Pup feeding habits of a mixed wolf-hybrid pack living in a human-modified landscape in Central Italy

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Short title
Wolf pup diet in a human-modified landscape

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Running title: Wolf pup diet in a human-modified landscape

Abstract
A medium-sized mammals selection as food diet for pup performed by adult wolves has been previously hypothesized. In this work the diet of wolf pups living in an anthropic area in Italy has been investigated to assess which prey potentially played a key role in their sustenance. Although wild ungulates were the main prey category for both pups and adults a significantly high occurrence of medium-size mammals (hare and coypu) and birds in pup diet has been recorded. Such result could confirm a selective food provision by adult wolves. Further investigations to assess the importance of this kind of prey for pup survival are desirable.

Keywords: *Canis lupus*, pup diet, coypu, European hare, human-dominated landscape

Within a generalist and opportunistic feeding habit, wolf diet in Italy is quite diversified and it is mainly based on wild ungulates (Meriggi et al., 2011). However the wolf shows a great adaptation to ever more anthropized ecological conditions, where it can benefit from a high availability of domestic prey (Meriggi et al., 2011, Torres et al., 2015). While of adult wolf diet has been widely investigated (Meriggi et al., 2011, Ferretti et al., 2019; Bassi et al., 2020) no data on pup diet in Italy are yet available and few studies were performed in Central-Eastern Europe and in North America (Bryan et al., 2006, Sidorovich et al., 2017, Mysłajek et al., 2019; Roffler et al., 2023).
The survival of pups strongly depends on the efficiency of adults in delivering food, which is mediated by the density of potential prey (Harrington et al., 1983). In Poland and Canada, although consuming the same prey species, a higher occurrence of medium mammals in pup diet than in adults has been recorded, allowing to hypothesize a selective food provisioning by adults (Bryan et al., 2006, Sidorovich et al., 2017; Mysłajek et al., 2019). The aim of this work was to preliminarily assess which

prey categories are more consumed by pups in Italy and if a selective provision of food to developing pups is operated by adults.

Wolf scats were collected from 2018 to 2021 in a suburban area of 1295 ha in the lower Pisan hills (43.551370°-10.551708°), Tuscany, Italy (Figure 1). The area is inhabited by a wolf pack composed by both Italian wolves and hybrids (Coppola et al., 2022) monitored with camera-traps since 2016. Evident skin disease attributable to mange ectoparasite (*Sarcoptes* sp.) infestation was observed only in the reproductive female and pups. In such area the wolf is the only large carnivore present and no stray dogs were recorded. The area is a fragmented agro-ecosystem, in which several productive and recreational activities occur. Animal husbandry is widely performed and is mainly characterized by extensive and semi-extensive medium-small sheep and cow farms.

Adult scats were sampled along 4 transects for a total length of 12 km (Figure 1), randomly chosen in the area. Each transect was covered twice a week from October to May, and three times a week from June to September, and a total of 768 km and 576 km walked, respectively. Pup scats were sampled in a rendez-vous site in July 2021 where presence of 7 pups of about two-months-old had been documented by camera traps and direct observations. Adult and pup scats were collected along transects and in the rendez-vous site, discriminating them from those of pet and shepherd dog following Peters and Mech (1975) procedures. "Video-scat" analysis was also used as additional wolf scat identification tool (Figure 2). For a subsample of scats (n= 4 of pups and n=23 of adults) identification of pure wolf individual was confirmed (100%) through DNA genotyping (Coppola et al., 2022 and unpublished data).

Samples were stored for 5 days at -80° to inactivate Taeniidae eggs and then at – 20° until analysis. Each scat was washed using two sieves with decreasing mesh sizes (1.5-0.1 mm) to separate indigested material. Hairs were washed in alcohol and observed under microscope (10x, 20x and 40x). Hairs were identified based on cortical scales, medulla, and root characteristics, using the key of Teerink (1991). Prey species were grouped in seven categories: livestock, wild ungulates, medium-sized mammals, birds, invertebrates, plant materials and rubbish. According to Kruuk and Parish (1981) to each consumed species and food category a volumetric class (< 1%; 1–5%; 6–25%; 26–50%; 51–75%; 76–95%; > 95%) was assigned and then converted in a final percentage volume: 0.5%; 2.5%; 15.5%; 38%; 63%; 85,6% and 98% respectively (Meriggi et al., 2015). The mean percent volume (MV%) of each category and species was calculated (Imbert et al., 2016). Frequency of occurrence (FO%) of each food category was calculated as number of scats in which each prey species was detected within the total quantity of scats analyzed (Imbert et al., 2016; Torretta et al., 2017).
The Brillouin index (1956) was applied to calculate the minimum number of scats necessary to study the wolf diet:

$$Hb = \ln N! - \sum \ln * n_i! / N$$

where Hb is the diversity of prey in the sample, N is the total number of prey taxa in all samples, and n_i is the number of prey taxa of the i^{th} category (Brillouin, 1956).
The variability of the diet was calculated with the B index (Levins, 1968):

$$B = 1/\sum p_i^2$$

where p_i is the contribution of every prey category to the total biomass of food consumed by wolf. Pianka (1973) index (α) was used to calculate the similarity of diet composition between pups and adult wolves:

$$\alpha_{jk} = \frac{\sum_i^n Pij * Pik}{\sqrt{\sum_i^n Pij^2 \ \sum_i^n Pik^2}}$$

where α_{jk} is Pianka index of niche overlap between pups (j) and adults (k), Pij is the proportion of prey categories i^{th} on the total resources used by pups, Pik is the proportion of prey categories i^{th} on the total resources used by adults.

Differences in FO% of each prey category and species in adult wolf diet in autumn-winter and spring-summer and between adults and pups in spring-summer were tested using the Chi-square test (χ^2). Statistical analyses were performed with R studio (v 4.1.2; R Core Team 2022).

Overall, 55 pup scats and 132 adult scats, of which 49 in spring-summer and 83 in autumn-winter, belonging to a pack of Italian wolf and hybrids were collected and analyzed. The influence of hybridisation on wolf ecology is still largely debated (Stronen et al., 2022) and a recent study performed in Italy suggests that there are no significant differences in hybrid diet composition compared to that of pure wolf (Bassi et al., 2017).

Brillouin index indicated that a minimum value of 18 and 14 scats for adults and pups, respectively, as well as the minimum value of 14 adult scats in autumn-winter and 13 in spring-summer were sufficient to perform diet analysis and to compare diet between the two seasons.

In this investigation both prey categories and food niche found in pup diet matched those found in adults (α=0.96; pup B = 0,26; adult B = 0,29). Conversely, prey species occurrence significantly differed. This result is in accordance with those previously reported in Poland, Canada and Belarus by Bryan et al., (2006), Sidorovich et al., (2017) and Mysłajek et al., (2019).

In this study pup and adult diet was mainly based on wild boar and roe deer (Table 1). Similar results were obtained by Bryan et al., (2006), Meriggi et al., (2011), Sidorovich et al., (2017), Mysłajek et al., (2019) on healthy packs in different countries. Such evidence indicates that predation performances and diet habits of the investigated pack were not influenced by the mange infestation.

In this study no differences were recorded in wild boar consumption between pups and adults. Conversely, roe deer occurred in pup diet with a significantly higher frequency compared to adults. Although adult consumption of roe deer in spring-summer was higher than in autumn-winter, these results are probably due to the presence of fawns easier to prey and transport with less energy expenditure (Meriggi et al., 2015).

Medium-size mammals, mainly hare and coypu, occurred in adult diet with a significantly higher frequencies during autumn-winter than spring-summer supporting results obtained by Ferretti et al., (2019). During summer hare and coypus were also present with a significantly lower frequencies in adults than pups. Also, a significant higher frequency of birds in pups diet compared to adults occurred. These results may suggest a key role of these three prey categories in pup diet. Similarly, in North American and Eurasian countries the beaver (*Castor fiber*) plays a key role for pups (Sidorovich et al., 2017, Mysłajek et al., 2019). In this investigation, differences recorded in roe deer and medium-size mammals and birds could hint at a potential selective provision operated by adults to developing pups, hypothesized by Bryan et al., (2006) and Mysłajek et al., (2019). Such adult behaviour could be driven by: I) ease of predation and transport of fawns, hares and coypus with less energy expenditure, II) to getting pups used to consume whole prey including liver, lungs, and heart, as higher nutrient parts, III) ease consumption of bones, as a releaser of calcium (Stockman et al., 2021), and IV) the size of such preys probably being insufficient for adult feeding requirements.

The badger, the crested porcupine and the *Mustela* spp. had a marginal role in the diet of both pups and adult wolves (Table 1).

in this study, livestock was a relevant prey for both pups and adults, being easily accessible. Specifically, sheep occurrence in pup and adult diet resulted similar and a higher sheep consumption was recorded in adult diet in spring-summer than in autumn-winter. In the study area some semi-extensive sheep farms overlap both the pack territory and the rendez-vous site, making this prey more accessible and easily transported especially during spring-summer, also leading to a peak of predation in such season (Coppola et al., 2022). Goat occurrence did not show significant differences in adult diet throughout the year but resulted significantly lower in pup diet compared to adults. This result could be due to the absence of goat farms in the surroundings of the pack rendez-vous site that make the transport of this prey more expensive.
Plant materials and invertebrates also occurred in the diet of both pups and adults with low mean percent volume values. Invertebrates in adult scats were detected with a significantly higher frequency in spring-summer than autumn-winter. Within the invertebrates, larvae of necro-saprophage Calliphoridae were the most frequently found in scats of both pups (66.7%, n=6) and adults (75%, n=8), probably due to scavenging, rather than predation.

In conclusion, although wolf pup and adult diets did not differ in prey categories, adults may have operated a selective provision mainly of hare, coypu, and minorly of roe deer and birds for pups. Authors are aware that this study focused on one pack only and further investigations to assess the relevance of these prey for pup sustenance are desirable.

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Table 1. Diet composition of adult and pup wolves living in a highly anthropized area in Pisa Province (Tuscany). For each category and prey species frequencies of occurrence (FO%) and mean percentage volume (MV%) are reported. Statistical differences (χ^2; p-value) of prey species FO% between spring-summer (SS) and autumn-winter (AW) for adults and between adults and pups are reported and marked in bold.

		ADULTS							**PUPS**		
Prey categories	**Species**	**Spring-summer**		**Autumn-winter**		**Total**		**SS *vs* AW (χ^2; p-value)**	**Spring-summer**		**Adult *vs* PUPS (χ^2; p-value)**
		FO%	MV%	FO%	MV%	FO%	MV%		FO%	MV%	
Livestock	*Ovis aries*	24.49	19.89	13.25	10.28	17.42	13.84	**4.12; <0.05**	20	7.28	0.58
	Capra hircus	14.29	9.31	14.46	8.99	14.39	9.11	0.00	3.64	0.97	**6.95; <0.01**
Total		31.65	27.37	26.50	19.27	28.79	22.28		23.64	8.25	
Wild ungulates	*Capreolus capreolus*	36.73	25.29	18.07	11.11	25	16.37	**8.75; <0.01**	67.28	48.62	**18.70; <0.01**
	Sus scrofa	57.14	30.83	68.67	48.40	64.39	41.88	2.84	65.45	16.57	1.45
Total		77.55	58.11	79.52	59.52	78.79	59.00		81.82	65.62	
Medium size mammals	*Lepus europeaus*	4.08	0.63	7.31	4.95	6.06	3.34	1.05	14.54	8.61	**6.48; <0.01**
	Myocastor coypus	2.04	0.05	9.64	6.86	6.82	4.33	**5.25; <0.05**	9.09	8.46	**4.73; <0.05**
	Meles meles	2.04	1.75	0	0	0.76	0.66	2.06	3.64	2.06	0.46
	Hystrix cristata	0	0	2.41	0.06	1.52	0.04	0.0	1.82	0.01	0.00
	Mustela spp.	2.04	2	0	0	0.76	0.74	0.00	0	0	0.00
Total		10.20	4.43	18.07	11.87	15.15	9.10		27.27	19.15	
Birds		2.04	0.32	1.20	0.19	1.52	0.23	0.22	12.73	0.32	**8.35; <0.01**
Invertebrates		12.24	0.15	2.41	0.02	6.06	0.17	**7.12; <0.01**	10.91	0.09	0.09
Plant		69.39	7.17	65.06	6.30	66.67	6.63	0.42	85.45	4.56	**7.38; <0.01**
Rubbish		4.08	0.33	3.61	1.22	3.79	0.89	0.03	1.82	0.28	0.89

Figure 1. Map of the location of the study area. Samplings were performed along four transects (black lines: T1-T4) and in the rendez-vous site (black dot). The image of the study area was elaborated using Qgis 2.18 software.

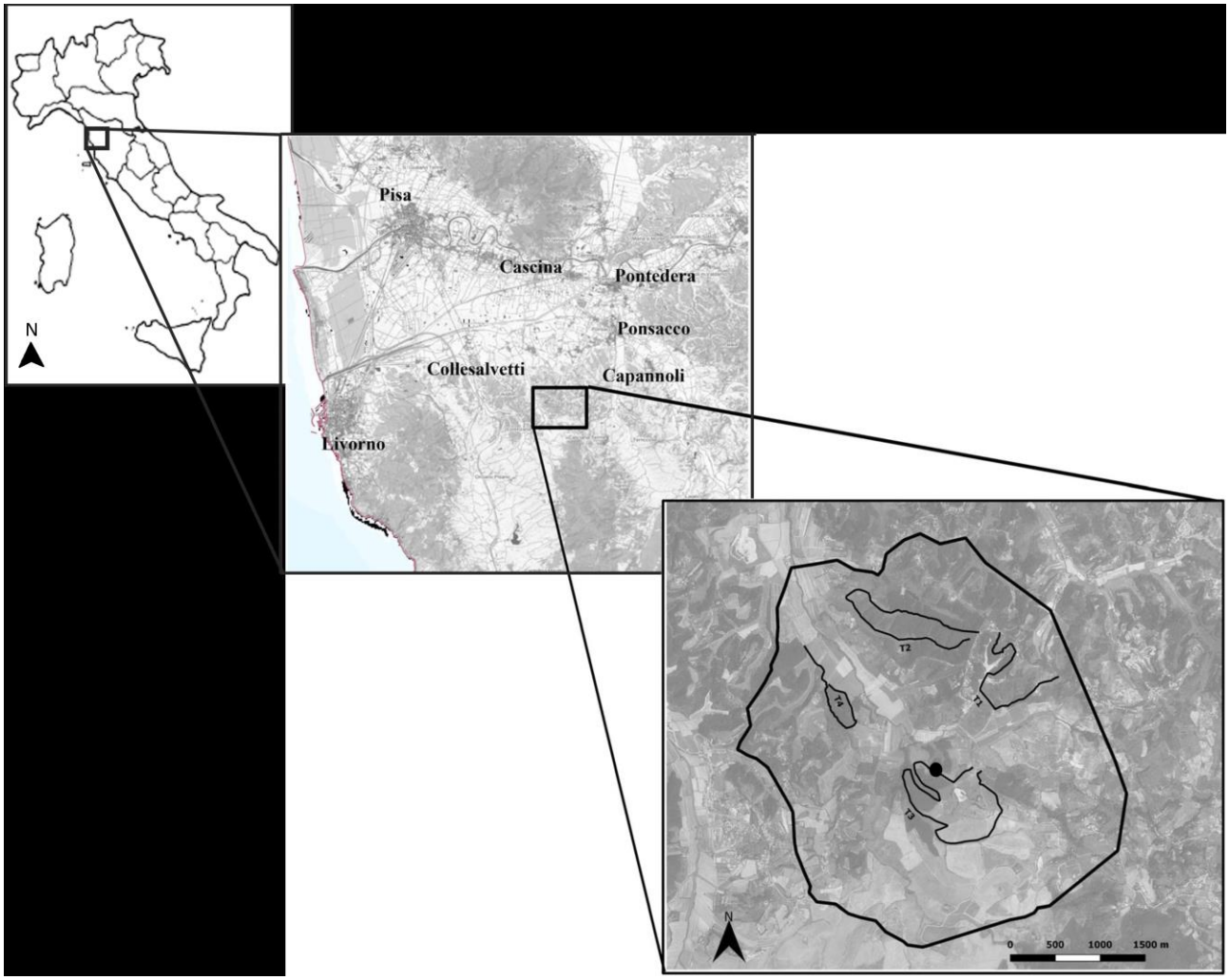

Figure 2. The adult reproductive female recognizable by the presence of a black coat (left) and a pup with evident skin disease attributable to mange ectoparasites infestation (right) while defecate in a marking site and in the rendez-vous site, respectively.