

1 **Title:** Rodenticides now ubiquitous in wildlife of Italian landscapes

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24 therefore not yet been accepted on a peer-reviewed journal. Be careful in interpreting and citing its  
25 results.

24 **Abstract**

25 Second Generation Anticoagulant Rodenticides (SGARs) are used to control synantropic rodents and  
26 therefore also affect non-target wildlife. However, no study tested for their presence across a whole  
27 assemblage of wildlife, limiting our understanding of their overall circulation and exposure pathways.

28 We tested for SGARs in a large sample of terrestrial mammals (n = 403) and birds (n = 189), that were  
29 recovered in Northern Italy. Species included herbivores, facultative scavengers as well as small,  
30 medium and large predators. Almost all small carnivores, owls, diurnal raptors, mesocarnivores and  
31 large carnivores tested positive to at least one SGAR. We also detected SGARs in non-target rodents,  
32 such as the crested porcupine (*Hystrix cristata*), the coypu (*Myocastor coypus*), the edible dormouse  
33 (*Glis glis*) and the red squirrel (*Sciurus vulgaris*).

34 Our findings indicate that SGARs could now be widespread between different mammals and birds in  
35 Europe. While early studies pinpointed their presence in predators, we detected them in different  
36 functional groups, including facultative scavengers and herbivores. This exposure also affects areas  
37 with limited human settlements.

38 This pervasive exposure to SGARs can potentially destabilize interspecific relationships, such as  
39 prey-predator dynamics, and have cascading effects on ecosystems. It is therefore necessary to  
40 estimate its demographic impacts on target and non-target species, while also implementing rules i)  
41 restricting the use of SGARs to professional pest control operators, ii) obliging competent authorities  
42 to monitor their application, selectivity and circulation in the food chain and iii) prioritizing  
43 alternative measures under an integrated pest management framework.

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## 48 **1. Introduction**

49 Commensal and invasive rodents can inflict severe economic damage, pose significant risks for public  
50 health and threaten native biodiversity, making their management a global priority (Capizzi et al.,  
51 2014). To mitigate these critical and otherwise often unmanageable impacts, anticoagulant  
52 rodenticides (ARs), particularly Second-Generation Anticoagulant Rodenticides (SGARs), have  
53 become the most ubiquitous method for rodent control worldwide, across urban and agricultural  
54 landscapes (Jacob and Buckle, 2018), a phenomenon that contributed to the emergence of  
55 anticoagulant-resistant rodent populations carrying *Vkorc1* mutations (Reggiani et al., 2025).

56 Despite the implementation of mitigation measures and theoretical assumptions regarding the spatial  
57 behavior of intoxicated rodents—which are intended to minimize non-target risks (Walther et al.,  
58 2021)—SGARs are increasingly detected in non-target small mammals and throughout the whole  
59 food chain (Shore and Coeurdassier, 2018). This widespread contamination occurs not only through  
60 the direct accidental consumption of baits or the malicious use of SGARs against larger wildlife  
61 (Musto et al., 2024), but fundamentally through the delayed mode of action of these toxicants. Since  
62 anticoagulants take several days to induce mortality, intoxicated rodents often continue to feed at the  
63 baiting points, accumulating multiples of the lethal dose in their tissues. These highly contaminated  
64 individuals then disperse into the surrounding environment, where they are preyed or scavenged  
65 (Elmeros et al., 2019), effectively transferring massive amounts of SGARs up the food chain. SGARs  
66 are also increasingly detected in freshwater (Regnery et al., 2019; Gómez-Canela et al., 2014) and the  
67 soil (Acosta-Dacal et al., 2021), posing significant challenges in terms of remediation.

68 In the last two decades, a growing number of studies focused on the impact of SGARs on bird and  
69 mammal predators (López-Perea and Mateo, 2017; Nakayama et al., 2019). The level of  
70 contamination by ARs is generally deemed to be particularly severe among small and medium-sized  
71 diurnal raptors and owls, due to their specialization on rodents (Carromeu-Santos et al., 2025; Garcês  
72 et al., 2023; Gomez et al., 2022; Elliott et al., 2024). This has sometimes reduced their populations  
73 (Coeurdassier et al., 2019; Young et al., 1997), an aspect which can potentially have important

74 cascading consequences over rodent populations, ecosystems and human activities (Donázar et al.,  
75 2016; Formoso et al., 2025). As for mammals, SGARs are also commonly detected in small- and  
76 medium-sized carnivores, such as Mustelidae, Felidae or Canidae (Keating et al., 2024).  
77 Recent studies, however, highlighted important levels of SGARs also in several species of large  
78 carnivores (Coeurdassier et al., 2026; Musto et al., 2024; Serieys et al., 2015; Yovovich et al., 2024),  
79 as well as in large raptors and vultures (Herring et al., 2022; Oliva Vidal et al., 2024). This issue is  
80 particularly concerning for the conservation of these species, which remain among the most  
81 endangered vertebrates globally, but also for the ecosystem services they deliver. In facts, large  
82 carnivores and raptors regulate ecosystems by controlling prey and mesopredators, as well as by  
83 altering their distribution and foraging patterns (Ripple et al., 2016; Terraube and Bretagnolle, 2018;  
84 Wilmers et al., 2025), while vultures deliver irreplaceable ecosystem services as scavengers (Carucci  
85 et al., 2022).

86 SGARs poisoning in wildlife it is associated with both acute mortality events and sublethal effects  
87 (Serieys et al., 2018). Therefore, although considerable uncertainty still surrounds their impact on  
88 mortality and demographic trends of non-target species, understanding the extent to which ARs affect  
89 different groups of predators is crucial to anticipate the complexity, scale and magnitude of their  
90 potential large-scale ecological impacts. For example, a reduction in large predators could negatively  
91 affect small vertebrates and their ecosystem functions, by increasing mesopredators (Prugh et al.,  
92 2009) or by reducing safe areas in the landscape of fear (Burgas et al., 2021; Virgós et al., 2021).  
93 Moreover, many predator assemblages are characterized by important interspecific dynamics  
94 (Kuijper et al., 2024; Lourenço et al., 2011; Sergio and Hiraldo, 2008; Virgós et al., 2020), which  
95 could be destabilized by mortality from ARs. Comparing the positivity to ARs between different  
96 groups of predators is also important to better understand potential pathways of exposure (Musto et  
97 al., 2024), also with respect to landscape anthropization, as recent literature emphasized its role over  
98 shifts in the feeding ecology of small, medium and large predators (Boal et al., 2018; Caspi et al.,

99 2025; Cerri et al., 2026; Eeraerts et al., 2022; Hisano et al., 2021; Majumder et al., 2025; Mikkola and  
100 Sieradzki, 2025; Tornberg et al., 2026).

101 While meta-analyses can summarize the incidence of SGARs between different species of wildlife  
102 (e.g. Nakayama et al., 2019), their results can be biased by differences in laboratory methodologies for  
103 detecting SGARs, which can differ between study areas and have changed through time (Valverde et  
104 al., 2021). There is therefore a need for empirical studies comparing SGARs in different functional  
105 groups from the same geographical area and time, through harmonized laboratory protocols, to  
106 appreciate group-specific exposures.

107 In this study we aim to address this gap by simultaneously considering the incidence of SGARs in a  
108 large sample of large rodents (n = 12), small carnivores (n = 27), diurnal raptors (n = 71), owls (n =  
109 83), mesocarnivores (n = 164) and large carnivores (n = 200), that were recovered or found dead in  
110 Northern Italy between 2022 and 2024. Our findings highlight an extremely concerning scenario,  
111 where: *i*) the positivity to SGARs is widespread between different groups of wildlife, *ii*) there is no  
112 clear effect of landscape naturalness over the presence of SGARs, which is high everywhere. This  
113 emphasizes the pervasiveness of exposure to SGARs across landscape and their circulation across  
114 food webs, ultimately calling for stricter regulations concerning their use.

115

## 116 **2. Materials and Methods**

### 117 **2.1 Study area and data collection**

118 The study area encompasses the Emilia-Romagna region, in Northern Italy, spanning over 22,510  
119 km<sup>2</sup> and hosting a population of 4,482,977 residents (199.15 residents/km<sup>2</sup>). The study area includes a  
120 wide range of temperate ecosystems, with mountainous and hilly areas in the Apennines and extensive  
121 lowlands across the Po Valley. Urbanization and intensive agriculture are mainly concentrated in the  
122 plains, while more natural and semi-natural habitats prevail in the uplands (Fig. 1). Therefore, rodent  
123 control activities involving anticoagulant compounds are widespread in lowlands, particularly in rural  
124 and/or urban areas. Rodent control in the study area is regulated under European Commission

125 Regulations No. 582/2012, No. 1107/2009, and No. 1062/2014, which authorize the use of both first-  
126 generation (coumatetralyl) and second-generation (SGARs, brodifacoum, bromadiolone,  
127 chlorophacinone, chloralosiso, difenacoum, difethialone, diphacinone, flocoumafen) anticoagulants.  
128 During 2022-2024 wildlife Emilia Romagna regional monitoring plan activities we tested for SGARs  
129 in 189 birds and 403 mammals. Birds included the little Owl (*Athene noctua*, n = 46), the long-eared  
130 owl (*Asio otus*, n = 12), the common buzzard (*Buteo buteo*, n = 21), raptors from the genus *Falco* sp.  
131 which were either common or lesser kestrel (n = 76), the scops owl (*Otus scops*, n = 17), the tawny owl  
132 (*Strix aluco*, n = 8) and the barn owl (*Tyto alba*, n = 9). Mammals included the golden jackal (*Canis*  
133 *aureus*, n = 6), the gray wolf (*Canis lupus*, n = 194), the European hedgehog (*Erinaceus europaeus*, n  
134 = 15), the crested porcupine (*Hystrix cristata*, n = 7), the coypu (*Myocastor coypus*, n = 5), individuals  
135 of *Martes* sp. (n = 12), the European badger (*Meles meles*, n = 57) and the red fox (*Vulpes vulpes*, n =  
136 107). We subjected this group of species to statistical analyses, as we had more than 5 records per  
137 species (see Section 2.3).

138 Moreover, we also tested for SGARs other birds and mammals which were found in smaller numbers.  
139 These included the Golden Eagle (*Aquila chrysaetos*, n = 1), the goshawk (*Accipiter gentilis*, n = 2),  
140 the sparrowhawk (*Accipiter nisus*, n = 3), the marsh harrier (*Circus aeruginosus*, n = 2), the common  
141 cuckoo (*Cuculus canorus*, n = 1), the hooded crow (*Cornix cornix*, n = 1), the short-toed snake eagle  
142 (*Circaetus gallicus*, n = 1), the edible dormouse (*Glis glis*, n = 1), seagulls from *Larus* sp. (n = 2), the  
143 honey buzzard (*Pernis apivorus*, n = 2), European polecat (*Mustela putorius*, n = 1) the magpie (*Pica*  
144 *pica*, n = 1), the red squirrel (*Sciurus vulgaris*, n = 3) and moles from *Talpa* sp. (n = 1).

145

## 146 **2.2 Collection of biological samples and laboratory analyses**

147 Biological samples were collected from wildlife specimens that had been recovered at wildlife  
148 rehabilitation centres, and subsequently died, whenever the veterinary suspected that the animal had  
149 died from intoxication. Moreover, biological samples were collected from wolves that were found  
150 dead in the study area and reported to the Istituto Zooprofilattico della Lombardia e dell'Emilia-

151 Romagna. Upon arrival at the laboratory operators reported the location of discovery (reported as  
152 GPS coordinates for wolves or municipality for other wildlife) and the sex of each specimen. For  
153 wolves we estimated age based on dental development, body size, and weight (Brasington et al.,  
154 2023). Individuals were classified into three age categories: class 1 (<12 months), class 2 (1–2 years),  
155 and class 3 (>2 years). Biometric data and phenotypic characteristics were also recorded. After  
156 completion of the external examination, carcasses were positioned in lateral recumbency and skinned.  
157 A complete necropsy was then performed, with all organs systematically examined and assessed  
158 before being sampled for further laboratory analyses.

159 The determination of anticoagulants (coumatetralyl, warfarin, coumatetralyl, coumachlor,  
160 bromadiolone, diphacinone, chlorophacinone, chloralosis, difenacoum, brodifacoum, flocoumafen,  
161 difethialone) was carried out on liver by means of a LC-MS/MS method (Vandenbroucke et al., 2008;  
162 Fourel et al., 2017; Bertero et al., 2020). Laboratory operators used the entire liver, if this was small or  
163 a few pieces if large enough. The liver was well homogenized by cutting the parenchyma and the  
164 serosa before analysis, the sample (typically 40 g) was extracted by vigorous stirring with acetone  
165 (100 mL); after filtration on paper with anhydrous sodium sulfate, an aliquot (2 mL) was dried under  
166 gentle nitrogen flow at 40 °C. The residue was reconstituted with 2 mL of 2 % ammonia solution in  
167 acetonitrile. Three defatting steps with n-hexane (2 mL) followed. Finally, an aliquot (1 mL) was  
168 stripped to dryness and reconstituted with 0,4 mL of acetonitrile. A 1 µL volume was injected into an  
169 LC-MS/MS system (Agilent QQQ 6460, equipped with an Agilent 1290 Infinity II UPLC).  
170 Chromatographic column was Zorbax Eclipse Plus C18 (2,1 ×50 mm, 1,8 µm). Column temperature  
171 was set at 40 °C. Chromatographic separation was performed through a linear gradient using as  
172 aqueous phase a 0,1 % formic acid solution and as organic phase 0,1 % formic acid solution in  
173 acetonitrile. Flow rate was set at 0,4 mL/ min. Run time was 11 min, with a post-time reconditioning  
174 of 2 min. Quantification was carried out by the external standard method in MRM mode (ESI  
175 negative) acquiring two proper and typical transitions, quantifier, and qualifier, for each analyte.  
176 MS/MS parameters were set as follows: capillary 4000 V, gas temperature 300°C, gas flow 10 L min–

177 1, nebulizer 35 psi, sheath gas temperature 300 °C, sheath gas flow 12 L/min. The limit of  
178 quantification (LOQ) was 1 µg /Kg for all analytes, with a mean recovery >80% and each analysis  
179 batch had 1 positive and one negative control. A concentration found ≥1 µg/Kg indicated a positive  
180 sample, while a concentration <1 µg /Kg denoted a negative sample.

181

### 182 **2.3 Statistical analyses**

183 For statistical analyses we aggregated different species into common functional groups, based on their  
184 ecology. Namely, we divided species into large rodents (n = 12), small carnivores (n = 27), owls (n =  
185 88), diurnal raptors (n = 97), mesocarnivores (n = 164) and large carnivores (n = 200). We believed  
186 this classification meaningful to study patterns in the incidence of SGARs, due to differences in the  
187 feeding ecology of the various species and therefore in their functional role in the ecosystem, which  
188 affects pathways of exposure.

189 Large rodents included the coypus (*Myocastor coypus*) and the crested porcupine (*Hystrix cristata*).  
190 These species are almost entirely herbivores (Mori et al., 2017; Prigioni et al., 2005) and too large to  
191 access bait dispensers. The presence of SGARs should therefore arise mostly from primary exposure,  
192 either from the inappropriate deployment of baits in the environment, or by deliberate poisoning,  
193 possibly in response to damages caused by feeding or burrowing (Musto et al., 2024). Small  
194 carnivores included species from the genus *Martes* sp. (*Martes foina* and *Martes martes*), as well as  
195 the European hedgehog. In these species contamination from SGARs should happen mostly through  
196 secondary exposure, as martens prey on rodents and hedgehogs feed almost entirely on invertebrates  
197 (Fieschi-Méric et al., 2026), which could also feed on rodenticides baits (Martín Cruz et al., 2025;  
198 Williams et al., 2023). Owls and raptors were also considered, as all these species in Italy largely base  
199 their diet on small mammals or insects (Berlusconi et al., 2025; Lovari et al., 1974; Marchesi and  
200 Sergio, 2004; Panzeri et al., 2014; Ramellini et al., 2024) and are subjected mostly to secondary  
201 exposure. We divided owls from raptors as scavenging in owls seems to be occasional (Allen et al.,  
202 2019), while it is common in species like buzzards. For mesocarnivores we considered the European

203 badger and the red fox, two species which can be exposed through predation or scavenging on small  
204 mammals and invertebrates, but which can also consume significant amounts of anthropogenic food  
205 waste (Neuman and Buesching, 2026; Soe et al., 2017). Finally, we classified the golden jackal and  
206 the gray wolf as large carnivores. These two species, albeit trophically plastic and capable to consume  
207 rodents (Canova and Meriggi, 2025; Lange et al., 2021; Travain et al., 2025), base a significant share  
208 of their diet in Europe on wild ungulates (Hatlauf and Lanszki, 2024; Newsome et al., 2016). From a  
209 functional viewpoint, our groups therefore included herbivores (large rodents), active predators  
210 showing little scavenging (small carnivores, owls) and predators that can be facultative scavengers  
211 (raptors, mesocarnivores, large carnivores). We will use the term “functional groups” hereinafter.  
212 We compared the proportion of individuals that were positive to 1, 2, or 3 or more (3+) compounds,  
213 between the various species and these three groups. We predicted ( $H_1$ ) that raptors, owls and small  
214 carnivores, which base most of their diets on small mammals and insects, have a higher incidence of  
215 SGARs than large rodents, mesocarnivores and large carnivores. We did not compare proportions  
216 through null-hypothesis testing, but rather graphically, because our data were opportunistic and this  
217 would have biased p-values (Kruschke and Liddell, 2018).  
218 Moreover, we used Bayesian Generalized Additive Models for ordered outcomes (Bürkner et al.,  
219 2019), to test for the effect of landscape naturalness over the incidence of 1, 2 or 3+ compounds. As  
220 rodent control is carried out mostly around human settlements, we predicted that: *i*) individuals found  
221 in more natural areas, with fewer human settlements, have a lower probability of testing positive to  
222 ARs ( $H_2$ ), *ii*) this effect to be stronger for species that base their diet on rodents and insects, such as  
223 owls, diurnal raptors and small carnivores ( $H_3$ ).  
224 As the coordinates of recoveries were only available for wolves, we quantified landscape naturalness  
225 by considering the municipality of each record. We used official administrative boundaries from the  
226 Italian Institute for Statistics (ISTAT, [https://www.istat.it/notizia/confini-delle-unita-amministrative-](https://www.istat.it/notizia/confini-delle-unita-amministrative-a-fini-statistici-al-1-gennaio-2018-2/)  
227 [a-fini-statistici-al-1-gennaio-2018-2/](https://www.istat.it/notizia/confini-delle-unita-amministrative-a-fini-statistici-al-1-gennaio-2018-2/)). Then, for each municipality we downloaded the density of  
228 human buildings at a resolution of 100 m

229 ([https://human-settlement.emergency.copernicus.eu/ghs\\_buS2023.php](https://human-settlement.emergency.copernicus.eu/ghs_buS2023.php)) and computed the Gini  
230 index. The Gini index ranges from 0, when all units have the same value of a certain quantity, to 1,  
231 when only one unit concentrates all the quantity of interest. Therefore, the Gini index, by being  
232 directly proportional to the spatial concentration of human buildings, became a proxy for landscape  
233 naturalness: municipalities with higher values had fewer and more concentrated settlements (Cerri et  
234 al., 2026).

235 In our Bayesian GAM, we predicted the probability that an individual tested positive for a different  
236 number of SGARs based on its species, the level of landscape naturalness of the municipality where it  
237 had been found, and their interaction. We selected models based on a stepwise-forward model  
238 selection approach, based on leave-one-out cross validation (Vehtari et al., 2017). For non-linear  
239 terms we used Gaussian Processes (Wood, 2017). Models were implemented in STAN (Carpenter et  
240 al., 2017) through the brms package (Bürkner et al., 2017) in R (R Core Team, 2026). A completely  
241 reproducible software code, with its accompanying dataset, is available at: <https://osf.io/yxu6m>

## 243 **Results**

244 We found high levels of positivity to at least one SGAR in all the various species from the six  
245 functional groups (Fig. 2; see also Fig. S1 in Appendix 1). The most common compounds were always  
246 Brodifacoum and Bromadiolone (Fig. 3). Moreover, a significant number of other species tested  
247 positive to at least 1 AR. These included also non-target rodents such as the red squirrel and the edible  
248 dormouse (Table 1).

249 Incidences were particularly high for the barn owl, the golden jackal and for martens (*Martes* sp.), for  
250 which all individuals tested positive to SGARS. As well as for the red fox, for which only 5  
251 individuals out of 107 tested negatives to SGARS. Conversely, we did not find any positive case for  
252 the scops owl, and only 1 individual of crested porcupine tested positive to SGARS. Small and  
253 mesocarnivores had a higher percentage of positive cases to a higher number of compounds ( $H_1$  was  
254 accepted).

255 Model selection (Fig. S3, Appendix 1) retained a model where a Gaussian Process predicted a  
256 different effect of landscape anthropization between the six different groups of wildlife. The inclusion  
257 of a random intercept, to account for differences in the overall incidence of SGARs between different  
258 municipalities, did not improve model fitness. The best candidate model showed a good fit to the data  
259 (Fig. S2, Appendix 1).

260 Contrary to our prior expectations we did not observe any general decrease in the positivity to SGARs  
261 in areas with higher landscape naturalness ( $H_2$  was rejected). Moreover, the effect of landscape  
262 naturalness over positivity to SGARs was not higher for small carnivores, raptors and owls ( $H_3$  was  
263 rejected). Indeed, positivity to 3+ SGARs slightly declined with landscape naturalness only for  
264 mesopredators. But even in this latter group effect size was extremely low and credibility intervals  
265 large (Fig. 4).

266

## 267 **Discussion**

268 The impact of human activities on ecosystems includes structural changes to the landscape, but also  
269 the release of potentially toxic compounds that can interfere with the physiology of living organisms  
270 (Sigmund et al., 2023). Exposure to toxic substances does not merely cause acute effects and direct  
271 mortality in wildlife, but can also lead to sublethal consequences, such as physiological,  
272 immunological, and behavioral changes (Rattner et al., 2014). These can go unnoticed for long  
273 periods of time, become widespread and exert long-lasting impacts on animal populations (Desforges  
274 et al., 2018), also by interacting with other drivers of change (e.g., climate change, Williams et al.,  
275 2025). Similar concerns also involve SGARS, which are increasingly regarded as a serious threat for  
276 wildlife conservation (Walther et al., 2021), particularly for predators and scavengers, but whose  
277 overall circulation across the food chain is still unclear. While some reviews compared positivity to  
278 SGARs between different groups of predators (Keating et al., 2024; Nakayama et al., 2019), to the  
279 best of our knowledge no study quantified their incidence in different wildlife species from the same  
280 area. Our results, based on a large dataset of wildlife specimens collected across a gradient of

281 landscape naturalness, ranging from intensive croplands to mountain areas (Fig. 1), emphasize four  
282 concerning points.

283 The first one is the pervasiveness of exposure to SGARs in different groups of wildlife, a fact which  
284 highlights multiple pathways of exposure and therefore significant flaws in rodenticide management  
285 practices. While our results align with previous studies about raptors, owls or carnivores (Carromeu-  
286 Santos et al., 2025; Elliott et al., 2024; Garcês et al., 2023; Gomez et al., 2022; Keating et al., 2024),  
287 positivity in insectivore species and in herbivorous rodents was much higher than expected. The high  
288 incidence of SGARs observed in the European hedgehog is likely driven by the contamination of its  
289 prey—primarily insects and slugs—which frequently feed on baits placed within bait stations (e.g. see  
290 Williams et al., 2023). Invertebrate contamination also explains positivity in the red squirrel and the  
291 edible dormouse, two species that occasionally supplement their diet with insects (Capizzi & Santini,  
292 2007). Secondary exposure in non-target rodents and hedgehogs, from the gradual intake of  
293 contaminated invertebrate prey, is supported by the fact that accumulated concentrations were not  
294 sufficient to cause the direct mortality of individuals around traps. Conversely, no contamination was  
295 detected in another insectivorous species, the scops owl, whose diet consists predominantly of  
296 orthopterans. Future studies should investigate which invertebrates feed on rodenticide baits and then  
297 quantify their dispersal around baiting sites and their consumption by insectivorous species. This  
298 would provide information on an extremely important, yet understudied, pathway of exposure.

299 The detection of SGARs in large herbivorous rodents, such as the crested porcupine and the coypu,  
300 which are too large to access standard bait stations, indicates that exposure in non-target wildlife is  
301 also driven by the deliberate use of ARs to manufacture poisoned baits for wildlife (Musto et al.,  
302 2024), the unauthorized deployment of baits in open agricultural areas and the improper deployment  
303 of legally purchased biocides against commensal rodents. To circumvent bait neophobia and increase  
304 bait uptake, users frequently leave baits partially or completely outside of tamper-resistant baiting  
305 stations, making them freely accessible to larger non-target wildlife. We believe that future studies,

306 based on detection dogs locating AR baits in the environment (Deák et al., 2021), should quantify the  
307 real level of compliance with existing regulations about pest control.

308 The second point is the high frequency of individuals that tested positive to multiple compounds (Fig.  
309 2 – 3), which was extreme for raptors, owls and mustelidae (e.g., *Martes* sp.). This point is not  
310 surprising per-sé, as some ARs have a long hepatic half-life (Horak et al., 2018, see below) and  
311 individuals of these species can accumulate different compounds in a few weeks simply by  
312 continuously preying on contaminated rodents, which constitute most of their diet. However, such of  
313 a widespread presence of multiple compounds raises serious questions about the potential  
314 amplifications of their non-lethal effects. Synergistic effects of multiple toxicants have received some  
315 attention in pest management and rodent control (Blažić et al., 2024), however this topic been  
316 completely ignored for non-target wildlife. Considering that some raptors, owls and mustelidae are  
317 already declining, due to several threats (Ascensao et al., 2024; Corregidor-Castro et al., 2023; Guíl  
318 and Pérez-García, 2022; Newton et al., 2017; Wright et al., 2022), their systematic exposure to  
319 multiple ARs could be a emerging threat with severe long-term implications.

320 The third point is the dominance of specific active ingredients across all the surveyed functional  
321 groups, most notably brodifacoum and bromadiolone (Fig. 3). The disproportionate prevalence of  
322 these two specific SGARs in non-target wildlife is driven by their pharmacokinetics and their  
323 commercial availability. The widespread detection of brodifacoum is primarily linked to its extreme  
324 physiological persistence in rodents: the hepatic half-life of brodifacoum in mice (*Mus musculus*) is  
325 exceptionally long (approximately 307 days), vastly exceeding that of other compounds such as  
326 difenacoum (62 days), chlorophacinone (35 days), or bromadiolone (28 days) (Horak et al., 2018;  
327 Vandenbroucke et al., 2008). This severe makes brodifacoum highly likely to biomagnify up the food  
328 chain even months after the primary consumption of the bait. Conversely, the high incidence of  
329 bromadiolone, characterized by a much shorter hepatic half-life, highlights the critical role of market  
330 dynamics. Although official market statistics were unavailable, sales data provided through personal  
331 communications with major rodenticide manufacturers operating in the study area (Lombardy and

332 Emilia-Romagna) indicate that bromadiolone was the most widely sold active ingredient (over 50% of  
333 total sales). Conversely, brodifacoum and difenacoum represented 25% and 20%, respectively, while  
334 the proportion of other active ingredients (including difethialone and flocoumafen) was substantially  
335 lower. The combination of massive environmental inputs of bromadiolone, driven by market  
336 preferences, coupled with the extreme physiological persistence of brodifacoum, relentlessly expose  
337 wildlife to these two compounds.

338 The last point concerns the lack of any spatial pattern in SGARs exposure: in our study area wildlife  
339 has the same incidence of SGARs in anthropized and in highly-natural areas. This lack of a clear  
340 spatial pattern is partially due to the fact that quantifying landscape naturalness at the municipality  
341 level represents a relatively coarse scale. This resolution may be inadequate for both highly mobile  
342 species, such as wolves and diurnal raptors, and more sedentary ones, like the European hedgehog.  
343 Indeed, a municipality classified as having high naturalness in our scale, may still contain small  
344 agricultural pockets or human settlements where the use of anticoagulant rodenticides is intensive.  
345 This may have masked potential spatial relationships, leading to the absence of clear statistical trends.  
346 However, this can also indicate that the availability of contaminated rodents (e.g. *Apodemus* sp., *Mus*  
347 sp., *Clethrionomys* sp., *Rattus* sp.) around human settlements is so high that scavengers and predators  
348 manage to continuously find a sufficient amount to test positive to SGARs, even in areas with small  
349 villages. This pattern also raises the question on whether, particularly in areas with very small human  
350 settlements, predators and scavengers deliberately select baiting sites, due to their availability of  
351 rodents (Engler and Krone, 2026). As the location of baiting sites can be fixed in time, these can  
352 provide wildlife communities with a predictable yet toxic food source, resulting into an ecological  
353 trap.

354 Overall, our findings emphasize an extremely concerning situation. SGARs in our study area, which  
355 can be compared to a wide range of European landscapes, at least in terms of its gradient of  
356 anthropization, are ubiquitous between wildlife species. This non-stop exposure to multiple SGARs at  
357 a time, raises important questions about the long-term impact of rodent control on wildlife

358 populations, trophic interactions and ecosystems. If invertebrates are systematically contaminated, to  
359 what extent they might affect important ecological processes delivered by insectivorous birds and  
360 mammals (da Silva et al., 2014; Platt et al., 2017)? If SGARs circulate in the food web of highly  
361 natural areas, can they disrupt the population dynamics of rodents, which play a crucial role in forest  
362 ecosystems (Čepelka and Dokulilová, 2025), and their predators? If SGARs are common in predators,  
363 how does intraguild predation, which is common in mammals and birds (Sergio et al., 2008), affect  
364 tertiary exposure?

365 Given the pervasive contamination from SGARs observed even in non-target species — including  
366 herbivores and insectivores- we believe that their application should be proportionate and targeted,  
367 restricted exclusively to scenarios where rigorous monitoring confirms that rodent populations have  
368 exceeded critical thresholds for economic damage or public health risks. To mitigate their  
369 environmental impact, current regulations should strongly enforce: *i*) limiting the use of SGARs  
370 strictly to professional pest control operators; *ii*) obliging public and private entities to monitor their  
371 application, including the actual selectivity of bait dispensers, and *iii*) prioritizing alternative  
372 measures, such as trapping, the reduction of environmental carrying capacity, and the implementation  
373 of nature-based solutions, whose effectiveness is increasingly demonstrated (Bontzorlos et al., 2024).

374

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381

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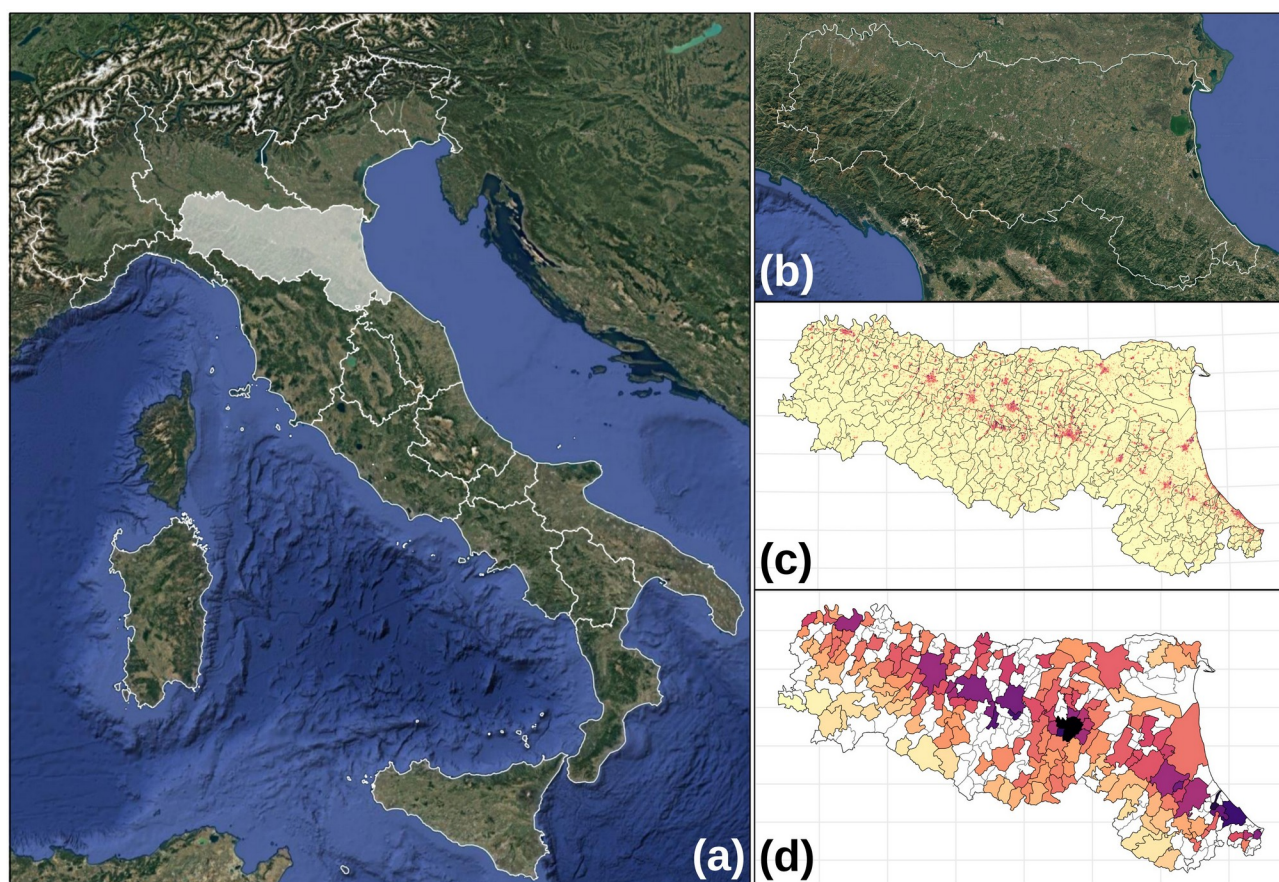
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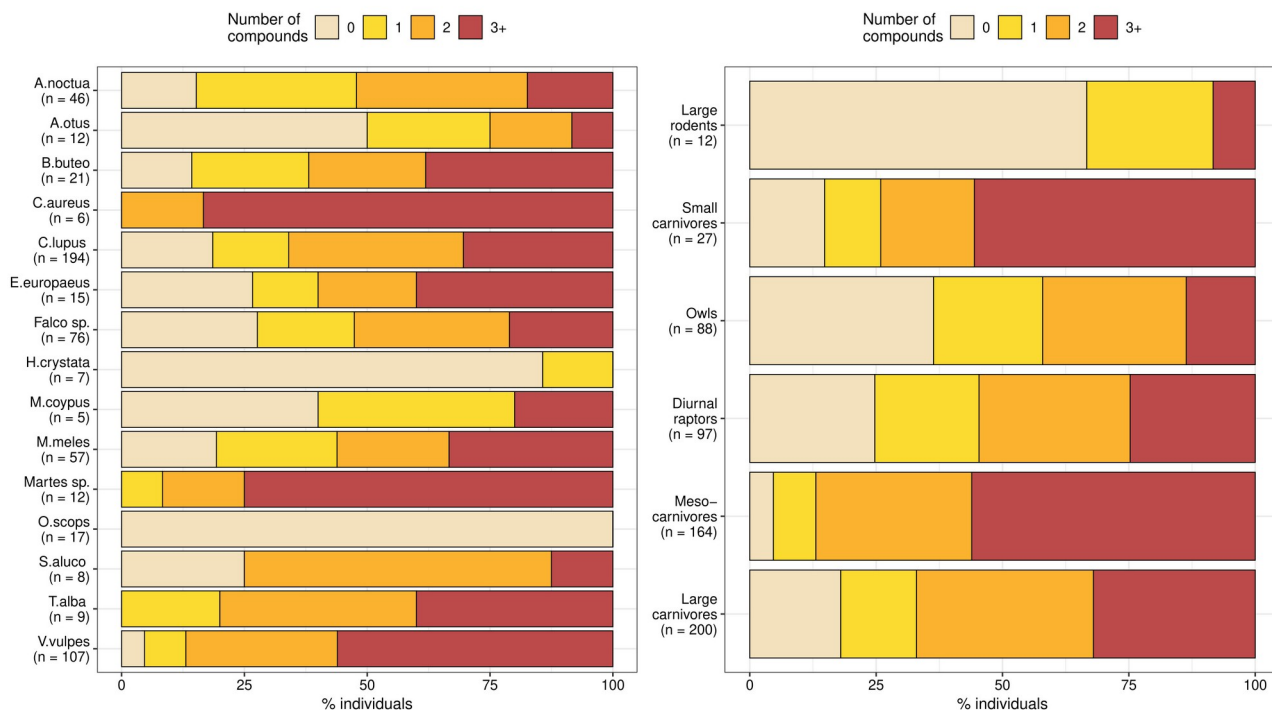


675 Fig. 1. Map of the study area: (a) position of the Emilia-Romagna region within Italy, (b) aerial image  
676 of the Emilia-Romagna region, (c) density of buildings in the various municipalities of the study area,  
677 at a 100 m resolution (darker areas have a higher density of buildings), (d) overview of landscape  
678 naturalness in the municipalities of the study area: municipalities with a darker color have a  
679 widespread presence of buildings and a lower naturalness. Aerial images come from Google Satellite,  
680 while the density of human buildings from the Global Human Settlement Layer (see the Methods  
681 section).

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689 Fig. 2. Percentage of individuals positive to 1, 2 or 3 or more ARs, between the various species that  
690 were tested (left), and between the six different groups of wildlife (right). For species-specific counts  
691 see Table 1.

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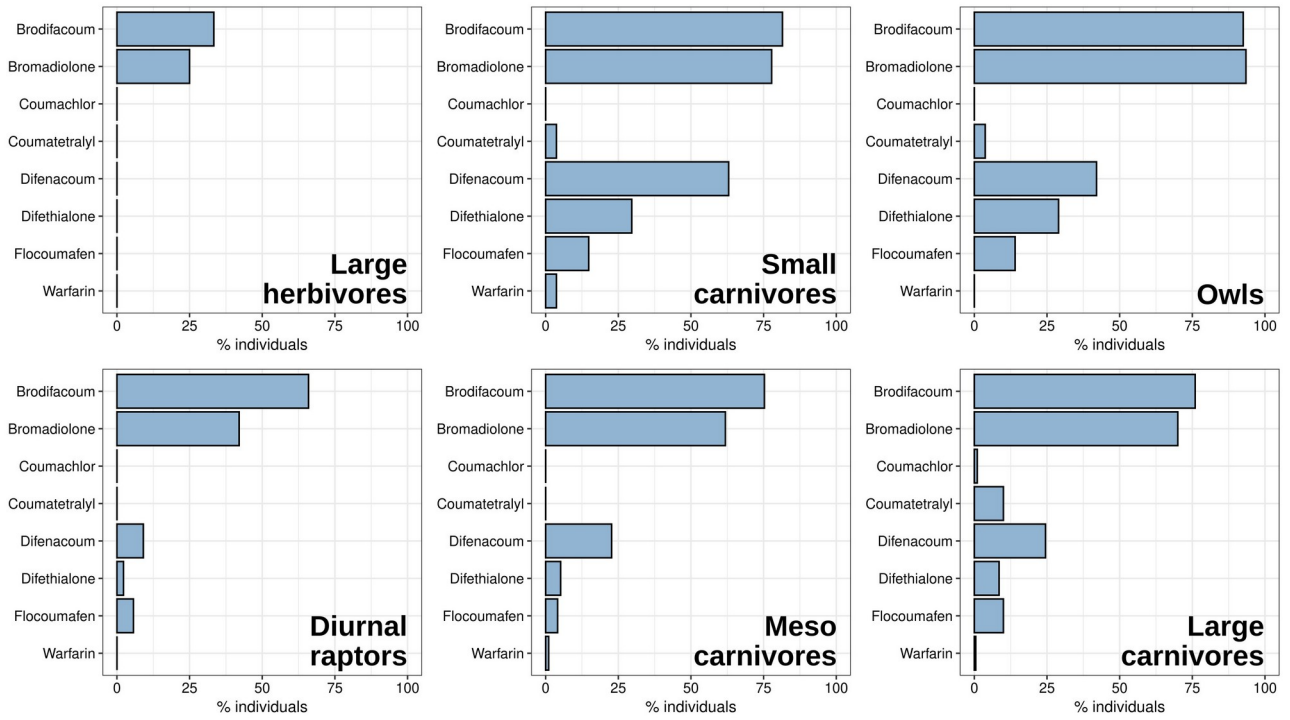
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707 Fig. 3. Percentage of individuals that tested positive to the various ARs, between the six different  
708 groups of wildlife. See the Methods section for a description of the species.

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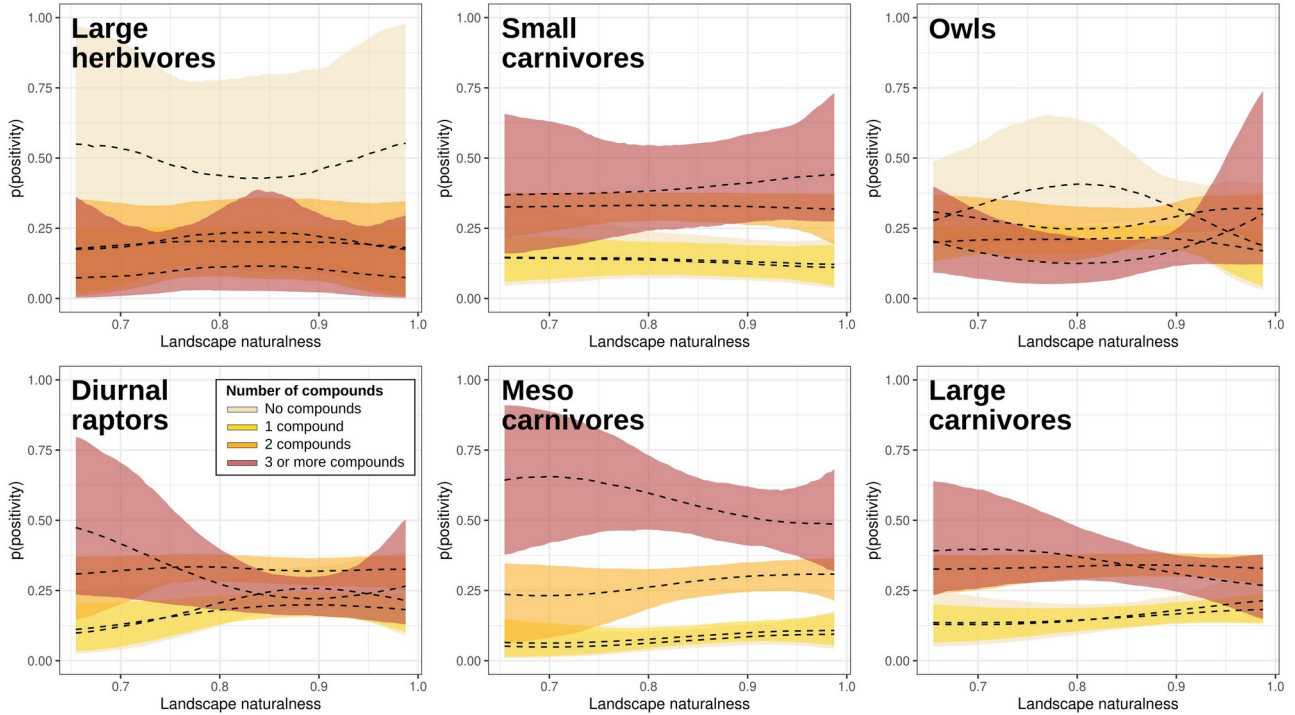
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722 Fig. 4. Marginal effect plots of the best candidate model, representing the probability of testing  
723 negative to ARs, or testing positive to 1, 2 or 3+ compounds, based on landscape naturalness, between  
724 the six groups of wildlife. Landscape naturalness was calculated as the Gini index of the density of  
725 buildings at 100 m, in the municipality where tested animals were recovered or found. Therefore, high  
726 values of the Gini index correspond to areas with high naturalness. Shaded areas represent 95%  
727 Bayesian credibility intervals.

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Species used for statistical modeling	Number of ARs detected			
	No compounds	1 compound	2 compounds	3+ compounds
<i>Athene noctua</i>	7	15	5	8
<i>Asio otus</i>	6	3	2	1
<i>Buteo buteo</i>	3	5	5	8
<i>Canis aureus</i>	0	0	1	5
<i>Canis lupus</i>	36	30	69	59
<i>Erinaceus europaeus</i>	4	2	3	6
<i>Falco sp.</i>	21	15	24	16
<i>Hystrix cristata</i>	6	1	0	0
<i>Myocastor coypus</i>	2	2	0	1
<i>Meles meles</i>	11	14	13	19
<i>Martes sp.</i>	0	1	2	9
<i>Otus scops</i>	17	0	0	0
<i>Strix aluco</i>	2	0	5	1
<i>Tyto alba</i>	0	1	2	2
<i>Vulpes vulpes</i>	5	9	33	60
Other species	No compounds	1 compound	2 compounds	3+ compounds
<i>Aquila chrysaetos</i>	0	0	0	1
<i>Accipiter gentilis</i>	0	0	0	2
<i>Accipiter nisus</i>	0	1	1	1
<i>Circus aeruginosus</i>	1	0	1	0
<i>Cuculus canorus</i>	1	0	0	0
<i>Cornix cornix</i>	0	0	1	0
<i>Circaetus gallicus</i>	0	0	0	1
<i>Glis glis</i>	1	0	1	0
<i>Larus sp.</i>	0	1	1	0
<i>Mustela putorius</i>	0	1	0	0
<i>Pernis apivorus</i>	1	1	0	0
<i>Pica pica</i>	1	0	0	0
<i>Sciurus vulgaris</i>	2	1	0	0
<i>Talpa sp.</i>	0	1	0	0

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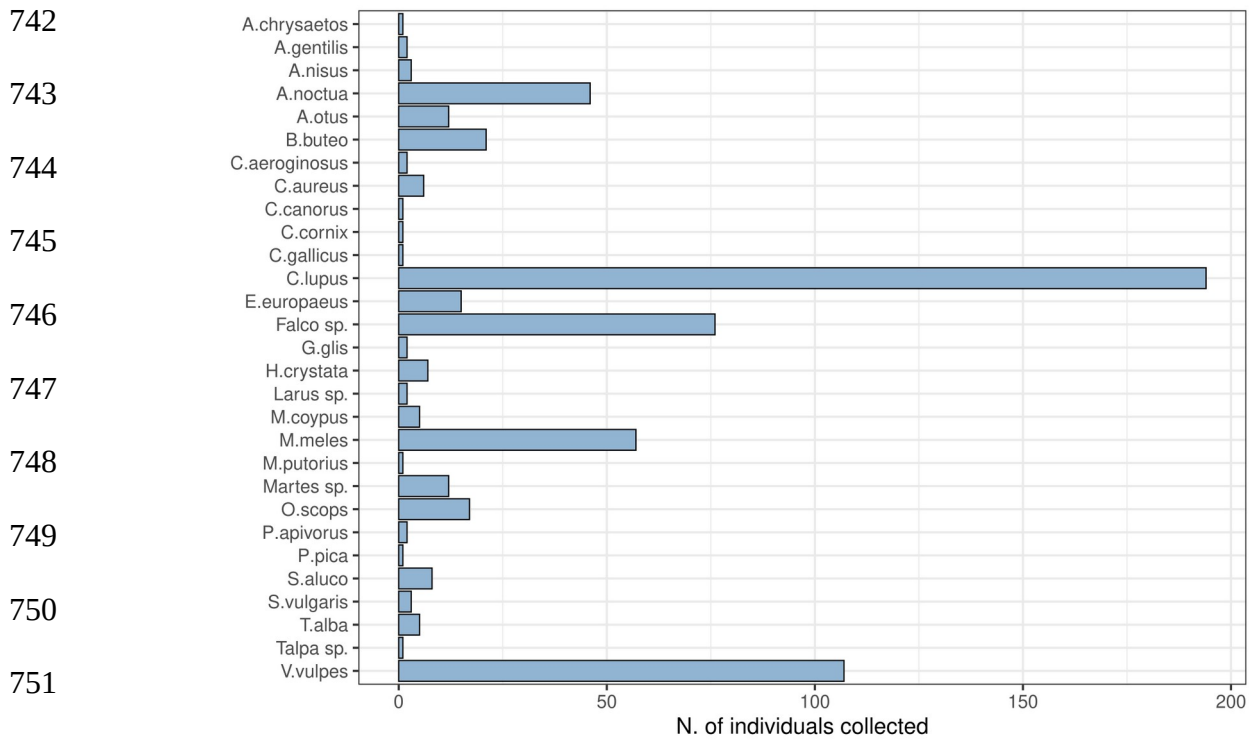
736 Table 1. Number of individuals that tested positive to different number of ARs, between the various  
737 species. “Other species” refers to species that were not used for statistical analyses.

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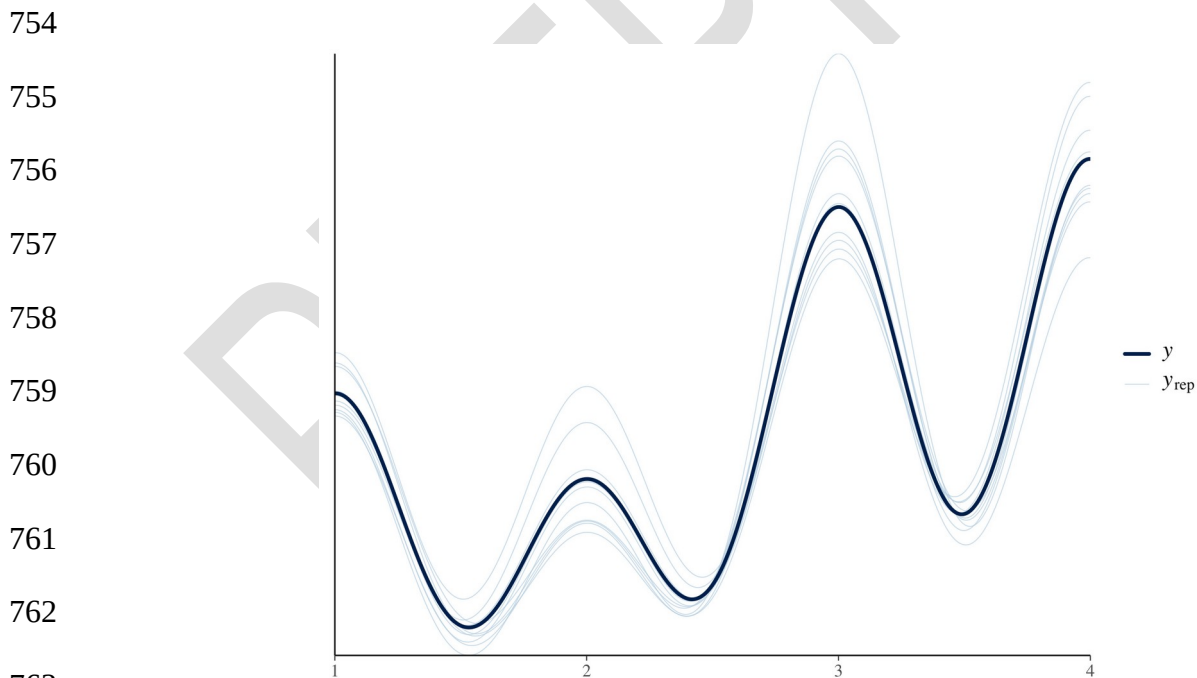
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741 **Appendix 1**



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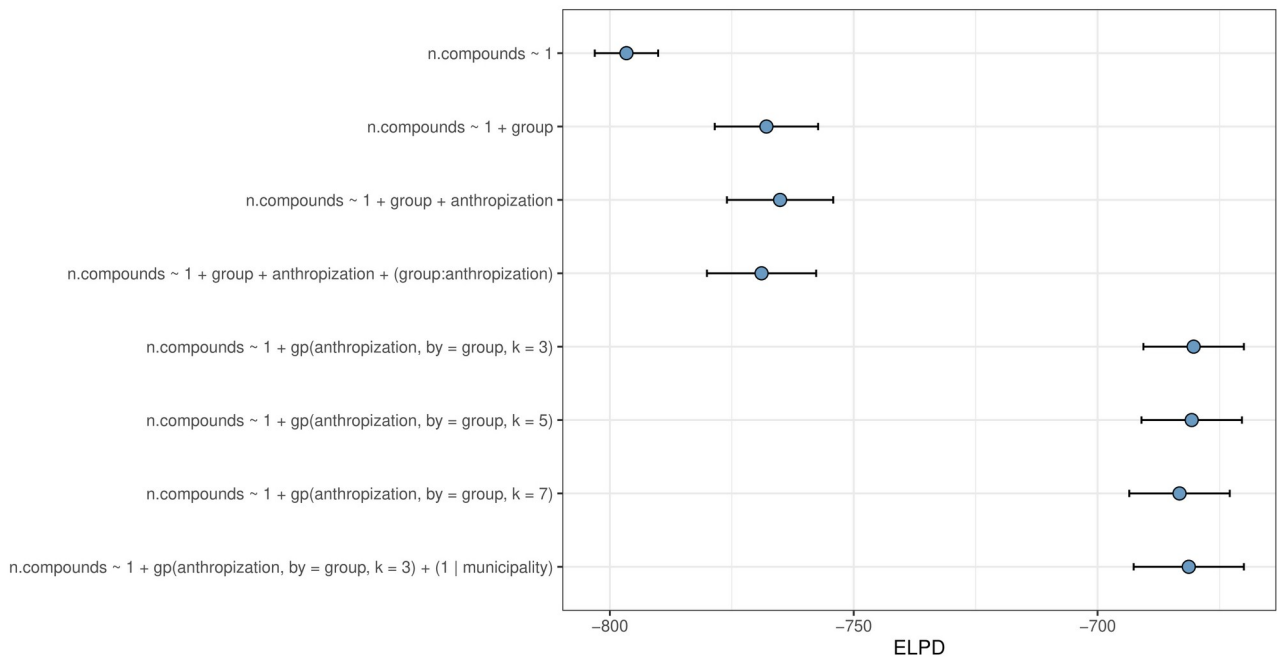
753 Fig. S1. Number of individuals that were collected, between the various species



764 Fig. S2. Posterior predictive checks, representing the goodness-of-fit of the best candidate model, in  
765 terms of distribution of the response variable. For further information see:  
766 <https://mc-stan.org/bayesplot/reference/PPC-overview.html>

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769 Fig. S3. Overview of model selection, based on the Expected Log-Pointwise Density ([https://mc-](https://mc-stan.org/loo/reference/loo_compare.html)  
770 [stan.org/loo/reference/loo\\_compare.html](https://mc-stan.org/loo/reference/loo_compare.html)). Model formula follows the following notation: the “:”  
771 term indicates an interaction, the “gp” terms indicate a Gaussian Process  
772 (<https://paulbuerkner.com/brms/reference/gp.html>), while “(1|x)” indicates a random intercept model  
773 (<https://paulbuerkner.com/brms/reference/brmsformula.html>).

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