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# 1                    **Neonicotinoids: Still present in farmland birds despite their ban**

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18

19 **Abstract**

20

21 Neonicotinoids (neonics) are the most widely used insecticides worldwide and are considered  
22 to be of low risk to non-target organisms such as vertebrates. Further, they are reported to  
23 be rapidly excreted and metabolized, reducing their potential toxicity. Nevertheless, growing  
24 evidence of adverse effects of neonics on farmland bird species raise questions about the  
25 purported harmless nature of these pesticides. We attempted to search for pesticide residues  
26 in species of different trophic levels and at different life stages, by using multiple bird  
27 monitoring programs on a Long-Term Socio-Ecological Research (LTSER) platform. Three  
28 passerine birds—the blackbird (*Turdus merula*), ciril bunting (*Emberiza cirilus*), and common  
29 nightingale (*Luscinia megarhynchos*)—that feed on seeds and invertebrates were monitored  
30 during their reproductive period, and the grey partridge (*Perdix perdix*) that feeds on seeds  
31 was monitored during its wintering period. We also monitored chicks of an apex predator—  
32 the Montagu’s harrier (*Circus pygargus*)—that preys mostly upon common voles but also upon  
33 insects. We found that the birds’ blood samples showed presence of residues of five neonics:  
34 three banned since 2018 in France—clothianidin, thiacloprid, and thiamethoxam—and two—  
35 dinotefuran and nitenpyram—used for veterinary purposes only. While none of these neonics  
36 was detected in blackbirds, all were present in grey partridges. Clothianidin was detected in  
37 all species, except blackbirds. Concentrations of the three banned neonics were similar or  
38 higher than concentrations found in birds monitored elsewhere before the ban. These findings  
39 raise questions about the persistence of neonics within the environment and the mode of  
40 exposure to wild fauna. Future investigations on the sublethal effects of these neonics on life-  
41 history traits of these farmland birds may help in providing a better understanding of the

42 effects of exposure of bird populations to these insecticides, and also to the consequent effect  
43 on human health.

44

45 Keywords:

46 Biomonitoring, farmland birds, passerines, pesticides, raptors.

## 47 **1. Introduction**

48

49           During the last century, the need for feeding the growing human population worldwide  
50 has led to an intensification of agricultural practices, including an extensive use of pesticides.  
51 Despite their use for millennia, several pieces of evidence implicate pesticides in the global  
52 decline in biodiversity (Wood & Goulson, 2017; Stanton et al., 2018; Moreau et al., 2022a).  
53 Moreover, other studies identify them as the cause of certain diseases in humans (Köhler &  
54 Triebkorn, 2013). As all living organisms in a given area share the same environment,  
55 protecting biodiversity by reducing pesticides inputs means also reducing humans' exposure  
56 to pesticides, and this is necessary to ensure our own health and safety (*One Health* concept).  
57 One way is thus to capitalize on wildlife monitoring schemes to better understand the risk of  
58 pesticide exposure for humans (Moreau et al., 2022a). In that respect, wild bird species are  
59 valuable candidates as they are well-monitored worldwide, being involved in long-term  
60 banding programs over decades or more, which have highlighted a global declining trend in  
61 several taxa and especially in farmland birds, e.g., in France, Sweden, United Kingdom, US,  
62 Europe, and North America (Wretenberg et al., 2006; Comolet-Tirman et al., 2015; Stanton et  
63 al., 2018; Rosenberg et al., 2019; Li et al., 2020; Burns et al., 2021; DEFRA, 2021). Pesticide use  
64 has been often identified as a major component responsible for this decline (Campbell et al.,  
65 1997; Geiger et al., 2010; Mineau & Whiteside, 2013; Chiron et al., 2014; Tassin de Montaignu  
66 & Goulson, 2020). Recent studies, for instance, showed a negative relationship between the  
67 abundance of northern bobwhites (*Colinus virginianus*) and their exposure to neonicotinoid  
68 insecticides (neonics) from 1978 to 2012 (Ertl et al., 2018a). Similarly, a wide-panel dataset  
69 regarding neonics use and birds' population trends revealed a significant negative impact of

70 this family of pesticides on avian biodiversity from 2003 to 2010 in the Netherlands (Hallmann  
71 et al., 2014) and from 2008 to 2014 in USA (Li et al., 2020).

72         Neonics are insecticides developed in the 1970s, with the first patent dating back to  
73 1977 for nithiazine (rapidly abandoned due to its poor stability), followed by patents for  
74 imidacloprid and thiacloprid in 1985, nitenpyram in 1988, acetamiprid and clothianidin in  
75 1989, thiamethoxam in 1992, and dinotefuran in 1994 (Tomizawa & Casida, 2005). Neonics  
76 target the neural function and are competitive agonists of nicotinic acetylcholine receptors  
77 (nAChR), which increase specificity to insects and not vertebrates (Tomizawa & Casida, 2003).  
78 In fact, in contrast to other insecticides such as carbamates and organophosphorous that also  
79 target the neural function by inhibiting the acetylcholinesterase (AChE) enzyme—an  
80 ubiquitous enzyme in the animal kingdom (review in Grue et al., 1997; Story & Cox, 2001;  
81 Walker, 2003; Mitra et al., 2011)—neonics were supposed to have minimal effects and a low  
82 toxicological impact on vertebrates and consequently on birds, due to the lower number of  
83 nAChRs they have and the lower sensitivity of their nAChRs compared to those of insects  
84 (Tomizawa & Casida, 2003, 2005; Ihara et al., 2017; Casida, 2018). Moreover, they were  
85 claimed to be rapidly metabolized and excreted, in a few hours (Bishop et al., 2018, 2020;  
86 Casida, 2018; Bean et al., 2019; English et al., 2021; Pan et al., 2022), but some reports  
87 question their fate along the trophic chain as they have been found in insectivorous birds,  
88 granivorous birds, piscivorous birds, and birds of prey (see references in the supplementary  
89 materials Table S1). Under experimental conditions, they seem to accumulate in the liver  
90 (Lopez-Antia et al., 2015a) and to be detectable in different organs and tissues, although they  
91 (at least for imidacloprid and thiamethoxam) seem to be rapidly cleared from birds' organism  
92 (Bean et al., 2019; Pan et al., 2022). Several studies showed various effects of neonics  
93 exposure on birds at different physiological levels (review in Gibbons et al., 2015 and Moreau

94 et al., 2022a). Although neonics do not act directly on AChE, they may induce neuronal  
95 degeneration, which affects AChE activity (Abu Zeid et al., 2019; Rawi et al., 2019), altering  
96 more complex functions such as learning and migration behaviour (Eng. et al., 2017) but also  
97 having sublethal effects on important functions of the organism such as the haematocrit,  
98 antioxidant defences, immunity, or fecundity (Lopez-Antia et al., 2013, 2015a, 2015b;  
99 Tokumoto et al., 2013; Hoshi et al., 2014; Mohanty et al., 2017; Humann-Guillemot et al.,  
100 2019; Lv et al., 2020).

101 Since the 1990s, the use of neonics has become widespread, making them the most  
102 widely used class of insecticide worldwide, mostly for coating seeds, despite being shown to  
103 impact non-target species, including humans (Tomizawa & Casida, 2005; Casida & Durkin,  
104 2013; Gibbons et al., 2015; Simon-Delso et al., 2015; Henry et al., 2015; Wood & Goulson,  
105 2017; Casida, 2018; Thompson et al., 2020; Zhang et al., 2022). In Europe, despite an EU  
106 moratorium in 2014, neonics were still detectable in bee-attractive crop nectar until 2018 at  
107 least (Wintermantel et al., 2020). Indeed, their degradation in soil (measured by DT<sub>50</sub> which is  
108 the Detection Time 50% representing the time to detect a 50% decrease in pesticide  
109 concentration) can take quite a long time, up to more than 6900 days (i.e., 19 years for  
110 clothianidin; see Table 2 in Thompson et al., 2020). Therefore, banning harmful neonics does  
111 not necessarily eradicate the problem of exposure. In EU, dinotefuran and nitenpyram have  
112 never been considered for use in phytopharmaceutical products (PPP; July 2022, EU Pesticides  
113 database: [https://food.ec.europa.eu/plants/pesticides\\_en](https://food.ec.europa.eu/plants/pesticides_en)) but are commonly used in  
114 veterinary medicine. In France, neonics are banned for outdoor use and in PPP since  
115 September 2018 (Décret n° 2018-675, July 2018), except for emergency authorized use of  
116 thiamethoxam and imidacloprid on sugar beet crops in production areas (EFSA, 2021).  
117 Nonetheless, the use of neonics is still allowed in veterinary medicine.

118 In the present study, we use different bird monitoring programs on a long-term socio-  
119 ecological research (LTSER) platform and a multiresidue analysis (Rodrigues et al., 2023) to  
120 evaluate the presence of neonics in an intensive farmland area where acetamiprid,  
121 clothianidin, imidacloprid, thiacloprid, and thiamethoxam were banned for 3 years for  
122 agricultural use. Nitenpyram and dinotefuran were also included in the screening to control  
123 for potential exposure of wild fauna to veterinary products. As the method was not specifically  
124 developed for neonics detection, acetamiprid and imidacloprid, were not detectable among  
125 the other 104 pesticide compounds. We selected five different species for their different  
126 ecology: (i) three passerine birds during their reproductive period: the blackbird (*Turdus*  
127 *merula*), ciril bunting (*Emberiza cirilus*), and common nightingale (*Luscinia megarhynchos*) that  
128 feed on seeds and invertebrates; (ii) the grey partridge (*Perdix perdix*) caught during its  
129 wintering period when it feeds only seeds; and (iii) an apex predator species, namely, the  
130 Montagu's harrier (*Circus pygargus*) that preys mostly upon common voles but also upon  
131 orthopterans. For this fifth species, we focused on chicks that are fed by their parents during  
132 the rearing period. Beyond the interest of their contrasted ecologies, farmland bird species  
133 including buntings, blackbirds or raptors have been shown to be exposed to neonics, however,  
134 a limited number were subject of measures in blood samples (Lennon et al., 2020a).  
135 Additionally, grey partridge is recognised to be a focal species for pesticide risk assessment  
136 (Millot et al., 2017; Bonneris et al., 2018). Here, we aimed at monitoring potential exposure  
137 of multiple bird species in an area where there is presumably no use of neonics for agricultural  
138 purposes for 3 years and where nitenpyram used in veterinary medicine has never been  
139 assessed to our knowledge. We thus collected blood samples from all individuals to determine  
140 the presence and measure the level of exposure to neonics. We selected blood (whole blood,  
141 i.e., red blood cells and plasma) for the analyses in order to focus on the effects of short-term



142 exposure only (Espín et al., 2016), so that potential exposure of the migratory species (i.e.,  
143 common nightingales, Montagu's harriers, and blackbirds) at wintering areas can be  
144 neglected.

145

## 146 **2. Materials and methods**

147

### 148 **2.1. Study area**

149

150 The study site is located in southwestern France (46°11'N, 0°28'W, Figure 1), in the Long-Term  
151 Socio-Ecological Research Zone Atelier Plaine & Val de Sèvres (LTSER ZAPVS), a 450 km<sup>2</sup> area  
152 where the soil occupancy and the agricultural practices are monitored each year since 1994  
153 (Bretagnolle et al., 2018). In this intensive farming area, winter cereal crops accounted for  
154 ~41% (wheat: 33.8% and corn: 9.6%) of the area under cultivation; in addition, there were  
155 sunflower (10.4%), oilseed rape (8.3%), pea (2%), and meadows (13.5%) (average coverage  
156 between 2009 and 2016, Bretagnolle et al., 2018). In this area, organic farming (no pesticide  
157 use) is carried out in 18% of the agricultural area. Detailed data on pesticide applications was  
158 not available, however, until 2018 imidacloprid was used on cereal crops in the study area,  
159 thiacloprid, thiamethoxam and clothianidin for their part were mainly used in maize, oilseed  
160 rape and cereal crops. Different monitoring studies performed in this area showed the  
161 transfer of neonics in several compartments. For instance, imidacloprid was found in soils,  
162 oilseed rape nectar, earthworms, and small mammals in this area (Henry et al., 2015;  
163 Wintermantel et al., 2020; Pelosi et al., 2021; Fritsch et al. 2022). Thiacloprid was detected in  
164 soils, earthworms and small mammals' hair while thiamethoxam was detected in nectar and

165 soils, and clothianidin in nectar and small mammals' hair (Wintermantel et al., 2020; Pelosi et  
166 al., 2021; Fritsch et al. 2022). Acetamiprid has been detected in small mammals' hair (Fritsch  
167 et al., 2022) despite its use is mainly for market gardening such as tomatoes, squash, and  
168 melon cultures which are not present in a substantial surface of the study area. All five neonics  
169 are banned for agricultural use in France since 2018, except imidacloprid and thiamethoxam  
170 for emergency authorizations on sugar beet crops. Still, sugar beet crops are only present in  
171 this area for their first year (seed production), and the use of neonics is banned for this  
172 purpose. Therefore, at the moment when birds were caught (2020–2022, see section 2.2.1),  
173 no neonics were being used for agricultural purposes. However, imidacloprid, dinotefuran,  
174 and nitenpyram may be in domestic use as veterinary treatment for domestic animals (cats,  
175 dogs, ferrets, and bunnies). There is no known screening of nitenpyram in the study area and  
176 dinotefuran has only been investigated in one previous study but was not detected in small  
177 mammals' hair sampled (Fritsch et al., 2022).

178

## 179 **2.2. Model species and blood collection**

180

### 181 2.2.1. Model species

182

#### 183 2.2.1.1. *Adult passerine birds*

184

185 The passerine species were sampled during the reproductive period from mid-April to end of  
186 June 2021. Birds were caught using net-trapping, following the same methodology as Moreau  
187 et al. (2022b). Among the 17 species trapped in this area, we selected three, namely, ciril  
188 buntings (N = 31), blackbirds (N = 64), and common nightingales (N = 34) because these species

189 were among the biggest of those captured, which allowed us to draw enough blood for  
190 analysis without risking any individual's health. They were also the most caught in the study  
191 area allowing us to make a large spatial screening.

192

#### 193 2.2.1.2. *Adult grey partridges*

194

195 Grey partridges were caught during two consecutive years from November 2020 to February  
196 2021 (winter 2020–2021) and from December 2021 to March 2022 (winter 2021–2022) in the  
197 study area. These individuals, even when caught in the wild, are gamebirds and have been  
198 probably raised in captivity before being released for hunting purposes; however, knowing  
199 the proportion of captive-born vs. wild-born partridges is almost impossible as banding before  
200 release is not mandatory. In winter, grey partridges are mostly herbivorous and granivorous,  
201 living in large coveys in winter crops and sleeping in ploughed fields at night. Thermal  
202 binoculars were used to spot them at nightfall and birds were then captured in the dark by  
203 dazzling them and using a landing net (i.e., a method inspired by Eurasian Woodcock catching  
204 technique; Williams, 2015). Blood samples were then collected, and birds were immediately  
205 released. A total of 23 and 31 partridges were caught during the 2020–2021 and 2021–2022  
206 winters, respectively.

207

#### 208 2.2.1.3. *Montagu's harrier chicks*

209

210 The Montagu's harrier has been monitored in the ZAPVS since 1994 (Bretagnolle et al., 2018).  
211 In this intensive agricultural area, they nest mainly on the ground of cereal crops and lay up  
212 to eight eggs (Arroyo et al., 1998; Millon et al., 2008). The incubation period lasts 29 days, and

213 the rearing period is between 30 and 35 days (Arroyo et al., 2007). The mean productivity in  
214 this site is 2.05 fledglings per breeding attempt (Arroyo et al., 2004), this success depending  
215 mainly on the availability of its main prey, the common vole (*Microtus arvalis*) although in case  
216 of poor vole availability, harriers may also feed on orthopterans (Salamolard et al., 2000; Butet  
217 & Leroux, 2001). Blood samples of chicks that were  $26 \pm 2$  days old were collected from June  
218 2021 to early August 2021. Fifty-five chicks from 22 nests were sampled.

219

#### 220 2.2.2. Blood sampling procedure

221

222 For all species, blood samples were collected in 2021, and in 2020 and 2022 for grey  
223 partridges, on wild individuals included in different monitoring programs (see details in  
224 section 2.2.1). For all of them, blood sampling was conducted by puncturing the brachial vein  
225 using a sterile needle and using heparinized capillaries to collect 50  $\mu$ L of blood. Blood samples  
226 were placed in Eppendorf tubes and kept refrigerated (0–5°C), before being returned to the  
227 laboratory where they were stored at -20°C for further analyses.

228

### 229 **2.3. Neonics analysis**

230

231 Neonics extractions were conducted following the method reported by Rodrigues *et al.*  
232 (2023; see also Table S2 in supplementary materials for a description of the neonics). Briefly,  
233 blood samples were defrosted and weighed, and a mixture of 2 mL of dichloromethane and  
234 ethyl acetate (1:1) was added to each sample, followed by homogenisation by using a vortex  
235 for 1 min. Extracts were then sonicated for 10 min. This sonication step was repeated three  
236 successive times. After each sonication step, a centrifugation step of 5 min was performed,

237 and supernatants were collected, pooled, and then gently evaporated under a fume hood until  
238 a final volume of 500  $\mu$ L. The extract was collected and stored at -20°C until the analyses to  
239 determine pesticide levels were performed by liquid chromatography coupled to tandem  
240 mass spectrometry (LC/MSMS) using multiple reactions monitoring (MRM) for quantification.

241 LC/MSMS analyses were conducted with a Thermo Scientific TSQ Quantum Access  
242 Triple Quadrupole Mass Spectrometer operating in heated positive electrospray ionization  
243 mode (HESI+) coupled with a Thermo Accela 1250 pump and a Thermo Combi Pal  
244 autosampler. Analyses were performed on a Nucleodur C<sub>18</sub> Pyramid column (150 mm  $\times$  3 mm,  
245 3  $\mu$ m). Samples were analysed in the gradient mode using a mobile phase composed of water  
246 and acetonitrile with both containing 0.1% formic acid.

247 The multiresidue analysis comprised detection and quantification of 104 pesticide  
248 molecules, including five neonics—clothianidin, dinotefuran, nitenpyram, thiacloprid, and  
249 thiamethoxam—performed using the MRM detection mode. The source was operated in the  
250 positive ionization mode with a spray voltage of 4500 V and the same spray and capillary  
251 temperatures of 300 °C each. Nitrogen was used as the sheath and auxiliary gas (20 and 10  
252 arbitrary units), while argon was used as the collision gas (1.5 arbitrary units). Two precursor  
253 product ion transitions for each analyte and internal standards were used for quantification.  
254 The transitions selected for MSMS analysis and retention times are shown in supplementary  
255 materials (Table S3). Data were acquired and processed using Excalibur software.

256 The limits of detection (LOD) and quantification (LOQ) represent three and ten times  
257 the ratio of the average noise height on either side of a known amount of a compound's peak  
258 to the peak height, respectively. The objective was to determine the minimum peak heights  
259 that can be used to distinguish a compound's peak from the noise on either side of the peak.

260 The LOD and LOQ determined for each sample type are presented in Table S3. LODs varied  
261 from 0.001 to 0.012  $\text{pg}\cdot\mu\text{L}^{-1}$  and LOQs from 0.005 to 0.041  $\text{pg}\cdot\mu\text{L}^{-1}$ .

262

### 263 **3. Results**

264

265 No neonics were detected in blackbirds. For the other species, all 5 neonics searched, namely,  
266 clothianidin, dinotefuran, nitenpyram, thiacloprid, and thiamethoxam were detected. A  
267 summary of the concentrations and occurrence of these neonics in our sampled bird species  
268 is provided in Table 1, and detailed neonicotinoid distribution among each individual is  
269 provided in Figure 2. Clothianidin was found in all species (except blackbirds), with average  
270 concentrations ranging from 0.05  $\text{pg}\cdot\mu\text{L}^{-1}$  in common nightingales to 951.60  $\text{pg}\cdot\mu\text{L}^{-1}$  in  
271 Montagu's harriers. The number of individuals that exhibited concentrations higher than the  
272 LOQ ranged from ~5% among Montagu's harriers up to ~26% among grey partridges sampled  
273 during the 2021–2022 winter. No clothianidin was detected in grey partridges sampled in the  
274 2020–2021 winter. Thiacloprid was detected in all circl buntings and common nightingales but  
275 always at concentrations below the LOQ. It was detected in one Montagu's harrier chick (89.58  
276  $\text{pg}\cdot\mu\text{L}^{-1}$ ) and one grey partridge (0.07  $\text{pg}\cdot\mu\text{L}^{-1}$ ) in the 2020–2021 winter. Thiamethoxam was  
277 detected in both passerine species and in grey partridges from both winters, with  
278 concentrations ranging from 0.06  $\text{pg}\cdot\mu\text{L}^{-1}$  in a common nightingale to 23.73  $\text{pg}\cdot\mu\text{L}^{-1}$  in a grey  
279 partridge from the 2020–2021 winter. The number of individuals that exhibited  
280 concentrations higher than the LOD ranged from ~4% among common nightingales up to 12%  
281 among circl buntings. Dinotefuran and nitenpyram were only detected in grey partridges: In  
282 the first winter (2020–2021), ~13% and ~87% of the individuals exhibited dinotefuran and  
283 nitenpyram concentrations higher than the LOD, with the average concentrations being 6.20

284 and 23.10  $\text{pg}\cdot\mu\text{L}^{-1}$ , respectively. In the second winter (2021–2022), dinotefuran and  
285 nitenpyram were detected with concentrations higher than the LOD in ~32 and ~94% of the  
286 individuals, respectively, with the average concentrations being 7.85 and 18.29  $\text{pg}\cdot\mu\text{L}^{-1}$ ,  
287 respectively.

288

## 289 **4. Discussion**

290

291 In the last few decades, neonics have attracted considerable interest (Klingelhöfer et al.,  
292 2022), especially because of the rising concern about their effects on non-target species such  
293 as honeybees (Wood & Goulson, 2017) and humans (Cimino et al., 2017; Han et al., 2018).  
294 Because of their chemical properties such as their half-life in the soil ( $DT_{50}$ ), solvability, and  
295 leaching potential, and because of their extensive use for agricultural purposes, the  
296 assessment of their presence in farmlands is imperative (Thompson et al., 2020). Considering  
297 that no neonics were used on plants since 2018 in France and that the analytical method used  
298 here provided good sensitivity results (Rodrigues et al., 2023), we expected to find no or very  
299 low concentrations in bird blood samples as this matrix reflects short-term exposure (Espín et  
300 al., 2016). However, in the present study, we not only detected five out of seven neonics,  
301 three of which have been banned since 2018 in France (clothianidin, thiacloprid, and  
302 thiamethoxam) and two others (dinotefuran and nitenpyram) that are supposedly being used  
303 only for treating domestic pets (Table S2), but in some cases, at rather high concentrations.  
304 The remaining two neonics (imidacloprid and acetamiprid) are not discussed here as these  
305 compounds were not detectable with the multiresidue method used in this study. However,  
306 knowing the historical background of the study area and results from studies in the same area  
307 before neonics ban (see section 2.1), if titration had been possible, we would have expected

308 to find large amounts of imidacloprid in birds' blood and probably no or very low  
309 concentrations of acetamiprid.

310

#### 311 **4.1. Monitoring neonics in wild birds**

312

313 Neonics were increasingly studied during the last decade and several recent studies have  
314 focused on quantifying the level of exposure in wild birds. However, these studies differ from  
315 our work in several aspects. First, in these studies, several biological matrices—blood,  
316 carcasses, eggs, faecal pellets and cloacal fluids, feathers, liver, and/or gizzard—were  
317 considered (see Table S1 for detailed references). Correlations between neonics  
318 quantifications from different matrices can be found but are not systematic (reviewed in Espín  
319 et al., 2016). Indeed, pesticides are distributed in biological tissues with different temporal  
320 patterns. For instance, pesticide molecules are integrated into feathers during the moulting  
321 period (Humann-Guillemint et al., 2022). The distribution of such neonic molecules in  
322 internal tissues such as the liver also depends on the chemical nature of the molecules and  
323 can be degraded if samples are taken on carcasses (Espín et al., 2016, and references therein).  
324 Then, a time lag might be observed between the exposure and the detection depending on  
325 the matrices used (Lennon et al., 2020b). Therefore, the results obtained from different  
326 matrices cannot be directly compared.

327 Our results can thus be reasonably compared to those of five previous studies that  
328 tested for neonics in whole blood (or plasma) samples from wild birds (Taliensky-Chamudis et  
329 al., 2017; Byholm et al., 2018; Hao et al., 2018; Lennon et al., 2020b; and Humann-Guillemint  
330 et al., 2021; Table S4). For clothianidin, our concentration range was similar to those found by  
331 Lennon et al. (2020b), although the maximum value was lower: they found concentrations



332 ranging from 0.5 (dunnocks) to 69300  $\text{pg}\cdot\mu\text{L}^{-1}$  (yellowhammers), whereas in our study, the  
333 values were 0.009 (common nightingale) to 2451.78  $\text{pg}\cdot\mu\text{L}^{-1}$  (Montagu's harrier chick). The  
334 maximum clothianidin concentration found by Humann-Guillemint et al. (2021) was 0.34  
335  $\text{pg}\cdot\mu\text{L}^{-1}$  in alpine swifts, which is similar to the lower values in the concentration range we  
336 obtained. Thiacloprid concentrations in all ciril buntings and common nightingales were below  
337 the LOQ, but concentrations in the grey partridges and the Montagu's harrier chicks were far  
338 higher than those found in honey buzzards by Byholm et al. (2018) and in white-crowned  
339 sparrows by Hao et al. (2018). They ranged from 0.012 to 0.031 and 0.0025 to 0.0031  $\text{pg}\cdot\mu\text{L}^{-1}$   
340 for honey buzzards and white-crowned sparrows, respectively, and from 0.073 to 89.58  $\text{pg}\cdot\mu\text{L}^{-1}$   
341 <sup>1</sup> in our study (Table S4). This result suggests that the grey partridges and the Montagu's  
342 harrier chicks were exposed to high, or recent quantities of thiacloprid (see section 4.2 for  
343 further details). For thiamethoxam, the concentrations we found were above those obtained  
344 by Hao et al. (2018): they ranged from 0.06 (common nightingales) to 23.73 (grey partridges)  
345  $\text{pg}\cdot\mu\text{L}^{-1}$ , whereas Hao et al. reported 0.0051–0.0337  $\text{pg}\cdot\mu\text{L}^{-1}$ . Taliensky-Chamudis et al. (2017)  
346 detected imidacloprid in one Eurasian eagle owl (*Bubo bubo*) chick among the 30 sampled,  
347 but did not detect any other neonics, so comparisons with our results are not possible. The  
348 difference in the results despite all studies using blood samples may be because some of the  
349 studies considered whole blood samples (Taliensky-Chamudis et al., 2017; Byholm et al., 2018;  
350 our study), while others used only plasma (Hao et al., 2018; Lennon et al., 2020b; Humann-  
351 Guillemint et al., 2021), inducing differences in the detectability of some molecules whose  
352 levels vary according to their water/lipid solubility, and affinity to different proteins  
353 (Rodrigues et al., 2023; Zhang et al., 2023). Moreover, all these studies fundamentally differ  
354 in their sensitivity as they rely on their own developed chemical analysis methods [although  
355 they are all derived from the same method, i.e., Quick, Easy, Cheap, Effective, Rugged, and

356 Safe (QuEChERS); Anastassiades et al., 2003]. If we consider clothianidin for instance, LOD and  
357 LOQ in our study (0.01 and 0.04  $\text{pg}\cdot\mu\text{L}^{-1}$ , respectively; Table S3) were 5 (LOQ) and 15 (LOD)  
358 times lower, respectively, than those (0.15 and 0.21  $\text{pg}\cdot\mu\text{L}^{-1}$ , respectively) of Lennon et al.  
359 (2020b), while LOQ was similar to that (0.05  $\text{pg}\cdot\mu\text{L}^{-1}$ ) reported by Humann-Guillemot et al.  
360 (2021). This may induce a difference in the number of neonic positive samples between  
361 studies, hence differences in average concentrations as well. For instance, applying the LOD  
362 of clothianidin from the study of Lennon et al. (2020b) would have led to non-detection of  
363 positive samples in common nightingales while we found 8% of individuals with  
364 concentrations above our LOD. It is not therefore easy to compare the results provided by  
365 the authors, especially when not all statistical values, such as the percentage of neonic  
366 detection among individuals or the mean concentration with its standard deviation, were  
367 obtained on the same basis (Table S4). As the use of the same methodology in all studies does  
368 not seem to be feasible, researchers should systematically report these values to allow direct  
369 comparisons.

370

#### 371 **4.2. Exposure of wild fauna to neonics**

372

373 Previous studies reported the limitations of feather and internal tissue samples for  
374 determining timing of exposure to contaminants, so blood is considered most suitable for  
375 determining recent exposure (Espín et al., 2016; Lennon et al., 2020b). Neonics are supposedly  
376 “rapidly” excreted (in hummingbirds, English et al., 2021) and cleared from blood (24h in  
377 quails), according to data regarding imidacloprid (Bean et al., 2019) and thiamethoxam (Pan  
378 et al., 2022); however, extrapolation to the behaviour of other neonics may not be reliable.  
379 Based on blood samples used for this study we cannot estimate long-term exposure,

380 therefore, possible exposure of the migratory species at their wintering areas may be  
381 disregarded. However, thiamethoxam/clothianidin were found in grey partridges' eggs in  
382 another study (Bro et al., 2016), so in the case of the Montagu's harrier chicks, we cannot  
383 exclude the possibility of maternal transfer of neonics, i.e., from the mother to the eggs. In  
384 other words, all tested positive individuals in the present study were probably recently  
385 exposed to neonics. This is important because the birds were sampled three and four years  
386 after EU banned neonics for PPP, and no derogation is known in our study area conversely to  
387 other regions in France. Nonetheless, we found three out of the five PPP neonics in our  
388 samples, with concentrations similar to those in birds sampled elsewhere before the ban  
389 (Byholm et al., 2018). Taken together, our results strongly suggests that clothianidin,  
390 thiacloprid, and thiamethoxam are still present in farmlands and raises questions about the  
391 mode of exposure to birds. Thiamethoxam is metabolized into clothianidin in animals, plants,  
392 and soil (Nauen et al., 2003, review in Simon-Delso et al., 2015; Pan et al., 2022) potentially  
393 explaining why it is more commonly present than thiamethoxam. These neonics' DT<sub>50</sub> ranged  
394 from few days for thiacloprid up to several years for clothianidin (Table S2) in the soil, meaning  
395 that they can still be incorporated into the diet of several detritivores such as earthworms  
396 (Pelosi et al., 2022). If so, at least some blackbirds, which are omnivorous and feed on different  
397 invertebrate species during the breeding season, including earthworms which are known to  
398 bioaccumulate pesticides (Pelosi et al., 2022; 2021), should have tested positive, but were not.  
399 This surprising result could have been first seen as a technical failure; however, other pesticide  
400 molecules than neonics were detected in blackbirds' blood, so that an analysis problem can  
401 be discarded. The absence of neonics in blackbirds' blood might be the consequence of several  
402 processes. One of them could be a higher detoxification capacity of blackbirds, mediated by  
403 their ability at monopolizing high amounts of carotenoids, which are antioxidants, involved in

404 sexual selection and known for their role in reducing oxidative stress (Møller et al., 2000, see  
405 Moreau et al., 2022a). Further analyses would be needed to explore this explanation.  
406 Granivorous birds, including grey partridges (as they were caught during the winter), ciril  
407 buntings, and common nightingales, which are omnivorous (mostly insectivorous during  
408 reproduction), tested positive. This suggests that the mode of exposure may include  
409 contaminated seeds from previous treated crops (Wintermantel et al., 2018) or organisms  
410 feeding on contaminated seeds. The higher concentrations found in Montagu's harriers, which  
411 are apex predators (mostly preying upon common voles and orthopterans), might be a result  
412 of biomagnification, i.e., the accumulation of toxic neonics along the trophic chain (Badry et  
413 al., 2020). In fact, clothianidin and thiacloprid, two compounds quantified in Montagu's  
414 harriers' blood, were found in hairs of small mammals from the area, reinforcing that view  
415 (Fritsch et al., 2022). Further investigations would be needed, and one way to do so would be  
416 using chicks' food pellets. These differences among species may also be attributable to the  
417 choices of individuals regarding their habitat and feeding resources for avoiding contaminated  
418 sources, as has been highlighted previously (McKay et al., 1999; Ruuskanen et al., 2020; Addy-  
419 Orduna et al., 2022). Another explanation relies on the detoxification processes in wild birds  
420 that are still poorly investigated and deserved further investigations (Moreau et al., 2022a).  
421 Indeed, some individuals could be more efficient than others at protecting themselves against  
422 toxic substances (Arnold et al., 2015).

423 Another important issue observed in our results is the high prevalence of dinotefuran  
424 and nitenpyram in grey partridges, although wildlife fauna is not supposed to be exposed to  
425 these as they are used only for pets and not farm animals, and consequently not for outdoor  
426 use in Europe, including France (Table S2). Even if grey partridges caught in this study area  
427 may originate mainly from captive breeding stocks (released for hunting purpose), the six

428 registered veterinary medicines containing dinotefuran and nitenpyram neonics that are  
429 authorized for use in France are not intended for treating any bird species or farm animal  
430 (<http://www.ircp.anmv.anses.fr/index.aspx>). Imidacloprid is included in 36 different speciality  
431 medications, making this neonic potentially more common even if not detectable here (see,  
432 for example, Perkins et al., 2021). Neonics are either used for topical applications on the skin  
433 (dinotefuran) or *per os* (nitenpyram) to treat flea infestations in cats and dogs typically;  
434 further, nitenpyram is expected to be eliminated within 48 h (Jeschke & Nauen, 2005; Rust,  
435 2017). In Europe, veterinary regulatory processes are governed by the European Medicines  
436 Agency, which states that products for non-food animals are not supposed to be of major  
437 environmental concerns since these animals are treated individually with low concentrations  
438 of active neonics (CVMP/VICH, 2000). To our knowledge, these two neonics, investigated in  
439 few studies (Table S1), have only been detected in one sample of hummingbirds' feather  
440 rinsate (i.e., not within the organism; Graves et al., 2019), suggesting contact and not  
441 ingestion. In the present study, the substantial concentrations of dinotefuran and nitenpyram  
442 found in grey partridges but not in any other species may indicate possible exposure during  
443 their stay in farms before release. This implies either an illegal use of these substances in  
444 farms, as they are not included in veterinary medicines for farm animals, or an unintentional  
445 contact of partridges through multiple potential pathways. One of these could be direct  
446 contact and/or ingestion of farmer's dogs or cats' urine, or indirect through contaminated  
447 drinking water. However, neonics being presumably rapidly excreted and blood reflecting  
448 short-term exposure, individuals sampled here, if contaminated in farms before release,  
449 should not test positive. Thus, we cannot exclude in our case that partridges once in the wild  
450 could have directly ingested contaminated water with cat and/or dog urine and/or after pet  
451 baths (Teerlink et al., 2017; Diepens et al., 2023). Besides, partridges that commonly use field

452 margins contrary to the other species, where pets walk and urinate, may have ingested these  
453 neonics while preening as the external surface of feathers are often contaminated and might  
454 even accumulate compounds (Pacyna-Kuchta, 2023). Indeed, there is growing evidence of  
455 veterinary products' transfer to the environment from dogs' hair and urine, and of secondary  
456 transfer to wildlife through nesting material or contaminated water (Diepens et al., 2023).  
457 Even though we cannot ascertain by which route the partridges were exposed to these  
458 neonics, our results indicate in line with Diepens et al. (2023), the urgent need for monitoring  
459 all pesticide or medicine compounds, irrespective of their intended use. Although treating  
460 domestic animals against ectoparasites might be of sanitary importance for public health  
461 (human and animal), these treatments may have the same adverse effects on wildlife as PPP  
462 (reviewed in Moreau et al., 2022a).

463         It should be noted that the interpretations given to the results provided in the present  
464 study are only assumptions and that the origin of birds' contamination remain unknown.  
465 Ongoing studies on neonics levels in soils and invertebrates of the study area since 2018  
466 should help to provide further clarifications on birds' contamination pathways. Moreover, the  
467 method of multiresidue analysis used here could be improved to allow the detection of all  
468 neonics, including imidacloprid and acetamiprid, especially as imidacloprid was extensively  
469 applied in the crops of the study area and is still highly used in veterinary medicine.

470

### 471 **4.3. Conclusions**

472

473 Our results highlight several problems with the use of a class of chemicals that are among the  
474 most used worldwide. First, banning neonics for outdoor use does not prevent the exposure  
475 of wildlife fauna to them, at least a few years after ban. Although illegal use cannot be

476 disregarded, it cannot explain on its own their ubiquity in wild birds from a wide ecological  
477 range, caught at the scale of the study site. This is an important element to consider in  
478 countries where neonics are still massively applied, considering their impact on both animals  
479 and humans (reviewed in Moreau et al., 2022a). Second, the detection of neonics used for  
480 domestic animals in wildlife fauna raises questions regarding the manner in which risk  
481 assessment for such applications is performed (Perkins et al., 2021; Diepens et al., 2023). For  
482 instance, to our knowledge, few studies have investigated the effects of dinotefuran and  
483 nitenpyram on wildlife fauna (Wang et al., 2018). Although clothianidin, dinotefuran,  
484 nitenpyram, and thiamethoxam are considered to be of low toxicity to birds, as indicated by  
485 their acute oral LD<sub>50</sub> (Table S2), thiacloprid, which was also found to be quite ubiquitous, is  
486 highly toxic to birds (Table S2). However, LD<sub>50</sub> is indicative of acute lethal toxicity under  
487 laboratory conditions for model species and is not necessarily an appropriate estimate of  
488 sublethal effects, as shown in the numerous studies that investigated the adverse effects of  
489 neonics on birds (Moreau et al., 2022a). Considering that very low residual levels of pesticides  
490 may have considerable sublethal effects on birds' reproduction (see Moreau et al., 2021, for  
491 example), the consequences of these exposures on bird populations as well as on human  
492 health (*One Health* concept) should be carefully considered.

493

#### 494 **Ethical statement**

495 This study was conducted conforming the French guidelines for the ethical use of animals in  
496 research (APAFIS#18557-2019010822312199v2, APAFIS#9465e201703101551625). We are  
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502

### 503 **Declaration of competing interests**

504 The authors declare that they have no conflicts of interest.

505

### 506 **Credit author statement**

507 EF, AG, AR, MM, VB, JM and KM conceived the ideas and designed the methodology; AG, JM  
508 and KM collected the data; EF, AR, MM and KM analysed the data; EF, AG, VB, JM and KM led  
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969 **Caption for figures**

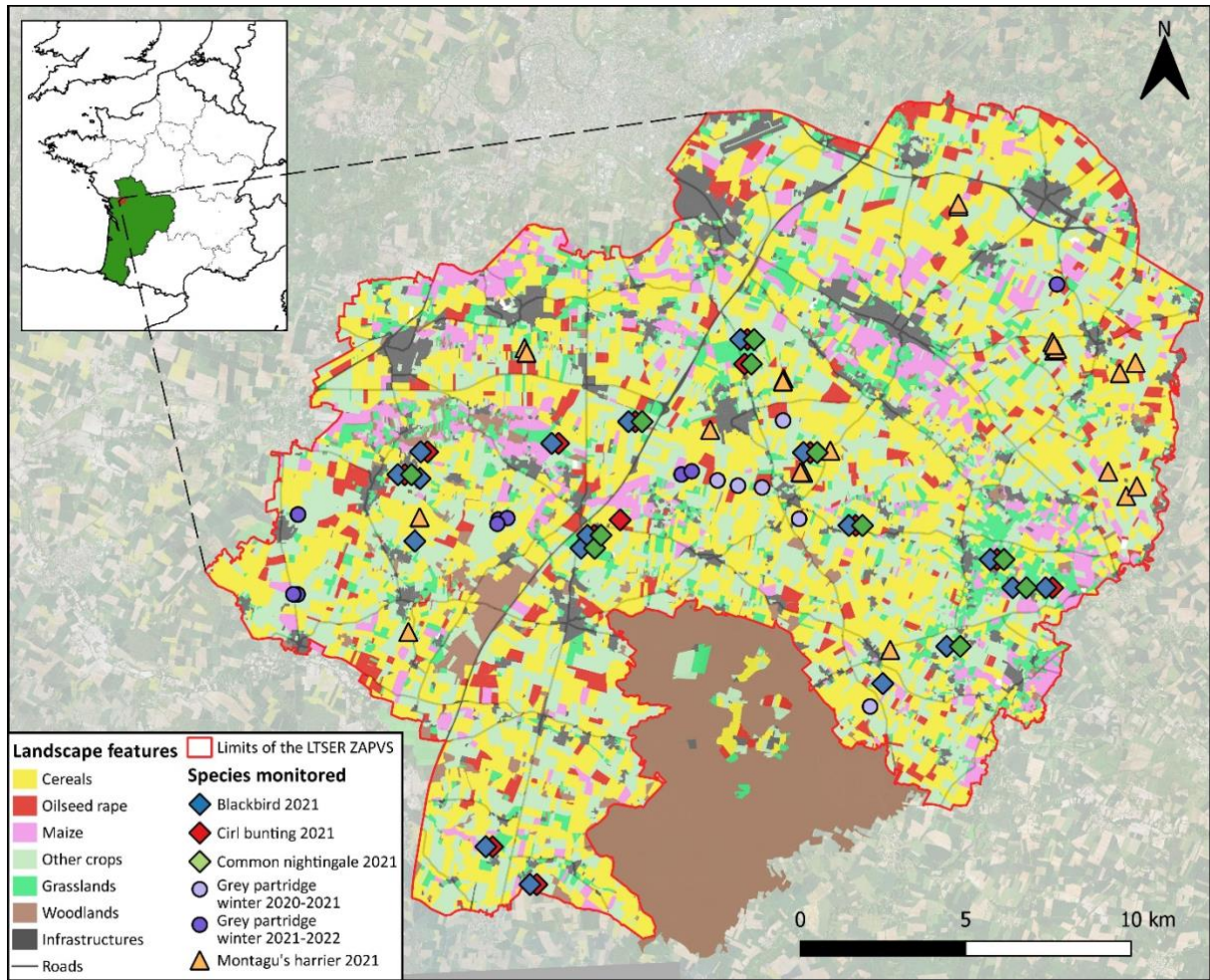
970

971 **Figure 1.** Spatial distribution of birds' catching sites across the Long-Term Socio-Ecological  
972 Research Zone Atelier Plaine & Val de Sèvres (LTSER ZAPVS). Landscape features provided  
973 correspond to data available for 2021 from our GIS database. Infrastructures correspond to  
974 buildings, sport fields, cemeteries, locks, and bridges present in the study area. The five bird  
975 species monitored in the study area are blackbird (*Turdus merula*,  $N_{2021} = 64$ ), ciril bunting  
976 (*Emberiza cirilus*,  $N_{2021} = 31$ ), common nightingale (*Luscinia megarhynchos*,  $N_{2021} = 34$ ), grey  
977 partridge (*Perdix perdix*,  $N_{2020-2021} = 23$  and  $N_{2021-2022} = 31$ ), and chicks of Montagu's harrier  
978 (*Circus pygargus*,  $N_{2021} = 55$ ). N corresponds to the number of adults sampled, except in the  
979 case of Montagu's harrier where N corresponds to the number of chicks sampled.

980

981 **Figure 2.** Wild farmland birds' exposure to 5 neonicotinoids. Distribution of the different  
982 neonicotinoid molecules for each individual of each species are represented as one stacked  
983 bar. Distribution in percent was obtained from raw concentrations of each molecule in birds  
984 blood sampled.

985



986

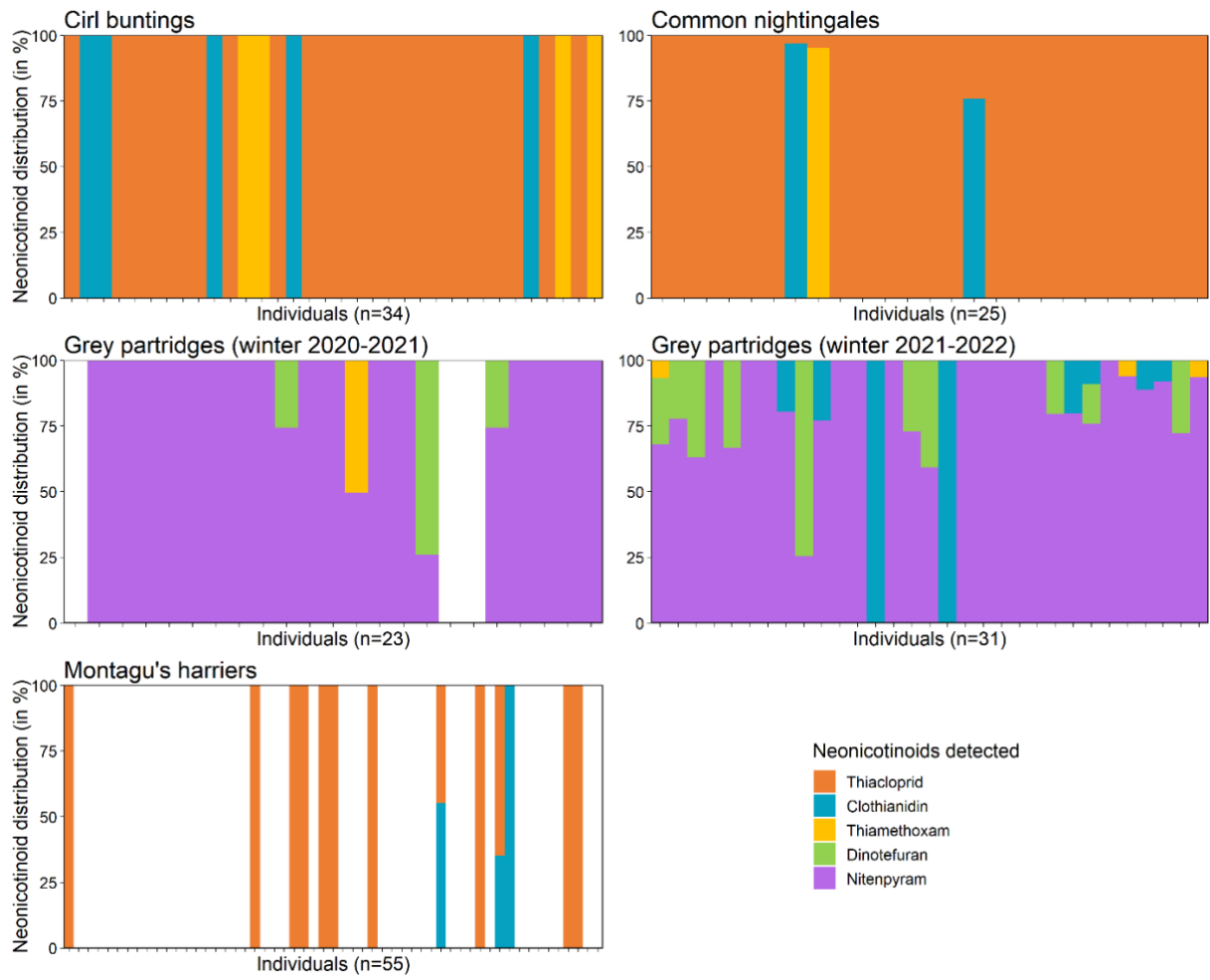
987

Figure 1.

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Figure 2.

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993 **Caption for tables**

994

995 **Table 1.** Summary of the mean concentrations [c] with standard deviations (SD) and range in  $\text{pg}\cdot\mu\text{L}^{-1}$  with their sample size (n) obtained by LC-  
 996 MS/MS above the limits of detection (LOD) for each species. The sample size for each species is also provided (N). The percentage of samples  
 997 above the LOD (n/N) is given in brackets (rounded to the nearest percent). Blackbirds are not referenced as no neonic was detected in the  
 998 sampled individuals.

	<b>Clothianidin</b>	<b>Dinotefuran</b>	<b>Nitenpyram</b>	<b>Thiacloprid</b>	<b>Thiamethoxam</b>
<b>Cirl buntings (N = 34)</b>					
n	5 (15%)	0	0	34 (100%)	4 (12%)
[c] ± SD	2.28±1.55	-	-	LOD < [c] < LOQ	2.59±1.37
range	1.04–4.87	-	-	-	1.61–4.56
<b>Common nightingales (N = 25)</b>					
n	2 (8%)	0	0	25 (100%)	1 (4%)
[c] ± SD	0.05±0.06	-	-	LOD < [c] < LOQ	0.06
range	0.009–0.093	-	-	-	-



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**Grey partridges (N = 54)**

<i>Winter 2020/2021 (N = 23)</i>	n	0	3 (13%)	20 (87%)	1 (4%)	1 (4%)
	[c] ± SD	-	6.20±3.62	23.10±11.33	0.07	23.73
	range	-	2.14–9.10	1.24–41.03	-	-

<i>Winter 2021/2022 (N = 31)</i>	n	8 (26%)	10 (32%)	29 (94%)	0	3 (10%)
	[c] ± SD	5.26±4.10	7.85±4.07	18.29±10.84	-	1.64±0.46
	range	1.92–14.26	3.32–16.61	1.78–43.53	-	1.23–2.14

**Montagu's harriers (N = 55)**

	n	3 (5%)	0	0	1 (2%)	0
	[c] ± SD	951.60±1299.21	-	-	89.58	-
	range	194.48–2 451.78	-	-	-	-

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