Bird Conservation International

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Research Article

Cite this article: Posillico M, Costanzo A, Bottoni S, Altea T, Opramolla G, Pascazi A, Panella M, Ambrosini R (2023). Reported mortality of Griffon Vulture *Gyps fulvus* in central Italy and indications for conservation and management. *Bird Conservation International*, **33**, e68, 1–11
https://doi.org/10.1017/S0959270923000199

Received: 26 July 2022
Revised: 05 May 2023
Accepted: 13 May 2023

Keywords:
anti-poisoning strategy; conservation; diclofenac; illegal killing; scavenger

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CAMBRIDGE UNIVERSITY PRESS

Reported mortality of Griffon Vulture *Gyps fulvus* in central Italy and indications for conservation and management

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Summary

Vultures are long-lived species sensitive to human-caused mortality that has already determined a widespread collapse in Asian and African populations. They provide significant ecosystem services (regulatory and cultural) consuming livestock carcasses and saving greenhouse gas emissions, favouring nutrient recycling, environmental sanitation, and providing financial revenue. Appraising the incidence and causes of mortality could help to improve management and conservation actions. We compiled records of reported mortalities for the reintroduced Griffon Vulture *Gyps fulvus* population of the central Apennines in Italy (123 cases, July 1994–December 2020). The average mortality was 4.69 vultures per year (± 1.14 SE), with no significant temporal trend. The peak of mortality events, estimated by harmonic regression analysis, was in March, while the minimum occurred in October. No differences were found among age classes and sex ratio mortality was established at 1.43:1 (M: F, $N = 68$). Out of 103 (83.7%) vultures which underwent a post-mortem and toxicological screening, 53% were poisoned, mainly by carbamates, and 27% died of unknown causes. Overall, direct or indirect anthropogenic mortality caused 67% of deaths. Even considering an inherent bias associated with reported mortality as to the prevalence of causes of death and estimation of mortality rates, the overwhelming relevance of poisoning highlights that existing anti-poisoning efforts should be refined and incorporated into a coordinated multidisciplinary strategy. A standardised approach, from vulture carcass discovery to post-mortem procedures and toxicological analysis, should be applied to reduce uncertainty in the determination of causes of death, increasing effectiveness in the prosecution of wildlife crimes. As most of the poisoning cases affecting the Griffon Vulture population in the central Apennines likely represent a side (though illegal) effect of retaliatory efforts to defeat livestock predators, effective strategies in reducing human–wildlife conflicts should be applied.

Introduction

As obligate scavengers at the top of the trophic web and *K*-selected species, vultures can be particularly affected by either direct or indirect anthropogenic mortality, both at a local level and worldwide (Lambertucci 2010, Margalida 2012, Ogada *et al.* 2012, Pain *et al.* 2008).

Direct persecution, whereby vultures are victims of deliberate illegal killing driven by dislike or superstition (Botha *et al.* 2017), is not the main driver of global vulture decline but can become locally frequent and have a large impact on several species (Brochet *et al.* 2019, Cailly Arnulphi *et al.* 2017, Daboné *et al.* 2023, Oppel *et al.* 2021). Indirect anthropogenic mortality also represents a major threat to vultures (Arrondo *et al.* 2020, Berny *et al.* 2015). For instance, secondary poisoning by veterinary pharmaceuticals administered to livestock, especially non-steroidal anti-inflammatory drugs (NSAIDs) such as flunixin, nimesulide, and carprofen, is implicated in the global collapse of many vulture species (Botha *et al.* 2017). In Asia, three formerly widespread species suffered a severe, rapid decline due to the use of diclofenac (Green *et al.* 2004), and recent simulations suggested its high potential impact even on Griffon Vulture *Gyps fulvus* populations in Europe (Green *et al.* 2016). Other major causes of decline are represented by the use of pesticides in poison baits, targeted at killing wild mammalian predators and feral dogs (Diekmann and Strachan 2006), and by lead poisoning, through the consumption of spent ammunition fragments in game carcasses and gut piles (Bassi *et al.* 2021, Monclús *et al.* 2020, Plaza *et al.* 2020b). Finally, impact with wind turbines and death by collisions with power lines or electrocution represents another significant life threat for large soaring birds and raptors (de Lucas *et al.* 2012, Martin *et al.* 2012, Sarrazin *et al.* 1994).

Although the Griffon Vulture occurs in several European countries, few studies have investigated extensively its death causes or mortality rates in Europe so far (Arrondo *et al.* 2020, Berny *et al.* 2015, Cano 2016, Xirouchakis 2004). In particular, no published information is reported for Italy, where five Griffon Vulture populations exist, mainly originating from resource-demanding conservation translocations (Genero 2009). This species, however, is still considered "Near Threatened" at the national level (Gustin *et al.* 2021), both because of the low number of breeding pairs and the persistence of high (though unquantified) rates of anthropogenic mortality (Brambilla *et al.* 2011, Genero 2009, Gustin *et al.* 2009).

To support management actions and conservation efforts, we collected and analysed records on reported Griffon Vulture mortality in the central Apennines from 1994 to 2020. Specifically, we aimed at investigating patterns of mortality according to year and month. Moreover, we explored the association between mortality, gender, and age class. Finally, we investigated the prevalence of the causes of death as well as the temporal trend of mortality causes. Resulting information should help in planning and enforcing appropriate conservation measures, thus aiding in preventing or decreasing further losses from non-natural causes, and prioritising management actions.

Methods

Study area

The study area, mainly located in L'Aquila province, Abruzzo region, is in the central Apennines (42°10'N 13°20'E), a mountainous zone, with ridges oriented from north-west to south-east, and elevations exceeding 2,000 m a.s.l. (maximum elevation 2,914 m in the Gran Sasso Massif) (Figure 1). Climate is predominantly Mediterranean-montane, with cold snowy winters and drier, warm summers (Piovesan *et al.* 2003). Livestock breeding is common: goats, sheep, cattle, and equids (in decreasing order of abundance), with a total of >95,500 heads per year in the whole L'Aquila province (Banca Dati Nazionale dell'Anagrafe Zootecnica 2022). Equids and cattle are usually left unattended in pastures. In some areas, livestock roam freely in winter, although at lower elevations. Sheep, goats, and cattle are usually kept on farms during winter. Transhumance, which represents a seasonally important source of food for vultures (Margalida *et al.* 2018), is still widely practised in the area, with thousands of livestock heads spread across pastures from mid-June to early October.

Study species

Griffon Vulture is a large colonial vulture of the Accipitridae family (weight 6–11 kg, wingspan 2.3–2.8 m). It is distributed from the Iberian peninsula and northern Morocco to the western Himalayan range, also occurring through the south-western Arabian peninsula, Ethiopia, Eritrea, and Sudan (Campbell 2015). Its life expectancy could be up to 41 years in captivity (Carey and Judge 2000), and the onset of senescence has been estimated at 28 years in the wild (Chantepie *et al.* 2016). Sexual maturity is attained at four years (Møller 2006) and the monogamous pairs lay one egg per breeding season (Cramp and Simmons 1980).

The last evidence of Griffon Vulture breeding in the central Apennines dates back to the mid-sixteenth century (Pandolfi and Zanazzo 1994). The current population in the study area originated from 93 individuals donated by the Spanish Government to the Italian Ministry of Agriculture-Forest Service. Birds were released from 1994 to 2002 mainly in the Monte Velino Reserve (MVR) (Potena *et al.* 2009). Currently, six breeding sites occur around the Fucino plain (Figure 1). The average ± SD reproductive success and number of fledglings per year from 2003 to 2015 were 0.77 ± 0.21 and 21.5 ± 5.9, respectively, and the breeding population is increasing (Altea *et al.* 2016).

Data sources

Overall, 148 occurrences of dead (N = 123, range: 0–27 individuals per year) (Figure 2) and impaired birds (N = 25) were recorded from July 1994 to December 2020. Sixty (48.8%) dead and 14 rescued vultures were marked birds, two of which were rescued twice. Records about mortality or rescues of impaired vultures were compiled from the archives of MVR, the office responsible for monitoring Griffon Vultures in the area. Dead and impaired vultures were mainly discovered by local people during outdoor activities, hikers, Forest Service, and subsequently Carabinieri, provincial police, and park wardens. Eight vultures were found thanks to satellite telemetry. Records covered information about the date, location, age class, whether the bird was GPS-tagged or otherwise marked, photographs, maps, description of the remains, and their perceived preservation status; in addition, death records also contained information on the causes of death from the associated necropsy report. As it was not always possible to collect all these data for every dead/rescued vulture, the sample size changed in different statistical analyses.

Annual trend of mortality

Based on the approximate date of death estimated during necropsy or on the detection date, we were able to assign 120 out of 123 mortality events to a given year. The first mortality event was documented soon after the first five vultures were released (mid-July 1994), while the last known death occurred in November 2020. Since mortality data for 1994 were limited to six months (July–December), we analysed inter-annual variation in mortality only from 1995 onwards. We also investigated the variation in occurrence of mortality events at within-year periods, considering only those records where necropsy or evidence allowed estimation of time of death to a given month (Table 1).

Age class and sex

The age class of vultures (juveniles, subadults, and adults) was assigned according to standard phenotypic characteristics, including moult patterns (Duriez *et al.* 2011, Zuberogoitia *et al.* 2013). Age classification was made by experienced and trained personnel while handling birds. We were never able to retrieve dead nestlings, though we were aware that some mortality occurred. Age class distribution of the vulture population was drawn from 303 different individuals live-captured 631 times (some individuals were captured more than once) during 30 capture sessions from 2010 to 2020.

The sex of dead vultures was determined starting from 2010 onwards from both a morphological examination of the gonads during necropsy or from DNA analyses. Molecular sexing of dead and live-captured individuals from 2010 to 2011 was run from tissue samples using P2/P8 primers (Griffiths *et al.* 1998).

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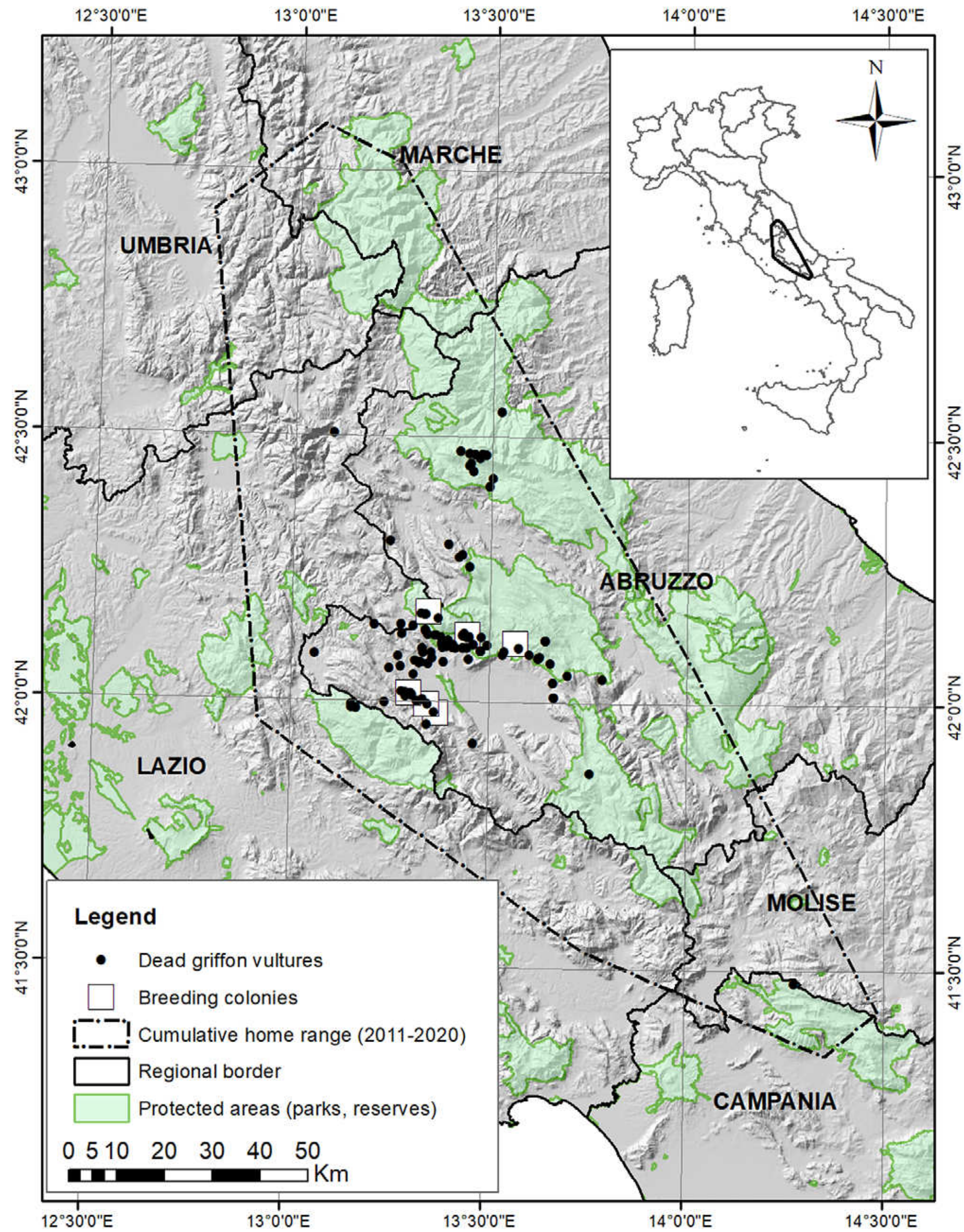

Figure 1. Distribution of dead Griffon Vultures *Gyps fulvus* (N = 123) in the central Apennines from 1994 to 2020, with respect to protected areas. The cumulative home range (minimum convex polygon drawn around all telemetry fixes) of GPS-instrumented resident vultures (2010–2020 data) is also shown.

Conversely, from 2011 to 2020, the individuals were sexed from plucked feathers following Garofalo *et al.* (2016).

Mortality causes

Post-mortem necropsy and toxicological analyses of 95 carcasses were carried out at the Istituto Zooprofilattico Sperimentale (Experimental Zooprophylactic Institute – IZS) per l'Abruzzo e il Molise (IZSAM, Teramo and Avezzano laboratories), while four carcasses were examined at IZS Lazio e Toscana (IZSLT), two at the Forensic Laboratory of the Veterinary Faculty, University of Teramo (VFTU), one at the Gran Sasso and Monti della Laga National Park (PNGSL), and another one at the IZS del Mezzogiorno (IZSDMP, Portici laboratory). In 20 cases (16%), carcasses consisted of too few remains and were not delivered for necropsy.

The causes of death were defined as "undetermined" if the necropsy was not conclusive, and "not-determinable" when paucity of the remains did not allow further analyses. When the report mentioned the occurrence of more than one likely cause of death, we questioned the pathologist for further clarification and identification of the ultimate cause. All vultures whose cause of death was related to human activities or infrastructures were further classified as died from direct (poisoning, shooting, and an instance where a vulture was likely beaten to death) or indirect (collision with wind turbines, suspended wires, vertical structures, and electrocution) anthropogenic causes, as opposite to natural causes (disease). For three vultures from two sites (distance 3.5 km) recovered within eight days, no toxic substances were detected from carcass samples.

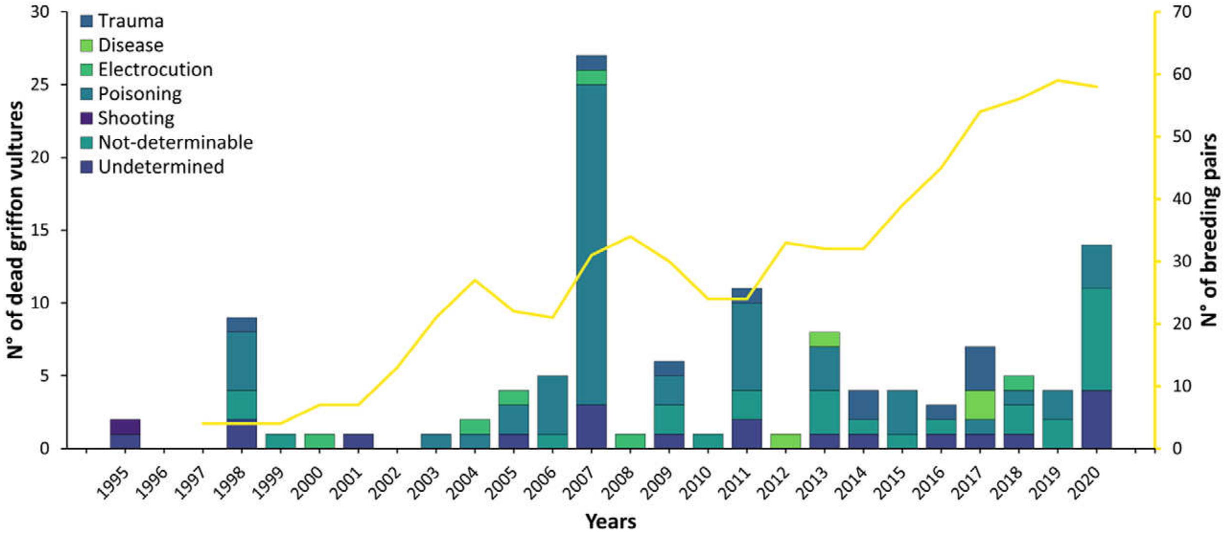

Figure 2. Annual reported number of dead Griffon Vultures *Gyps fulvus* from 1995 to 2020 in the central Apennines. Different colours represent different mortality causes. The yellow line represents the number of breeding pairs in each year.

Table 1. Number of dead Griffon Vultures *Gyps fulvus* from the central Apennines (1994–2020), according to gender and age class by month and breeding phenology.

		Incubation			Chick rearing				Non-breeding				
Gender and age class	Whole study period	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Females	28	2	6	9	3	2	2	0	1	0	1	0	2
Males	40	1	12	5	6	3	3	0	1	0	2	3	4
Undetermined	55	2	4	6	12	4	6	3	6	2	3	3	1
Adults	49	3	7	10	4	3	4	3	4	1	1	4	4
Subadults	48	1	8	5	13	5	5	0	3	0	4	1	2
Juveniles	9	0	2	3	2	0	0	0	0	1	0	0	1
Undetermined	17	1	5	2	2	1	2	0	1	0	1	1	0

Nevertheless, we considered them as poisoned based on evidence from carcass status (Fajardo and Zorrilla 2016), and because a bait containing a carbamate insecticide (methomyl) was found a few days after the vultures, 1 km away from one of the birds. Harmed birds that died soon after their retrieval for the same reason causing their impairment (N = 5) were included in the sample of dead vultures.

Reporting of toxicological analyses by IZSAM has been standardised in its current array since 2000. Accordingly, to quantify the prevalence of detected toxic substances, we selected only toxicological reports of vultures which underwent post-mortem from 2000 onwards yielding positive results (N = 48). Depending upon preservation status, vultures were tested against diseases subject to mandatory sanitary surveillance (i.e. avian influenza, West Nile disease, Usutu virus, and Newcastle disease). Diagnoses of pathologies were carried out depending on the outcome of the necropsy inspection. From 2011 to 2017 a subsample of vultures (N = 11), three of which were also tested for embedded shots by X-ray, was analysed for lead concentration according to Bassi *et al.* (2021). The occurrence of NSAIDs was assessed from 2017 to 2020 for 13 vultures. The presence of toxic substances commonly employed to poison wildlife and domestic animals was assayed (Table S1). The occurrence of further single toxics was tested for by pathologist's examination of remains thanks to evidence from specific inquiries. Due to the paucity of analysable matter, some panels were not fully implemented.

Statistical methods

We aimed at modelling temporal variation in mortality, expressed as the proportion of individuals found dead, but the number of individuals in the population in each year was unknown. We, therefore, used the number of breeding pairs in each year (Altea *et al.* 2016, MVR unpublished data) as a proxy for population size (Van Beest *et al.* 2008), and modelled the annual number of casualties in a generalised linear model (GLM) model assuming a negative binomial distribution of the data where the log-number of breeding pairs was entered as an offset (see Supplementary material 2 for further details on this model).

https://doi.org/10.1017/S0959270923000199 Published online by Cambridge University Press

To test for within-year variations in mortality, we used harmonic analysis (periodic regression), whereby we transformed months into angles (January = 30°; December = 360°) and then included the sines and cosines of these angles, and their interaction, into a generalised linear mixed model (GLMM) model as predictors. With this formulation, the sines contrast spring vs. autumn mortality, while cosines contrast summer vs. winter mortality. The GLMM model assumed a negative binomial distribution of the data and included the year as a random grouping factor and the sine of the month (transformed as above) as a random slope within year (see Supplementary material 3 for a mathematical description of the model and code). The diagnostic of the model indicated that the model did not show zero-inflation nor overdispersion (details not shown).

We used a multinomial logit model for modelling the relative proportion of adults, subadults, and juveniles found dead in each year, while logistic regressions were used to investigate annual variation in the sex ratio of individuals found dead.

Given the small sample size, only descriptive statistics are shown for impaired vultures. The analyses were performed in R 3.2.2 (R Core Team 2016) with the glmmTMB (Magnusson *et al.* 2017) and VGAM libraries (Yee *et al.* 2015).

Results

Annual trends of mortality

The average (mean ± SE) annual mortality was 4.69 ± 1.14 vultures per year. We found no significant temporal trend in reported annual mortality during the 26 years of study (model coefficient ± SE: -0.03 ± 0.03, z_{21} = 0.96, P = 0.34). Harmonic analysis showed that the number of reported mortality events peaked in March (day 73 = 14 March), whereas the minimum was in October (day 289 = 16 October) (Table 2). Out of the 120 mortality events classified according to season, 76.7% occurred in winter (N = 48) and spring (N = 44), while 75.8% occurred during the incubation (January–March, N = 48) and chick-rearing periods (April–July, N = 43) (Figure 3).

Table 2. Result of the negative binomial generalised linear mixed model (GLMM) fitting a harmonic regression model to the yearly variation in Griffon Vulture *Gyps fulvus* mortality. Year was included as a random grouping factor and the sine of month as a random slope within year. According to the harmonic analysis, sine, and cosine contrasted spring vs. autumn and summer vs. winter, respectively. Variances of the random effects were 0.83 for year and 1.25 for sine of the month within year. SE = standard error.

Predictors	Estimate	SE	*z*	*P*
Intercept	–1.80	0.31	5.76	<0.001
Sine (month)	0.97	0.40	2.40	0.02
Cosine (month)	–0.04	0.21	0.17	0.87
Sine × Cosine	–0.33	0.41	0.81	0.42

Age class and sex effects

Mortality in different age classes could be determined for 106 out of 123 dead vultures (86%) (Table 1). The frequency of occurrence of age classes of dead vultures in 2010–2020 did not differ from that observed in individuals captured during the same period (G = 0.78, df = 2, P = 0.68). The multinomial logit model showed that on

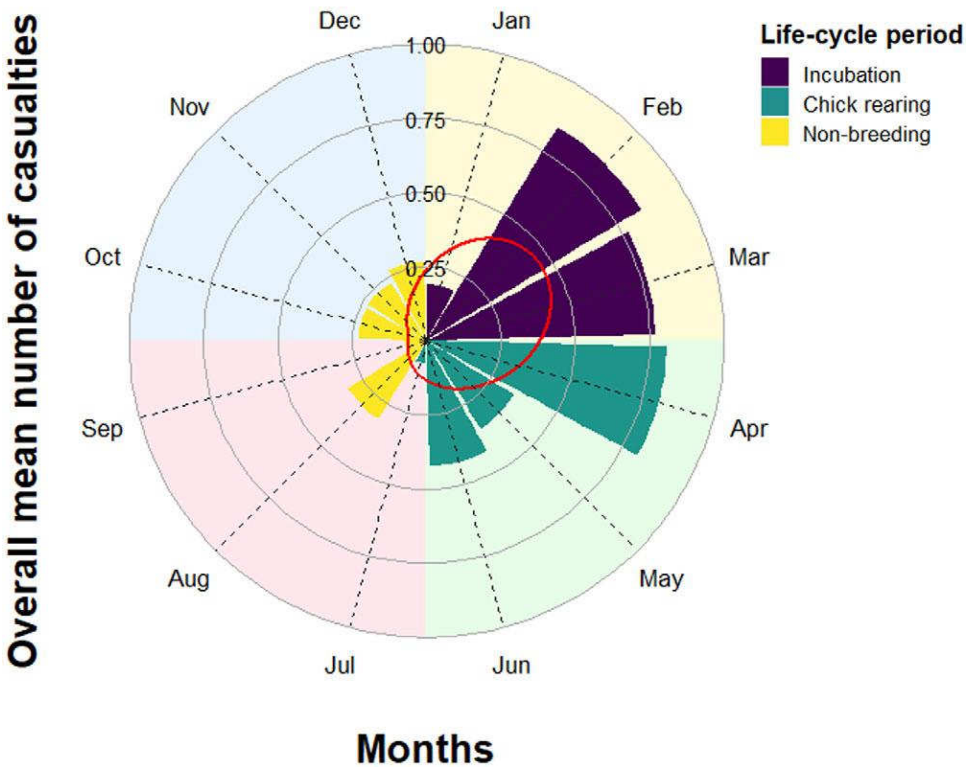

Figure 3. Mean overall number of dead Griffon Vultures *Gyps fulvus* per month (N = 120). Different periods of the annual life cycle are highlighted. Background colours represent the four seasons. The red line represents the fitted values of the harmonic regression model.

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average, juveniles (N = 9) were found dead less often than adults (N = 49) (coef: -1.70 ± 0.37, z = 4.61, P <0.001), while adults and subadults (N = 48) were found in similar proportions on average (z = -0.13, P = 0.90) (Table 1). In addition, the proportion of dead subadults and juveniles did not decrease over the years compared with adults (z ≥-1.43, P ≥0.14).

Sex could be assessed for 68 dead vultures (54.8% of the total sample), out of which 40 were males and 28 females (1.43:1), which did not differ significantly from 1:1 (exact binomial text, P = 0.18) (Table 1). A binomial regression model showed that the sex ratio of dead vultures did not vary through the years (z = -0.35, P = 0.73).

Mortality causes

A post-mortem and a toxicological screening were carried out on 103 (83.7%) dead vultures. Poisoning was by far the most important mortality factor, affecting 55 Griffon Vultures and representing 53.4% of all mortality events and 73.3% of all cases for which mortality causes could be determined (N = 75). Natural mortality occurred in only four vultures that died from diseases, all the remaining events represented direct (N = 57, 55.3%) or indirect (N = 15, 14.6%) anthropogenic mortality. Shooting occurred just once, while electrocution (N = 6, 5.8%) and collision with wind turbines (N = 9, 8.7%) represented the most important causes of deaths due to human infrastructures. For 19 (18.4%) birds, the cause of death was unknown.

From 2000 to 2020, 92 vultures were sent for necropsy. The preservation status of carcasses allowed for toxicological analyses in 84 cases. Toxicological tests were carried out screening 49 substances overall, though not all were tested for in all cases. On average 17.3 toxic agents (± 12.6 SD, range: 4–36) were tested for each vulture. Most vultures (92%) were tested for carbamates, while 54% and 52% were tested for organochlorines and organophosphates, respectively. Plant-derived toxins (mostly strychnine, and coumarin in three cases) were investigated in 44% of birds, while inorganic compounds (i.e. zinc phosphide and arsenic) were tested in 23% of cases. Rodenticides (8%) and molluscicides (metaldehyde, 2% of vultures) were the least tested substances (Table S1); 48 vultures were positive at such tests. Overall, 14 toxic substances (29% of the total tested) were detected. One to six substances were identified in each poisoned bird (mean ± SD = 1.44 ± 0.99). Carbamates recurred most frequently, as they were detected 35 times in 34 poisoned vultures, followed by organochlorine pesticides and strychnine, detected 27 and 4 times in 12 and 4 birds, respectively (Table 3).

In 2020, a Griffon Vulture that died of carbofuran poisoning resulted positive for diclofenac (7% of 13 birds tested for NSAIDs). However, its concentration was by far lower than the one known to be lethal (L. Giannetti–IZSLT, pers. comm.).

None of the Griffon Vultures screened by X-ray yielded embedded or ingested lead shots (E. Bassi, pers. comm.). Two birds (18%) showed subclinical to clinical lead concentration (E. Bassi, pers. comm.). In these latter cases, mortality may have occurred due to the level of lead concentration in the bones.

Discussion

Annual and seasonal trends of mortality

We did not detect an annual change in the number of dead vultures. As our sample was mostly made from reported casualties, annual random variation in search effort could have biased our results. However, a fair number of vultures have been reported by hikers, farmers, and hunters, from which we assume their regular presence in the study area. Moreover, local Forest Service station officers and park wardens regularly patrol assigned areas. Hence, we are confident that this bias was negligible. In addition, by using the breeding population size as a proxy for the total population, we statistically

Table 3. Toxic substances detected from 48 Griffon Vultures *Gyps fulvus* poisoned from 2000 to 2020 in the central Apennines. Occurrence (%) of toxic agents is reported with respect to total poisoned vultures (N = 48, sum >100%) and to the total occurrences of toxic substances (N = 69, sum =100%) in dead vultures. Up to six substances were detected in a single bird.

Class	Name	*N* occurrences	Relative frequency (%)	
			Poisoned vultures (*N* = 48)	Occurrence of toxic agents (*N* = 69)
CARBAMATES	Aldicarb	10	20.83	14.49
	Carbaryl	2	4.17	2.9
	Carbofuran	22	45.83	31.88
	Methomyl	1	2.08	1.45
ORGANOCHLORINES	Dieldrin	6	12.5	8.7
	Hexachlorobenzene	3	6.25	4.35
	HCH (beta)	4	8.33	5.8
	Lindane	1	2.08	1.45
	P,P-DDD	1	2.08	1.45
	P,P-DDE	12	25	17.39
ORGANOPHOSPHATES	Chlorpyriphos ethyl	1	2.08	1.45
	Phorate	1	2.08	1.45
PLANT TOXINS	Strychnine	4	8.33	5.8
INORGANIC ZINC	Zinc phosphide	1	2.08	1.45

https://doi.org/10.1017/S0959270923000199 Published online by Cambridge University Press

controlled for the potential bias due to a positive association between the occurrence of mortality events and the increase in Griffon Vulture population size over the years (Altea *et al.* 2016).

A decline in the annual breeding success and population growth, and an increase in mortality of young age classes have been reported in Spain for the Griffon Vulture as a consequence of a severe food shortage related to the implementation of sanitary legislation after the bovine spongiform encephalopathy (BSE) outbreak in 2009 (Donázar *et al.* 2009, Margalida *et al.* 2011, Zuberogoitia *et al.* 2010). In our area, we did not observe an annual increase in detected mortality after 2009, nor a decrease in the breeding population (Altea *et al.* 2016). Hence, survival in our population seems not to be related to the availability of food provisioning. However, mortality peaked at the end of winter (in between incubation and the chick-rearing period), while it was at its minimum in mid-autumn (during the non-breeding season). These mortality patterns are similar to those observed in other species such as Cinereous Vulture (*Aegypius monachus*) in Spain (Hernández and Margalida 2008), Oriental White-backed Vulture (*Gyps bengalensis*) in Pakistan (Gilbert *et al.* 2002), and Andean Condor (*Vultur gryphus*) in Argentina (Estrada Pacheco *et al.* 2020, but see Margalida *et al.* 2008). Both resource distribution and meteorological conditions could explain our results. During winter, food resources occur within a more restricted range than in summer as the abundant transhumant herds are no longer spread across pastures and many resident herds are kept at low elevations. Moreover, meteorological conditions in winter are less favourable for soaring, the typical low energy-demanding flight of large scavenging raptors (Ruxton and Houston 2004). In addition, winter rigours combined with high energy investment in reproduction for breeding individuals require a greater energy expenditure, making Griffon Vultures more susceptible to illness and death. Conversely, during the warmer season, although chicks demand a huge food provision, energy expenditure for thermoregulation is lower, and the abundance of livestock is higher because of incoming transhumant herds (Margalida *et al.* 2018). Another, non-mutually exclusive explanation of the intra-annual pattern of mortality that we observed could be related to the timing when poisoned baits are used. Horses and cattle are usually left unattended in pastures, even during the season when the young are born, between March and May. This might result in an increase in livestock losses due to predation on calves and foals by wild and domestic predators in late winter/spring (Cozza *et al.* 1996, our study area), and preventive and retaliatory reactions by stakeholders might be the main cause for poisoning (Ntemiri *et al.* 2018). Wolves (*Canis lupus*) and an undetermined number of feral dogs and wolf–dog hybrids have steadily inhabited the whole Apennines totalling c.2,400 individuals (La Morgia *et al.* 2022). Notwithstanding its protected status, unresolved issues of wolf–livestock conflict generate reactions by local farmers and residents to control wolf numbers through illegal means. Indeed, human-related mortality, including poisoning, is responsible for more than 70% of wolf casualties in the central and northern Apennines (Lovari *et al.* 2007, Musto *et al.* 2021).

Age class and sex effects

Age class was known for 86% of Griffon Vultures in our sample, however only a significantly smaller portion was represented by juveniles, while subadults and adults appeared in similar proportions, reflecting the age class distribution in our study population. Conversely, Berny *et al.* (2015) found a higher proportion of juveniles in their sample of dead vultures, and the same occurred for Oriental White-backed Vultures in Pakistan (Gilbert *et al.* 2002). As suggested by Harel *et al.* (2016), juveniles have reduced skills in managing soaring flights compared with experienced vultures, showing lower soaring–gliding efficiency and spending more energy per time unit. Although their lower flying performance could be buffered by social facilitation while foraging (Jackson *et al.* 2008), their survival could be affected because they are more prone to traumas due to flight accidents. Moreover, their later arrival at carrion sites predisposes them to suboptimal food intake in terms of both quantity and quality, as observed at feeding stations (Bosè *et al.* 2012, Bosé and Sarrazin 2007), which consequently affects survival. In addition, juveniles and immature vultures are more likely to undertake long trips and disperse at several hundred kilometres from their natal range than subadults and adults (Alarcón and Lambertucci 2018), and during such forays, many are likely to die. In such cases, well outside the study area, dead juveniles could rarely be retrieved or even reported. Such a circumstance could partly explain why the number of reported dead juveniles was lower in the central Apennines compared with other study sites.

Although both the breeding population of Griffon Vultures and the number of fledged chicks increased over the years (Altea *et al.* 2016), both the proportion of dead juveniles and the overall detected mortality did not. As the survival rate of Griffon Vulture increases with age until adulthood (Chantepie *et al.* 2016), and the proportion of adults in the population increased progressively after translocations were completed, we could conclude that the growth of the breeding population was not paralleled by an increase in the overall reported mortality. On the other hand, the small number of dead juveniles and their higher dispersal rates could have played a role in obscuring possibly higher juvenile mortality from reported occurrences.

The sex ratio of dead individuals (1.43:1) was not significantly different from the typical 1:1 ratio for Griffon Vultures (Bosé *et al.* 2007). Such a mortality pattern was similar to that found for Andean Condors (Estrada Pacheco *et al.* 2020), Oriental White-backed Vultures in Punjab, Pakistan (Gilbert *et al.* 2002), Bearded Vultures in Europe (Margalida *et al.* 2008), and Griffon Vultures in France (Berny *et al.* 2015). This is not surprising as the monogamous Griffon Vulture is sexually monomorphic (Bosé *et al.* 2007), and both sexes equally invest in nesting and parental care (Cramp and Simmons 1982). However, despite what was previously reported (Altea *et al.* 2013, Monsarrat *et al.* 2013), Morant *et al.* (2023) recently found significant differences in movement patterns of breeding Griffon Vultures in different populations in Spain. There, females showed larger home ranges and lower monthly fidelity than males (Morant *et al.* 2023).

Mortality causes

Worldwide, poisoning represents the most frequent threat for vultures (Botha *et al.* 2017, Plaza *et al.* 2019b). Secondary and unintentional poisoning has been recognised as the most recurrent vulture intoxication type in Europe (Hernández and Margalida 2008). The central Apennines population is no exception, as poisoning incidents accounted for 83.7% of mortality cases where the cause was identified. Poisoning events could affect high numbers of vultures (so-called massive poisoning), and repeated massive poisoning incidents could lead to local extinction (Ogada *et al.* 2016). In our study area, two massive poisoning events were reported, in 1998 and 2007. In addition, even if not directly lethal, poisoning could impair flight ability, which could predispose vultures to deadly accidents like, for instance, collision with suspended wires or wind blades and electrocution (Mineau *et al.* 1999).

In our study, carbamates were by far the most recurrent toxic component determining the death of reported vultures. Other pesticides (organochlorines) and strychnine were detected as well, though less frequently. Moreover, rodenticides were found in only 8% of tested birds, a low prevalence that is comparable with that found in the population of Griffon Vultures in the Pyrenees (Oliva-Vidal *et al.* 2022b). The prevalence of carbamates (among which carbofuran and aldicarb dominated, although both have been banned since 2007–2008) is a recurrent trait in both scavengers and wildlife poisoning issues and these substances have been found to affect several species in different geographical areas (Estrada Pacheco *et al.* 2020, Guitart *et al.* 2010, Hernández and Margalida 2009, Plaza *et al.* 2019b, Safford *et al.* 2019). The prevalence of specific toxic substances in wildlife poisoning is usually linked to factors like lethality, cost, and availability of stocks before their prohibition (Plaza *et al.* 2019a), and less dependent upon the legal status of such substances, as underscored by the abundant and recurrent use of carbamates in western Europe, most of which have been banned as pesticides for more than 15 years (Poledník *et al.* 2011). Although strychnine has been banned in Italy since the 1970s, its use in baits might have been sustained by illegal trade from abroad. Similarly, the illegal trade of carbofuran and aldicarb could also represent, in the long term, a continuous source of such pesticides, which could be halted only by a global ban (López-Bao and Mateo-Tomás 2022). In our opinion, most of the poisoning episodes in our study represent secondary or indirect poisoning events (see also Oliva-Vidal *et al.* 2022a), as suggested, in some cases, by the association of dead dogs, wolves, foxes, and corvids with vulture casualties, as well as Canidae remains found within the gizzard or gastrointestinal content of vultures. Evidence of accidental contamination has never been found by personnel in charge of the investigation, nor do landfills or garbage dumps occur where vultures could have been contaminated. Despite our small sample of NSAID- and lead-tested vultures being restricted in time, our effort allowed the reporting of the first-ever diclofenac-intoxicated Griffon Vulture in Italy, and the second intoxicated vulture in Europe, after the death of a juvenile Cinereous Vulture in Spain (Herrero-Villar *et al.* 2021). In one case, clinical lead concentration was reported in the bones, which could have caused or contributed to death. The recorded prevalence of diclofenac and lead at subclinical to clinical levels in a small sample of vultures, although hampering robust conclusions on their impact at a population level, suggests the need for a continued and integrated effort to monitor the occurrence of those substances (Margalida *et al.* 2014), not to overlook their influence on Griffon Vulture conservation.

Management and conservation implications

Addressing the gaps and uncertainties in the existing information on mortality events of Griffon Vultures could assist in developing protocols to handle them efficiently. Our findings showed that most deaths in the central Apennines are due to anthropogenic causes, similar to what happens for other vultures in Europe and worldwide (Berny *et al.* 2015, Botha *et al.* 2017, Green *et al.* 2016, Hernández and Margalida 2008, López-Bao and Mateo-Tomás 2022, Margalida 2012, Ogada *et al.* 2016, Smits and Naidoo 2018). However, the main cause of death may vary with geographical area and target species (e.g. Ogada *et al.* 2012), and thorough post-mortem protocols are necessary to reduce the number of cases where necropsy fails to identify the cause of death (38% in our study). Considering the prevalence of mortality causes in our small sample of GPS-tagged birds, the proportion of deaths from unknown reasons falls to 12.5%, although non-natural (i.e. human-related) mortality still accounts for most (62.5%) of the casualties. Consequently, a huge effort is necessary to monitor a larger number of individuals with GPS tags, allowing an unbiased estimation of mortality rates (Arrondo *et al.* 2020, Monti *et al.* 2022), faster retrieval of carcasses (Peshev *et al.* 2022), and, possibly, the prosecution of those responsible. Procedures to investigate the proximate and ultimate causes of death would imply formal coordination and common objectives among (1) public veterinary bureaux in charge of carrying out post-mortem investigations, (2) agencies responsible for wildlife management and conservation, and (3) public bodies with responsibility for carrying out investigations and crime prosecutions (Poledník *et al.* 2011). We also recommend a uniform and enhanced panel of toxic substances to be analysed, always including lead, as well as screening for the occurrence of potential new threats after veterinary use of NSAIDs (Herrero-Villar *et al.* 2020). Thus, the occurrence of NSAIDs should be routinely monitored in all potentially affected raptor species during management programmes or for those individuals entering wildlife recovery centres, as well as in livestock carcasses, both freely available for scavengers and provisioned in feeding stations. This would provide information to foster management actions, along with awareness-raising campaigns aimed at preventing the inappropriate use of such veterinary drugs (Moreno-Opo *et al.* 2021), and eventually leading to a ban on diclofenac use in livestock (Margalida *et al.* 2021). Notwithstanding that Griffon Vultures in Abruzzo mostly feed on livestock carrion (>90%, MP pers. obs.), lead poisoning from spent ammunition deserves a thorough monitoring complemented by an extensive survey of embedded shots through X-ray given its potential impact on wildlife populations.

Intended conservation strategies should also emphasise human–wildlife conflicts, mostly on livestock losses from wild and domestic predators, i.e. wolves and dogs (Mateo-Tomás *et al.* 2012, Sánchez-Barbudo *et al.* 2012) or, more recently, on the presumed predatory behaviour of Griffon Vultures attacking livestock (Lambertucci *et al.* 2021, Oliva-Vidal *et al.* 2022a), both deserving sound mitigation measures. Existing technical opportunities for wildlife crime prosecution should be readily implemented, and also by enforcing further anti-poison dog units (Poledník *et al.* 2011) prioritising poisoning hot spots. Other anthropogenic threats should also be addressed, namely the impact of wind farms and electrocution. Although only 6.5% of reported casualties were determined by accidents that occurred at wind farms, attention should be paid to possible underestimation of such a threat (Carrete *et al.* 2009), as well as to the application of mitigation and compensation measures to prevent, decrease, and compensate such mortality (Cole and Dahl 2013, de Lucas *et al.* 2012, Tomé *et al.* 2017). Similarly, we advocate urgently addressing electrocution by the identification and prioritisation of areas at higher risk and subsequently securing relevant structures (Eccleston and Harness 2018, Tintó *et al.* 2010).

The provision of food to Griffon Vultures for conservation purposes, though not fully justified in the central Apennines, could reasonably alleviate food shortage during critical periods of the year when mortality is higher (e.g. winter, breeding), likely increasing survival. Although buffering low natural-food availability could be beneficial, providing an objective support to food supplementation should be mandatory, as there are implications and drawbacks linked to food subsidies which should not be overlooked (Cortés-Avizanda *et al.* 2016, Moreno-Opo *et al.* 2015). Re-implementing

https://doi.org/10.1017/S0959270923000199 Published online by Cambridge University Press

food supplementation in the Apennines could exert some benefits if guided by a sound, biologically based strategy, taking advantage of local opportunities and following examples such as the farm-based feeding stations in Sardinia (Aresu *et al.* 2021).

Currently, more than 70% of vulture species worldwide are prone to extinction by human activities (Buechley and Şekercioğlu 2016, IUCN 2019), because their feeding behaviour exposes them to contaminants and pathogens (Plaza *et al.* 2020a), often in large numbers because of their gregarious foraging behaviour (Ogada *et al.* 2012). In addition, vultures are long-lived species that occupy the highest trophic levels and are therefore predisposed to bio-accumulation (Smits and Naidoo 2018). For these reasons, deliberate or incidental poisoning (Ragothaman and Chirukandoth 2011) represent the most widespread reasons for the decline of obligate scavengers worldwide (Plaza *et al.* 2019a,b). As reported by Monti *et al.* (2022) for 20 Griffon Vultures monitored by GPS tracking in the central and southern Apennines, poisoning affected 40% of dead vultures, and thus reasonably exerts quite an impact on vulture survival in our study area. We hope that the overwhelming occurrence of poisoning through time reported in this study and its high potential impact on survival (Monti *et al.* 2022) could represent a starting point for a widespread and common conservation strategy not only for vultures, but also for other protected and threatened wildlife in Mediterranean countries such as, for instance, the wolf, Brown Bear (*Ursus arctos*), and Golden Eagle (*Aquila chrysaetos*), whose ranges largely overlap those of vultures in the study area.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0959270923000199.

Acknowledgements. We are grateful to the many persons facilitating our collection of records, especially those from Corpo Forestale dello Stato/Carabinieri Forestali from Comando Regionale Abruzzo, Comando Provinciale/Gruppo L'Aquila, Reparto Carabinieri Biodiversità Pescara, the many Comando Stazione Forestale, and colleagues from Riserva Naturale Orientata Monte Velino. Parco Nazionale Gran Sasso Laga, Parco Regionale Sirente-Velino, Riserva Regionale Montagne della Duchessa, and Parco Regionale dei Monti Simbruini, also contributed data. Experimental Zooprophylactic Institutes of Abruzzo and Molise, Lazio and Toscana, del Mezzogiorno Portici, Veterinary Faculty of Teramo and the Veterinary Services of Avezzano (Sandro Bisciardi and Vera De Angelis), Sulmona (Marco Amicarella), L'Aquila (Massimo Ciuffetelli and Livio Giammaria), and Rieti (Fernando Salvi and Mauro Grillo), as well as the Centro Recupero Animali Selvatici-Rome, provided invaluable support. Francesca Manzia, Pietro Badagliacca, Stefania Salucci, Daria Di Sabatino, Francesco Tancredi, and Nicola D'Alessio shared information on procedures for necropsies and toxicological screening. Enrico Bassi allowed the results of lead analysis to be quoted. Antoni Margalida, Paolo Ciucci, and anonymous referees made valuable comments enhancing the manuscript. AC was partially financially supported by grant FSE-REACT EU, DM 10/08/2021 n. 1062.

References

Alarcón, P. A. E. and Lambertucci, S. A. (2018) A three-decade review of telemetry studies on vultures and condors. *Mov. Ecol.* **6:** 1–13.

Altea, T., Panella, M., De Sanctis, A., Morini, P., Opramolla, G., Bartolo, R., Pascazi, A., et al. (2013) Home range e spostamenti del grifone *Gyps fulvus* nell'Appennino centrale. Pp. 21–29 in F. Mezzavilla and F. Scarton eds. *Atti Secondo convegno Italiano rapaci diurni e notturni. Treviso, 12–13* ottobre 2012. Quaderni Faunistici 3. Venice, Italy: Associazione Faunisti Veneti.

Altea, T., Panella, M., Opramolla, G. and Posillico, M. (2016) Alimentazione suppletiva del grifone nella Riserva Naturale Monte Velino: andamento temporale, utilizzo dei carnai e relazioni con la riproduzione. *Gazz. Ambient.* **2:** 37–46.

Aresu, M., Rotta, A., Fozzi, A., Campus, A., Muzzeddu, M., Secci, D., Fozzi, I., et al. (2021) Assessing the effects of different management scenarios on the conservation of small island vulture populations. *Bird Conserv. Internatn.* **31:** 111–128.

Arrondo, E., Sanz-Aguilar, A., Pérez-García, J. M., Cortés-Avizanda, A., Sánchez-Zapata, J. A. and Donázar, J. A. (2020) Landscape anthropization shapes the survival of a top avian scavenger. *Biodivers. Conserv.* **29:** 1411–1425.

Banca Dati Nazionale dell'Anagrafe Zootecnica. (2022) Dati forniti dalla BDN dell'Anagrafe Zootecnica istituita dal Ministero della Salute presso il CSN dell'Istituto "G. Caporale" di Teramo. Accessed online November 2022 from https://www.vetinfo.it/j6_statistiche/index.html#/.

Bassi, E., Facoetti, R., Ferloni, M., Pastorino, A., Bianchi, A., Fedrizzi, G., Bertoletti, I., et al. (2021) Lead contamination in tissues of large avian scavengers in south-central Europe. *Sci. Total Environ.* **778:** 146130.

Van Beest, F., Van Den Bremer, L., De Boer, W. F., Heitkönig, I. M. A. and Monteiro, A. E. (2008) Population dynamics and spatial distribution of Griffon Vultures (*Gyps fulvus*) in Portugal. *Bird Conserv. Internatn.* **18:** 102–117.

Berny, P., Vilagines, L., Cugnasse, J.-M., Mastain, O., Chollet, J.-Y., Joncour, G. and Razin, M. (2015) VIGILANCE POISON: illegal poisoning and lead intoxication are the main factors affecting avian scavenger survival in the Pyrenees (France). *Ecotoxicol. Environ. Saf.* **118:** 71–82.

Bosè, M., Duriez, O. and Sarrazin, F. (2012) Intra-specific competition in foraging Griffon Vultures *Gyps fulvus*: 1. Dynamics of group feeding. *Bird Study* **59:** 182–192.

Bosé, M., Le Gouar, P., Arthur, C., Lambourdiere, J., Choisy, J. P., Henriquet, S., Lecuyer, P., et al. (2007) Does sex matter in reintroduction of griffon vultures *Gyps fulvus*? *Oryx* **41:** 503–508.

Bosé, M. and Sarrazin, F. (2007) Competitive behaviour and feeding rate in a reintroduced population of Griffon Vultures *Gyps fulvus*. *Ibis* **149:** 490–501.

Botha, A. J., Andevski, J., Bowden, C. G. R., Gudka, M., Safford, R. J., Tavares, J. and Williams, N. P. (2017) *Multi-species action plan to conserve African-Eurasian vultures.* CMS Raptors MOU Technical Publication No. 5. Abu Dhabi, United Arab Emirates: Coordinating Unit of the CMS Raptors MOU.

Brambilla, M., Gustin, M. and Celada, C. (2011) Defining favourable reference values for bird populations in Italy: setting long-term conservation targets for priority species. *Bird Conserv. Internatn.* **21:** 107–118.

Brochet, A.-L., Jbour, S., Sheldon, R. D., Porter, R., Jones, V. R., Al Fazari, W., Al Saghier, O., et al. (2019) A preliminary assessment of the scope and scale of illegal killing and taking of wild birds in the Arabian peninsula, Iran and Iraq. *Sandgrouse* **41:** 154–175.

Buechley, E. R. and Şekercioğlu, Ç. H. (2016) The avian scavenger crisis: looming extinctions, trophic cascades, and loss of critical ecosystem functions. *Biol. Conserv.* **198:** 220–228.

Cailly Arnulphi, V. B., Lambertucci, S. A. and Borghi, C. E. (2017) Education can improve the negative perception of a threatened long-lived scavenging bird, the Andean condor. *PLoS One* **12:** e0185278.

Campbell, M. O. (2015) *Vultures: their evolution, ecology and conservation*. Boca Raton, FL, USA: CRC Press.

Cano, C. (2016) *El veneno en España: evolución del envenenamiento de fauna silvestre (1992–2013)*. Madrid, Spain: WWF/Adena.

Carey, J. and Judge, D. (2000) *Longevity records: life spans of mammals, birds, reptiles, amphibians and fish*. Odense, Denmark: Odense University Press.

Carrete, M., Sánchez-Zapata, J. A., Benítez, J. R., Lobón, M. and Donázar, J. A. (2009) Large scale risk-assessment of wind-farms on population viability of a globally endangered long-lived raptor. *Biol. Conserv.* **142:** 2954–2961.

Chantepie, S., Teplitsky, C., Pavard, S., Sarrazin, F., Descaves, B., Lecuyer, P. and Robert, A. (2016) Age-related variation and temporal patterns in the survival of a long-lived scavenger. *Oikos* **125:** 167–178.

Cole, S. G. and Dahl, E. L. (2013) Compensating white-tailed eagle mortality at the Smøla wind-power plant using electrocution prevention measures. *Wildl. Soc. Bull.* **37:** 84–93.

Cortés-Avizanda, A., Blanco, G., DeVault, T. L., Markandya, A., Virani, M. Z., Brandt, J. and Donázar, J. A. (2016) Supplementary feeding and endangered avian scavengers: benefits, caveats, and controversies. *Front. Ecol. Environ.* **14:** 191–199.

Cozza, K., Fico, R., Battistini, M.-L. and Rogers, E. (1996) The damage-conservation interface illustrated by predation on domestic livestock in central Italy. *Biol. Conserv.* **78:** 329–336.

Cramp, S. and Simmons, K. (1980) *The birds of the western Palearctic. Vol.* **II**. *Hawks to bustards*. Oxford, UK: Oxford University Press.

Cramp, S. and Simmons, K. E. L. eds. (1982) *Gyps fulvus Eurasian griffon vulture. Handbook of the birds of Europe, the Middle East and North Africa*. Oxford, UK: Oxford University Press.

Daboné, C., Ouéda, A., Thompson, L. J., Adjakpa, J. B. and Weesie, P. D. M. (2023) Trade in vulture parts in West Africa: Burkina Faso may be one of the main sources of vulture carcasses. *Bird Conserv. Internatn.* **33:** e8.

Diekmann, M. and Strachan, A. (2006) Saving Namibia's most endangered bird. WAZA Mag. 16–19.

Donázar, J. A., Margalida, A., Carrete, M. and Sánchez-Zapata, J. A. (2009) Too sanitary for vultures. *Science* **326:** 664.

Duriez, O., Eliotout, B. and Sarrazin, F. (2011) Age identification of Eurasian Griffon Vultures *Gyps fulvus* in the field. *Ringing Migr.* **26:** 24–30.

Eccleston, D. T. and Harness, R. E. (2018) Raptor electrocutions and power line collisions. Pp. 273–302. in J. N. Sarasola, J. M. Grande and J. J. Negro eds. *Birds of prey. Biology and conservation in the XXI century*. Dordrecht, The Netherlands: Springer.

Estrada Pacheco, R., Jacome, N. L., Astore, V., Borghi, C. E. and Piña, C. I. (2020) Pesticides: the most threat to the conservation of the Andean condor (*Vultur gryphus*). *Biol. Conserv.* **242:** 1–7.

Fajardo, I. and Zorrilla, I. (2016) *Manual de técnica policial ambiental: identificación in situ de causas de muerte en fauna silvestre. Consejería de Medio Ambiente y Ordenación del Territorio*. Seville, Spain: Junta de Andalucía.

Garofalo, L., Fanelli, R., Opramolla, G., Polidori, M., Tancredi, F., Altea, T., Posillico, M., et al. (2016). Comparison between two molecular protocols for sex determination in birds, with implications for the management and conservation of the Eurasian Griffon vulture *Gyps fulvus*. *Avocetta* **40:** 17–22.

Genero, F. (2009) Estado actual y conservación de las aves carroñeras en Italia. *Munibe. Supl.* 178–191.

Gilbert, M., Virani, M. Z., Watson, R. T., Oaks, J. L., Benson, P. C., Khan, A. A., Ahmed, S., et al. (2002) Breeding and mortality of Oriental White-backed Vulture *Gyps bengalensis* in Punjab Province, Pakistan. *Bird Conserv. Internatn.* **12:** 311–326.

Green, R. E., Donazar, J. A., Sánchez-Zapata, J. A. and Margalida, A. (2016) Potential threat to Eurasian griffon vultures in Spain from veterinary use of the drug diclofenac. *J. Appl. Ecol.* **53:** 993–1003.

Green, R. E., Newton, I. A. N., Shultz, S., Cunningham, A. A., Gilbert, M., Pain, D. J. and Prakash, V. (2004) Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent. *J. Appl. Ecol.* **41:** 793–800.

Griffiths, R., Double, M. C., Orr, K. and Dawson, R. J. G. G. (1998) A DNA test to sex most birds. *Mol. Ecol.* **7:** 1071–1075.

Guitart, R., Sachana, M., Caloni, F., Croubels, S., Vandenbroucke, V. and Berny, P. (2010) Animal poisoning in Europe. Part 3: wildlife. *Vet. J.* **183:** 260–265.

Gustin, M., Brambilla, M. and Celada, C. (2009) Valutazione dello stato di conservazione dell'avifauna italiana. *Ministero dell'Ambiente, della Tutela del Territorio e del Mare*. Rome, Italy: Lega Italiana Protezione Uccelli/BirdLife International.

Gustin, M., Nardelli, R., Brichetti, P., Battistoni, A., Rondinini, C. and Teofili, C. (2021) Lista Rossa IUCN degli uccelli nidificanti in Italia 2021. *Ministero dell'Ambiente, della Tutela del Territorio e del Mare*. Rome, Italy: International Union for Conservation of Nature Italy.

Harel, R., Horvitz, N. and Nathan, R. (2016) Adult vultures outperform juveniles in challenging thermal soaring conditions. *Sci. Rep.* **6:** 1–8.

Hernández, M. and Margalida, A. (2008) Pesticide abuse in Europe: effects on the Cinereous vulture (*Aegypius monachus*) population in Spain. *Ecotoxicology* **17:** 264–272.

Hernández, M. and Margalida, A. (2009) Poison-related mortality effects in the endangered Egyptian vulture (*Neophron percnopterus*) population in Spain. *Eur. J. Wildl. Res.* **55:** 415–423.

Herrero-Villar, M., Delepoulle, É., Suárez-Regalado, L., Solano-Manrique, C., Juan-Sallés, C., Iglesias-Lebrija, J. J., Camarero, P. R., et al. (2021) First diclofenac intoxication in a wild avian scavenger in Europe. *Sci. Total Environ.* **782:** 146890.

Herrero-Villar, M., Velarde, R., Camarero, P. R., Taggart, M. A., Bandeira, V., Fonseca, C., Marco, I., et al. (2020) NSAIDs detected in Iberian avian scavengers and carrion after diclofenac registration for veterinary use in Spain. *Environ. Pollut.* **266:** 115157.

IUCN. (2019) *International Union for Conservation of Nature annual report 2019*. Gland, Switzerland: International Union for Conservation of Nature.

Jackson, A. L., Ruxton, G. D. and Houston, D. C. (2008) The effect of social facilitation on foraging success in vultures: a modelling study. *Biol. Lett.* **4:** 311–313.

Lambertucci, S. A. (2010) Size and spatio-temporal variations of the Andean condor *Vultur gryphus* population in north-west Patagonia, Argentina: communal roosts and conservation. *Oryx* **44:** 441–447.

Lambertucci, S. A., Margalida, A., Speziale, K. L., Amar, A., Ballejo, F., Bildstein, K. L., Blanco, G., et al. (2021) Presumed killers? Vultures, stakeholders, misperceptions, and fake news. *Conserv. Sci. Pract.* **3:** e415.

López-Bao, J. V. and Mateo-Tomás, P. (2022) Wipe out highly hazardous pesticides to deter wildlife poisoning: the case of carbofuran and aldicarb. *Biol. Conserv.* **275:** 109747.

Lovari, S., Sforzi, A., Scala, C. and Fico, R. (2007) Mortality parameters of the wolf in Italy: does the wolf keep himself from the door? *J. Zool.* **272:** 117–124.

de Lucas, M., Ferrer, M., Bechard, M. J. and Muñoz, A. R. (2012) Griffon vulture mortality at wind farms in southern Spain: distribution of fatalities and active mitigation measures. *Biol. Conserv.* **147:** 184–189.

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., van Bentham, K., et al. (2017) Package 'glmmtmb.' R Package. Version 0.2. 0.

Margalida, A. (2012) Baits, budget cuts: a deadly mix. *Science* **338:** 192.

Margalida, A., Bogliani, G., Bowden, C. G. R., Donázar, J. A., Genero, F., Gilbert, M., Karesh, W. B., et al. (2014) One health approach to use of veterinary pharmaceuticals. *Science* **346:** 1296–1298.

Margalida, A., Colomer, M. À. and Sanuy, D. (2011) Can wild ungulate carcasses provide enough biomass to maintain avian scavenger populations? An empirical assessment using a bio-inspired computational model. *PLoS One* **6:** e20248.

Margalida, A., Green, R. E., Hiraldo, F., Blanco, G., Sánchez-Zapata, J. A., Santangeli, A., Duriez, O., et al. (2021) Ban veterinary use of diclofenac in Europe. *Science* **372:** 694–695.

Margalida, A., Heredia, R., Razin, M. and Hernández, M. (2008) Sources of variation in mortality of the Bearded Vulture *Gypaetus barbatus* in Europe. *Bird Conserv. Internatn.* **18:** 1–10.

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

Martin, G. R., Portugal, S. J. and Murn, C. P. (2012) Visual fields, foraging and collision vulnerability in *Gyps* vultures. *Ibis* **154:** 626–631.

Mateo-Tomás, P., Olea, P. P., Sánchez-Barbudo, I. S. and Mateo, R. (2012) Alleviating human–wildlife conflicts: identifying the causes and mapping the risk of illegal poisoning of wild fauna. *J. Appl. Ecol.* **49:** 376–385.

Mineau, P., Fletcher, M. R., Glaser, L. C., Thomas, N. J., Brassard, C., Wilson, L. K., Elliott, J. E., et al. (1999) Poisoning of raptors with organophosphorus and carbamate pesticides with emphasis on Canada, US and UK. *J. Raptor Res.* **33:** 1–37.

Møller, A. P. (2006) Sociality, age at first reproduction and senescence: comparative analyses of birds. *J. Evol. Biol.* **19:** 682–689.

Monclús, L., Shore, R. F. and Krone, O. (2020) Lead contamination in raptors in Europe: a systematic review and meta-analysis. *Sci. Total Environ.* **748:** 141437.

Monsarrat, S., Benhamou, S., Sarrazin, F., Bessa-Gomes, C., Bouten, W. and Duriez, O. (2013) How predictability of feeding patches affects home range and foraging habitat selection in avian social scavengers? *PLoS One* **8:** e53077.

Monti, F., Serroni, P., Rotondaro, F., Sangiuliano, A., Sforzi, A., Opramolla, G., Pascazi, A., et al. (2022) Survival of a small reintroduced griffon vulture population in the Apennines: insights from Global Positioning System tracking. *Avian Biol. Res.* **16**. doi: 17581559221137309.

Morant, J., Arrondo, E., Sánchez-Zapata, J. A., Donázar, J. A., Cortés-Avizanda, A., De La Riva, M., Blanco, G., et al. (2023) Large-scale movement patterns in a social vulture are influenced by seasonality, sex, and breeding region. *Ecol. Evol.* **13:** e9817.

https://doi.org/10.1017/S0959270923000199 Published online by Cambridge University Press

Moreno-Opo, R., Carapeto, R., Casimiro, R., Rubio, C., Muñoz, B., Moreno, I. and Aymerich, M. (2021) The veterinary use of diclofenac and vulture conservation in Spain: updated evidence and socio-ecological implications. *Sci. Total Environ.* **796:** 148851.

Moreno-Opo, R., Trujillano, A., Arredondo, Á., González, L. M. and Margalida, A. (2015) Manipulating size, amount and appearance of food inputs to optimize supplementary feeding programs for European vultures. *Biol. Conserv.* **181:** 27–35.

La Morgia, V., Marucco, F., Aragno, P., Salvatori, V., Gervasi, V., De Angelis, D., Fabbri, E., et al. (2022) *Stima della distribuzione e consistenza del lupo a scala nazionale 2020/2021*. Relazione tecnica realizzata nell'ambito della convenzione. .Rome, Italy: Italian Institute for Environmental Protection and Research.

Musto, C., Cerri, J., Galaverni, M., Caniglia, R., Fabbri, E., Apollonio, M., Mucci, N., et al. (2021) Men and wolves: anthropogenic causes are an important driver of wolf mortality in human-dominated landscapes in Italy. *Glob. Ecol. Conserv.* **32:** e01892.

Ntemiri, K., Saravia, V., Angelidis, C., Baxevani, K., Probonas, M., Kret, E., Mertzanis, Y., et al. (2018) Animal mortality and illegal poison bait use in Greece. *Environ. Monit. Assess.* **190:** 1–16.

Ogada, D., Shaw, P., Beyers, R. L., Buij, R., Murn, C., Thiollay, J. M., Beale, C. M., et al. (2016) Another continental vulture crisis: Africa's vultures collapsing toward extinction. *Conserv. Lett.* **9:** 89–97.

Ogada, D. L., Keesing, F. and Virani, M. Z. (2012) Dropping dead: causes and consequences of vulture population declines worldwide. *Ann. N. Y. Acad. Sci* **1249:** 57–71.

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

Oliva-Vidal, P., Martínez, J. M., Sánchez-Barbudo, I. S., Camarero, P. R., Colomer, M. À., Margalida, A. and Mateo, R. (2022b) Second-generation anticoagulant rodenticides in the blood of obligate and facultative European avian scavengers. *Environ. Pollut.* **315:** 120385.

Oppel, S., Arkumarev, V., Bakari, S., Dobrev, V., Saravia-Mullin, V., Adefolu, S., Sözüer, L. A., et al. (2021) Major threats to a migratory raptor vary geographically along the eastern Mediterranean flyway. *Biol. Conserv.* **262:** 109277.

Pain, D. J., Bowden, C. G. R., Cunningham, A. A., Cuthbert, R., Das, D., Gilbert, M., Jakati, R. D., et al. (2008) The race to prevent the extinction of South Asian vultures. *Bird Conserv. Internatn.* **18:** S30–S48.

Pandolfi, M. and Zanazzo, G. (1994) Una avifauna dell'Appennino centrale nel'500: il trattatello "Cognizione degl'uccelli et animali pertinenti al'aere" e le lettere di Costanzo Felici da Piobbico ad Ulisse Aldrovandi. *J. Integre. Biogeogr.* **17:** 509–530.

Peshev, H., Mitrevichin, E., Stoyanov, G., Grozdanov, A. and Stoynov, E. (2022) GPS tracking data relates vulture mortality due to acute intoxication in a considerable distance from the site of poisoned bait consumption. *Forensic Sci. Int. Anim. Environ.* 100052.

Piovesan, G., Bernabei, M., Di Filippo, A., Romagnoli, M. and Schirone, B. (2003) A long-term tree ring beech chronology from a high-elevation old-growth forest of Central Italy. *Dendrochronologia* **21:** 13–22.

Plaza, P. I., Blanco, G. and Lambertucci, S. A. (2020a) Implications of bacterial, viral and mycotic microorganisms in vultures for wildlife conservation, ecosystem services and public health. *Ibis* **162:** 1109–1124.

Plaza, P. I., Blanco, G., Madariaga, M. J., Boeri, E., Teijeiro, M. L., Bianco, G. and Lambertucci, S. A. (2019a) Scavenger birds exploiting rubbish dumps: pathogens at the gates. *Transbound. Emerg. Dis.* **66:** 873–881.

Plaza, P. I., Martínez-López, E. and Lambertucci, S. A. (2019b) The perfect threat: pesticides and vultures. *Sci. Total Environ.* **687:** 1207–1218.

Plaza, P. I., Wiemeyer, G. and Lambertucci, S. A. (2020b) Comparison of lead contamination among related scavenging birds sharing food sources: a call for caution when using surrogate species. *Ibis* **162:** 1303–1311.

Poledník, L., Poledníková, K., Větrovcová, J., Hlaváč, V., Jansman, H., van Tulden, P., Pichler, C., et al. (2011) Regulation of carbofuran and its use to poison wildlife in the European Union and the rest of Europe. Pp. in 132–170 in N. Richards ed. *Carbofuran and wildlife poisoning: global perspectives and forensic approaches*. Hoboken, NJ, USA: John Wiley & Sons.

Potena, G., Panella, M., Sammarone, L., Altea, T., Spinetti, M., Opramolla, G. and Posillico, M. (2009) Il Grifone *Gyps fulvus* nell'Appennino centrale: status report 1994–2009. *Alula* **16:** 47–52.

R Core Team. (2016) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ragothaman, V. and Chirukandoth, S. (2011) Mitigating human-wildlife conflict and retaliatory poisonings in India to preserve biodiversity and maintain sustainable livelihoods. Pp. 99–131 in N. Richards ed. *Carbofuran and wildlife poisoning: global perspectives and forensic approaches*. Hoboken, NJ, USA: John Wiley & Sons.

Ruxton, G. D. and Houston, D. C. (2004) Obligate vertebrate scavengers must be large soaring fliers. *J. Theor. Biol.* **228:** 431–436.

Safford, R., Andevski, J., Botha, A., Bowden, C. G. R., Crockford, N., Garbett, R., Margalida, A., et al. (2019) Vulture conservation: the case for urgent action. *Bird Conserv. Internatn.* **29:** 1–9.

Sánchez-Barbudo, I. S., Camarero, P. R. and Mateo, R. (2012) Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. *Sci. Total Environ.* **420:** 280–288.

Sarrazin, F., Bagnolini, C., Pinna, J. L., Danchin, E. and Clobert, J. (1994). High survival estimates of Griffon Vultures (*Gyps fulvus fulvus*) in a reintroduced population. *Auk* **111:** 853–862.

Smits, J. and Naidoo, V. (2018) Toxicology of birds of prey. Pp.229–250 in J. N. Sarasola, J. M. Grande and J. J. Negro eds. *Birds of prey. Biology and conservation in the XXI century*. Dordrecht, The Netherlands: Springer.

Tintó, A., Real, J. and Mañosa, S. (2010) Predicting and correcting electrocution of birds in Mediterranean areas. *J. Wildl. Manage.* **74:** 1852–1862.

Tomé, R., Canário, F., Leitão, A. H., Pires, N. and Repas, M. (2017) Radar assisted shutdown on demand ensures zero soaring bird mortality at a wind farm located in a migratory flyway. Pp.119–133 in J. Köppel ed. *Wind energy and wildlife interactions. Presentations from the CWW2015 Conference.* Dordrecht, The Netherlands: Springer.

Xirouchakis, S. (2004) Causes of raptor mortality in Crete. Pp. 849–860 in R. D. Chancellor and B.-U. Meyburg eds. *Raptors in the modern world.* Berlin, Germany: World Working Group on Birds of Prey and Owls .

Yee, T. W., Stoklosa, J. and Huggins, R. M. (2015) The VGAM package for capture-recapture data using the conditional likelihood. *J. Stat. Softw.* **65:** 1–33.

Zuberogoitia, I., De La Puente, J., Elorriaga, J., Alonso, R., Palomares, L. E. and Martínez, J. E. (2013) The flight feather molt of Griffon Vultures (*Gyps fulvus*) and associated biological consequences. *J. Raptor Res.* **47:** 292–303.

Zuberogoitia, I., Martínez, J. E., Margalida, A., Gómez, I., Azkona, A. and Martínez, J. A. (2010) Reduced food availability induces behavioural changes in Griffon Vulture *Gyps fulvus*. *Ornis Fenn.* **87:** 52–60.

https://doi.org/10.1017/S0959270923000199 Published online by Cambridge University Press

Table S1. Number of times toxic substances (grouped in seven classes) were tested for their occurrence in 48 Griffon vultures affected by poisoning from 2000 to 2020 in the central Apennines. The percentage of vultures tested for each class of substance is also given.

Active substance	Times tested	% Birds tested
Aldicarb	40	83
Carbaryl	43	90
Carbofuran	42	88
Methiocarb	41	85
Methomyl	8	17
Carbamates	**174**	**92**
Arsenic	1	2
Zinc phosphide	11	23
Inorganic compounds	**12**	**23**
Metaldehyde	1	2
Molluscicides	**1**	**2**
Aldrin	14	29
Dieldrin	13	27
Endosulfan (alpha)	25	52
Endosulfan (alpha +beta)	12	25
Endosulfan (beta)	21	44
Hexachlorobenzene	14	29
HCH (alpha)	14	29
HCH (beta)	14	29
Heptachlor	14	29
Heptachlor Epoxide	24	50
Lindane	14	29
Methoxychlor	24	50
Mirex	24	50
O,P-DDD	24	50
O,P-DDE	14	29
O,P-DDT	24	50
P,P-DDD	24	50
P,P-DDE	19	40
P,P-DDT	24	50
Organochlorines	**356**	**54**
Azinphos ethyl	24	50
Azinphos methyl	24	50
Chlorpyriphos ethyl	6	13
Chlorpyriphos methyl	6	13
Diazinon	25	52
Dimethoate	14	29
Fenthion	24	50
Malathion	16	33
Mevinphos (Phosdrin)	24	50

Parathion ethyl	25	52
Parathion methyl	25	52
Phorate	25	52
Ronnel	13	27
Organophosphates	**251**	**52**
Coumarin	3	6
Strychnine	20	42
Plant Toxins	**23**	**44**
Crimidine	1	2
Brodifacoum	3	6
Bromadiolone	3	6
Coumachlor	3	6
Coumatetralyl	3	6
Difenacoum	3	6
Warfarin	3	6
Rodenticides (anticoagulants or not)	**19**	**8**

Supporting information 2. Description of the model used to estimate the annual variation in mortality according to population size.

Annual mortality is the ratio of individuals found dead over the number of individuals in the population, but the actual population size was unknown to us. We therefore assumed that population size in year t (N_t) was proportional to the number of breeding pairs in the same year (B_t):

$$N_t = kB_t.$$

We then modelled the temporal variation in mortality of the population with a negative binomial regression model with a log link function:

$$\ln(\mathrm{E}(D_t / kB_t)) = \mathrm{b}_0 + \mathrm{b}_1 \times \mathrm{year}$$

where D_t denotes the number of individuals found dead in each year, E is the expected value, and b_0 and b_1 are the intercept and the slope of the negative binomial regression.

This model can be re-written as:

$$\ln(\mathrm{E}(D_t)) = \mathrm{b}_0 + \mathrm{b}_1 \times \mathrm{year} + \ln(k) + \ln(B_t)$$

and posing $\mathrm{d}_0 = \mathrm{b}_0 + \ln(k)$, we have

$$\ln(\mathrm{E}(D_t)) = \mathrm{d}_0 + \mathrm{b}_1 \times \mathrm{year} + \ln(B_t)$$

This latter equation shows that a negative binomial regression model where the log-number of breeding pairs is entered as an offset is able to model annual variation in mortality.

Supporting information 3. Mathematical description of the model used to estimate within-year variation in mortality and the related R code.

Harmonic analysis was used to test for within-year variation in mortality. To do so, we first converted months into angles (March = 90°, June =180°, September = 270°, December = 360°) and then built a GLMM model whereby the number of individuals found dead in each month is the response variable, while the sines and cosines of the angles corresponding to the months, and their interaction are the predictors. The GLMM model assumed a negative binomial distribution of the data and included the year as a random grouping factor. Preliminary analyses also showed that the optimal random structure for this model included sine as a random slope within year. This procedure can be mathematically described as follows:

$$M_i = \textit{i-th month of each year}$$

$$x_i = \frac{2\pi}{12} M_i$$

$$y_{ij} = \textit{number of death vultures in the i-th month of the j-th year}$$

$$y_{ij} = NegBin(\mu_{ij}, k)$$

$$E(y_{ij}) = \mu_{ij} \textit{ and } Var(y_{ij}) = \mu_{ij} + \frac{\mu_{ij}^2}{k}$$

$$\log(\mu_{ij}) = b_0 + b_1\, sin(x_i) + b_2\, cos(x_i) + b_3\, sin(x_i)\, cos(x_i) + r_0 year_j + r_1\, sin(x_i)\, year_j$$

$$r_0 = N(0, \sigma_0^2)$$

$$r_1 = N(0, \sigma_1^2)$$

The R code used for the GLMM analysis was:

```
Model1 <- glmmTMB (y ~ sin*cos + (1+sin|year), data=data, family="nbinom2")
```