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NEW STRATEGIES AND NEW CHALLENGES FOR PESTICIDE STUDIES: HOW TO COMBINE THE PRESERVATION OF OUR ENVIRONMENT AND SUSTAINABLE AGRICULTURE?

Comparison of pesticide contamination between captive-reared and wild grey partridges: insights into environmental exposure disparities

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Abstract

Pesticide contamination is often cited as a key factor in the global decline of farmland birds. However, the majority of studies on pesticide exposure in non-target fauna are not representative of what happens in nature because they are limited to artificial conditions. The aim of this study was to define and compare, for the first time, pesticide contamination in grey partridges (*Perdix perdix*) from two different contexts, i.e., captivity vs. the wild. Blood samples taken from 35 captive and 54 wild partridges in 2021–2022 were analysed for 94 pesticides most commonly used in French agriculture. Captive partridges had 29 molecules detected in their blood (12 herbicides, 14 fungicides, and three insecticides) compared to wild partridges, which had 50 molecules (13 herbicides, 23 fungicides, and 14 insecticides). Of these pesticide compounds found in individuals, 26 were banned. Captive partridges had significantly fewer pesticide molecules than wild partridges, with one to 14 pesticides per captive individual and 8 to 20 pesticides per wild individual. Nineteen molecules were common to both groups, with concentrations up to three times higher in wild partridges than in captive partridges. Our results thus show multiple exposures for most of our individuals, especially in wild partridges, which can lead to cocktail effects, which are never considered. Furthermore, the difference in contamination between the wild and captive partridges reflects the multiple routes of contamination in nature, in particular, due to the use of a wide range of habitats by wild partridges.

Keywords Blood · Controlled environment · Farmland · Multiresidual analysis · *Perdix perdix* · Pesticide cocktails

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Introduction

In recent decades, agricultural landscapes worldwide have undergone major changes triggered by agricultural management and mechanisation, which have affected ecosystem functions and led to the decline of wild birds (Green et al. 2005; Inger et al. 2015; Stanton et al. 2018). Several factors, such as the reduction of food resources, the fragmentation and destruction of habitats, and the use of pesticides, are at the root of the progressive decline of farmland birds (Chiron et al. 2014; Stanton et al. 2018; Rigal et al. 2023). The role of pesticides in this decline is undoubtedly the most debated factor. Considered an environmental stressor, these agrochemicals can have direct and indirect effects at individual, population, and community levels, on target and non-target species (Guerrero et al. 2011; Sabatier et al. 2014; Mitra et al. 2018). Ingestion of pesticides can be fatal in the short term, but chronic exposure over the long term, even at low levels, can have sublethal effects on individuals (Moreau et al. 2022a). Pesticides can act at the intracellular level, affecting physiology (e.g., immune system disorders, Franco et al. 2009), behaviour (Mitra et al. 2018), and life history traits (i.e., reproduction and survival, Mitra et al. 2018; Lopez-Antia et al. 2018; Kumar et al. 2019). They can affect population dynamics (Potts 1986; Potts and Aebischer 1995; Rigal et al. 2023) and even disrupt community structure, for example at the level of the food chain (Hanazato 2001).

To grant marketing authorization for pesticides, toxicity tests on a few model species (e.g., Japanese quail or mallard duck for birds) are assessed by short-term tests carried out under controlled conditions by only one route of exposure, the oral route (i.e., considered to be the main route of exposure in birds) (Moreau et al. 2022a). Generally, individuals are exposed to different concentrations of a single contaminant over a period of a few weeks to a few months, depending on the life expectancy of the species and the half-life of the molecules tested. However, the use of these artificial conditions to study the effects of pesticides on organisms has been criticized as being unreliable for extrapolation to real life, i.e., in the wild (Moreau et al. 2022a). Indeed, individuals in the wild will cover a wider range of habitats than those in captivity, depending on their vital needs (e.g., feeding). They are thus exposed to pesticides by multiple routes, with some molecules being taken up by routes other than the oral route (e.g., dermal route, Vyas et al. 2007). Pesticides are dispersed in soil, water, and air, and individuals can be exposed by contact with the product or by eating contaminated food (Sánchez-Bayo 2021; Fritsch et al. 2022; Fuentes et al. 2023a, b). However, in the wild, the amount of pesticides present in each compartment such as soil, plants, and animals is unknown, so the concentrations used in the laboratory may not reflect the real exposure that species encounter in the field (Mineau 2005; Hilbers et al. 2018). Furthermore, non-target species are not exposed to a single molecule as in laboratory experiments, but to a cocktail of pesticides, with potential interactions between/among molecules and different effects on organisms (Moreau et al. 2022a). It is therefore difficult to reproduce the complexity of realistic environmental exposures, and this may explain why results from experimental studies and effects measured in nature are poorly correlated (Story and Cox 2001). Understanding the extent of bird contamination in different situations is therefore important and should provide guidance for pre-approval risk assessment testing.

To our knowledge, no study has compared the level of contamination in wild *versus* captive-bred birds (i.e., artificial conditions with a single habitat type and mostly a single source of contamination, food). We expect captive-bred birds to be less exposed to pesticides than wild birds, which use a wide range of habitats and consume natural diversified foods. To test this hypothesis, we take advantage of a species living in both conditions, the grey partridge, *Perdix perdix*, an iconic game bird of European farmland and a valuable candidate for assessing exposure to a pesticide cocktail in an agricultural context. Since 1950, this farmland species has declined significantly in Europe, mainly due to the intensification of agriculture with landscape modification and the massive use of pesticides (Kuijper et al. 2009). As sedentary birds living in agricultural areas, they have a rather small home range (i.e., less than 150 ha) and are exposed to a wide range of pesticides, mainly through their diet. Partridge chicks feed mainly on insects during the first 10 days of their life, after which their diet becomes increasingly composed of plant material and seeds. This corresponds to the main diet of adults, who consume leaves and seeds of cultivated plants such as winter cereals, oil-seed rape, and seeds of wild plants such as *Amaranthus retroflexus* and *Polygonum aviculare* (Browne et al. 2006; Orłowski et al. 2011). These two main plant food groups are particularly exposed to pesticides, through the use of herbicides to control weeds in crops, or by using insecticides and fungicides to control pests and fungal diseases in cereals (Gurr et al. 2003). Thus, for grey partridges, food from these crops is a significant source of exposure to pesticides. Experimental studies with captive grey partridges have notably shown sublethal effects on their physiology and behaviour after ingestion of conventional cereals intended for animal consumption, containing pesticide residues (Moreau et al. 2021; Gaffard et al. 2022a, b). Because of the decline of this species and its hunting value, several farms have been set up to produce individuals for release at the start of the hunting season. These individuals are reared in semi-field conditions (large pens), which is considered to be a homogeneous environment, and should therefore be less exposed to pesticide contamination than wild partridges, which live in a greater variety of habitats and are likely to be more exposed from different sources. By controlling their diet as much as possible, captive partridges can be considered a control group compared to wild individuals who are more likely to be exposed to contaminated food sources. To test our hypothesis, we sought to characterize and compare the pesticide profile for 94 pesticides most commonly used in agriculture in France in the blood of 35 captive-bred grey partridges from a commercial game farm and 54 grey partridges living in the wild.

Material and methods

Characterization of contamination in natural populations

The study was conducted on the Long-Term Socio-Ecological Research (LTSER) platform "Zone Atelier Plaine & Val de Sèvre" (ZAPVS, Deux-Sèvres) in western France (Fig. 1). The study area covered 435 km^2 and was characterized by intensive cereal production, with 60 organic farms and 350 conventional farms (Bretagnolle et al. 2018). During 2 consecutive years, from November 2020 to February 2021 and from December 2021 to March 2022, 54 wild grey partridges (29 males and 25 females) were captured on bare farmland. The captures took place after nightfall, in the dark,

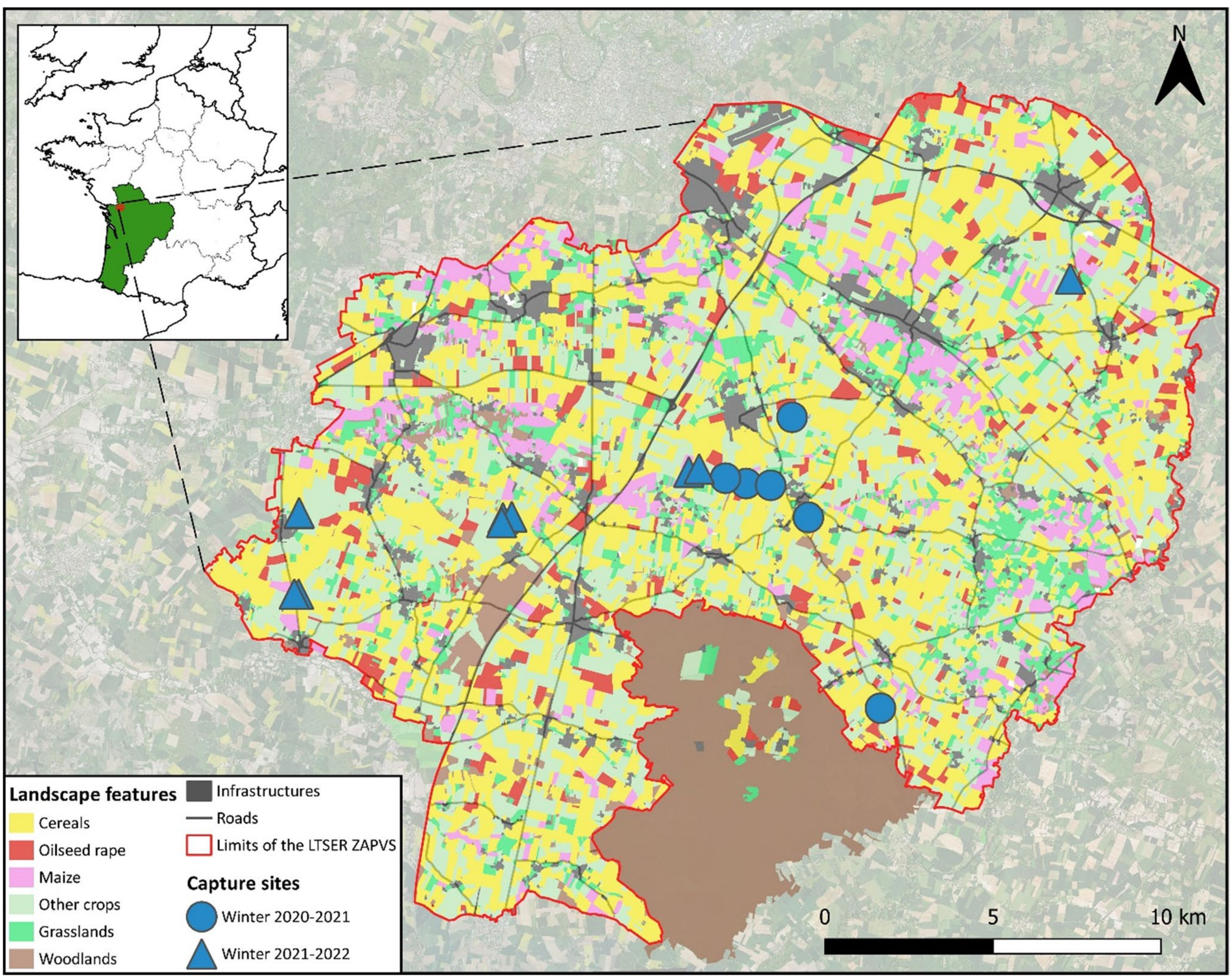

Fig. 1 Location of wild partridge traps in the Long-Term Socio-Ecological Research Zone Atelier Plaine & Val de Sèvres (LTSER ZAPVS). Partridges captured in winter 2020–2021 ($n=23$) are represented by blue circles; those captured in winter 2021–2022 ($n=31$) are represented by blue triangles. Landscape features are taken from the 2021 data in our GIS database, with infrastructure corresponding to buildings, sports fields, cemeteries, locks, and bridges

using thermal binoculars, dazzlers, and a landing net (i.e., a method inspired by the Eurasian woodcock trapping technique), when the weather was favourable (cloud cover, no moon) and individuals were sleeping in the plough. Partridges were captured at different times of the night, then placed together in transport boxes and brought back to the laboratory to spend the night with food (i.e., organic maize and wheat) and water *ad libitum*. The next morning at 9 am, each captured partridge was identified with an alphanumeric metal ring, a blood sample was taken, and the animal was released at the point of capture. Keeping them until the morning allowed us to standardise the time of blood sampling and to avoid the risk of predation, which is higher at night on individuals stressed by capture.

Characterization of contamination in captive partridges

The captive partridges came from a wild genetic strain (the F3 generation of wild captured birds) and were raised in an aviary (100 m × 15 m) located in a game farm in La Grossière (Deux-Sèvres, France). Thirty-five individuals, identified by an alphanumeric metal ring, were fed *ad libitum* on a mixture of cereals purchased from conventional farmers, containing equal amounts of wheat, maize, peas, and faba beans, to which various pesticides had been applied during the growing season. The aviary was considered a semi-field environment and was surrounded by conventionally grown fields. The birds were constantly monitored, watered, and fed daily by the farmer and kept under a natural light cycle. At 1 year of age, blood samples were taken from all individuals to quantify their pesticide exposure and diversity.

Multi-residual analyses of pesticides

For all individuals (wild and captive partridges), 50 µl of blood was collected from the brachial vein using sterile needles (Ø 0.06 mm), heparinized micro-capillary tubes, and directly reserved in Eppendorf tubes. All samples were stored at − 80 °C until multi-residual analysis. Following the method of Rodrigues et al. (2023), 94 pesticides were monitored for each individual. The limits of detection (LOD) and quantification (LOQ) for all molecules are provided in Tables S1 and S2 (Supplementary Information).

We assessed the pesticide load by using two metrics commonly found in ecotoxicology studies (e.g., Fritsch et al. 2022; Tartu et al. 2014): the number of pesticides detected ("N_P" hereafter) and the concentration (in pg.mg^{-1}) of each pesticide, The comparison of N_p between the two groups of grey partridges was assessed using a Wilcoxon test. Statistical analyses were performed with RStudio software (version 4.0.4, R Core Team 2021). For all tests, p-values were given at a 0.05 significance level.

Results

Overall, 60 of the 94 pesticides were detected in the blood of all partridges. Twenty-nine molecules were found in captive partridges, including 12 herbicides, 14 fungicides, and three insecticides (Fig. 2).

Four pesticides were present in the blood of more than 50% of captive individuals: diphenylamine and nitenpyram in 31 individuals, tolylfluanid in 21 individuals, and bifenox in 20 individuals (Fig. 2). In comparison, 50 pesticides were detected in wild-caught partridges, including 13 herbicides, 23 fungicides, and 14 insecticides (Fig. 3). Eleven pesticides were present in the blood of 50% or more of wild individuals, i.e., bifenox, carbendazim, chloridazone, cyproconazole, epoxyconazole, etridiazole, indoxacarb, metamitron, nitempyram, triadimenol, and trifluxustrobine (Fig. 3). Nineteen molecules were common to both groups, with nitempyram and bifenox present in more than half of the individuals in each group (Figs. 2 and 3). In captive partridges, the most prevalent molecules were those common with wild partridges, with molecules found only in them being detected in less than six individuals each time (Fig. 2). Conversely, 31 molecules were only found in wild partridges, with many of them having a very high prevalence in the individuals sampled, e.g., chloridazon, cyproconazole, etridiazole, indoxacarb, metamitron, triadimenol, and trifloxystrobin (Fig. 3).

Overall, captive partridges had significantly fewer pesticide molecules in their blood (median = 4 molecules) than wild-caught partridges (median = 14 molecules; Wilcoxon–Mann–Whitney test, $W = 32.5$, $p < 0.0001$; Fig. 4). The number of pesticides in the blood of captive partridges ranged from one to 14 molecules per individual, compared with wild partridges which had between eight and 20 pesticides each (Figs. 2 and 3). For the 19 molecules common to both groups, concentrations in wild-caught partridges were up to three times higher than those found in captive partridges (Figs. 2 and 3). For example, alachlor concentrations in the blood of captive partridges ranged from 0 to 72.561 pg.mg^{-1}, with an average of 5.86 ± 19.43 pg.mg^{-1}, whereas concentrations in wild-caught partridges ranged from 0 to 228.9 pg.mg^{-1}, with an average of 18.49 ± 46.55 pg.mg^{-1}. Similarly, epoxyconazole concentrations ranged from 0 to 0.12 pg.mg^{-1} with an average of 0.003 ± 0.02 pg.mg^{-1} in the blood of captive partridges, whereas the blood of wild partridges showed concentrations ranging from 0 to 414.3 pg.mg^{-1} with an average of 171.82 ± 77.55 pg.mg^{-1}.

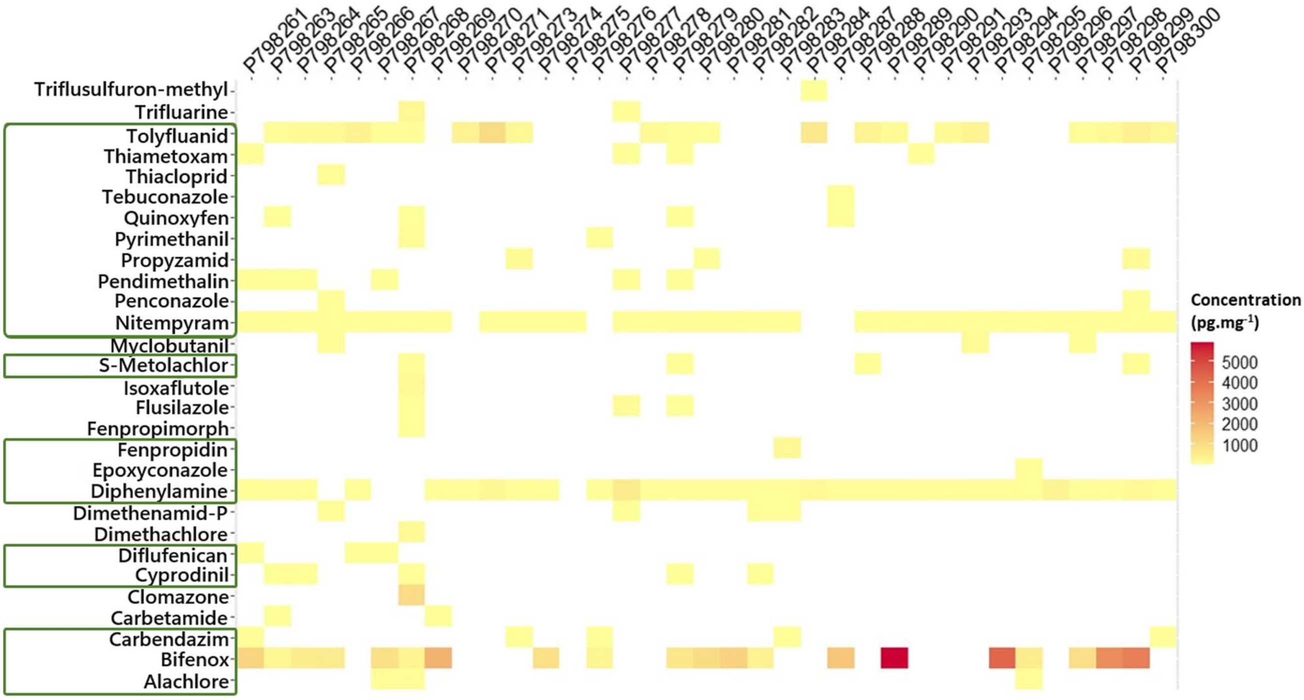

Fig. 2 Heat map illustrating the concentration (pg.mg^{-1}) of the 29 pesticide molecules found in the blood of 35 captive grey partridges. The redder the concentration, the higher the concentration. The molecules framed in green are those also found in wild partridges

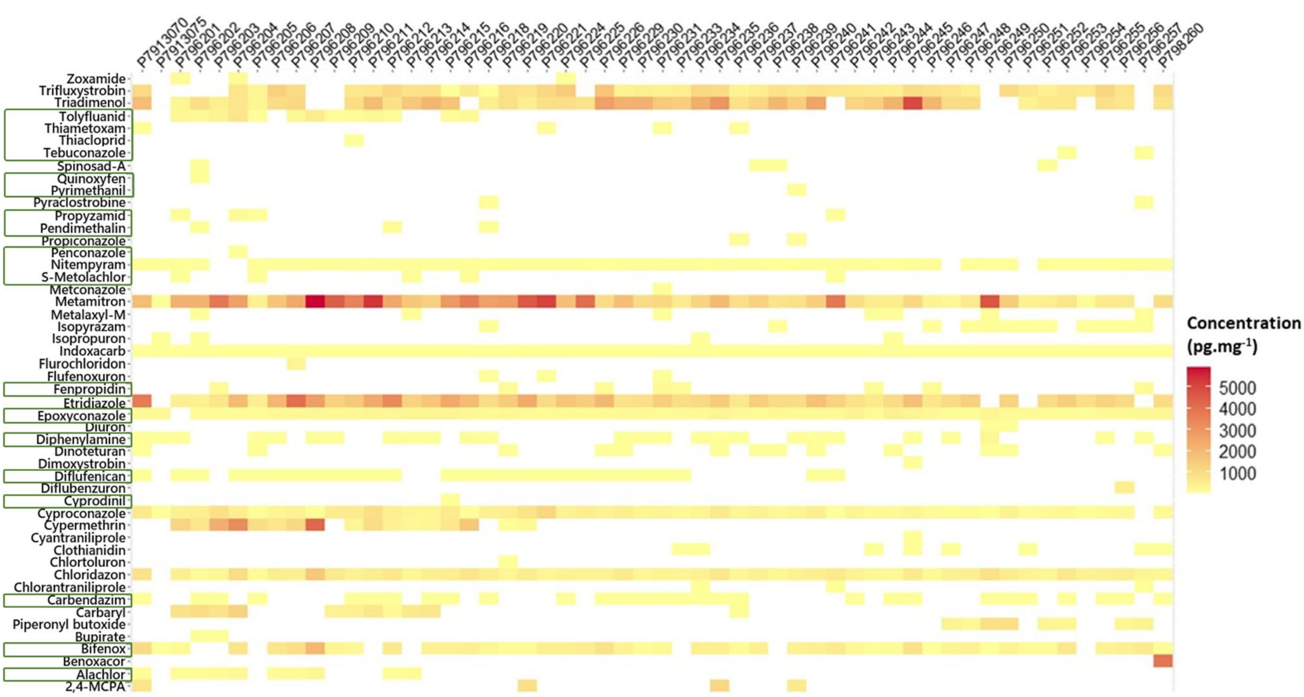

Fig. 3 Heat map illustrating the concentration (pg.mg^{-1}) of the 50 pesticide molecules found in the blood of 54 grey partridges captured in the wild. The redder the concentration, the higher the concentration. The molecules framed in green are those also found in captive partridges

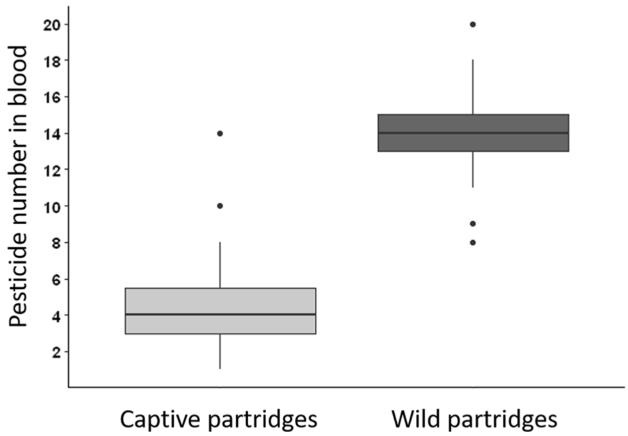

Fig. 4 Comparison of the number of pesticides found in the blood of captive partridges ($n=35$) and wild partridges ($n=54$). The horizontal line in the centre of the box corresponds to the median, the horizontal lines at the top and bottom of the box are the first and third quartiles, and the vertical line indicates the lower and upper values included in the confidence interval of 95%. Black circles correspond to outliers

Discussion

The present study compares, for the first time, the pesticide contamination of grey partridges from two different contexts, i.e., captive and wild individuals. Using a recent method for the multi-residual determination of pesticides in blood, we were able to study the exposure of individuals in the short term, reflecting local environmental contamination (Espín et al. 2016).

In contrast to experimental studies, which often evaluate the effect of a single molecule (Moreau et al. 2022a), we found a variety of molecules in our individuals, whether from captive or wild environments. The most prevalent molecules in captive partridges were also found in wild individuals, e.g., bifenox, diphenylamine, nitenpyram, or tolyfluanid. The presence of these molecules in both captive and wild partridges suggests that they are present in both environments, despite the distance between study sites (ca. 30 km). Although the aviary is considered to be a semi-homogeneous environment, it is surrounded by conventional agricultural fields. For this reason, pesticides applied in the surrounding fields could potentially contaminate the water, air, dust, plants, and soil in the enclosures. The fraction of applied pesticides that is dispersed into the air is estimated to be ca. 15–40% (Socorro et al. 2016), and the transport of molecules can even occur over long distances, up to thousands of kilometres in the atmosphere (Shen et al. 2005). In addition, previous studies on grey partridges present in the same type of aviary have highlighted their contamination by pesticide residues, some of which are found in the seeds ingested from conventional and even organic farming (Moreau et al. 2021; Gaffard et al. 2022a, b). However, this contamination remains lower than in wild partridges. About ten molecules were found only in captive partridges, with a very low prevalence (i.e., < 20% of individuals for each molecule). For wild partridges, the number of molecules specific to this group was three times higher, with 31 molecules detected, some of which were very common (i.e., > 50% of individuals). Some pesticides frequently detected in blood, such as chloridazon, etridiazole, or indoxacarb, have been banned although recently (see Table S2). More generally, pesticide levels in the blood of all partridges (captive and wild) revealed the presence of 26 molecules that have been banned for several years (Tables S1 and S2). If blood is supposed to reflect recent exposure (Espín et al. 2016), this would mean that these molecules are still present in the birds' diet and/or environment. For example, for pesticides banned from use for several years such as chloridazon (2018), diphenylamine (2012), or tolyfluanid (2007), we did not expect to find them in blood samples from birds. However, these molecules were found in the birds studied here, some with a high prevalence in our two groups of individuals (e.g., diphenylamine). These results show that many banned pesticides are still present in detectable and quantifiable concentrations in agricultural areas and especially in food networks (Fritsch et al. 2022). Those that persist in soil may be remobilised due to current agricultural practices and climate change, as shown for example by DDT stored in vineyard soils (Sabatier et al. 2014). Illegal use could also be one of the causes of partridge contamination, as reported in the ZAPVS for fipronil and lindane (Fritsch et al. 2022), which could have serious consequences for wildlife populations in Europe. Moreover, the detection of the two neonicotinoids dinotefuran and nitenpyram is also questionable since there are two insecticides that are only authorized for use on dogs and cats. This result would suggest that grey partridges may have come into unintentional contact with substances through several routes, for instance by ingesting water contaminated with cat or dog urine (see Fuentes et al. 2023a for more details).

Furthermore, despite the presence of several pesticides in captive partridges that appear to come from a source other than their diet, the differences in contamination levels between the two groups studied were significant. Captive partridges were expected to be less contaminated due to the homogeneous environment of semi-field conditions compared to wild individuals which are more likely to be contaminated from different sources due to the wide variety of habitats in which they live. Grey partridges living in the wild were at least twice as contaminated with pesticides as captive individuals, with almost twice as many fungicides and five times as many insecticides found in their blood. Overall, more molecules from different chemical families were found in wild partridges, with a proportion of individuals being more heavily contaminated. For the 19 pesticide molecules in common, the concentrations quantified in the blood of wild partridges could be up to three times higher than those found in captive partridges. A first explanation for this result could be related to the different detoxification/metabolisation capacities of the partridges. Indeed, a study on grey partridges showed that the enzymatic activity of cytochrome P450 involved in body detoxification could be different between wild and hand-reared individuals, with wild partridges having lower enzymatic activity in particular (Liukkonen-Anttila 2001). These individuals would therefore have a reduced ability to detoxify their bodies from pesticides. Another explanation could be that higher contamination of wild partridges in nature may occur through their use of a wide range of habitats and consumption of different food resources (i.e., leaves and seeds of cultivated plants or weeds), which are known to be chemically treated, such as the application of herbicides (Browne et al. 2006; Orłowski et al. 2011; Moreau et al. 2022a). Indeed, although their home range is relatively small (< 150 ha), grey partridges have complex and extensive habitat requirements (Potts 2012). Habitat heterogeneity appears to be of greater importance to them because this heterogeneity provides them with different resources to secure their vital needs, i.e., nesting sites and food (Potts 2012; Schöll et al. 2023). As a result, they may be exposed to pesticides in multiple locations and via multiple pathways within their home range. When pesticides are applied to target plants in the field, they can degrade into new chemicals (Marie et al. 2017) or move through transfer processes such as adsorption, leaching, volatilisation, spray drift, and runoff (Robinson et al. 1999; Tudi et al. 2021). Pesticide residues can be taken up into the air by inhalation or when birds preen (Mineau 2011; Sánchez-Bayo 2021). Chemical uptake can also occur through the dermal route, particularly through the skin on the bird's legs (Vyas et al. 2007), or through maternal transfer (Mineau 2005). Finally, in nature, other contaminated foods such as insects, water, foliage, seeds, and treated plants may be consumed (Mineau 2011; Syafrudin et al. 2021). To better understand how individuals are exposed, future research should therefore focus on habitat use by partridges. Characterizing the home range of wild partridges, for instance with GPS tags on individuals, should therefore be further investigated as well as determining habitat type and learning about pesticide use by farmers in the fields occupied by individuals to get an idea of their pesticide exposure. Furthermore, the inclusion of partridge chicks, which unlike adults feed on insects (Browne et al. 2006), would allow a better understanding of the contribution of ingested food to the transfer of pesticides in birds, accumulating pesticides via insects and contributing more to cocktail effects (Tison et al. 2024). Given the wide range of habitats used by wild birds and the multiple sources of contamination in nature, there is a real need for innovative research that integrates cocktail effects and realistic exposure doses to better understand the hidden processes at the root of declines in farmland bird populations.

Considering the number of pesticides found in the blood, only one captive partridge was found to have a single molecule, but most partridges were found to have several pesticides. Wild partridges had at least eight different molecules in their blood and up to 20 molecules, consistent with the multiple exposure explanation in nature and the diversity of habitats covered by wild individuals. Four pesticides (i.e., an insecticide, indoxacarb; an herbicide, chloridazon; two fungicides, cyproconazole, and epoxyconazole) were also present in all wild partridges. A diversity of molecules is found at the individual scale, which reflects the actual exposure of non-target fauna in the environment, as pesticides are often used in a mixture, with different molecules used on the same crop (Moreau et al. 2022a). This contamination by several pesticides was also found in the chicks of the Montagu's harrier living in the same study area ZAPVS using the same analytical method (Rodrigues et al. 2023; Fuentes et al. 2024). In these chicks, ten herbicides, 12 fungicides, and five insecticides were detected in the blood. Chloridazon was also one of the most prevalent (31% of chicks), and it was in the grey partridges in this study (almost all the wild partridges). In grey partridge, no study allows the same comparison to be made with the analytical method. However, several pesticide molecules have been found in grey partridge carcasses in the northwest of France, including in particular s-metolachlor and cypermethrin (Millot et al. 2015). The simultaneous presence of several pesticides can induce cocktail effects through chemical interactions depending on their own properties, concentrations, and location in the organism, leading to different effects on organisms (Cedergreen 2014; Hernández et al. 2017). Three main scenarios can be proposed (Hernández et al. 2017): (i) the molecules do not interact and have additive or independent effects, (ii) the molecules interact and act synergistically with increased effects relative to their respective actions, and (iii) the molecules interact and act antagonistically with decreased effects relative to their respective actions. These effects remain poorly studied in the literature, as the experimental designs used do not allow the detection of real interaction(s) between pesticides, at least beyond two molecules, which is the rule under field conditions (Moreau et al. 2022a). Given all the possibilities of interactions between molecules and even metabolites of certain compounds, the study of cocktail effects remains difficult experimentally but can be inferred from wildlife monitoring, for example, that of wild birds inhabiting contrasting habitats for pesticides used along an organic farming gradient could be valuable (e.g., in passerine birds, Moreau et al. 2022b or raptor species, Fuentes et al. 2023b). These interactions between molecules could then be taken into account when studying the impact of pesticides on bird health and other traits such as reproduction. In this study, the concentrations of pesticides found in the blood of partridges were all lower than the LD50, therefore probably not affecting their survival. However, chronic exposure to smaller amounts can elicit sublethal effects, i.e., long-term effects (Moreau et al. 2022a). Other studies on grey partridges fed with grains containing pesticide residues have demonstrated that ingesting low pesticides doses over a long period had consequences on physiological pathways (e.g., lower concentrations of carotenoids, higher activated immune system; Moreau et al. 2021) and at the transgenerational level, i.e., chicks of the partridges fed with contaminated grains had a lower body mass than chicks of partridges from the control group (i.e., without pesticide residues in grains) (Gaffard et al. 2022b). Therefore, due to these deleterious side effects of pesticides even at low concentrations, the overall contamination of partridges in our study should encourage regular monitoring of contamination in wild species, including health parameters (immune system, behaviour, microbiota, etc.), which may allow a better understanding of the role of pesticides in the decline of specialist birds in agricultural areas (Moreau et al. 2022a).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-024-34925-z.

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Author contribution JM, KM, OP, VB, and AG contributed to the study conception and design. Material preparation, data collection, and analysis were performed by AG, JM, AR, and MM. The original draft of the manuscript was written by LB. All authors provided editorial advice.

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Data availability Once the paper will be accepted, data will be deposited in the Dryad.

Declarations

Ethics approval All experiments complied with French laws on animal experimentation. Permission to capture and handle the study birds was given by the authority Préfecture Départementale des Deux-Sèvres (Number 2022/1).

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests The authors declare no competing interests.

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