055), predicts a loss of 20 breeding territories and similarly in adults, a loss of 66 breeding territories. Our results therefore suggest that even in a long-lived species such as the Bearded Vulture, population size and viability are very sensitive to changes in adult mortality (Saether & Bakke, 2000) but also productivity. Productivity also seems to exert a very strong effect on population viability in other species (see Genovart, Oro & Tenan, 2018). In addition, since population growth is very sensitive to adult survival rate, the extraction of floating adults should also have substantial demographic effects. It is therefore important to know the true effect of extractions in order to avoid levels which would leave a population vulnerable to damage from stochastic increases in mortality or reductions in productivity. It should be noted that our models assume that extractions of floating adults are homogenous across all age classes between 10 and 20 years. Therefore, some of the individuals extracted from the Pyrenean population could be potential breeders (mainly those between 10 and 14 years old), while others (from 15 to 20 years old) could be extracted with less demographic impact. However, because the priority of reintroductions is to establish a viable or self-sustaining population at the new site, it seems imperative to evaluate trade-offs between objectives, and the benefits through a structured decision-making framework when faced with such complex decisions (Seddon *et al.*, 2007; Converse *et al.*, 2013). For example, the experimental reintroduction of non-territorial 15- to 20-year-old individuals would have less demographic impact on the source population but these individuals would have low probability of breeding at the release site. The post-release dispersal behavior of individuals released as adults is unknown, and there is a possibility they could return immediately to the natal site. As such, there could be benefit in reintroducing 15–20 year old birds to improve knowledge about management options available to maximise site fidelity.

How many clutches and non-territorial adults could be removed?

Focusing on management by the removal of clutches and non-territorial adults, our results suggest that there are only three scenarios inside the 95% CI (i.e. those that do not affect the source population size after 30 years): (1) the removal of five clutches and five non-territorial adults during a single year; (2) the annual removal of five non-territorial adults during a 6-year period; and (3) the annual removal of five clutches during a 6-year period. We identify a limiting temporal removal scenario of 6 years in which two removal actions are combined (i.e. clutches and non-territorial adults) rather than extractions concentrated into a single year. All the rest of the combinations modelled will have detrimental effects on the dynamics of the source population, although some of the forecast trajectories showed population growth. This is because selective harvesting can indirectly increase recruitment, thereby potentially impacting population growth rate (Milner, Nilsen & Andreassen, 2007). Furthermore, our simulations show that the effects of extractions will not be detectable until 10–15 years after the start of such interventions. Therefore, applying the precautionary principle, interventions in the Pyrenean Bearded Vulture source population should be performed with caution. As an experimental project, the extractions could focus on floating individuals >15 years, which should have a lower demographic impact and allow an increase in the number of extractions without undue population effects. A next step would be to use GPS transmitters to monitor the behavior of translocated individuals and their success in settling into their new locations in order to fine-tune future management procedures. With respect to clutch extractions, we suggest there would be value in future work to evaluate the impact of removing clutches from high-quality versus low-quality habitat. For example, removal of clutches from low quality habitat potentially has a lower demographic impact (Margalida *et al.*, 2017*b*) and would allow an increase in the number of extractions without undue population effects.

Concluding remarks

Our simulation approach provides a vital tool for planning the management and conservation of a threatened long-lived population and for making objective decisions regarding trade-offs in future reintroduction projects (Converse *et al.*, 2013; Lloyd *et al.*, 2019; Panfylova, Ewen & Armstrong, 2019). Quantitative modeling provides explicit predictions about future population trends and the uncertainty surrounding forecast population trajectories under different extraction regimes and allows the pros and cons of different removal alternatives to be assessed. Our results will be of value to decision-makers, allowing them to anticipate and avoid detrimental impacts on source populations. However, due to the inevitable stochastic changes in demographic parameters and the potential limitations of computational models, it is essential to constantly update future model replications with current observed parameter values when using theoretical models to implement management actions for threatened species, in order to increase credibility, efficiency and objectivity (Thiele & Grimm, 2015; Margalida *et al.*, 2018).

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Definition of the parameters used in the PDP model.

correspondence

The shadow of diclofenac hangs over European vultures

To the Editor — Of the sixteen Old World vultures, 81% are globally threatened or near-threatened; four vulture species inhabit Europe, of which three are threatened or near-threatened. Since 1993, the EU and various national governments have invested significant financial resources in the conservation of vultures — including at least 76 LIFE projects related to these species — and between 1993 and 2014 spent €121.9 million, of which €59.7 million came from European funds. During this period, Spain, home to 90% of all European vultures, invested €72.8 million (€30.8 million received from EU) on 38 projects related to vulture conservation[1].

However, all these conservation efforts will be of little worth if the use of veterinary diclofenac, authorized in Spain since 2013, spreads. A mathematical model has estimated that annually diclofenac could cause 715–6,389 vulture deaths[2]. Thus, its potential ecological impact on ecosystem services is obvious.

In November 2014 the Conference of the Parties of the UNEP Convention on Migratory Species adopted a resolution aimed at providing the veterinary sector with guidance on how to prevent the poisoning of migratory birds and called for an evaluation of the risks that veterinary medicinal products pose to scavenging migratory bird species[3]. In December 2014, the European Medicines Agency's Committee for Medicinal Products for Veterinary Use proposed to the European Commission that the veterinary use of diclofenac in Europe should be regulated[4]. In June 2015, the Veterinary Pharmaceutical Committee (VPC) decided not to initiate a withdrawal of marketing authorizations for veterinary products containing diclofenac in Europe since, in the Committee's opinion, the management measures being applied by member states would be effective in keeping the risk to vultures and other necrophagous birds under control. On 4 July 2016, in a VPC meeting, member states were invited to provide an update of the situation in their territories. Their report indicates that (1) most member states say that they have not yet authorized the use of diclofenac; (2) those that have authorized the use of this product state that appropriate safety warnings are included in the product literature and that fitting measures have been put in place for the safe disposal of fallen stock and to provide birds of prey with carrion; (3) no member states have yet reported any deaths of vultures due to poisoning by veterinary medicinal products in their territories. However, the true situation is somewhat different: (1) the use of diclofenac has been authorized in at least five member states (Spain, Italy, Estonia, Czech Republic and Latvia) and currently there is a request for a permit in Portugal; (2) the safety warning in the product literature is insufficient and merely recommends "Do not administer to animals susceptible to enter the wild animal food chain," there is no information about the 'appropriate measures' to be applied for the safe disposal of carrion by avian scavengers or any supervision of these measures; (3) until 2016, there was no monitoring of NSAIDs (nonsteroidal anti-inflammatory drugs) contamination of ungulate cadavers available to vultures and other obligate and facultative avian scavengers. Nevertheless, to date at least one griffon vulture has been reported to have died as a result of ingesting flunixin[5], an NSAID with similar effects to diclofenac. Thus, it is just a question of time before deaths due to diclofenac begin to occur.

Currently, there is no complete ban on diclofenac in Europe and the potential risk it represents is still present[6]. Given that the VPC has no data for anywhere in Europe on veterinary medicine residues in carcasses available to scavengers, the current risk cannot be effectively assessed. Accordingly, the precautionary principle must be applied, which should entail the immediate ban on the use of diclofenac for livestock to avoid undesirable consequences to vulture populations[7] and the promotion of the use of safe alternatives such as meloxicam[8]. The catastrophic decline in Asian vulture populations[9,10] is sufficient warning of what could happen and, likewise, ought to make it unnecessary to have to wait until more dead vultures begin to appear. ❒

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Competing interests

The authors declare no competing financial interests.

Bearded vulture *Gypaetus barbatus* in the Huesca Pyrenees, Aragón (Spain)

Photo: Pilar Oliva-Vidal

Avian scavengers provide essential ecological, economic and cultural services; yet vultures are among the most threatened groups of birds worldwide and their populations have declined significantly in recent decades. Behind this global collapse lie a wide array of anthropogenic factors, which are accentuating this decline in our human-dominated world. Currently, the major threats to vulture populations – the illegal use of poisons, the ingestion of veterinary drugs and other toxic substances (e.g., pesticides), lead contamination, landscape transformation (e.g., 'landscape closure' processes due to rural abandonment) and changes in health policies – are all continuing to grow. In addition, in recent times human-vulture cohabitation has been negatively influenced by 'fake news' about the aggressive behaviour of vultures towards livestock that are jeopardizing conservation efforts for Old and New World vultures. Thus, integrative research linking different disciplines is essential for providing useful insights into the management and conservation of vulture populations in order to design and propose effective management actions.

This thesis aims to provide a multidisciplinary approach embracing a series of different disciplines including behavioural ecology, conservation biology, ecotoxicology and the social sciences to improve the efforts currently being made to conserve European scavenger birds. More specifically, it investigates how cascading anthropogenic (e.g., ecological, toxicological, socio-economic and political) changes affect avian scavengers and the ecosystem services they provide. In this context and under a changing environment scenario, this thesis first explores how changes in rural landscapes due to farmland abandonment affect the functioning of scavenger assemblages and scavenging dynamics (*Chapter 1*). Next, the human-wildlife conflict between vultures and livestock is addressed from a socio-economic ecological perspective (*Chapter 2*). Then, from an ecotoxicological point of view, this thesis assesses how avian scavengers exhibiting different foraging behaviour are exposed to anticoagulant rodenticides (*Chapter 3*). Finally, we apply bioinspired computational models to estimate the carrying capacity of an ecosystem on the basis of the availability of trophic resources, to quantify the spatio-temporal distribution of these resources and their relationship to transhumance (*Chapter 4*), and to forecast the demographic impact of translocations for reintroduction projects on the source Pyrenean bearded vulture population (*Chapter 5*). The conceptual framework of this thesis thus combines field studies, active monitoring programs, ecological modelling and adaptive management approaches to provide policy-makers, managers and conservationists with a set of evidence-based conservation tools in order to establish priority conservation lines and ensure the coexistence of avian scavengers and humans in a context of an ever-changing human-mediated world.