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Diet of Golden Eagle *Aquila chrysaethos* nestlings in Central Apennines, Italy

Gianluca Damiani[1,2], Andrea Mazzarano[3], Silvia Dancali[4] and Paolo Forconi[5]

[1]Ornis italica, Piazza Crati 15, 00199 Roma (RM), Italy
[2]Department of Ecological and Biological Sciences, University of Tuscia, Largo dell'Università snc, 01100 Viterbo (VT), Italy
[3]Via Ugo La Malfa 5, 00045 Genzano di Roma (RM), Italy
[4]Via Conti S. Maroto 25, 62032 Camerino (MC), Italy
[5]Studio Faunistico Chiros, Via Cardarelli 23, 62100 Macerata (MC), Italy

*corresponding author: Gianluca Damiani, gianlucadamianiph@gmail.com

GD 0000-0001-6225-6309, AM 0000-0002-3580-7811

Abstract - The diet of animal species reflects important evolutionary and behavioural adaptations that may affect the viability of populations. The reproductive success, the habitat selection, and the spatial distribution of individuals are often related to trophic resources. By studying the diet of a predator, it is possible to better understand the ecological interactions between different species at a local scale. We studied the nestlings' diet of six Golden Eagle *Aquila chrysaetos* breeding pairs in Central Apennines (Italy), through the analysis of pellets and prey remains (2000-2004) and integrated them with visual observations (2000-2022). While data from pellets and prey remains allowed for estimates in biomass and diet breadth, nest visual observations provided new qualitative insights into the species' hunting behaviour. We were able to identify 16 species of mammals, 14 species of birds and 2 species of reptiles among prey items. The application of the Levins index on the 21 families detected produced a value of 8.45, indicating a rather wide trophic niche. The dominant preys were hares (*Lepus europaeus* and *Lepus corsicanus*), with a 25% frequency and 43% of the total estimated biomass. Notably, it appears that wild boars and corvids are becoming more important for the diet of the golden eagle's nestlings, which is in agreement with the recent expansion of such species in the study regions, coupled with the decline of hares and Phasianids.

Keywords: birds of prey, prey remains, trophic niche, hare, dietary, environmental changes.

INTRODUCTION

The understanding of population dynamics is crucial for the conservation of animal populations (Begon et al. 2005). For predators, the availability of prey is a major limiting factor, both for survival and reproduction (Millon & Bretagnolle 2008). Among birds of prey, the Golden Eagle *Aquila chrysaetos* is an apex predator that feeds on a large variety of preys (Watson 2010). Although the Golden Eagle is considered a generalist predator (Nystrom et al. 2006, Whithfield et al. 2009, Bedrosian et al. 2017), a meta-analysis on its diet reported that generalization can lead to lower productivity (Watson 1998). The abundance and availability of preferred preys, such as hares (*Le-*

pus europaeus and *Lepus corsicanus*), are critical factors ensuring high productivity of Golden Eagle pairs (Whitfield et al. 2009). In general, when the preferred preys are readily available, the Golden Eagle's diet is rather specialized, while in their absence, Golden Eagles become more generalist (Watson 2010).

To date, there are several techniques to analyze the diet of a species, such as stomach contents or pellets analysis, prey remains, direct observations (Collopy 1983, Marti 1987), and isotopes ratio (e.g., Nadjafzadeh et al. 2016). Among these, isotopes analysis is expensive and does not provide an exact proportion of each prey type in the diet, but only its occurrence. Pellet analysis, prey remains, visual observations and video recording using camera traps or webcams (Skouen 2012) may be limited by certain biases (i.e. difficult in evaluating the precise number of prey items, underestimation of small prey, difficulties in the evaluation of the biomass consumed). Visual observation of prey delivered to the nests is considered the method that is less prone to systematic errors (Marti 1987). However, due to the observing distance (Watson 2010) and the rearrangement of preys operated by the eagles (e.g. plucked and dismembered), it is not always easy to recognize the prey species. Given that each of these methods has advantages and disadvantages, an integrated approach is suggested (e.g. Collopy 1986, Simmons et al. 1991, Seguin et al. 1998).

For the Central Apennines area (Italy), the available studies indicate a generalist feeding behaviour of the species throughout the year, with a marked preference for hares (Novelletto & Petretti 1980, Magrini et al. 1987). Here, we combined data from pellets and prey remains collected in proximity to the nest between 2000 and 2004 with visual observations between 2000 and 2022 with the aim to update the knowledge about Golden Eagle nestlings' diet in the Central Appennines.

MATERIALS AND METHODS

Study area

Central Apennines span from Sibillini mountains at the northernmost fringe to the Matese mountains at the southernmost one, with elevations ranging between 500 and 2,900 meters a.s.l., consisting of a surface of ca. 6,500 km^2 and 280 km of linear extension (Zocchi & Panella 1996). About 4,000 km^2 are included in three National Parks (Monti Sibillini National Park, Gran Sasso and Monti della Laga National Park and Abruzzo, Lazio and Molise National Park) and several Natural Parks and protected areas. The study area is located within the provinces of Macerata, Fermo, Ascoli Piceno, l'Aquila, Rieti, Roma and Frosinone.

The area hosts temperate, alpine, and Mediterranean climates (Cutini et al. 2021). The vegetation is composed by a mosaic of broad-leaved woods (*Quercus* spp., *Ostrya carpinifolia*, *Fagus sylvatica*), secondary grasslands (*Bromus erectus*, *Brachypodium* sp.) and primary grasslands (*Sesleria apennina*, *Carex kitaibeliana*, *Festuca violacea*), limited reforestation of conifers consisting of Black Pine *Pinus nigra* and Spruce *Picea abies* (Ballelli et al. 1981). The area is also characterized by scattered agricultural areas and human settlements, connected by an extensive road network.

In the Central Apennines the reported Golden Eagle density is about 1 pair/300 km^2 (Magrini et al. 2013, Mazzarano et al. 2024), and the distance between nesting sites is 14.09 km, SD = 6.44 km (Mazzarano et al. 2024).

Data collection and analysis

The diet of six Golden Eagles pairs was studied using two different approaches, collecting and analyzing 187 pellets and 33 collections of prey remains found around nests and perches within a 100 m radius from the nest, from May to September, between 2000 and 2004. To not disturb the breeding activity, the nests were visited at least 20 days after the juveniles fledged (Fig. 1).

The pellets were analyzed, separating the contents, under an optical microscope at 100, 400 and 1,000x.

To determine the hair of the prey, the medulla, cortex and cuticle were analyzed following Teerink

(1991), Debrot et al. (1982) and personal comparison collections. Feathers were identified via microscopy observation, based on knots morphology, villi presence and pigmentation, allowing to distinguish preys by order (Day 1966, Brom 1986). Sometimes, it was also possible to identify the species relying on the coloration of some characteristic feathers (Robertson et al. 1984), beaks, skulls, legs and nails, comparing them to personal collections and to those of the Museum of Natural Sciences of Camerino. A similar method was used to observe and determine reptile scales. We followed Hue (1907), Chaline et al. (1974) and Barone (1975) for bone recognition, and Harrison (1988) for skulls and feathers identification.

The number of preys was calculated by identifying the minimum number of individuals represented in each prey remain unit (Mollhagen et al. 1972, Seguin et al. 2001). Multiple remains of the same species that were found in different pellets in the same location and in the same year were considered as belonging to a single individual, unless a larger number could be identified by counting bone parts or nails. This approach possibly underestimated the number of preys counted. Consequently, in this study, each species detected in a single pellet is considered as a species occurrence, and not a single individual (e.g., Seguin et al. 1998).

The data from pellets and remains were analyzed separately and subsequently integrated by calculating the percent frequency of individuals and the percentage of biomass. For the prey biomass, the average weight of males and females was taken into consideration, referring, when possible, to data relating to the study area and season. For the carcasses of larger animals (i.e. mammalian species weighing at least 10 kg), an average weight of 5 kg was considered, in line with our observations on the size of the portion of prey delivered to the nest.

The weight classes of prey were considered following Watson (2010). The trophic niche was calculated by applying the Levins index (Levins 1968): $B = 1/ \sum p_i^2$ where p_i represents the proportion of taxon i in the diet. The values of this index vary from 1 to n; small values indicate a narrow trophic niche for specialized diets, while large values represent a generalized diet.

The results obtained, coming from different methods, were tested with the Chi-square test (X^2) using contingency tables, as well as the number of individuals in pellets and remains, with taxa grouped by families (Watson 2010).

Additionally, to extend the data obtained from pellets and remains, six breeding pairs within the same study area (two of which were also used for the analysis of prey remains), were monitored between 2000 to 2022 (from 2000 to 2007 and from 2014 to 2022), recording the prey delivered to the nest, through direct observations from May to September (Fig. 1). Prey deliveries were observed during 38 days of occasional observations throughout the nestling phase using binocular 10x42, spotting scope 20-60x, photographs or video recording with telescopic lens from mimetic hides.

RESULTS

From the analysis of pellets and remains, 123 and 125 prey items were identified, respectively (Table 1).

The application of the X^2 test, grouping some families, indicated a significative difference among the pellets and remains analysis ($X^2 = 43.49$, df = 8, *p-value* < 0.05).

By pooling the data together and considering the certain minimum number of individuals, a total of 197 identified and 9 unidentified prey items were estimated (Table 1). Overall, the data from pellets includes that of remains, mainly with reptiles, small passerines and rodents, while the remains provide more detailed data on age classes of some species and facilitate the biomass calculation: for hares (n = 42), 64.3% were adults and 35.7% were juveniles (based on femurs or other bone lengths), while for the red fox *Vulpes vulpes* (n = 11) eight young individuals were identified and for the remaining three the age could not be determined. For the wild boar *Sus scrofa* (n = 10), nine juveniles and one adult were identified.

The diet of Golden Eagle nestlings was obtained by

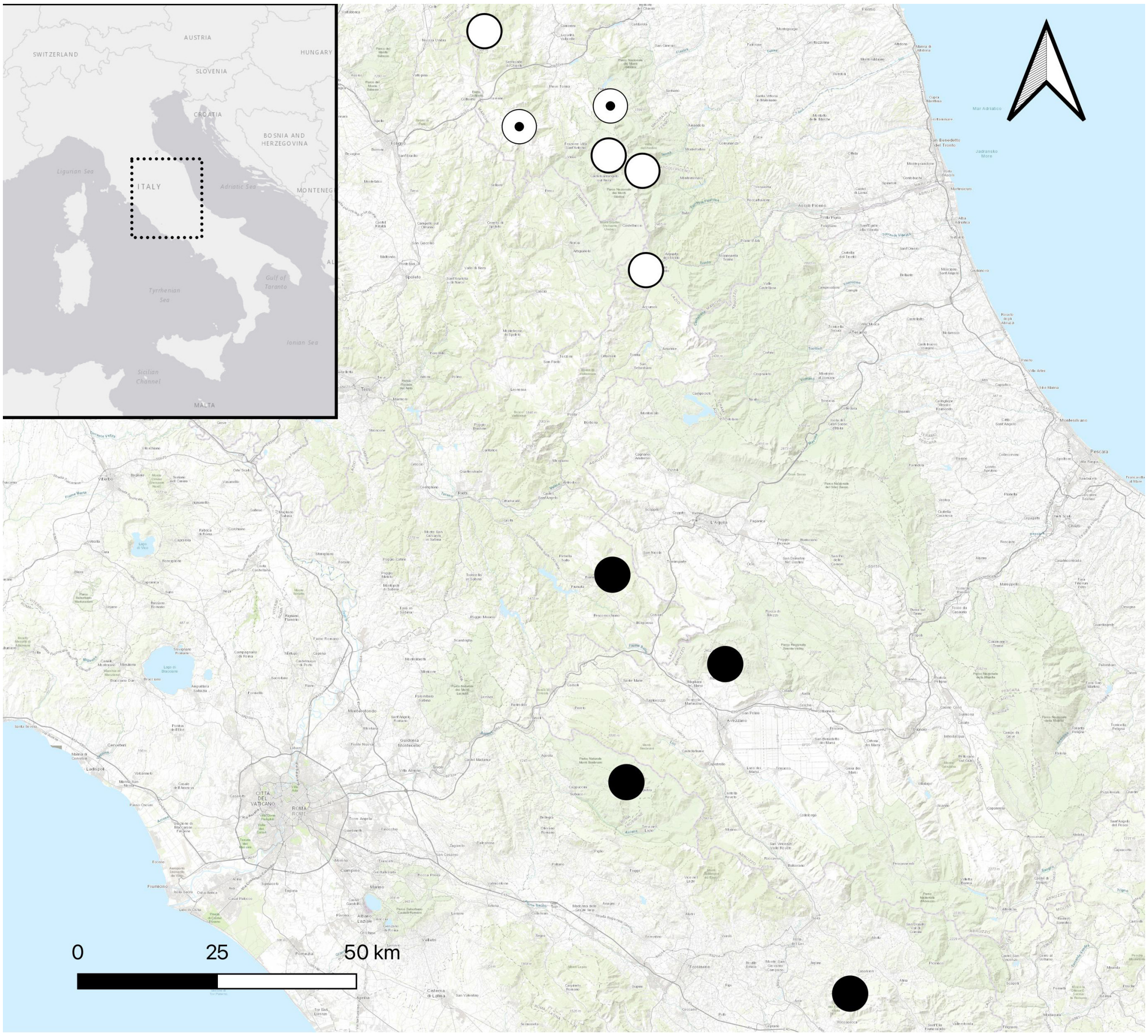

Figure 1. Study area, Central Apennines (Italy). White circles represent nests where we collected pellets and prey remains (n = 4) from 2000 to 2004; black circles represent nests where we carried out visual observations (n = 4) from 2000 to 2022; white circles with black dots (n = 2) are nests where both methods were used.

integrating remains and pellets analysis (Table 1). The calculated biomass was corrected using the percentage of waste estimates developed by Brown & Watson (1964). Considering the abundance of individuals, mammals (61.4%) were more abundant than birds (34.5%), while reptiles (4.1%) constituted a marginal part of the diet. Estimates of mammal biomass reached 89%, birds made up for 10% and reptiles 0.5% of the biomass in the diet. At the species level, hares were the most recurrent preys, followed by corvids, mainly Hooded Crow *Corvus cornix* and Eurasian Jay *Garrulus glandarius*. In terms of biomass, hares accounted for an even higher percentage of the total diet (43.3%), as well as red foxes, wild boars and sheeps *Ovis aries* (10% each). Corvids' biomass made up for only 3.5% of the overall diet. Despite the most frequent weight class being 2-4 kg (28.4%), the average prey weight was 1.37 kg, SD = 1.67. Moreover, the classes from 0.126 kg to 2 kg together represented over 50% (Fig. 2), in line with what was previously reported by Watson (2010).

Table 1. Diet of Golden Eagle nestlings from prey remains and pellet analysis. The percentage of waste, as established by Brown & Watson (1964): up to 1 kg (20%), from 1 to 4 kg (30%) and more than 4 kg (40%).

Class, Family, Species	(Prey remains) N. individuals	(Pellets) N. Individuals	(Pellets) Frequency of occurrence	N. total individuals	% Frequency Individuals	Average weight (g)	% Biomass	% corrected biomass with % of waste
MAMMALIA					61.42		89,55	88,34
Erinaceidae					1.01		0.49	0.56
Erinaceus europaeus (European Hedgehog)	1	2	5	2		800		
Talpidae					0.51		0.03	0.03
Talpa spp. (Mole)	0	1	1	1		95		
Leporidae					25.38		43.33	43.90
Lepus spp. (Hares)	47	17	114	50		3,330 ad. 2,000 juv.		
Sciuridae					4.57		0.81	0.94
Sciurus vulgaris (Red Squirrel)	2	8	18	9		297		
Gliridae					7.11		0.36	0.41
Eliomys quercinus (Garden Dormouse)	0	1	1	1		78		
Myoxus glis (European dormouse)	1	13	44	13		85		
Muridae					1.52		0.02	0.02
Apodemus sp. (Field Mouse)	0	1	1	1		18		
Muridae not determined	0	2	2	2		18		
Canidae					5.58		11.68	11.84
Vulpes vulpes (Red Fox)	7	9	33	11		3,500 juv.		
Mustelidae					3.55		3.48	3.53
Meles meles (European Badger)	1	1	9	1		3,630 juv.		
Martes foina (Beech Marten)	2	6	14	6		1,307		
Felidae					1.01		1.82	1.85
Felis silvestris catus (European Wildcat)	1	2	2	2		3,000		
Suidae					5.08		9.71	9.62
Sus scrofa (Wild Boar)	8	4	11	10		3,000 juv. 5,000 (carcass)		
Cervidae					0.51		1.12	1.14

Capreolus capreolus (Roe Deer)	0	1	3	1		3,700 juv.		
Bovidae					5.08		15.18	13.18
Ovis aries (Sheep)	7	4	9	8		5,000 (carcass)		
Capra hircus (Goat)	1	2	3	2		5,000 (carcass)		
Equidae					0.51		1.52	1.32
Equus caballus (Horse)	1	0	0	1		5,000 (carcass)		
AVES					34.52		9.92	11.05
Accipitridae					2.03		0.94	1.10
Buteo buteo (Common Buzzard)	2	1	2	2		778		
Accipitridae not determined	0	2	3	2		778		
Falconidae					2.54		0.26	0.31
Falco tinnunculus (Common Kestrel)	4	1	3	5		174		
Phasianidae					7.11		4.19	4.40
Alectoris graeca (Rock Partridge)	1	2	4	3		540		
Perdix perdix (Grey Partridge)	4	1	2	4		365		
Alectoris / *Perdix*	0	1	2	1		450		
Phasianus colchicus (Pheasant)	1	1	1	2		1,133		
Gallus domesticus (Domestic Chicken)	1	3	3	4		2,000		
Columbidae					2.54		0.63	0.73
Columba livia (Rock Dove)	1	0	0	1		320		
Columba palumbus (Wood Pidgeon)	2	0	0	2		479		
Columbidae	0	2	2	2		400		
Strigidae					1.52		0.31	0.36
Asio otus (Long-eared Owl)	1	0	0	1		237		
Strix aluco (Tawny Owl)	0	1	1	1		442		
Asio otus / *Strix aluco*	0	1	1	1		340		
Corvidae					16.75		3.53	4.08
Garrulus glandarius (European Jay)	8	9	33	10		160		
Pica pica (Eurasian Magpie)	1	0	0	1		180		
Corvus cornix (Hooded Crow)	14	10	44	16		509		
Corvidae not determined	4	4	11	6		283		
Passerine birds	0	4	11	4	2.03	50	0.06	0.07

REPTILIA					4.06		0.53	0.61
Lacertidae					0.51		0.01	0.01
Lacerta bilineata (Western Green Lizard)	0	1	1	1		30		
Colubridae					3.55		0.52	0.60
Hierophis viridiflavus (Green Whip Snake)	0	7	14	7		245		
TOTAL	**123**	**125**	**408**	**197**	**100**		**100**	**100**

Figure 2. Distribution of weight classes of prey. The X-axis reports the eight weight classes (grams). The Y-axis reports the percentage of each class in the diet. Weight classes follow Watson (2010) (1: 0 - 63 g; 2: 64 - 125 g; 3: 126 - 250 g; 4: 251 - 500 g; 5: 501 - 1.000 g; 6: 1.001 - 2.000 g; 7: 2.001 - 4.000 g; 8: > 4.001 g).

The application of the Levins index on the 21 families detected produced a value of 8.45, indicating a rather wide trophic niche.

Comparing the diet of the four pairs nesting within the Sibillini National Park (preys n = 95) with the two pairs outside protected areas (preys n = 102), we observed a different proportion of hares delivered to the nest, with 30.5% inside the National Park and 20.6% outside protected areas. On the contrary, the Glirids, the Mustelids, the Felids, the domestic chicken *Gallus domesticus*, Eurasian Jay and small passerines were more frequent in the diet outside the National Park; however, this difference was not statistically significant (X^2 = 6.72, df = 7, *p-value* = 0.5).

Among our direct observations of the prey delivered to the nests by the adults (Table 2) mammals were the most frequent preys (47.2%), with hares as the dominant prey (17%), followed by birds (43.4%) and reptiles (9.4%).

Table 2. Observed prey delivered to 6 nests of Golden Eagle in the Central Apennines (2000-2022). A total of 38 days of observation were conducted.

Class and Species	**N. ind.**	**% Frequency Individuals**
MAMMALIA		47.17
Lepus spp. (Hares)	9	
Sciurus vulgaris (Red Squirrel)	3	
Myoxus glis (European dormouse)	3	
Small mammal	1	
Vulpes vulpes (Red Fox)	2	
Meles meles (European Badger)	1	
Martes foina (Beech Marten)	1	
*Sus scrof*a (Wild Boar juv)	2	
Ovis aries (Sheep)	1	
Young ungulate	2	
AVES		43.40
Alectoris graeca (Rock Partridge)	2	
Perdix perdix (Grey Partridge)	2	
Gallus domesticus (Domestic Chicken)	1	
Columba palumbus (Wood Pidgeon)	3	
Pyrrhocorax pyrrhocorax (Red Chough)	1	
Corvus cornix (Hooded Crow)	4	
Corvidae chicks	3	
Birds unidentified (medium dimension)	7	
REPTILIA		9.43
Hierophis viridiflavus (Green Whip Snake)	2	
Colubridae indetermined	3	
TOTAL	**53**	

DISCUSSION

Our results are in agreement with previous studies on the species in the area of Central Appennines (Novelletto & Petretti 1980, Magrini et al. 1987), despite such studies also included data on the adults' diet throughout the year. Further, we highlight that hares remain the most preyed upon species in this population of Golden Eagles, despite a reduction in the hares' frequency compared to those reported in the previous studies, from 48.8% to 25.5% (Magrini et al. 1987). Phasianids family also shows a decline from 20.6% (Novelletto & Petretti 1980) to 16.1% (Magrini et al. 1987), until our result of 7.1%. In contrast, Corvids showed a threefold increase from 6.3%-10.1% (Novelletto & Petretti 1980; Magrini et al. 1987) up to 16.8% in this study. In our study, the *Suidae* family, which was not recorded in previous studies, represents 5.1% of the total Golden Eagle diet. Finally, a fair percentage of reptiles (4.1%) were recorded, mainly Colubrids.

Regarding biomass, according to Novelletto & Petretti (1980) and Magrini et al. (1987), the most relevant species were hares, domestic chicken and red fox, while in the present study the number of relevant species increased and included hares, sheep, red fox and wild boar.

A comparable number of Mustelids, Felids and medium-large birds were detected in both the prey remains and pellets (Table 1). However, the prey remains data likely underestimated small preys (reptiles, small birds and rodents), while the analysis of pellets could have underestimated the presence of large prey. In fact, Seguin et al. (1998), found a significant underestimation of micromammals and reptiles in prey remains compared to visual observations but no significant differences between pellets and visual observations analysis.

The frequency of different prey species reflects the breadth of the nestlings' diet, while the total biomass provides a more accurate indication of the importance of the different species within the diet. The trophic niche has become wider over time: the Levins index went from 3.43 (Novelletto & Petretti 1980) to 8.45 in the present study. Golden Eagles in Central Apennines likely shifted their predation to more available species over time (Whitfield et al. 2009). For example, the observed increase in predation on the Hooded Crow is in line with its constant increase in the study area (Giuliani 2019). We can assume that, as the prey became more abundant, Golden Eagles could exploit their availability, balancing predation energy cost with energy intake (Schluter 1981). Similarly, wild boars' abundance in the Central Apennines area increased in recent years (Rossetti et al. 2021) and has now become a new trophic source for Golden Eagles.

Despite the small sample size, our visual observations of prey deliveries in the last years may further support the decline of hares. In other studies, similar results were obtained, where the frequency of different preys was inversely related to the presence of hares in Golden Eagle diet (Collopy 1983, Steenhof & Kochert 1988).

Our study shows that hares are still the most dominant prey species, but their share of the overall diet decreased. Both the Italian and European hares, in fact, have shown a drastic decline in central Italy (Freschi et al. 2016, Naldi et al. 2020). Therefore, we suggest that a reduction of hunting pressure and an increase in sanitary checks in hares` restocked populations may be needed. Despite this decline in hares' density, the Central Apennines Golden Eagle population is steadily increasing since the '90s (Fasce & Fasce 2017).

At the same time, we found an increase in the total number of prey species, resulting in a wider trophic niche. The conspicuous presence of species such as Wood Pigeon *Columba palumbus*, Hooded Crow, Wild Boar and Red Fox, may points towards an increase in their availability in the Golden Eagle diet, but future studies should aim at quantifying this change. Our observational results are in line with the ones from pellets and prey remains and add further qualitative information, such as the predation on corvid nestlings and Red Chough *Pyrrhocorax pyrrhocorax*.

For a wider interpretation of Golden Eagle diet in

Central Apennines further research is necessary, including multiple seasons and different data collection techniques (pellets and prey remains analysis, direct observations, camera trapping, isotopes analysis) in order to reduce systematic errors. In fact, overestimation or underestimation errors must be evaluated in relation to different prey species, for which a specific correction factor relating to the applied technique must be calculated.

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