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First evidence of widespread positivity to anticoagulant rodenticides in grey wolves (*Canis lupus*)

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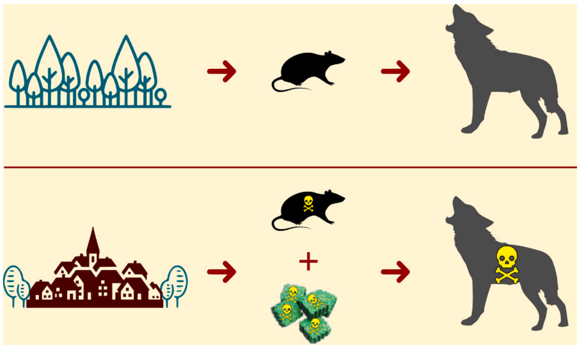
HIGHLIGHTS

- No study quantified exposure to rodenticides in European large carnivores.
- We tested wolves found dead in Italy ($n = 186$) and modelled trends in 2018–2022.
- Most wolves (61.8 %) tested positive for one, or more, rodenticides.
- Exposure increased with landscape anthropization and after 2020.
- Rodent control could jeopardize large carnivores in anthropized landscapes of Europe.

GRAPHICAL ABSTRACT



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ABSTRACT

Second-generation Anticoagulant Rodenticides (ARs) can be critical for carnivores, due to their widespread use and impacts. However, although many studies explored the impacts of ARs on small and mesocarnivores, none assessed the extent to which they could contaminate large carnivores in anthropized landscapes.

We filled this gap by exploring spatiotemporal trends in grey wolf (*Canis lupus*) exposure to ARs in central and northern Italy, by subjecting a large sample of dead wolves ($n = 186$) to the LC-MS/MS method.

Most wolves ($n = 115/186$, 61.8 %) tested positive for ARs (1 compound, $n = 36$; 2 compounds, $n = 47$; 3 compounds, $n = 16$; 4 or more compounds, $n = 16$). Bromadiolone, brodifacoum and difenacoum, were the most common compounds, with brodifacoum and bromadiolone being the ARs that co-occurred the most ($n = 61$).

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Brodifacoum
Bromadiolone

Both the probability of testing positive for multiple ARs and the concentration of brodifacoum, and bromadiolone in the liver, systematically increased in wolves that were found at more anthropized sites. Moreover, wolves became more likely to test positive for ARs through time, particularly after 2020.

Our results underline that rodent control, based on ARs, increases the risks of unintentional poisoning of non-target wildlife. However, this risk does not only involve small and mesocarnivores, but also large carnivores at the top of the food chain, such as wolves. Therefore, rodent control is adding one further conservation threat to endangered large carnivores in anthropized landscapes of Europe, whose severity could increase over time and be far higher than previously thought. Large-scale monitoring schemes for ARs in European large carnivores should be devised as soon as possible.

1. Introduction

The long-term conservation of large mammals in anthropized landscapes is often said to depend upon a combination of legal protection, sustainable exploitation, the availability of suitable habitat and trophic resources, human tolerance, and infrastructure development (Apollonio et al., 2017; Di Marco et al., 2014; Di Minin et al., 2016; Kauffman et al., 2021; Wolf and Ripple, 2016). Moreover, many studies highlighted the risk posed by infectious or parasitic diseases (Cunningham et al., 2017).

However, exposure of large mammals to anthropogenic chemicals has received proper attention only over the last few years (https://www.ewg.org/interactive-maps/pfas_in_wildlife/map/). This is despite the fact that persistent, bioaccumulative and toxic (PBT) chemicals can enter the trophic chain and alter the physiology, behavior, health, and reproduction of mammals (Torquetti et al., 2021; Saaristo et al., 2018; Zala and Penn, 2004; Köhler and Triebskorn, 2013), in turn impacting their populations (Desforges et al., 2018). The impact of PBTs can be particularly critical for large carnivores (Rodríguez-Estival and Mateo, 2019), whose populations in many parts of the Global North, although recovering (Ingeman et al., 2022), are still relatively limited and potentially susceptible to significant shrinkage.

Anticoagulant rodenticides (hereinafter ARs) are among the most problematic PBTs for predators, due to the possibility of secondary exposure through direct predation of rodents or the consumption of dead ones (Elmeros et al., 2019; Fernandez-de-Simon et al., 2019, 2022; Geduhn et al., 2015; López-Perea et al., 2019; Rattner and Harvey, 2021; Wright et al., 2022) given their likely impact on the immune system of mammals (Serieys et al., 2018). This is especially true for second-generation ARs which are more effective against rodents than first-generation compounds and more persistent in the environment. Although the European Union adopted regulations that progressively constrained the use of ARs, exposure in non-target mammal predators remained high (Elmeros et al., 2018), also due to different national laws and free trade agreements between member states (Eisemann et al., 2018).

The grey wolf (*Canis lupus*) has steadily expanded its distribution in Europe, over the last three decades, due to environmental change and increased legal protection (Cimatti et al., 2021). Despite wolves in Europe might be exposed to ARs since *i*) rodents are part of their diet (Newsome et al., 2016; Zlatanova et al., 2014) and *ii*) they are expanding into areas where rodent control is a routine activity, no study has explored this phenomenon This gap is surprising because ARs have recently been found in mesocarnivores and large carnivores living around human settlements, indicating that secondary exposure to these substances is no longer restricted to small carnivores (Serieys et al., 2018, 2019; McMillin et al., 2018; Rudd et al., 2018; Lestrade et al., 2021).

Here we want to fill this gap by exploring spatiotemporal trends in wolf exposure to ARs in central and northern Italy, by relying on a large sample of animals that were found dead between 2018 and 2022 and tested using standardized laboratory protocols.

2. Methods

2.1. Study area

The study area encompasses the Emilia-Romagna and Lombardy regions, as well as the northernmost portion of the Tuscany region (Fig. 1). This area is characterized by different temperate and Mediterranean ecosystems, with urbanization occurring mostly in the lowlands. The human population is estimated to be approximately 10.5 million people, across 46.039 km^2, with a density of 269.4 ± 167.6 inhabitants/km^2 (mean ± standard deviation).

Wolf densities range approximately from 4.9 and 13.2 wolves/100 km^2 (La Morgia et al., 2022), while the presence of wolves in Lombardy is still limited mostly to single dispersing individuals (Dondina et al., 2020). Over the last three decades, wolves have progressively colonized most of the study area, starting from the more undisturbed habitat patches in mountains and then reaching agricultural and peri-urban environments in lowlands (Bassi et al., 2015; Zanni et al., 2023).

While in the study area wolves prey mostly on wild ungulates, such as the roe deer (*Capreolus capreolus*) and the wild boar (*Sus scrofa*) (Bassi et al., 2017, 2020; Ferretti et al., 2019; Mori et al., 2017; Milanesi et al., 2012; Mattioli et al., 2011; Capitani et al., 2004), recent studies suggest that they can also include other types of prey in their diet, such as the coypu (*Myocastor coypus*, Ferretti et al., 2019).

In the study area rodent control is authorized according to Regulation n. 582/2012 (https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:167:0001:0123:en:PDF), Regulation n. 1107/2009 (https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:en:PDF) and Regulation n. 1062/2014 (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32014R1062) from the European Commission. In Italy, both first-generation (chlorophacinone, coumatetralyl) and second-generation ARs (brodifacoum, bromadiolone, difenacoum, difethialone, flocoumafen) are authorized. In Table 1, the number of anticoagulant rodenticide formulations available on the market in Italy, based on active ingredient is given. Rodent control targets almost exclusively synanthropic species, i.e., the house mouse (*Mus musculus*), the brown rat (*Rattus norvegicus*) and the black rat (*Rattus rattus*). Moreover, although this practice is illegal, empirical evidence indicates that ARs are commonly used to control coypus in croplands (see the Discussion for further details). Rodent management is performed mostly by pest control operators (hereinafter: PCO), which can purchase any active ingredient, with concentrations up to 50 ppm of the active principle (Sinergitech, 2020). It is noteworthy that rodenticides can also be purchased by private citizens, although only as small packages with concentrations of the active principle below 30 ppm (https://www.izs.it/IZS/Engine/RAServeFile.php/f/pdf_normativa/Biocidi-Rodenticidi/Biocidi_IZSTeramo.pdf). Rodenticides are typically used to control rodents in outdoor settings, since indoor interventions are usually based on trapping. Unlike other European countries (e.g., the United Kingdom), in Italy there are no restrictions on the use of the most toxic active principles (i.e., brodifacoum and flocoumafen) in outdoor interventions.

2.2. *Data collection and laboratory analysis*

Our final sample included 186 wolves (Fig. 1), which had been collected between 2018 and 2022 by local authorities and then subjected to a necropsy and a toxicological examination aimed at detecting ARs.

During necropsy, each animal underwent an external inspection aimed at assessing its nutritional status and development of genital organs, as well as to inspect and detect anomalies in its skeleton, skin, mucous membranes, ocular bulbs, ear lobes, oral cavity and nostrils. The age of each animal was estimated based on their dental development, body size and weight (Brasington et al., 2023). Individuals had a balanced sex ratio (53.6 % were males), and our sample included both young and adult wolves (1st year of age = 27.9 %; 2nd year of age = 34.6 %, 3rd year of age or higher = 37.7 %). Then, the carcass was placed in dorsal decubitus and skinned. The complete necropsy began

Table 1
Number of anticoagulant rodenticide formulations available on the market in Italy, based on active ingredient (data updated to 2020). These include rodenticides falling under Product-Type (PT) 14, i.e., formulations intended for Trained Professionals, Professionals, General public.

Active ingredient	n	Generation
Brodifacoum	121	2nd
Bromadiolone	98	2nd
Difenacoum	68	2nd
Chlorophacinone	4	1st
Coumatetralyl	4	1st
Difethialone	8	2nd
Flocoumafen	3	2nd
Bromadiolone+Difenacoum	3	2nd

Source: Sinergitech, 2020.

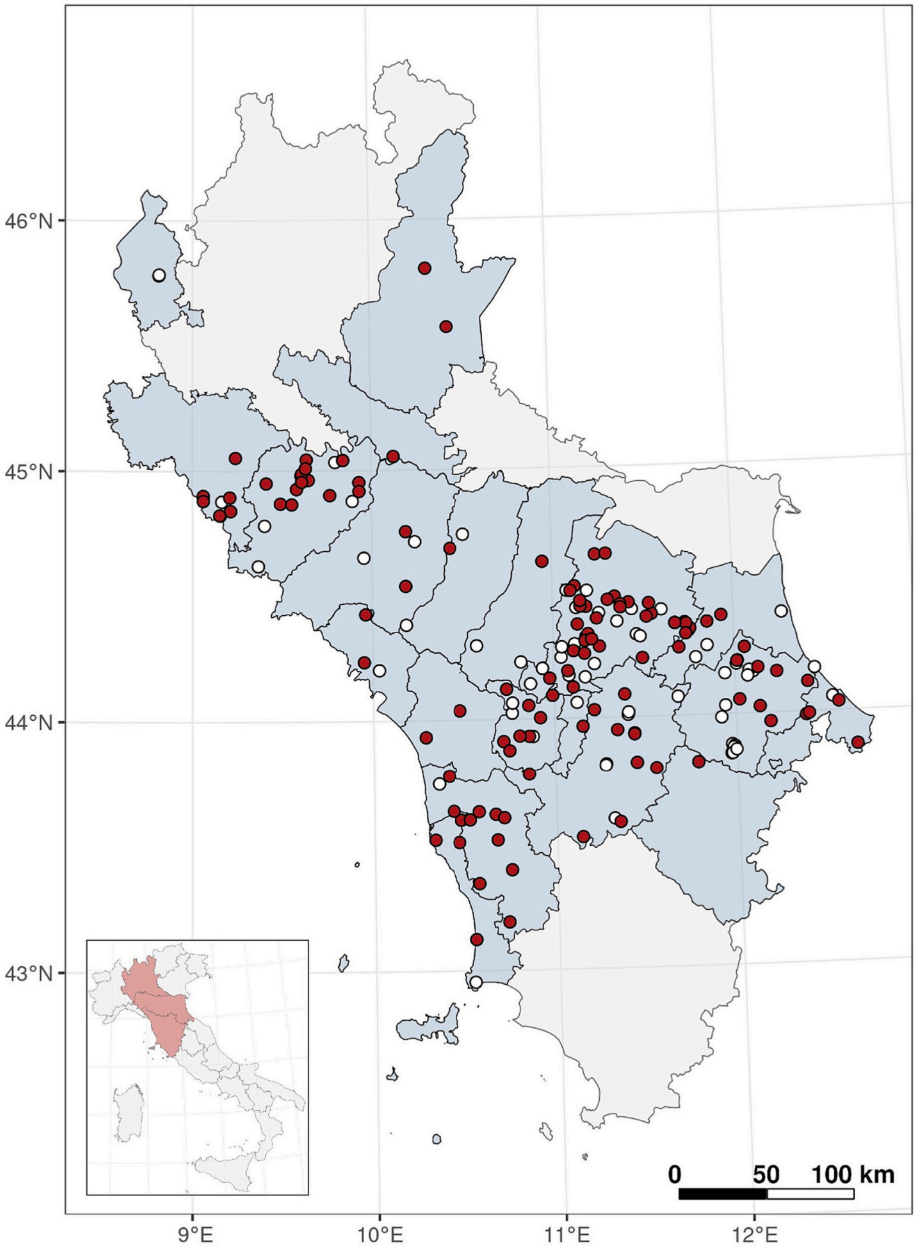

Fig. 1. Distribution of wolves that were found dead in the study area and were negative (white dots) or positive (red dots) to anticoagulant rodenticides (ARs). Provinces in the Emilia-Romagna, Lombardy, and Tuscany regions, that were covered by data collection are highlighted. The position of the study area in Italy is shown in the figure in the lower-left corner.

with the opening of the abdominal cavity, followed by the opening of the thoracic cavity and finally of the skull. At each of these steps, the cavities and organs were inspected and evaluated individually before being sampled for laboratory analysis.

The determination of anticoagulants (coumafuryl, warfarin, coumatetralyl, coumachlor, bromadiolone, difenacoum, brodifacoum, flocoumafen, difethialone) was carried out by means of a LC-MS/MS method (Vandenbroucke et al., 2008; Fourel et al., 2017; Bertero et al., 2020). In detail the liver was homogenized before analysis, the sample (typically 40 g) was extracted by vigorous stirring with acetone (100 mL); after filtration on paper, an aliquot (2 mL) was dried under gentle nitrogen flow at 40 °C. The residue was reconstituted with 2 mL of 2 % ammonia solution in acetonitrile. Three defatting steps with n-hexane (2 mL) followed. Finally, an aliquot (1 mL) was stripped to dryness and reconstituted with 0,4 mL of acetonitrile. A 1 μL volume was injected into an LC-MS/MS system (Agilent QQQ 6460, equipped with an Agilent 1290 Infinity II UPLC). Chromatographic column was Zorbax Eclipse Plus C18 (2,1 × 50 mm, 1,8 μm). Column temperature was set at 40 °C. Chromatographic separation was performed through a linear gradient using as aqueous phase a 0,1 % formic acid solution and as organic phase 0,1 % formic acid solution in acetonitrile. Flow rate was set at 0,4 mL/min. Run time was 11 min, with a post-time reconditioning of 2 min. Quantification was carried out by the external standard method in MRM mode (ESI negative) acquiring two proper and typical transitions, quantifier, and qualifier, for each analyte (Table S1). MS/MS parameters were set as follows: capillary 4000 V, gas temperature 300 °C, gas flow 10 L min–1, nebulizer 35 psi, sheath gas temperature 300 °C, sheath gas flow 12 L/min.

The limit of quantification (LOQ) was 1 μg /Kg for all analytes, with a mean recovery >80 % and each analysis batch had 1 positive and one negative control. A concentration found ≥1 μg/Kg indicated a positive sample, while a concentration < 1 μg /Kg denoted a negative sample. An overview of concentrations, for each compound, is available in Table S3.

To evaluate whether a positive anticoagulant test can be classified as intoxication with clinical signs, it is necessary to correlate analytical data, the anatomopathological picture and the anamnesis. In particular, it would be necessary to know the timing and level of exposure (how much anticoagulant was ingested and when) and the medical history of each wolf. However, as we relied on opportunistic sampling, we did not know neither the time and level of exposure, nor the medical history. Therefore, we only classified as poisoned those individuals for which the toxicological examination was positive (presence of anticoagulants) in association with an anatomopathological picture indicative of blood clotting disorders (e.g., petechiae, ecchymoses, hyphema, pale mucosa, nasal, vaginal and ear bleeding, pulmonary hemorrhages and hemothorax, pleural and pericardial bleedings, subdural and cerebral bleedings, gastrointestinal bleeding, hemoperitoneum, hemorrhages and hematomas in the renal parenchyma, bleedings, and hematomas of liver parenchyma (Valchev et al., 2008; Lombardo et al., 2013). In the case of subjects with polytrauma from impact, the coagulopathy is not attributed to positivity to ARs as it could have been caused by the trauma of the impact.

One factor that can complicate the interpretation of the data is the state of preservation of the carcass, which when suboptimal, can alter the anatomopathological pictures and interfere with the finding of the ARs.

In this study we did not model the relationship between exposure to ARs and symptoms such as coagulopathies. While available literature indicates that ARs can affect the health of large carnivores (Fraser et al., 2018; Serieys et al., 2018), we lacked adequate information about some potentially important confounders. These include the time elapsed between ARs intake and the moment when a certain wolf was found, the amount of ARs ingested, pre-existing health conditions, the physiological or nutritional state of each individual, as well as genetic differences. Without this information, any comparison relating the amount of ARs to coagulopathies, or other diseases, could have produced spurious findings.

2.3. Statistical analyses

We modelled how the effect of landscape characteristics, measured at the sites where wolves had been found, affected *i*) their probability of testing positive to 1, 2, 3 or more ARs (among brodifacoum, bromadiolone, coumatetralyl, difenacoum, difethialone, flocoumafen), *ii*) the presence of brodifacoum and bromadiolone, the two most common ARs (see below), detected in their livers.

Rodent control in the study area is associated with urban areas and farms. In these environments, we expected wolves to be positive to a higher number of ARs, due to the higher exposure to contaminated rodents, which would be scavenged or hunted.

However, in anthropized areas, wolves could test positive to a greater number of ARs, simply due to a greater availability of retailers and rodenticides on the market. Pest control operators often combine different products, believing this practice to attain a greater effectiveness against rodents. To rule out this second hypothesis, we also modelled the concentration of the two most common rodenticides, brodifacoum and bromadiolone, in the liver of wolves. We did so as ARs are metabolized quickly by the liver, therefore only a standing assumption of these compounds from rodents can assure their presence in this organ. If the consumption of contaminated rodents is the main path of exposure, as wolves regularly consume them in anthropized environments, the probability of finding a wolf which consumed a contaminated rodent soon before dying would be systematically greater. In turn, the concentration of ARs in the liver would be systematically higher.

The environmental characteristics of recovery sites were quantified by aggregating important environmental attributes with Partitioning Around Medoids cluster analysis (Kassambara, 2017). Rather than using only the presence of human infrastructures, we opted for creating a composite index, reflecting both human presence and other important topographic and land cover characteristics of the study area. These included *i*) the presence of human infrastructures, by using the Human Footprint Index (Venter et al., 2016) as a proxy, *ii*) the percentage of tree cover and iii) croplands at a 250 m scale, measured through the MODIS/Terra Vegetation Continuous Fields (https://lpdaac.usgs.gov/products/mod44bv006/), *iv*) the elevation, *v*) the roughness of the terrain at each point and *vi*) the topographic position index, indicating if a certain point was on a mountain top or at the bottom of a valley (Wilson et al., 2007). Environmental variables were calculated as median values in a buffer with a radius of 6 km around the point. This size corresponded to an area of approx. 113 km^2, reflecting the most recent home range estimatesfor the species in Italy (Mancinelli et al., 2018; Mattioli et al., 2018).

The silhouette method, the elbow method, and the gap statistic method supported the existence of two different environmental conditions (Fig. S1). By overlaying them with satellite imagery of the study area, and by exploring the distribution of environmental characteristics in the two groups (Fig. S2), we noticed that the first group corresponded to relatively undisturbed areas on hills and mountains, with high levels of tree cover and rough terrain, and low presence of human infrastructures. On the other hand, the second group corresponded to lowland areas with a high presence of human infrastructures and croplands.

We modelled the probability of testing positive to multiple ARs through a Bayesian ordered-logit formulation (Bürkner and Vuorre, 2019). On the other hand, we used a zero-altered Gamma regression (Zuur et al., 2017) to model the presence of brodifacoum and bromadiolone detected in the liver of tested wolves.

In our models, we also controlled for the sex and age class of each individual, two potentially confounding variables that were measured as ordered variables with polynomial contrasts. Anthropization was deemed to be a potentially important predictor of positivity to ARs, as rodent control in the study area is mostly concentrated around urban areas and farms. Moreover, in anthropized landscapes, wolves could face a higher exposure to anticoagulants as they can regularly include rodents in their diet, due to the lack of ungulates. Finally, young male wolves were assumed to be more at risk of exposure from ARs, as this group is the most involved in dispersal (Ausband, 2022; Morales-González et al., 2022; Caniglia et al., 2014), when individuals cannot rely on pack hunting, thus shifting to smaller prey, like rodents. We used bivariate thin-plates splines to measure the spatial correlation of observations and a cyclic cubic spline to measure cyclic temporal variations in recoveries (Wood, 2006). Exploratory analyses indicated that predictors did not have any association between them, nor any spatial, or temporal pattern.

2.4. *Comparison with other recovered wildlife*

To reach a more complete understanding of temporal trends in wildlife exposure, we compared our findings about wolves, with positivity to ARs in other wildlife that has been recovered and tested for these compounds in the Emilia-Romagna region. Contrary to wolves that have always been tested for AR, only those individuals that showed signs of acquired coagulopathies on pathological examination were tested for AR. This dataset ($n = 176$), included recoveries of multiple species, that could prey on or consume dead rodents, which occurred between 2018 and 2022, mostly red fox (*Vulpes vulpes*, $n = 67$), common buzzard (*Buteo buteo*, $n = 23$), Eurasian badger (*Meles meles*, $n = 13$), wild boar (*Sus scrofa*, $n = 9$), European hedgehog (*Erinaceus europaeus*, n = 9), coypu ($n = 7$), house mouse and rats (n = 7), stone and pine marten (*Martes* sp., $n = 4$), and other diurnal ($n = 15$) and nocturnal ($n = 8$) raptors. Individuals were subjected to the same laboratory analyses that were used for wolves, and then we modelled the temporal trends of positivity to coumatetralyl, brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen. This dataset was used as a "control", to detect any temporal change in the use of rodenticides, at least for part of the study area. A Bayesian Generalized Additive model, with a cyclic cubic spline, and a Bernoulli distribution of the response, was used to model temporal fluctuations in the probability that a recovered animal was positive to rodenticides.

Model selection for both wolves and recovered wildlife followed a stepwise approach, starting from a null model, and then evaluating the effect of each covariate on the predictive accuracy of candidate models, through leave-one-out cross-validation (Vehtari et al., 2017). Statistical analyses were carried out with the statistical software R (R Core Team, 2023) and with STAN (Carpenter et al., 2017), through the 'brms' package (Bürkner, 2017). A reproducible dataset and software code is available at the following link: https://osf.io/yqv4n/

3. Results

Our findings indicate that most wolves ($n = 115/186$, 61.8 %) tested positive for ARs (1 compound, $n = 36$; 2 compounds, $n = 47$; 3 compounds, $n = 16$; 4 or more compounds, n = 16). The most common compounds were bromadiolone ($n = 97$), brodifacoum ($n = 93$) and difenacoum ($n = 26$, Fig. S3).

Overall, brodifacoum and bromadiolone were the ARs that co-occurred the most ($n = 61$), followed by brodifacoum and difenacoum ($n = 20$; Table S2). The mean concentrations of the various ARs are reported in Table S3.

Of the 115 wolves that tested positive for ARs, 19 presented an anatomopathological picture attributable to coagulopathies with evident coagulation alterations, while 96 died of other causes such as vehicle collision, gunshot, intraspecific aggression, diseases, and tested positive for ARs, even if in the absence of characteristic pathological lesions.

Leave-one-out cross-validation retained anthropization and the time when wolves were found as meaningful covariates. Compared to wolves from more remote areas, those from anthropized areas had a lower probability to test negative for ARs or to test positive for a single compound. Conversely wolves from more anthropized areas had a higher probability to test positive for 2, or more, ARs (Fig. 2). Moreover, wolves had a higher risk of testing positive for ARs from late summer to late winter, and this probability became higher after 2020, particularly the probability of testing positive to 3, or more, ARs (Fig. 3).

Overall, the R^2 of the best ordered logit model was 0.62 (McKelvey and Zavoina, 1975). Model selection indicated that wolves from more anthropized areas had also a higher concentration of brodifacoum in their liver (Fig. 4), while the concentration of bromadiolone was not significantly higher.

As for other wildlife species, positivity to ARs was found to be particularly high for the red fox, where 60 individuals out of 67 (89.6 %) showed traces of rodenticides. Moreover, also 18 buzzards out of 23 (78.3 %) were positive. However, when considering the temporal distribution of positivity to ARs, among all the various wildlife species, no clear trend emerged (Fig. S4).

A complete overview of model selection is given in Appendix S1.

4. Discussion

The grey wolf is now widespread in Italy, with an estimated population of 2945–3608 individuals (La Morgia et al., 2022), and a conservation status that changed from "Vulnerable" to "Near Threatened" during the last decade (Rondinini et al., 2022). Nevertheless, our findings highlight a concerning situation regarding the exposure of this species to both first and second-generation ARs.

In our opinion, our findings should raise concerns about *i*) our true understanding of wolf ecology in human-dominated landscapes, *ii*) the extent to which grey wolves in Italy, and more generally in Europe, might be subjected to secondary exposure to ARs and *iii*) the lack of selectivity of rodent control through ARs and the need to update regulations concerning their use.

4.1. *Understanding of wolf ecology in anthropized landscapes*

More than half of the wolves in our sample tested positive for one, or more, ARs, particularly after 2020. While we expected some individuals to show traces of rodenticides (Di Blasio et al., 2020), due to the trophic flexibility of the species, such a magnitude was largely unexpected. Moreover, both the number of ARs and the presence of brodifacoum in the liver of wolves, increased in those individuals that had been found in anthropized environments.

In Europe, wolves, while capable of exploiting many different types of prey, have traditionally been regarded as relying on wild ungulates or livestock (Zlatanova et al., 2014). Our findings indicate that rodents might be consumed regularly by certain individuals and in certain environmental conditions, particularly when wild ungulates and livestock are scarce. This consumption might happen either through predation or scavenging and involve both coypus and rats.

Ferretti et al. (2019) reported invasive alien coypu as important prey in croplands of Central Italy, whose importance can be comparable to that of the roe deer. Adult coypus weigh between 5 and 10 kg and could attain high densities (16–55 individuals/km of water bodies; Balestrieri et al., 2016), providing wolves with a biomass comparable to that of wild ungulates. As coypus are a major pest in Central and Northern Italy (Cocchi and Bertolino, 2021), evidence from local newspapers indicates that they are subjected to illegal baiting with ARs by farmers (Table S4).

Nomadic wolves moving in unfamiliar landscapes ("floaters", sensu Fuller et al., 2003) are probably more prone to become exposed to ARs. By not hunting large prey in groups (MacNulty et al., 2012), floaters usually shift to easier prey, such as livestock (Imbert et al., 2016). However, in anthropized environments, where livestock is not common, they might be preying on coypus, which are targeted by ARs. Considering that wolves around human settlements exploit human food waste

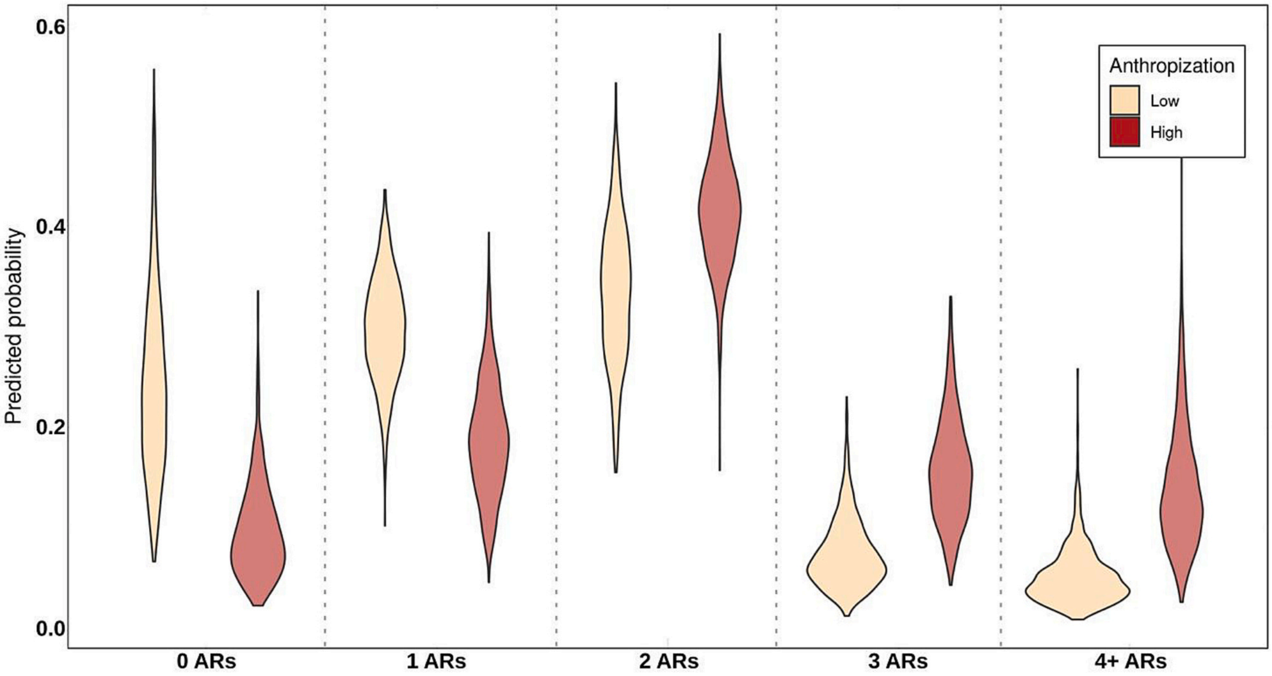

Fig. 2. Predicted probabilities that wolves tested positive for a certain number of anticoagulant rodenticides (ARs), between areas with different levels of anthropization. Conditional effect plot from the Bayesian ordered logit model, representing the posterior distribution: the largest section of the violin plot indicates values with the highest probability.

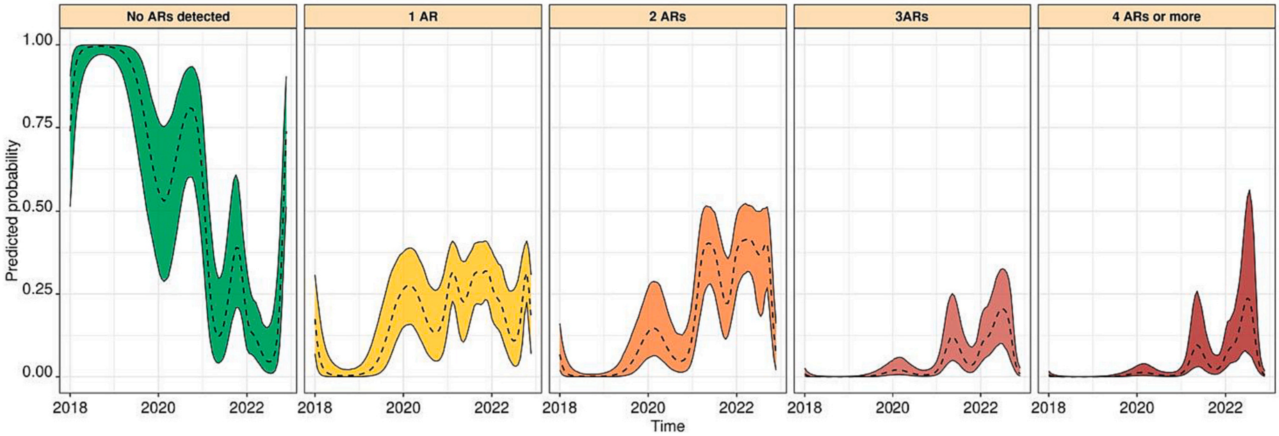

Fig. 3. Predicted probabilities that wolves tested positive for a certain number of anticoagulant rodenticides (ARs), through time. Conditional effect plot from the Bayesian ordered logit model.

and animal by-products, they could also prey on synanthropic rats, such as *R. rattus* or *R. norvegicus*, that also concentrate around these resources. Moreover, wolves are known for scavenge whenever they can, particularly when outside of a pack (Ciucci et al., 2020). Therefore, solitary wolves inhabiting anthropized areas might regularly scavenge on coypus and rats that have previously consumed ARs, and therefore bioaccumulate these compounds.

In our study area, as packs progressively saturated undisturbed habitats (Bassi et al., 2015), in the last 4–5 years floaters and young breeding pairs (Zanni et al., 2023) found new territories and settled mostly at anthropized areas in lowlands, and started relying heavily on rodents (Zanni et al., unpublished results). This pattern could have increased their exposure to ARs, and progressively led to the marked increase in positivity to ARs that was observed after 2020.

Interestingly, our temporal trends are only partially similar to those reported by Rial-Berriel et al. (2021) for the Canary Islands. Since 2018, when a restriction on the concentration of active ingredients in baits ($<$ 30 ppm) came into force, there has been a decrease in the concentration of these compounds in the liver of raptors but also an increase in their average number.

While we found both concentrations and the number of compounds increased in anthropized environments, we observed two different temporal trends. The number of compounds found in wolves increased after 2020, but we did not detect any temporal trend in concentrations. We have no clear explanation for this difference, but it could have been caused by an increased market availability and/or the hoarding of different ARs with different active principles. For example, Di Blasio et al. (2020) reported caches held by private citizens to be responsible for the presence of endosulfan in their samples, even years after this substance had been banned.

Thus, our study calls for a detailed assessment of wolf diet and movements in human-dominated landscapes, and how individuals

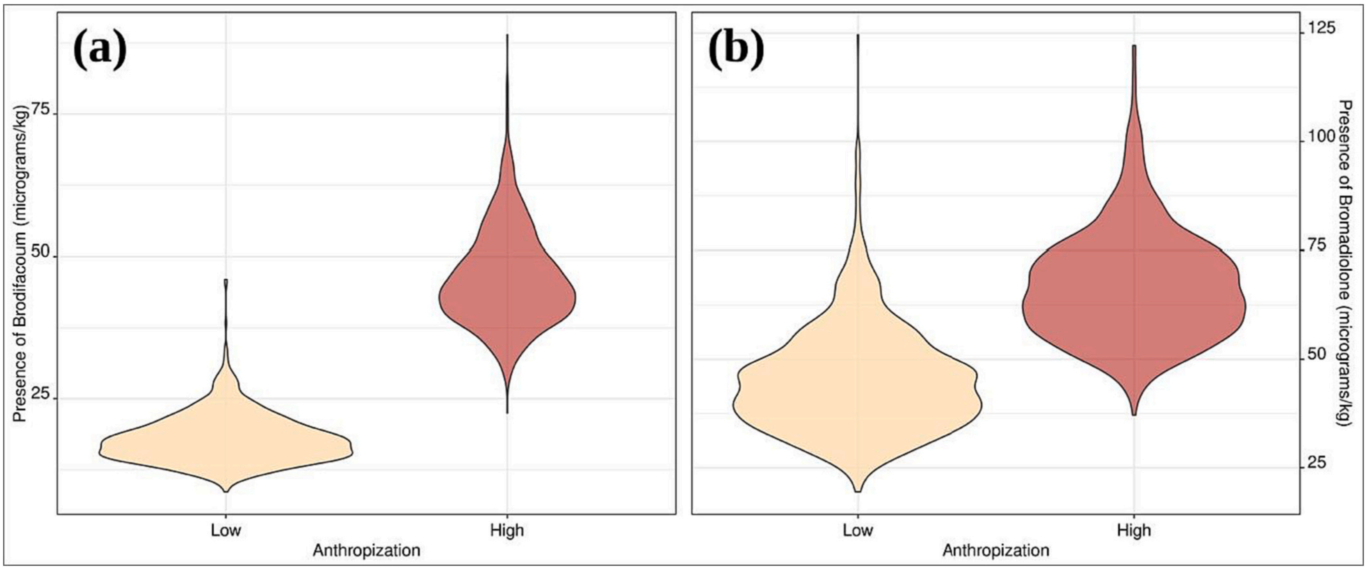

Fig. 4. Predicted concentrations of brodifacoum (a) and bromadiolone (b), expressed as micrograms per kg, between areas with different levels of anthropization. Conditional effect plot from the Bayesian zero-altered Gamma model, representing the posterior distribution: the largest section of the violin plot indicates values with the highest probability.

undergoing different life stages could change their diet. Moreover, our data is based on the opportunistic collection of dead wolves. While this method can be suitable to identify spatial patterns, it might suffer from the fact that we do not know how ARs are metabolized and excreted by wolves. Future research should explore the toxicokinetics of ARs in this species and develop methods accounting for temporal changes in the probability of detecting ARs (e.g. scat analysis, Prat-Mairet et al., 2017).

4.2. *Selectivity of chemical control of rodents*

After having detected for the first time a significant level of contamination of anticoagulants in wolves, our study is a warning on the penetration of anticoagulant rodenticides up to the top of the food chain of terrestrial ecosystem in Europe. Indeed, finding high frequencies of contamination in a species believed to prey mostly on ungulates raises serious concerns about the actual level of bioaccumulation that rodent control can determine, even in those species which are not specialized in rodents. While other studies previously suggested that ARs could be present in large carnivores, these were carried out on small samples. For example, Berny et al. (1997) tested a single individual of Eurasian lynx (*Lynx lynx*), while Riley et al. (2007) tested 4 mountain lions (*Puma concolor*). Our study, on the contrary, was carried out across a large sample of individuals and revealed a wide spatial spread, with significant temporal variations, in the use of rodenticides across the study area, affecting even some of the most persistent active ingredients.

Worldwide, rodenticides are the most widely used technique for rodent control (Capizzi et al., 2014). Empirical evidence suggests that rodenticides are used without adequate awareness and as a preventive measure, often resorting to so-called permanent baiting. Although this practice is explicitly banned in official EU documents, it still finds application in the daily practices of many professionals and amateurs engaged in rodent control, at least in Italy.

There is a need to identify integrated approaches to rodent control that can limit the use of rodenticides to those situations where these are truly needed, while prioritizing trapping and environmental sanitation elsewhere. Moreover, compounds with lower persistence and toxicity towards nontarget species should be preferred (e.g., cholecalciferol, Witmer, 2018). In regulating the use of these substances, environmental risk must be balanced with the social benefits of synanthropic rodent control (e.g., Van Den Brink et al., 2018).

Finally, to appreciate the real level of selectivity of ARs, further studies should also quantify the dispersal of contaminated rodents in the environment (Walther et al., 2021) and the extent to which they are predated, or scavenged, by large carnivores (Montaz et al., 2014).

4.3. *Exposure to ARs in expanding wolf populations and potential consequences for conservation*

The potential widespread positivity to ARs calls for the rapid creation of a pan-European surveillance network for toxic chemicals in recovering populations of large carnivores.

Our findings are based on a convenience sample that probably included more wolves from anthropized areas and/or undergoing nomadic behavior. Even if our level of exposure could hardly be taken as representative of the whole population in the study area, it reasonably indicates that exposure to ARs can involve a considerable number of individuals.

Although we believe that the consumption of contaminated rodents is the main pathway through which wolves become exposed to ARs, our widespread positivity could have also partially originated from deliberate poisoning attempts. In Italy, wolf persecution is not uncommon and sometimes carried out by means of poisonous baits (Musto et al., 2021), often containing ARs. While we believe that deliberate poisoning contributed marginally to our findings, due to the lack of spatial or temporal clustering that usually characterizes wildlife persecution (Faulkner et al., 2018) and the widespread positivity to ARs among foxes and diurnal raptors, future research about this phenomenon is needed.

Considering that rodent control is common in many other parts of Italy and Europe (Eisemann et al., 2018), where it already affects raptors (Gomez et al., 2022; Nakayama et al., 2019), smaller carnivores (Wright et al., 2022; Fernandez-de-Simon et al., 2019, 2022; Elmeros et al., 2018, 2019; López-Perea et al., 2019; Geduhn et al., 2015) and domestic pets (Calzetta et al., 2018; Berny et al., 2010) we believe that secondary exposure to ARs might be an overlooked phenomenon with regard to European wolf populations.

This could bear two consequences for wolves. The first one is toxicosis, which is suspected to be a relevant source of mortality for urban coyotes (*Canis latrans*) in North America (Poessel et al., 2015). This scenario might be realistic only for those wolves whose diet is largely based on rodents, and for old individuals, which might suffer from the progressive accumulation of AR residues (Rattner and Harvey, 2021). However, it is hard to make predictions about its impacts, as we currently do not have threshold values for ARs in the grey wolf.

On the other hand, there is evidence that ARs can amplify immune dysfunctions in carnivores, increasing their impact on mortality. For example, Serieys et al. (2015) found that bobcats (*Lynx rufus*), that had been exposed to ARs had a higher probability of having a severe level of mange. Subsequent studies (Fraser et al., 2018; Serieys et al., 2018) showed that this could be due to multiple impacts of ARs on the immune system, including on gene expression, that compromised the immune response of bobcats against mange. Moreover, ARs are suspected to affect pregnancies in domestic dogs (Fitzgerald et al., 2018) and their impacts can also be exacerbated by simultaneous exposure to multiple compounds (Serieys et al., 2015).

Of the 115 wolves tested positive for ARs, 19 presented an anatomopathological picture with blood clotting disorders as extensive, generalized, and multiple hemorrhages (Valchev et al., 2008; Lombardo et al., 2013). In detail, in the 19 wolves with pathological signs the most frequent gross findings were pulmonary hemorrhages and hemothorax, associated in some cases with: pleural and pericardial bleedings, subdural and cerebral bleedings, gastrointestinal bleeding and hemoperitoneum, hemorrhages in the renal parenchyma and visceral congestion. It should be noted that the majority of the positive wolves did not show an anatomopathological picture indicative of coagulation disorders. This could lead one to think that many positive wolves had sublethal concentrations which could have been a contributing cause of death. In facts, chronic exposure to ARs would have compromised the hepatic metabolism, coagulation, and behavior of wolves, undermining their capacity to react to dangerous situations (Fournier-Chambrillon et al., 2004; Valchev et al., 2008). Moreover, poisoned individuals, due to behavioral alteration and the incapacity to effectively hunt could be more prone to approach human settlements, remaining victims of car collisions or direct persecution (Musto et al., 2021). This may be somewhat of a shortcoming in the sampling strategy, but it is nonetheless something that is inevitably present in ecotoxicology studies based on the analysis of animals found dead, not affecting the consistency of the findings (Schwartz et al., 2020).

Although we still need to understand the extent to which ARs can affect the immune response in the grey wolf, their populations in Europe regularly experience infectious and parasitic diseases (Millán et al., 2016; Kołodziej-Sobocińska et al., 2014) and sometimes have low genetic variability (Hindrikson et al., 2017), two threats whose demographic impact could be magnified by sublethal exposure to ARs. Although it is unlikely that ARs affected wolves living in undisturbed areas, increased wolf mortality in anthropized landscapes could generate widespread and unpredictable source-sink dynamics. These scenarios are particularly concerning, given the increasing pressure in some areas of Europe for the lethal control of wolves, a practice whose long-term impact on wolf populations is still uncertain (Lennox et al., 2018; Treves et al., 2016), and the difficulties in monitoring wolf populations at a temporal and spatial resolution that would allow for adaptive management (Merli et al., 2023).

5. Conclusion

This study emphasizes one important fact: nowadays the use of ARs in European countries, like Italy, is so widespread that these compounds are affecting the entire food chain, and a significant number of non-target wildlife (Elliott et al., 2016), being a public health concern (Alabau et al., 2020; Di Blasio et al., 2020). Our data show that ARs can be found even in those species, like large carnivores, where they should be relatively uncommon. This fact bears three consequences for research, conservation and policymaking.

In terms of research, our findings emphasize the need to better understand the ecology of large carnivores inhabiting anthropized landscapes. Wolves are generally deemed to rely on wild and domestic ungulates, but their widespread exposure to ARs in anthropized areas might indicate that large rodents and rats are preyed or scavenged at a higher rate than previously thought. Therefore, we need to study the diet and movements of wolves inhabiting these environments, across their entire life cycle.

In terms of conservation, the widespread positivity to ARs in Italian wolves, calls for the urgent assessment of this phenomenon at a larger spatial scale. Rodent control is common in many European countries, and wolves living there might therefore be exposed to ARs as well. This could in turn amplify other threats like infectious/parasitic diseases and complicate the adaptive management of this species. Therefore, there is the urgent need to create a pan-European monitoring of ARs in wolves and other large carnivores, based on standardized anatomopathological and toxicological protocols.

Finally, our findings raise doubts about the real level of selectivity attained by rodent control, and the illegal use of ARs to kill coypus. In terms of policymaking, we believe that new regulations about the use of ARs should be defined and enforced to *i*) better monitor their use, *ii*) restrict their availability to citizens, *iii*) reduce permanent baiting and *iv*) encouraging integrated rodent control.

Ethical approval

Not applicable.

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CRediT authorship contribution statement

Carmela Musto: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jacopo Cerri:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Dario Capizzi:** Conceptualization, Supervision, Writing – review & editing. **Maria Cristina Fontana:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Silva Rubini:** Data curation, Writing – review & editing. **Giuseppe Merialdi:** Conceptualization, Data curation, Supervision, Writing – review & editing. **Duccio Berzi:** Conceptualization, Data curation, Writing – review & editing. **Francesca Ciuti:** Conceptualization, Data curation, Writing – review & editing. **Annalisa Santi:** Conceptualization, Data curation, Writing – review & editing. **Arianna Rossi:** Conceptualization, Data curation, Writing – review & editing. **Filippo Barsi:** Conceptualization, Data curation, Writing – review & editing. **Luca Gelmini:** Conceptualization, Data curation, Writing – review & editing. **Laura Fiorentini:** Conceptualization, Data curation, Writing – review & editing. **Giovanni Pupillo:** Conceptualization, Data curation, Writing – review & editing. **Camilla Torreggiani:** Conceptualization, Data curation, Writing – review & editing. **Alessandro Bianchi:** Conceptualization, Data curation, Writing – review & editing. **Alessandra Gazzola:** Conceptualization, Data curation, Writing – review & editing. **Paola Prati:** Conceptualization, Data curation, Writing – review & editing. **Giovanni Sala:** Conceptualization, Data curation, Writing – review & editing. **Marco Apollonio:** Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing. **Mauro Delogu:** Conceptualization, Supervision, Writing – review & editing. **Alberto Biancardi:** Conceptualization, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Laura Uboldi:** Conceptualization, Data curation, Writing – review & editing. **Alessandro Moretti:** Conceptualization, Data curation, Writing – review & editing. **Chiara Garbarino:** Data curation, Supervision, Writing – original draft, Writing – review &

editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data available via Open Science Framework Digital Repository: https://osf.io/yqv4n/

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at: https://osf.io/yqv4n/ and https://doi.org/10.1016/j.scitotenv.2024.169990.

References

Alabau, E., Mentaberre, G., Camarero, P.R., Castillo-Contreras, R., Sánchez-Barbudo, I.S., Conejero, C., Fernández-Bocharán, M.S., López-Olvera, J.R., Mateo, R., 2020. Accumulation of diastereomers of anticoagulant rodenticides in wild boar from suburban areas: implications for human consumers. Sci. Total Environ. 738, 139828 https://doi.org/10.1016/j.scitotenv.2020.139828.

Apollonio, M., Belkin, V.V., Borkowski, J., Borodin, O.I., Borowik, T., Cagnacci, F., Yanuta, G., 2017. Challenges and science-based implications for modern management and conservation of European ungulate populations. Mammal res. 62, 209–217. https://doi.org/10.1007/s13364-017-0321-5.

Ausband, D.E., 2022. Inherit the kingdom or storm the castle? Breeding strategies in a social carnivore. Ethology 128 (2), 152–158. https://doi.org/10.1111/eth.13250.

Balestrieri, A., Zenato, M., Fontana, E., Vezza, P., Remonti, L., Caronni, F.E., Saino, N., Prigioni, C., 2016. An indirect method for assessing the abundance of introduced pest *Myocastor coypus* (Rodentia) in agricultural landscapes. J. Zool. 298, 37–45. https://doi.org/10.1111/jzo.12284.

Bassi, E., Gazzola, A., Bongi, P., Scandura, M., Apollonio, M., 2020. Relative impact of human harvest and wolf predation on two ungulate species in Central Italy. Ecol. Res. 35 (4), 662–674. https://doi.org/10.1111/1440-1703.12130.

Bassi, E., Willis, S.G., Passilongo, D., Mattioli, L., Apollonio, M., 2015. Predicting the spatial distribution of wolf (*Canis lupus*) breeding areas in a mountainous region of Central Italy. PloS One 10 (6), e0124698. https://doi.org/10.1371/journal.pone.0124698.

Bassi, E., Canu, A., Firmo, I., Mattioli, L., Scandura, M., Apollonio, M., 2017. Trophic overlap between wolves and free-ranging wolf×dog hybrids in the Apennine Mountains, Italy. Glob. Ecol. Conserv. 9, 39–49. https://doi.org/10.1016/j.gecco.2016.11.002.

Berny, P.J., Buronfosse, T., Buronfosse, F., Lamarque, F., Lorgue, G., 1997. Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) by bromadiolone, a 4-year survey. Chemosphere 35 (8), 1817–1829. https://doi.org/10.1016/S0045-6535(97)00242-7.

Berny, P., Caloni, F., Croubels, S., Sachana, M., Vandenbroucke, V., Davanzo, F., Guitart, R., 2010. Animal poisoning in Europe. Part 2: companion animals. Vet. J. 183 (3), 255–259. https://doi.org/10.1016/j.tvjl.2009.03.034.

Bertero, A., Chiari, M., Vitale, N., Zanoni, M., Faggionato, E., Biancardi, A., Caloni, F., 2020. Types of pesticides involved in domestic and wild animal poisoning in Italy. Sci. Total Environ. 707, 136129 https://doi.org/10.1016/j.scitotenv.2019.136129.

Brasington, T.J., Hadley, J.M., Stahler, D.R., Stahler, E.E., Cassidy, K.A., 2023. A visual guide to wolf dentition and age determination. Wildl. Biol. 1–27. https://www.researchgate.net/publication/374921532_A_Visual_Guide_to_Wolf_Dentition_and_Age_Determination_For_Researchers_and_Wildlife_Professionals.

Bürkner, P.C., 2017. brms: an R package for Bayesian multilevel models using Stan. J. Stat. Softw. 80, 1–28. https://doi.org/10.18637/jss.v080.i01.

Bürkner, P.C., Vuorre, M., 2019. Ordinal regression models in psychology: a tutorial. Adv. Meth. Pract. Psychol. Sci. 2 (1), 77–101. https://doi.org/10.18637/jss.v080.i01.

Calzetta, L., Roncada, P., Piras, C., Soggiu, A., Liccardi, G., Mattei, M., Pistocchini, E., 2018. Geographical characteristics influencing the risk of poisoning in pet dogs: results of a large population-based epidemiological study in Italy. Vet. J. 235, 63–69. https://doi.org/10.1016/j.tvjl.2018.04.003.

Caniglia, R., Fabbri, E., Galaverni, M., Milanesi, P., Randi, E., 2014. Non-invasive sampling and genetic variability, pack structure, and dynamics in an expanding wolf population. J. Mammal. 95 (1), 41–59. https://doi.org/10.1644/13-MAMM-A-039.

Capitani, C., Bertelli, I., Varuzza, P., Scandura, M., Apollonio, M., 2004. A comparative analysis of wolf (*Canis lupus*) diet in three different Italian ecosystems. Mamm. Biol. 69 (1), 1–10. https://doi.org/10.1078/1616-5047-112.

Capizzi, D., Bertolino, S., Mortelliti, A., 2014. Rating the rat: global patterns and research priorities in impacts and management of rodent pests. Mamm. Rev. 44, 148–162. https://doi.org/10.1111/mam.12019.

Carpenter, B., Gelman, A., Hoffman, M.D., Lee, D., Goodrich, B., Betancourt, M., Riddell, A., 2017. Stan: a probabilistic programming language. J. Stat. Softw. 76 (1) https://doi.org/10.18637/jss.v076.i01.

Cimatti, M., Ranc, N., Benítez-López, A., Maiorano, L., Boitani, L., Cagnacci, F., Santini, L., 2021. Large carnivore expansion in Europe is associated with human population density and land cover changes. Divers. Distrib. 27 (4), 602–617. https://doi.org/10.1111/ddi.13219.

Ciucci, P., Mancinelli, S., Boitani, L., Gallo, O., Grottoli, L., 2020. Anthropogenic food subsidies hinder the ecological role of wolves: insights for conservation of apex predators in human-modified landscapes. Glob. Ecol. Cons. 21, e00841 https://doi.org/10.1016/j.gecco.2019.e00841.

Cocchi, R., Bertolino, S., 2021. Piano di gestione nazionale della nutria (*Myocastor coypus*). Istituto Superiore per la Protezione e la Ricerca Ambientale. https://terraevita.edagricole.it/wp-content/uploads/sites/11/2022/01/piano_gestione_nutria_10-2021.pdf.

Cunningham, A.A., Daszak, P., Wood, J.L., 2017. One health, emerging infectious diseases and wildlife: two decades of progress? Philos. Trans. R. Soc. Lond. B Biol. Sci. 372 (1725), 20160167 https://doi.org/10.1098/rstb.2016.0167.

Desforges, J.P., Hall, A., McConnell, B., Rosing-Asvid, A., Barber, J.L., Brownlow, A., Dietz, R., 2018. Predicting global killer whale population collapse from PCB pollution. Science 361 (6409), 1373–1376. https://doi.org/10.1126/science.aat1953.

Di Blasio, A., Bertolini, S., Gili, M., Avolio, R., Leogrande, M., Ostorero, F., Zoppi, S., 2020. Local context and environment as risk factors for acute poisoning in animals in northwest Italy. Sci. Total Environ. 709, 136016 https://doi.org/10.1016/j.scitotenv.2019.136016.

Di Marco, M., Boitani, L., Mallon, D., Hoffmann, M., Iacucci, A., Meijaard, E., Rondinini, C., 2014. A retrospective evaluation of the global decline of carnivores and ungulates. Conserv. Biol. 28 (4), 1109–1118. https://doi.org/10.1111/cobi.12249.

Di Minin, E., Slotow, R., Hunter, L.T., Montesino Pouzols, F., Toivonen, T., Verburg, P.H., Moilanen, A., 2016. Global priorities for national carnivore conservation under land use change. Sci. Rep. 6 (1), 1–9. https://doi.org/10.1038/srep23814.

Dondina, O., Orioli, V., Torretta, E., Merli, F., Bani, L., Meriggi, A., 2020. Combining ensemble models and connectivity analyses to predict wolf expected dispersal routes through a lowland corridor. PloS One 15 (2), e0229261. https://doi.org/10.1371/journal.pone.0229261.

Eisemann, J.D., Fisher, P.M., Buckle, A., Humphrys, S., 2018. An international perspective on the regulation of rodenticides. In: Anticoagulant Rodenticides and Wildlife. Springer, Cham, pp. 287–318. https://doi.org/10.1007/978-3-319-64377-9_11.

Elmeros, M., Lassen, P., Bossi, R., Topping, C.J., 2018. Exposure of stone marten (*Martes foina*) and polecat (*Mustela putorius*) to anticoagulant rodenticides: effects of regulatory restrictions of rodenticide use. Sci. Total Environ. 612, 1358–1364. https://doi.org/10.1016/j.scitotenv.2017.09.034.

Elliott, J.E., Rattner, B.A., Shore, R.F., Van Den Brink, N.W., 2016. Paying the pipers: mitigating the impact of anticoagulant rodenticides on predators and scavengers. BioScience 66 (5), 401–407. https://doi.org/10.1093/biosci/biw028.

Elmeros, M., Bossi, R., Christensen, T.K., Kjær, L.J., Lassen, P., Topping, C.J., 2019. Exposure of non-target small mammals to anticoagulant rodenticide during chemical rodent control operations. Environ. Sci. Pollut. Res. 26 (6), 6133–6140. https://doi.org/10.1007/s11356-018-04064-3.

Faulkner, S.C., Stevens, M.C., Romañach, S.S., Lindsey, P.A., Le Comber, S.C., 2018. A spatial approach to combatting wildlife crime. Conserv. Biol. 32 (3), 685–693. https://doi.org/10.1111/cobi.13027.

Fernandez-de-Simon, J., Coeurdassier, M., Couval, G., Fourel, I., Giraudoux, P., 2019. Do bromadiolone treatments to control grassland water voles (*Arvicola scherman*) affect small mustelid abundance? Pest Manag. Sci. 75 (4), 900–907. https://doi.org/10.1002/ps.5194.

Fernandez-de-Simon, J., Díaz-Ruiz, F., Jareño, D., Domínguez, J.C., Lima-Barbero, J.F., de Diego, N., Viñuela, J., 2022. Weasel exposure to the anticoagulant rodenticide bromadiolone in agrarian landscapes of southwestern Europe. Sci. Total Environ. 155914 https://doi.org/10.1016/j.scitotenv.2022.155914.

Ferretti, F., Lovari, S., Mancino, V., Burrini, L., Rossa, M., 2019. Food habits of wolves and selection of wild ungulates in a prey-rich Mediterranean coastal area. Mamm. Biol. 99, 119–127. https://doi.org/10.1016/j.mambio.2019.10.008.

Fitzgerald, S.D., Martinez, J., Buchweitz, J.P., 2018. An apparent case of brodifacoum toxicosis in a whelping dog. J. Vet. Diagn. Invest. 30 (1), 169–171. https://doi.org/10.1177/1040638717741664.

Fourel, I., Damin-Pernik, M., Benoit, E., Lattard, V., 2017. Core-shell LC-MS/MS method for quantification of second-generation anticoagulant rodenticides diastereoisomers in rat liver in relationship with exposure of wild rats. J. Chromatogr. B Biomed. Appl. 1041-1042, 120–132. https://doi.org/10.1016/j.chroma.2019.460848.

Fournier-Chambrillon, C., Berny, P.J., Coiffier, O., Barbedienne, P., Dasse, B., Delas, G., Galineau, H., Mazet, A., Pouzenc, P., Rosoux, R., Fournier, P., 2004. Evidence of secondary poisoning of free-ranging riparian mustelids by anticoagulant rodenticides in France: implications for conservation of European mink (*Mustela lutreola*). J. Wildl. Dis. 40, 688–695. https://doi.org/10.7589/0090-3558-40.4.688.

Fraser, D., Mouton, A., Serieys, L.E., Cole, S., Carver, S., Vandewoude, S., Wayne, R., 2018. Genome-wide expression reveals multiple systemic effects associated with detection of anticoagulant poisons in bobcats (*Lynx rufus*). Mol. Ecol. 27 (5), 1170–1187. https://doi.org/10.1111/mec.14531.

Fuller, T.K., Mech, L.D., Cochrane, J.F., 2003. Wolf population dynamics. In: Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, IL, USA. Pacific climatic effects on ungulate recruitment, 481, pp. 161–191. https://www.wrrb.ca/sites/default/files/Fuller%202003.pdf.

Geduhn, A., Jacob, J., Schenke, D., Keller, B., Kleinschmidt, S., Esther, A., 2015. Relation between intensity of biocide practice and residues of anticoagulant rodenticides in red foxes (*Vulpes vulpes*). PloS One 10 (9), e0139191. https://doi.org/10.1371/journal.pone.0139191.

Gomez, E.A., Hindmarch, S., Smith, J.A., 2022. Conservation letter: raptors and anticoagulant rodenticides. J. Raptor Res. 56 (1), 147–153. https://doi.org/10.3356/JRR-20-122.

Hindrikson, M., Remm, J., Pilot, M., Godinho, R., Stronen, A.V., Baltrūnaité, L., Saarma, U., 2017. Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management. Biol. Rev. 92 (3), 1601–1629. https://doi.org/10.1111/brv.12298.

Imbert, C., Caniglia, R., Fabbri, E., Milanesi, P., Randi, E., Serafini, M., Torretta, E., Meriggi, A., 2016. Why do wolves eat livestock?: factors influencing wolf diet in northern Italy. Biol. Conserv. 195, 156–168. https://doi.org/10.1016/j.biocon.2016.01.003.

Ingeman, K.E., Zhao, L.Z., Wolf, C., Williams, D.R., Ritger, A.L., Ripple, W.J., Stier, A.C., 2022. Glimmers of hope in large carnivore recoveries. Sci. Rep. 12 (1), 10005 https://doi.org/10.1038/s41598-022-13671-7.

Kassambara, A., 2017. Practical guide to cluster analysis in R: unsupervised machine learning (Vol. 1). Sthda. http://www.sthda.com/english/articles/25-clusteranalysis-in-r-practical-guide/.

Kauffman, M.J., Aikens, E.O., Esmaeili, S., Kaczensky, P., Middleton, A., Monteith, K.L., Goheen, J.R., 2021. Causes, consequences, and conservation of ungulate migration. Annu. Rev. Ecol. Evol. Syst. 52, 453–478. https://doi.org/10.1146/annurev-ecolsys-012021-011516.

Köhler, H.R., Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341 (6147), 759–765. https://doi.org/10.1126/science.1237591.

Kołodziej-Sobocińska, M., Zalewski, A., Kowalczyk, R., 2014. Sarcoptic mange vulnerability in carnivores of the Białowieża Primeval Forest, Poland: underlying determinant factors. Ecol. Res. 29, 237–244. https://doi.org/10.1007/s11284-013-1118-x.

La Morgia, V., Marucco, F., Aragno, P., Salvatori, V., Gervasi, V., De Angelis, D., Fabbri, E., Caniglia, R., Velli, E., Avanzinelli, E., Boiani, M.V., Genovesi, P., 2022. Stima della distribuzione e consistenza del lupo a scala nazionale 2020/2021. Relazione tecnica realizzata nell'ambito della convenzione ISPRA-Ministero della Transizione Ecologica "Attività di monitoraggio nazionale nell'ambito del Piano di Azione del lupo". https://www.isprambiente.gov.it/it/attivita/biodiversita/monitoraggio-nazionale-del-lupo/file-monitoraggio/report-nazionale-lupo-20_21.pdf.

Lennox, R.J., Gallagher, A.J., Ritchie, E.G., Cooke, S.J., 2018. Evaluating the efficacy of predator removal in a conflict-prone world. Biol. Conserv. 224, 277–289. https://doi.org/10.1016/j.biocon.2018.05.003.

Lestrade, M., Vergne, T., Guinat, C., Berny, P., Lafitte, J., Novella, C., Le Loc'h, G., 2021. Risk of anticoagulant rodenticide exposure for mammals and birds in Parc national des Pyrénées, France. J. Wildl. Dis. 57, 637–642. https://doi.org/10.7589/JWD-D-20-00125.

Lombardo, A., Ambrogi, C., Corrias, F., Ragona, G., Fico, R., Brajon, G., 2013. Anatomopathological surveys in the case of intentional poisoning in the provinces of Florence, Prato and Pistoia [conference poster]. In XV Congresso Nazionale SI Di. LV, Monreale (PA), Italia, 23-25 ottobre 2013 (pp. 310-311). Società Italiana di Diagnostica di Laboratorio Veterinaria (SIDiLV). https://www.cabdirect.org/cabdirect/abstract/20173354941.

López-Perea, J.J., Camarero, P.R., Sanchez-Barbudo, I.S., Mateo, R., 2019. Urbanization and cattle density are determinants in the exposure to anticoagulant rodenticides of non-target wildlife. Environ. Pollut. 244, 801–808. https://doi.org/10.1016/j.envpol.2018.10.101.

MacNulty, D.R., Smith, D.W., Mech, L.D., Vucetich, J.A., Packer, C., 2012. Nonlinear effects of group size on the success of wolves hunting elk. Behav. Ecol. 23 (1), 75–82. https://doi.org/10.1093/beheco/arr159.

Mancinelli, S., Boitani, L., Ciucci, P., 2018. Determinants of home range size and space use patterns in a protected wolf (*Canis lupus*) population in the central Apennines, Italy. Can. J. Zool. 96 (8), 828–838. https://doi.org/10.1139/cjz-2017-0210.

Mattioli, L., Capitani, C., Gazzola, A., Scandura, M., Apollonio, M., 2011. Prey selection and dietary response by wolves in a high-density multi-species ungulate community. Eur. J. Wildl. Res. 57 (4), 909–922. https://doi.org/10.1007/s10344-011-0503-4.

Mattioli, L., Canu, A., Passilongo, D., Scandura, M., Apollonio, M., 2018. Estimation of pack density in grey wolf (*Canis lupus*) by applying spatially explicit capture-recapture models to camera trap data supported by genetic monitoring. Front. Zool. 15 (1), 1–15. https://doi.org/10.1186/s12983-018-0281-x.

McKelvey, R.D., Zavoina, W., 1975. A statistical model for the analysis of ordinal level dependent variables. J. Math. Soc. 4, 103–120. https://doi.org/10.1080/0022250X.1975.9989847.

McMillin, S.C., Poppenga, R.H., Chandler, S.C., Clifford, D.L., 2018. Anticoagulant rodenticide residues in game animals in California. https://digitalcommons.unl.edu/icwdm_usdanwrc/2537/.

Merli, E., Mattioli, L., Bassi, E., Bongi, P., Berzi, D., Ciuti, F., Luccarini, S., Morimando, F., Viviani, V., Caniglia, R., et al., 2023. Estimating Wolf population size and dynamics by field monitoring and demographic models: implications for management and conservation. Animals 13, 1735. https://doi.org/10.3390/ani13111735.

Milanesi, P., Meriggi, A., Merli, E., 2012. Selection of wild ungulates by wolves Canis lupus (L. 1758) in an area of the northern Apennines (North Italy). Ethol. Ecol. Evol. 24 (1), 81–96. https://doi.org/10.1080/03949370.2011.592220.

Millán, J., López-Bao, J.V., García, E.J., Oleaga, Á., Llaneza, L., Palacios, V., Esperón, F., 2016. Patterns of exposure of Iberian wolves (*Canis lupus*) to canine viruses in human-dominated landscapes. Ecohealth 13 (1), 123–134. https://doi.org/10.1007/s10393-015-1074-8.

Montaz, J., Jacquot, M., Coeurdassier, M., 2014. Scavenging of rodent carcasses following simulated mortality due to field applications of anticoagulant rodenticide. Ecotoxicology 23, 1671–1680. https://doi.org/10.1007/s10646-014-1306-7.

Morales-González, A., Fernández-Gil, A., Quevedo, M., Revilla, E., 2022. Patterns and determinants of dispersal in grey wolves (*Canis lupus*). Biol. Rev. 97 (2), 466–480. https://doi.org/10.1111/brv.12807.

Mori, E., Benatti, L., Lovari, S., Ferretti, F., 2017. What does the wild boar mean to the wolf? Eur. J. Wildl. Res. 63 (1), 1–5. https://doi.org/10.1007/s10344-016-1060-7.

Musto, C., Cerri, J., Galaverni, M., Caniglia, R., Fabbri, E., Mucci, N., Bonilauri, P., Maioli, G., Fontana, M.C., Gelmini, L., Prosperi, A., Rossi, A., Garbarino, C., Fiorentini, L., Ciuti, F., Berzi, D., Merialdi, G., Delogu, M., 2021. Men and wolves: anthropogenic causes are an important driver of wolf mortality in human-dominated landscapes in Italy. Glob. Ecol. Cons. 32, e01892 https://doi.org/10.1016/j.gecco.2021.e01892.

Nakayama, S.M., Morita, A., Ikenaka, Y., Mizukawa, H., Ishizuka, M., 2019. A review: poisoning by anticoagulant rodenticides in non-target animals globally. J. Vet. Med. Sci. 81 (2), 298–313. https://doi.org/10.1292/jvms.17-0717.

Newsome, T.M., Boitani, L., Chapron, G., Ciucci, P., Dickman, C.R., Dellinger, J.A., Ripple, W.J., 2016. Food habits of the world's grey wolves. Mamm. Rev. 46 (4), 255–269. https://doi.org/10.1111/mam.12067.

Poessel, S.A., Breck, S.W., Fox, K.A., Gese, E.M., 2015. Anticoagulant rodenticide exposure and toxicosis in coyotes (*Canis latrans*) in the Denver metropolitan area. J. Wildl. Dis. 51 (1), 265–268. https://doi.org/10.7589/2014-04-116.

Prat-Mairet, Y., Fourel, I., Barrat, J., Sage, M., Giraudoux, P., Coeurdassier, M., 2017. Non-invasive monitoring of red fox exposure to rodenticides from scats. Ecol. Indic. 72, 777–783. https://doi.org/10.1016/j.ecolind.2016.08.058.

R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rattner, B.A., Harvey, J.J., 2021. Challenges in the interpretation of anticoagulant rodenticide residues and toxicity in predatory and scavenging birds. Pest Manag. Sci. 77, 604–610. https://doi.org/10.1002/ps.6137.

Rial-Berriel, C., Acosta-Dacal, A., Zumbado, M., Henríquez-Hernández, L.A., Rodríguez-Hernández, Á., Macías-Montes, A., Boada, L.D., Travieso-Aja, M.D.M., Martin-Cruz, B., Suárez-Pérez, A., Cabrera-Pérez, M.Á., Luzardo, O.P., 2021. Epidemiology of animal poisonings in the Canary Islands (Spain) during the period 2014-2021. Toxics 14 (9(10)), 267. https://doi.org/10.3390/toxics9100267.

Riley, S.P.D., Bromley, C., Poppenga, R.H., Uzal, F.A., Whited, L., Sauvajot, R.M., 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban Southern California. J. Wildl. Manag. 71, 1874–1884. https://doi.org/10.2193/2005-615.

Rodríguez-Estival, J., Mateo, R., 2019. Exposure to anthropogenic chemicals in wild carnivores: a silent conservation threat demanding long-term surveillance. Curr. Opin. Environ. Sci. Health 11, 21–25. https://doi.org/10.1016/j.coesh.2019.06.002.

Rondinini, C., Battistoni, A., Teofili, C., 2022. Lista Rossa IUCN dei vertebrati italiani 2022 Comitato Italiano IUCN e Ministero dell'Ambiente e della Sicurezza Energetica, Roma. http://www.iucn.it/pdf/Lista-Rossa-vertebratiitaliani-2022.pdf.

Rudd, J.L., McMillin, S.C., Kenyon Jr., M.W., Clifford, D.L., Poppenga, R.H., 2018. Prevalence of first and second-generation anticoagulant rodenticide exposure in California mountain lions (*Puma concolor*). In: Proceedings of the Vertebrate Pest Conference (Vol. 28, No. 28). https://doi.org/10.5070/V42811046.

Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., Arnold, K.E., 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. Proc. Royal Soc. B P ROY SOC B-BIOL SCI 285 (1885), 20181297. https://doi.org/10.1098/rspb.2018.1297.

Schwartz, A.L.W., Shilling, F.M., Perkins, S.E., 2020. The value of monitoring wildlife roadkill. Eur. J. Wildl. Res. 66, 18. https://doi.org/10.1007/s10344-019-1357-4.

Serieys, L.E., Armenta, T.C., Moriarty, J.G., Boydston, E.E., Lyren, L.M., Poppenga, R.H., Crooks, K.R., Wayne, R.K., Riley, S.P., 2015. Anticoagulant rodenticides in urban bobcats: exposure, risk factors and potential effects based on a 16-year study. Ecotoxicology 24 (4), 844–862. https://doi.org/10.1007/s10646-015-1429-5.

Serieys, L.E., Lea, A.J., Epeldegui, M., Armenta, T.C., Moriarty, J., VandeWoude, S., Carver, S., Foley, J., Wayne, R.K., Riley, S.P.D., Uittenbogaart, C.H., 2018. Urbanization and anticoagulant poisons promote immune dysfunction in bobcats. Proc. Royal Soc. B P ROY SOC B-BIOL SCI 285 (1871), 20172533. https://doi.org/10.1098/rspb.2017.2533.

Serieys, L.E., Bishop, J., Okes, N., Broadfield, J., Winterton, D.J., Poppenga, R.H., Viljoen, S., Wayne, R.K., O'Riain, M.J., 2019. Widespread anticoagulant poison exposure in predators in a rapidly growing South African city. Sci. Total Environ. 666, 581–590. https://doi.org/10.1016/j.scitotenv.2019.02.122.

Sinergitech, 2020. In: Sinergitech (Ed.), Dossier roditori. Ricognizione sulla presenza nel mercato italiano delle formulazioni di uso ratticida, 63 pp. https://www.aidpi.it/news/disponibile-dossier-rodenticidi/.

Torquetti, C.G., Guimarães, A.T.B., Soto-Blanco, B., 2021. Exposure to pesticides in bats. Sci. Total Environ. 755, 142509 https://doi.org/10.1016/j.scitotenv.2020.142509.

Treves, A., Krofel, M., McManus, J., 2016. Predator control should not be a shot in the dark. Front. Ecol. Environ. 14 (7), 380–388. https://doi.org/10.1002/fee.1312.

Valchev, I., Binev, R., Yordanova, V., Nikolov, Y., 2008. Anticoagulant rodenticide intoxication in animals – a review. Turk. J. Vet. Anim. Sci. 32, 4. https://journals.tubitak.gov.tr/veterinary/vol32/iss4/1.

Van Den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A., 2018. Anticoagulant rodenticides and wildlife: introduction. In: Van Den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A. (Eds.), Anticoagulant Rodenticides and Wildlife, pp. 1–9. https://doi.org/10.1007/978-3-319-64377-9_1.

Vandenbroucke, V., Desmet, N., De Backer, P., Croubels, S., 2008. Multi-residue analysis of eight anticoagulant rodenticides in animal plasma and liver using liquid chromatography combined with heated electrospray ionization tandem mass spectrometry. J. Chromatography B. 869, 101–110. https://doi.org/10.1016/j.jchromb.2008.05.011.

Vehtari, A., Gelman, A., Gabry, J., 2017. Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Stat. Comput. 27 (5), 1413–1432. https://doi.org/10.1007/s11222-016-9696-4.

Venter, O., Sanderson, E.W., Magrach, A., Allan, J.R., Beher, J., Jones, K.R., Watson, J.E., 2016. Global terrestrial human footprint maps for 1993 and 2009. Sci. Data. 3 (1), 1–10. https://doi.org/10.1038/ncomms12558.

Walther, B., Ennen, H., Geduhn, A., Schlötelburg, A., Klemann, N., Endepols, S., Schenke, D., Jacob, J., 2021. Effects of anticoagulant rodenticide poisoning on spatial behaviour of farm dwelling Norway rats. Sci. Total Environ. 787, 147520 https://doi.org/10.1016/j.scitotenv.2021.147520.

Wilson, M.F., O'Connell, B., Brown, C., Guinan, J.C., Grehan, A.J., 2007. Multiscale terrain analysis of multibeam bathymetry data for habitat mapping on the continental slope. Mar. Geod. 30 (1–2), 3–35. https://doi.org/10.1080/01490410701295962.

Witmer, G.W., 2018. Perspectives on existing and potential new alternatives to anticoagulant rodenticides and the implications for integrated pest management. In: Van Den Brink, N.W., Elliott, J.E., Shore, R.F., Rattner, B.A. (Eds.), Anticoagulant Rodenticides and Wildlife, pp. 357–378. https://doi.org/10.1007/978-3-319-64377-9_13.

Wolf, C., Ripple, W.J., 2016. Prey depletion as a threat to the world's large carnivores. Royal Soc. Open Sci. 3 (8), 160252 https://doi.org/10.1098/rsos.160252.

Wood, S.N., 2006. Generalized Additive Models: An Introduction With R. Chapman and Hall/CRC. https://doi.org/10.1201/9781315370279.

Wright, P.G., Croose, E., Macpherson, J.L., 2022. A global review of the conservation threats and status of mustelids. Mamm. Rev. https://doi.org/10.1111/mam.12288.

Zala, S.M., Penn, D.J., 2004. Abnormal behaviours induced by chemical pollution: a review of the evidence and new challenges. Anim. Behav. 68 (4), 649–664. https://doi.org/10.1016/j.anbehav.2004.01.005.

Zanni, M., Brogi, R., Merli, E., Apollonio, M., 2023. The wolf and the city: insights on wolves conservation in the Anthropocene. Anim. Conserv. https://doi.org/10.1111/acv.12858.

Zlatanova, D., Ahmed, A., Valasseva, A., Genov, P., 2014. Adaptive diet strategy of the Wolf (*Canis lupus* L.) in Europe: a review. Acta zool. Bulg. 66 (4), 439–452.

Zuur, A.F., Ieno, E.N., Saveliev, A.A., 2017. Spatial, temporal and spatial-temporal ecological data analysis with R-INLA. Highland Statistics Ltd, 1. https://www.highstat.com/index.php/books2?view=article&id=11&catid=18.