Ecology, physiology and behaviour

What do wolves eat? Insights into diet variation of an apex predator in Central and Southern Europe using faecal DNA metabarcoding

De Barba, Marta[1,2*]; Konec, Marjeta[1,2]; Boljte, Barbara[2]; Pazhenkova, Elena[1,2]; Hrga, Nuša[2]; Majić Skrbinšek, Aleksandra[1,2]; Simčič, Gregor[2]; Brenner, Wiebke[3]; Kozyra-Zyskowska, Katarzyna[4]; Sharma, Sandeep[5]; Malić Limari, Snježana[6]; Skrbinšek, Tomaž[1,2]

[1] DivjaLabs Ltd., Ljubljana, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia
[3] Rewilding Oder Delta e.V., Rothenklempenow, Germany
[4] Praca Przyrodnika Sp. z o.o., Szczecin, Poland
[5] German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany
[6] WWF Adria, Zagreb, Croatia
* marta.debarba@gmail.com

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Trophic interactions, which encompass the diet of a species and its dynamics in natural systems, shape the distribution of species and their ecological niche, and influence population dynamics, viability, and responses to environmental changes as well as ecosystem function. Apex predators, such as grey wolf (*Canis lupus*), play critical roles in ecosystems, but their diets can be influenced by human activities, also exacerbating human-wildlife conflicts. Accurate knowledge of diet composition and feeding behaviour of apex predators is therefore instrumental for effective management and implementation of conflict mitigation measures for conservation.

Using a DNA metabarcoding approach we investigated the diet of wolves in three European regions with differing socio-economic and environmental conditions: Alps in Slovenia, the Dinaric region in Croatia, and the Oder Delta across Germany and Poland. Faecal samples (n = 70, 60, and 78, respectively) collected in 2019-2024 were analysed using a metabarcode marker to identify vertebrate/mammalian prey. Results revealed clear spatial patterns in diet composition driven by prey availability. Wild ungulates, particularly red deer (*Cervus elaphus*) and European roe deer (*Capreolus capreolus*), emerged as primary prey across all regions, reflecting known preference by wolves when available. Wild boar (*Sus scrofa*) was also frequently detected, especially in Croatia, in areas where the preferred natural prey abundance declined. Occurrence of domestic animals (e.g. cattle, goat, sheep) in the samples was generally low, but increased with higher incidence of human presence and activities. In addition, the same DNA extracts processed for diet analysis were also used for individual genotyping with potential for deciphering individual feeding habits, but also providing an example of cost-effective use of faecal samples typically collected in genetic monitoring programs for multiple purposes.

This study provides high-resolution dietary data and a deeper understanding of the ecological role of wolves important for informing management in different parts of Europe. It also demonstrates the power of DNA metabarcoding for efficient non-invasive diet assessment which is crucial for enabling large scale ecological studies, such as to explore spatiotemporal variation in diet through environmental gradients or as a consequence of anthropogenic activities and global changes, ultimately enhancing future conservation efforts.