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First evidence of age-dependent decreases in non-persistent pesticide mixtures in nestlings of a farmland raptor

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ABSTRACT

Intensive use of synthetic pesticides in conventional agriculture may harm non-target organisms through sub-lethal effects on life-history traits. Farmland birds are exposed throughout their life cycle, but the fate of non-persistent pesticide mixtures in wild birds remains unknown. In this study, we investigated changes in pesticide contamination levels in Montagu's harrier (*Circus pygargus*) nestlings during their growth. In total, 35 chicks were sampled twice during the rearing period, and blood was tested for 116 pesticides to assess pesticide load through two proxies; the number of pesticides detected and the sum of pesticide concentrations. Body mass and tarsus length were also measured to estimate body condition. Across the two sampling times, nine herbicides, five insecticides and four fungicides were detected. Contamination levels decreased significantly with nestling age irrespective of sampling date, and there was no relationship between pesticide load and body condition. Moreover, concentrations of chlorpyrifos-methyl, fenpropidin, metamitron, picloram and S-metolachlor, all detected throughout the rearing period, were unrelated to any of the explanatory variables. However, ethofumesate concentrations decreased significantly with chick age. This study provides the first evidence that non-persistent pesticide mixtures can decrease with age in wild nestlings. This has implications for understanding how chicks are contaminated and provides new insights on pesticide fate within organisms.

1. Introduction

In agroecosystems, the intensification of agriculture has led to landscape homogenisation and increasing mechanisation and chemical inputs (Matson et al., 1997; Stanton et al., 2018). Among chemical inputs, pesticides are applied to prevent and control pests and weeds that may cause diseases or compete with crop plants, and include herbicides, fungicides, insecticides and rodenticides. Multiple pesticides are applied in fields simultaneously or sequentially, adsorbed by target and non-target species, and then supposed to be naturally degraded in the environment through a variety of processes, leaving by-product residues (Al-Mamun, 2017). However, the drift and persistence of some pesticides may lead to them remaining in soils and aquatic environments, possibly far from their application location, and even in untreated crops, sometimes years after their last application (Humann-Guilleminot et al., 2019; Wintermantel et al., 2020; Geissen et al., 2021). Consequently, even without direct exposure to some banned compounds, various

species belonging to multiple trophic levels are contaminated with pesticide mixtures, highlighting the transfer of pesticides in all environmental compartments (Pelosi et al., 2021; Fritsch et al., 2022; Fuentes et al., 2023a, 2024a; Merleau et al., 2024). Some currently used pesticides are cleared from non-target organisms within a few hours to days (Bean et al., 2019; Pan et al., 2022). However, toxicokinetics is typically only conducted under controlled conditions for single compounds in one or a few model species, limiting knowledge extrapolation (Bean et al., 2019; Moreau et al., 2022; Pan et al., 2022). Little is known about the extent to which wild species are exposed to pesticides *in natura* (but see Bariod et al., 2024 for a captive vs. field comparison) and even less about how it changes over the season or during animal growth and development because very few studies have investigated variation in pesticide contamination levels over time in the field (but see Merleau et al., 2024). Thus, the fate of synthetic pesticide mixtures within wild organisms remains unknown, which is a concern because they are considered a major factor responsible for biodiversity decline, especially

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among farmland birds that have declined sharply across Europe (Geiger et al., 2010; Rigal et al., 2023). Indeed, whether currently used or banned, pesticides are known to have lethal and sublethal effects in non-target organisms such as wild birds, even at low doses (see Mitra et al., 2011; Moreau et al., 2022; Fritsch et al., 2024).

In wild birds, studies on heavy metals and persistent organic pollutants (POPs) reported differences in contamination levels of adults at different breeding stages, with higher concentrations from pre-laying to chick rearing periods (Nordstad et al., 2012), in nestlings depending on the growth stage (Lemmettyinen et al., 1982; Honda et al., 1986; Ackerman et al., 2011), and across the lifetime, with moderate changes in heavy metal concentrations (Bauerová et al., 2020). For nestlings, there can be a reduction in contamination levels with ageing, or the reverse, depending on the pollutant (Lemmettyinen and Rantamäki, 1980; Honda et al., 1986; Bustnes et al., 2013), U-shaped variation (Ackerman et al., 2011), or no variation with age (Monteiro and Furness, 2001; Bustnes et al., 2003). In fact, during nestling growth, pollutant concentrations may vary with a reduction in lipid content, and with distribution in growing tissues and feathers (Honda et al., 1986; Becker and Sperverlage, 1989). Fat tissues are rich in lipophilic pollutants, particularly those provided by the females through egg yolk (Charnetski, 1976; Mineau, 1982); therefore, the decline in lipid content during chick growth coupled with both an increase in tissue mass (muscle, liver) acting as storage and excretion in growing feathers, may reduce the circulating concentrations of pollutants (Charnetski, 1976; Goutner et al., 2001; Dauwe et al., 2006). This process, known as a dilution effect, is larger in faster-growing chicks (Goutner et al., 2001), although in some cases dietary intake of pollutants can exceed the dilution (Charnetski, 1976; Honda et al., 1986; Ackerman et al., 2011). Nevertheless, to the best of our knowledge, changes in contamination levels of multiple non-persistent (i.e., not included in POPs) pesticides in birds, and their associations with fat stores during growth, have not been investigated.

Montagu's harrier (*Circus pygargus*) is a migratory specialist raptor, feeding on voles and nesting on the ground in cereal crops. Chicks are particularly valuable as bio-indicators of local environmental contamination levels because they are exposed to pesticide mixtures during their development (Fuentes et al., 2024a). They can be contaminated through multiple pathways, notably contact (with the soil, vegetation and rain), inhalation (contaminated air) and the consumption of contaminated prey brought by parents. Another possible origin is the maternal transfer of compounds from adult females to eggs (e.g., Bellot et al., 2022).

In this study, we explored temporal changes in pesticide loads (using total pesticide number and the sum of their concentrations) in Montagu's harrier chicks and potential relationships between their contamination levels and a reduction in their lipid stores during growth. Chicks were blood-sampled twice during growth to assess pesticide loads for 116 compounds. This matrix was expected to reflect both recent contamination from a few hours to days, depending on the compounds (Espín et al., 2016; Katagi and Fujisawa, 2021), and concentrations in other tissues assuming an equilibrium between blood and soft tissues, as shown for some compounds (Bustnes et al., 2013; Espín et al., 2016). Besides the lack of studies on the redistribution of non-persistent pesticides between blood and other tissues, temporal variation in contamination levels within organisms seemingly vary according to both species and pollutant, making difficult to predict variations of non-persistent pesticide mixtures. Nevertheless, we predicted that pesticide contamination levels would decrease with chick age due to a dilution effect, which should be reflected by a positive relationship between contamination load and chick fat stores.

2. Material and methods

2.1. Study area and model species

2.1.1. Zone Atelier Plaine & Val de Sevre (ZAPVS)

The ZAPVS study area is located in southwestern France (46°11'N, 0°28'W). The 450 km² area is covered mainly by intensive agricultural fields (Fig. S1 in Supplementary Materials), with cereal crops covering ~41% of the area under cultivation between 2009 and 2016 (Bretagnolle et al., 2018). Crops under organic farming (i.e., not using synthetic pesticides and fertilisers; Regulation EU, 2018/848) accounted for ~11% of the ZAPVS surface in 2018 (Fig. S1).

2.1.2. Montagu's harriers

Montagu's harriers are migratory raptors, and their reproduction has been monitored in the ZAPVS area since 1994 (Bretagnolle et al., 2018). They nest on the ground in cereal fields, generally laying up to six eggs (Arroyo et al., 1998). Incubation and rearing periods last 29 days and 30–35 days, respectively (Arroyo et al., 2007). Breeding success depends mainly on the availability of its main prey, the common vole (*Microtus arvalis*), although orthopterans and passerine birds are also preyed upon (Salamolard et al., 2000). In our study site, breeding success is ~2.05 fledglings per breeding attempt (Arroyo et al., 2004), with brood sizes varying between one and five nestlings (see Fuentes et al., 2023b for an overview of several years). Males have home ranges of ~14 km² in the study area (Salamolard, 1997) and provision incubating females and chicks, while females may contribute to food provisioning later in the rearing period, hunting close to the nest (Garcia and Arroyo, 2005). During winter, Montagu's harriers from the studied population are located in western Africa (Garcia and Arroyo, 1998).

2.2. Data collection

2.2.1. Ethical statement

Handling of chicks was performed under license from the Centre de Recherches sur la Biologie des Populations d'Oiseaux (CRBPO; Museum National d'Histoire Naturelle; licence #1308). All experiments were conducted following French guidelines for the ethical use of animals in research (APAFIS#18557–2019010822312199v2).

2.2.2. Sampling design

In 2018, Montagu's harrier nests were searched for, located and recorded using global positioning system (GPS) coordinates (Fig. S1). Nests were visited twice for blood sampling during the chick-rearing period (mean duration between samplings 10 ± 3 days). Morphometric estimation of age was performed *a posteriori* using wing length (Arroyo, 1995; calculator available at <https://busards.com/index.php/Outils/biometry>), thus chick age varied between 10 and 23 days (mean ± standard deviation: 16 ± 3 days) during the first visit and between 23 and 29 days (26 ± 2 days) during the second visit. Chicks were banded with a unique coded aluminium ring provided by the Museum National d'Histoire Naturelle de Paris (France) and sexed according to iris colour, brown for females and grey for males (Leroux and Bretagnolle, 1996) during the first sampling visit. During this visit, chicks were carefully handled in the shortest time possible to collect morphometric data and blood samples, before being placed back in the nest. The same procedure was followed during the second nest visit, providing blood samples from two different ages for each chick. Nestlings were sampled between mid-June and the end of July 2018. Although 81 chicks (24 nests) were monitored in 2018, blood samples for pesticide titration were only available for 35 (22 nests), which are included in the present study. No sex-ratio bias among chicks was observed in 2018 (36 females and 45 males; Binomial test, $p = 0.37$), even when considering only the 35 chicks studied (17 females and 18 males; Binomial test, $p = 1.00$).

2.2.3. Morphometric measurements

Nestlings were weighed using a Pesola 500 g spring scale (accuracy ± 5 g). Tarsus length was measured with a digital calliper (accuracy ± 0.1 mm). Wing and tail length were measured with a ruler (accuracy ± 1 mm). As the right limbs of chicks were measured twice at first and second visits while left limbs were measured twice only for the second visit, mean tarsus length and mean wing length were used in further analyses.

2.2.4. Scale mass index (SMI)

SMI is a residual body mass, calculated as follows (Peig and Green, 2009):

$$SMI_i = Mass_i \times \left(\frac{\text{Mean tarsus length of the group}}{\text{Mean tarsus length}_i} \right)^{b_{sma}}$$

where b_{sma} is the slope of the major axis regression of body mass (log-transformed) on the mean tarsus length (log-transformed), using the standard major axis method from the 'smatr' package (Warton et al., 2012) in R v.4.2.2 software (R Core Team, 2022). Males and females were separated to calculate SMI, accounting for expected differences in growth patterns. The SMI of chicks is assumed to reflect their lipid stores (Peig and Green, 2009) and is thus expected to decrease with age due to the pre-fledging reduction of body reserves to attain the optimum mass for flight (Mauck and Ricklefs, 2005; Wright et al., 2006).

2.2.5. Blood sampling

Individual blood samples (50 μ L) were collected by puncturing the brachial vein using a sterile needle and heparinized capillaries. Each sample was collected in a plastic microcentrifuge tube, transported to the laboratory in a cooler (0–5 °C), and stored at –20 °C for further analyses.

2.3. Analytical methods

Following Rodrigues et al. (2023), 50 μ L samples of whole blood (i.e., red blood cells and plasma) were used for multiresidue analyses. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and automated thermal desorption gas chromatography coupled to tandem mass spectrometry (ATD-GC-MS/MS) were performed to detect and quantify 116 compounds in multiple reactions monitoring detection mode for both instrumentations. These 116 compounds included herbicides, fungicides, insecticides, a safener that improves herbicide selectivity towards weeds rather than crop plants, and a synergist that enhances pesticide action, all among the most used in France. To the 104 compounds validated in Rodrigues et al. (2023), 12 compounds were added for multiresidue analysis after optimisation and validation. Limit of detection (LOD) and limit of quantification (LOQ) for each compound detected are provided in Supplementary Materials Table S1. LODs varied between 0.001 and 0.124 pg mg^{-1} , and LOQs varied between 0.004 and 0.436 pg mg^{-1} .

2.4. Statistical analyses

For each chick, contamination levels were estimated as the total number of pesticides detected in blood (N_{pesti}) and the total sum of pesticide concentrations ($\sum \text{Conc}$). These metrics are commonly used in ecotoxicology studies to reflect levels of contamination (Jenssen et al., 2010; Tartu et al., 2014; Fritsch et al., 2022; Fuentes et al., 2024b). N_{pesti} and $\sum \text{Conc}$ were correlated (Pearson's correlation test $\rho = 0.60$, 95% confidence interval = 0.45; 0.73), however, the presence of compounds (i.e., the number) and their quantity (i.e., the concentrations) might have different dynamics over time, thus we included both metrics in further analyses. Neither of the variables satisfied normality and homoscedasticity, therefore prior to model implementation, $\sum \text{Conc}$ was square root-transformed and used as a response variable in a linear

mixed effect model (LMM). Meanwhile, N_{pesti} was used as a response variable in a generalised linear mixed-effects model fitted with a Poisson distribution (PGLMM). For all models described hereafter, we used the identity of chicks nested within the nest identity as random effects (chicks were sampled twice and may belong to the same nest).

To investigate changes in contamination levels with chick age and relationship with chick lipid stores, we constructed one LMM ($\sum \text{Conc}$ as response variable) and one PGLMM (N_{pesti} as response variable) using sampling date (in Julian days), age (days) and SMI (grams) of chicks as explanatory variables. Sampling date is relevant because any environmental (contamination levels, weather) and/or phenotypic (breeding onset) variation during the season might explain within-individual temporal patterns. Although SMIs of chicks are expected to decrease with age (Fig. S2 in Supplementary Materials), these variables were not included as interacting effects in the model because this led to multicollinearity (checked using Variance Inflation Factor, VIF). We did not include the sex of nestlings in models as contamination levels did not differ between males and females (Wilcoxon rank sum test: $W = 753$, $p = 0.10$ for the sum of pesticide concentrations, and $W = 618$, $p = 0.95$ for the number of pesticides detected), in line with previous results on Montagu's harrier chicks of the same population (Fuentes et al., 2024b). When a pesticide was detected during both sampling occasions, its temporal variation was investigated through LMM using its concentration (square root-transformed) as a response variable and using sampling date, age and SMI of chicks as explanatory variables. In all cases, the significance of each effect within the model was assessed using likelihood-ratio Chi-square tests (Fox and Weisberg, 2019). All analyses were performed in R v.4.2.2 software (R Core Team, 2022) using the packages 'car' (Fox and Weisberg, 2019) for VIF checking and likelihood-ratio Chi-square tests, 'DHARMA' (Hartig, 2022) to check model residuals, and 'lme4' (Bates et al., 2015) to implement mixed-effects models.

3. Results

In total, 18 pesticides were detected (i.e., concentrations $>$ LOD) in blood samples from 35 chicks (70 blood samples), including 9 herbicides, 5 insecticides and 4 fungicides (Fig. 1 and Table S1 in Supplementary Materials). Chicks had on average (mean \pm standard deviation) 5.60 ± 1.52 pesticides in their blood (N_{pesti}) and total concentrations of 980.97 ± 594.31 pg mg^{-1} ($\sum \text{Conc}$) during the first sampling. At the second sampling, mean N_{pesti} was 3.54 ± 2.55 and mean $\sum \text{Conc}$ was 580.00 ± 735.34 pg mg^{-1} . Contamination levels of chicks decreased with increasing age, with the effect of age significant for both N_{pesti} and $\sum \text{Conc}$ (Table 1; Fig. 2; Table S2 in Supplementary Materials). Neither N_{pesti} nor $\sum \text{Conc}$ were influenced by the sampling date or SMI of chicks (Table 1 and Table S2, Figs. S3 and S4 in Supplementary Materials).

Seven pesticides were detected in the first samples alone, compared with five in second samples alone, and six in both samples (Fig. 1; Table S1). The concentrations of five of the pesticides present at both sampling occasions (chlorpyrifos-methyl, fenpropidin, metamitron, picloram, S-metolachlor) were not influenced by any of the explanatory variables (Table S3 and Fig. S5 in Supplementary Materials). Ethofumesate concentrations were significantly decreased with chick age, while sampling date and chick SMI had no significant effect (Table S3).

4. Discussion

The results of the present study provide the first evidence of a reduction in both the number and total concentrations of non-persistent pesticide mixtures with age in wild farmland bird nestlings. However, we did not find here a relationship between pesticide contamination levels in chick blood and body condition, suggesting that a mechanism other than a reduction in fat stores is responsible for the lower contamination levels as chicks age. When assessing the concentrations of pesticides present throughout chick growth, we observed a reduction

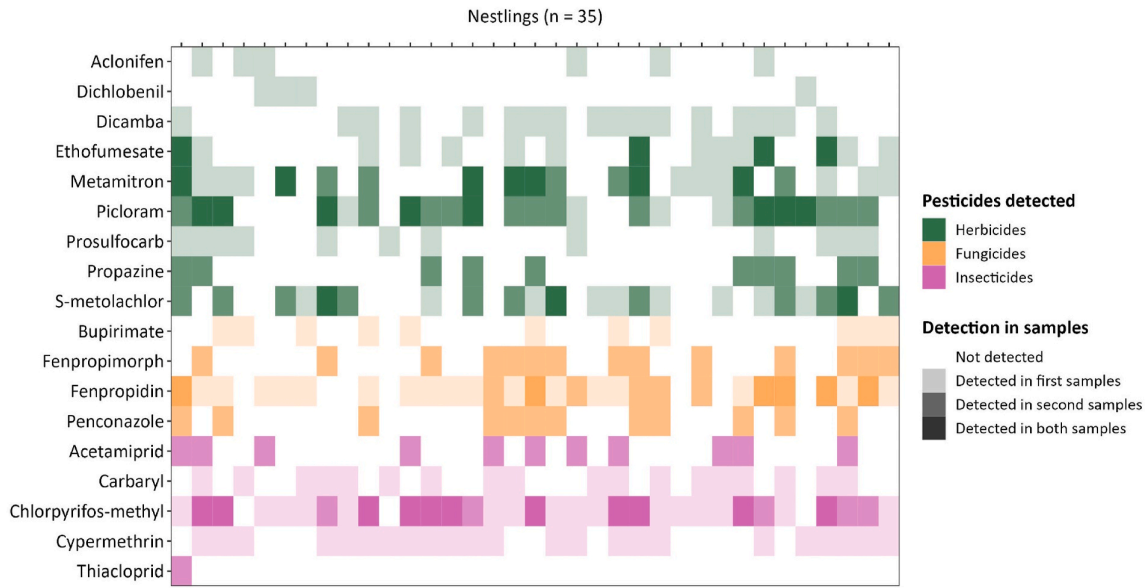


Fig. 1. Pesticides detected in Montagu's harrier (*Circus pygargus*) chicks of 10–23 days old in first samples and 23–29 days old in second samples. Columns represent chicks and rows represent pesticides. Each coloured cell (green for herbicides, orange for fungicides and pink for insecticides) indicates a detected pesticide, and shading levels indicate if detection occurred in first or second samples only, or both.

Table 1
Effects of sampling date (in Julian days), age (days) and body condition (grams) of Montagu's harrier chicks on pesticide contamination levels. The statistical significance of each variable was assessed by an analysis of deviance based on likelihood-ratio Chi-square tests (statistical values, degrees of freedom and *p*-values). Tests were performed on a linear mixed-effects model (LMM) for the square root-transformed sum of concentrations ($\sum Conc$) and on a generalised LMM fitted with a Poisson distribution (PGLMM) for the number of pesticides detected (N_{pesti}). Significant effects ($p < 0.05$) are depicted in bold.

Variables	χ^2	df	<i>p</i>
N_{pesti}			
Sampling date	0.027	1	0.868
Age	5.573	1	0.018
SMI	0.141	1	0.707
$\sum Conc$			
Sampling date	0.002	1	0.964
Age	7.958	1	0.005
SMI	0.370	1	0.543

with ageing only for ethofumesate, indicating that the reduced contamination loads with age are mainly attributable to the clearance of the seven pesticides detected only in younger nestlings, despite the detection of five other pesticides in older nestlings.

Although a dilution effect may be expected to occur during chick growth, we did not detect any link between contamination levels and body condition, despite a significant decrease in pesticide loads with aging. However, half of the compounds detected were not particularly lipophilic, as reflected by their octanol-water partition coefficients (log *P* values < 3; see Table S1 in Supplementary Materials). They might thus have a lower affinity for fat tissues and respond differently from POPs such as dichlorodiphenyltrichloroethane (DDT), known for its ability to be stored in fat tissues including egg yolk (log *P* = 6.91; see Blus, 2011), except cypermethrin that may bioaccumulate in fatty tissues (log *P* > 5; EPA, 2012). This may explain the lack of a relationship between pesticide load and body condition. Nonetheless, our study suggests that younger nestlings have a higher pesticide load that may be due to maternal detoxification into eggs (e.g., Bargar et al., 2001; Bellot et al., 2022), and during the latter part of the rearing period, the detoxification

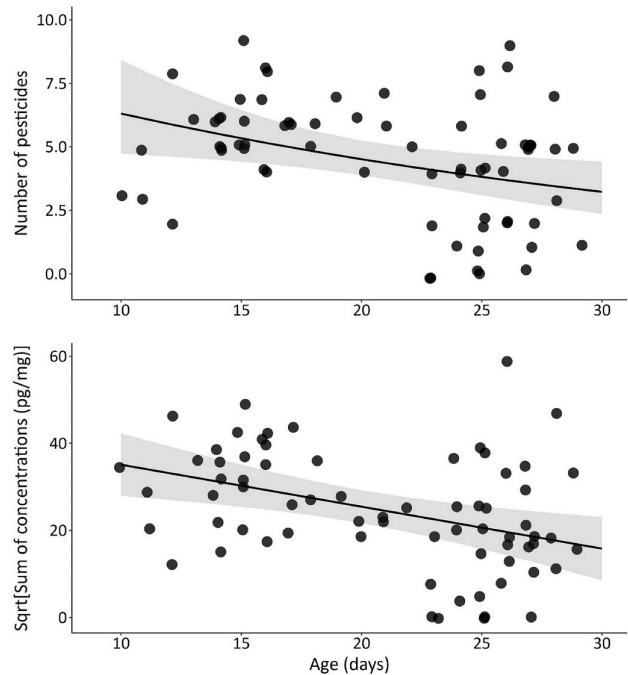


Fig. 2. Age-dependent decrease in pesticide contamination levels, both the number of pesticides (top) and the sum of pesticide concentrations (bottom), in Montagu's harrier (*Circus pygargus*) chicks. Lines and shading represent the values predicted for the effect of chick age and 95% confidence intervals from the linear mixed-effects model (LMM) fitted with a Poisson distribution (number of pesticides) and the LMM (sum of concentrations square root-transformed).

capabilities of chicks might allow decreased pesticide loads. Indeed, pesticide intake from the diet remains steady, as food provisioning is not likely to be decreased during the nestling period, but rather increased

(Kitowski, 2003; Wieringa et al., 2019). Therefore, a higher pesticide excretion rate is needed in Montagu's harrier nestlings to result in the lower contamination levels observed. For instance, in Japanese quail chicks, the activity of esterases (enzymes involved in organophosphate detoxification) increased with age, indicating a higher capacity of synthesis of enzymes with chick ageing (Narváez et al., 2016). Studies on the development of detoxification mechanisms during bird growth are scarce, but they suggest that embryos and hatchlings have lower enzymatic activities, resulting in older nestlings having stronger metabolic and excretion capacities (Veini et al., 1986; Liu et al., 2019; Caviedes-Vidal and Karasov, 2001). Early-life changes in the gut microbiota might also explain the reduction in contamination levels with age because it is involved in detoxification processes (Grond et al., 2018; Liu et al., 2020) and displays a strong temporal variation during bird development (Teyssier et al., 2018; Zhu et al., 2021). Overall, temporal variation in pesticide mixtures in Montagu's harrier chicks is probably governed by the ontogenetic development of physiological mechanisms not explored herein that increase metabolic potential and counterbalance pesticide uptake.

Although a general trend in the reduction of pesticide load with age was observed, single pesticides might differ from one another in their temporal dynamics within individuals. Compounds detected in both samples may indicate the persistence of maternally transferred molecules in the organism (from fat tissues then delivered in blood) and/or permanent exposure during the rearing period. If the former was correct, the six compounds detected at both sampling periods should decrease with chick ageing, but no trends in concentration changes with age were observed for five of them (Fig. S5), which indicates an absence of metabolism/detoxification and/or continuous high-level contamination of chicks with these pesticides throughout growth. The fungicide fenpropidin and the insecticide chlorpyrifos-methyl detected at both sampling periods may have been heavily used on cereal crops before sampling nestlings as cereals are predominant crops in the study area (Table S1; Figs. S1 and S6). Ethofumesate and metamiltron are two herbicides that can be applied to seed carriers of industrial and fodder beet in the study area. However, only very few fields of these crops are present in the study area, which questions the origin of the contamination of nestlings with these substances. The herbicides picloram and S-metolachlor can be applied to oilseed rape and maize, respectively, both of which are major crops in the study area, covering 8.3% and 9.6%, respectively (Bretagnolle et al., 2018; Fig. S1), with treatment periods spanning part of the nestling rearing period (Fig. S6). Pesticide treatments may have resulted in direct exposure during application, through particle drift, via contact with adults and/or due to persistence of pesticides within these crops (plants and/or soil), resulting in contaminated eggs and nestlings and/or prey (none of these pathways are mutually exclusive). Metamiltron, S-metolachlor and metabolites of chlorpyrifos-methyl have been detected in hairs of small mammals in the study area, supporting repeated exposure through the diet, while ethofumesate, fenpropidin and picloram were not searched for (Fritsch et al., 2022). Additionally, as males can hunt several kilometres away from the nest, crops outside the study area may influence the pesticide contamination of nestlings, which may result in a mismatch between the pesticides detected and the predominant crops in the study area (Fuentes et al., 2024a).

On the other hand, the detection of some compounds only in first samples might reflect early contamination, possibly through eggs, while detection only in second samples might reflect food or contact-mediated contamination later in the rearing period. For instance, dichlobenil and carbaryl, banned in France since 2008 and 2007, respectively (Fig. 2 and Table S1 in Supplementary Materials) were detected only in first samples, similar to the highly lipophilic cypermethrin, supporting the aforementioned explanation. The maximum reported half-life in soil (DT50, the time taken to reduce by 50% the dose applied) is six months for dichlobenil and 329 days for carbaryl (Lewis et al., 2016), while chicks were sampled ten years after they were banned. Thus, they could

have been ingested by females in wintering areas, transferred to eggs, found in nestlings only in the first weeks post-hatching, and then excreted and/or stored in their growing tissues or feathers. Nonetheless, we detected propazine, a herbicide prohibited in France since 2002, in second samplings. Moreover, in a previous study on Montagu's harrier chicks aged ~26 days old from the same population, banned molecules were also detected (Fuentes et al., 2024a). This suggests either that maternally transferred compounds persist until chicks fledge (notably in liver and fat tissues) and are released in the bloodstream during the growing period, and/or that there is fraudulent use of such pesticides (Fuentes et al., 2024a). We cannot discount this last scenario based on detection of propazine only in second samplings, and because our first samplings probably occurred too late (≥ 10 days post-hatching) to reflect only maternal transfer without a dietary input. Titration of pesticides in unhatched eggs of clutches could prove interesting because it might provide information on compounds deposited in eggs before chick hatching.

Regarding the five pesticides detected only in older nestlings, food or contact-mediated contamination is supported for penconazole, acetamiprid and thiamethoxam, as these have also been detected in small mammals and other environmental compartments in the study area (Wintermantel et al., 2020; Pelosi et al., 2021; Fritsch et al., 2022), while propazine has not been detected (Fritsch et al., 2022) and fenpropimorph was not investigated. Pesticide titration in food pellets collected at nests could provide novel insight into dietary contamination pathways.

Although the within individual temporal pattern is quite clear for Montagu's harrier chicks, the generality of our findings is complicated to assert for other species. Additionally, for a given species, the population studied might influence pesticide temporal patterns, not only due to differences in environmental pesticide loads, but also differences in diet and ecology. Indeed, as suggested previously (Fuentes et al., 2023b; 2024b), alternative prey in the diet of Montagu's harriers might be important due to differences in nutritional composition such as carotenoids and other antioxidants involved in detoxification processes (Møller et al., 2000; Sternalski et al., 2012), which could affect pesticide uptake and detoxification. Thus, additional studies are needed, including dietary and metabolic analyses, to better understand the fate of pesticide cocktails within organisms, and more broadly within the environment.

5. Conclusion

The present study revealed a reduction in both the number of pesticides detected and the sum of pesticide concentrations in the blood of wild nestlings of a farmland raptor with increasing age. Although the mechanisms leading to decreased contamination levels remain unclear, this provides a first step in understanding the fate of non-persistent pesticide mixtures within wild birds. The next step is to determine if these contamination levels can impact the development of Montagu's harrier nestlings, as this might unveil the factors underpinning the decline of this farmland bird.

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CRedit authorship contribution statement

Elva Fuentes: Writing – original draft, Visualization, Formal analysis, Conceptualization. **Jérôme Moreau:** Writing – review & editing, Supervision, Conceptualization. **Maurice Millet:** Writing – review & editing, Investigation, Formal analysis. **Vincent Bretagnolle:** Writing – review & editing, Conceptualization. **Karine Monceau:** Writing –

review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2024.120179>.

Data availability

Data will be made available on request.

References

- Ackerman, J.T., Eagles-Smith, C.A., Herzog, M.P., 2011. Bird mercury concentrations change rapidly as chicks age: toxicological risk is highest at hatching and fledging. *Environ. Sci. Technol.* 45, 5418–5425. <https://doi.org/10.1021/es200647g>.
- Al-Mamun, A., 2017. Pesticide degradations, residues and environmental concerns. In: *Pesticide Residue in Foods*. Springer, Cham, pp. 87–102. https://doi.org/10.1007/978-3-319-52683-6_5.
- Arroyo, B., Bretagnolle, V., Leroux, A., 2007. Interactive effects of food and age on breeding in the Montagu's harrier *Circus pygargus*. *Ibis* 149, 806–813. <https://doi.org/10.1111/j.1474-919X.2007.00716.x>.
- Arroyo, B., García, J.T., Bretagnolle, V., 2004. Montagu's harrier. – BWP update. *The Journal of the Birds of the Western Palearctic* 6, 41–55.
- Arroyo, B., Leroux, A., Bretagnolle, V., 1998. Patterns of egg and clutch size variation in the Montagu's harrier. *J. Raptor Res.* 32, 136–142.
- Arroyo, B., 1995. Breeding Ecology and Nest Dispersion of Montagu's Harrier *Circus pygargus* in Central Spain. University of Oxford, UK. PhD thesis.
- Bargar, T.A., Scott, G.I., Cobb, G.P., 2001. Uptake and distribution of three PCB congeners and endosulfan by developing white leghorn chicken embryos (*Gallus domesticus*). *Arch. Environ. Contam. Toxicol.* 41, 508–514. <https://doi.org/10.1007/s002440010278>.
- Bariod, L., Gaffard, A., Rodrigues, A., Millet, M., Bretagnolle, V., Pays, O., Monceau, K., Moreau, J., 2024. Comparison of pesticide contamination between captive-reared and wild grey partridges: insights into environmental exposure disparities. *Environ. Sci. Pollut. Control Ser.* <https://doi.org/10.1007/s11356-024-34925-z>.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Bauerová, P., Krajčingrová, T., Těšický, M., Velová, H., Hrančák, J., Musil, S., et al., 2020. Longitudinally monitored lifetime changes in blood heavy metal concentrations and their health effects in urban birds. *Sci. Total Environ.* 723, 138002. <https://doi.org/10.1016/j.scitotenv.2020.138002>.
- Bean, T.G., Gross, M.S., Karouna-Renier, N.K., Henry, P.F., Schultz, S.L., Hladik, M.L., Kuivila, K.M., Rattner, B.A., 2019. Toxicokinetics of imidacloprid-coated wheat seeds in Japanese quail (*Coturnix japonica*) and an evaluation of hazard. *Environ. Sci. Technol.* 53, 3888–3897. <https://doi.org/10.1021/acs.est.8b07062>.
- Becker, P.H., Sperverlage, H., 1989. Organochlorines and heavy metals in herring Gull (*Larus argentatus*) eggs and chicks from the same clutch. *Bull. Environ. Contam. Toxicol.* 42, 721–727. <https://doi.org/10.1007/BF01700394>.
- Bellot, P., Brischoux, F., Fritsch, C., Goutte, A., Alliot, F., Rocchi, S., Angelier, F., 2022. Evidence of environmental transfer of tebuconazole to the eggs in the house sparrow (*Passer domesticus*): an experimental study. *Chemosphere* 308, 136469. <https://doi.org/10.1016/j.chemosphere.2022.136469>.
- Blus, L.J., 2011. DDT, DDD, and DDE in birds. In: *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Lewis Publishers, pp. 425–444.
- Bretagnolle, V., Berthet, E., Gross, N., Gaufré, B., Plumejeaud, C., Houté, S., et al., 2018. Towards sustainable and multifunctional agriculture in farmland landscapes: lessons from the integrative approach of a French LTSE platform. *Sci. Total Environ.* 627, 822–834. <https://doi.org/10.1016/j.scitotenv.2018.01.142>.
- Bustnes, J.O., Bårdsen, B.J., Herzke, D., Johnsen, T.V., Eulaers, I., Ballesteros, M., et al., 2013. Plasma concentrations of organohalogenated pollutants in predatory bird nestlings: associations to growth rate and dietary tracers. *Environ. Toxicol. Chem.* 32, 2520–2527. <https://doi.org/10.1002/etc.2329>.
- Bustnes, J.O., Bakken, V., Skaare, J.U., Erikstad, K.E., 2003. Age and accumulation of persistent organochlorines: a study of arctic-breeding glaucous gulls (*Larus hyperboreus*). *Environ. Toxicol. Chem.* 22, 2173–2179. <https://doi.org/10.1897/02-456>.
- Caviedes-Vidal, E., Karasov, W.H., 2001. Developmental changes in digestive physiology of nestling house sparrows, *Passer domesticus*. *Physiol. Biochem. Zool.* 74, 769–782. <https://doi.org/10.1086/322966>.
- Charnetski, W.A., 1976. Organochlorine insecticide residues in ducklings and their dilution by growth. *Bull. Environ. Contam. Toxicol.* 16, 138–144. <https://doi.org/10.1007/BF01685219>.
- Dauwe, T., Jaspers, V.L., Covaci, A., Eens, M., 2006. Accumulation of organochlorines and brominated flame retardants in the eggs and nestlings of great tits, *Parus major*. *Environ. Sci. Technol.* 40, 5297–5303. <https://doi.org/10.1021/es060747a>.
- EPA, 2012. Sustainable futures/P2 framework manual EPA-748-B12-001. Section 5: estimating physical/chemical and environmental fate properties with EPI suite. Available at <https://www.epa.gov/sustainable-futures/sustainable-futures-p2-framework-manual>.
- Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., van den Brink, N.W., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? *Ecotoxicology* 25, 777–801. <https://doi.org/10.1007/s10646-016-1636-8>.
- Fox, J., Weisberg, S., 2019. *An R Companion to Applied Regression*, third ed. Sage Publications, Thousand Oaks CA.
- Fritsch, C., Berny, P., Crouzet, O., Le Perchec, S., Coeurdassier, M., 2024. Wildlife ecotoxicology of plant protection products: knowns and unknowns about the impacts of currently used pesticides on terrestrial vertebrate biodiversity. *Environ. Sci. Pollut. Res.* 1–63. <https://doi.org/10.1007/s11356-024-33026-1>.
- Fritsch, C., Appenzeller, B., Burkart, L., Coeurdassier, M., Scheffler, R., Raoul, F., et al., 2022. Pervasive exposure of wild small mammals to legacy and currently used pesticide mixtures in arable landscapes. *Sci. Rep.* 12, 15904. <https://doi.org/10.1038/s41598-022-19959-y>.
- Fuentes, E., Moreau, J., Rodrigues, A., Millet, M., Bretagnolle, V., Monceau, K., 2024a. Pesticide contamination patterns in Montagu's harrier (*Circus pygargus*) chicks. *Environ. Sci. Pollut. Control Ser.* <https://doi.org/10.1007/s11356-024-34937-9>.
- Fuentes, E., Moreau, J., Millet, M., Bretagnolle, V., Monceau, K., 2024b. Organic farming reduces pesticide load in a bird of prey. *Sci. Total Environ.* 930, 172778. <https://doi.org/10.1016/j.scitotenv.2024.172778>.
- Fuentes, E., Gaffard, A., Rodrigues, A., Millet, M., Bretagnolle, V., Moreau, J., Monceau, K., 2023a. Neonicotinoids: still present in farmland birds despite their ban. *Chemosphere* 321, 138091. <https://doi.org/10.1016/j.chemosphere.2023.138091>.
- Fuentes, E., Moreau, J., Teixeira, M., Bretagnolle, V., Monceau, K., 2023b. Effects of conventional vs. organic farming practices on raptor nestling health: neither black nor white. *Agric. Ecosyst. Environ.* 358, 108719. <https://doi.org/10.1016/j.agee.2023.108719>.
- García, J.T., Arroyo, B.E., 2005. Food-niche differentiation in sympatric hen *Circus cyaneus* and Montagu's harriers *Circus pygargus*. *Ibis* 147, 144–154. <https://doi.org/10.1111/j.1474-919X.2004.00377.x>.
- García, J.T., Arroyo, B.E., 1998. Migratory movements of western European Montagu's Harrier *Circus pygargus*: a review. *Hous. Theor. Soc.* 45, 188–194.
- Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., et al., 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic Appl. Ecol.* 11, 97. <https://doi.org/10.1016/j.baae.2009.12.001>.
- Geissen, V., Silva, V., Lwanga, E.H., Beriot, N., Oostindie, K., Bin, Z., Ritsema, 2021. Cocktails of pesticide residues in conventional and organic farming systems in Europe—Legacy of the past and turning point for the future. *Environ. Pollut.* 278, 116827. <https://doi.org/10.1016/j.envpol.2021.116827>.
- Goutner, V., Furness, R.W., Papakostas, G., 2001. Mercury in feathers of Squacco Heron (*Ardeola ralloides*) chicks in relation to age, hatching order, growth, and sampling dates. *Environ. Pollut.* 111, 107–115. [https://doi.org/10.1016/S0269-7491\(99\)00329-2](https://doi.org/10.1016/S0269-7491(99)00329-2).
- Gronk, K., Sandercock, B.K., Jumpponen, A., Zeglin, L.H., 2018. The avian gut microbiota: community, physiology and function in wild birds. *J. Avian Biol.* 49, e01788. <https://doi.org/10.1111/jav.01788>.
- Hartig, F., 2022. DHARMA: residual diagnostics for hierarchical (Multi-Level/Mixed) regression models. R package version 0.4.6. <https://CRAN.R-project.org/package=DHARMA>.
- Honda, K., Min, B.Y., Tatsukawa, R., 1986. Distribution of heavy metals and their age-related changes in the eastern great white egret, *Egretta alba modesta*, in Korea. *Arch. Environ. Contam. Toxicol.* 15, 185–197. <https://doi.org/10.1007/BF01059967>.
- Humann-Guillemot, S., Binkowski, L.J., Jenni, L., Hille, G., Glauser, G., Helfenstein, F., 2019. A nation-wide survey of neonicotinoid insecticides in agricultural land with implications for agri-environment schemes. *J. Appl. Ecol.* 56, 1502–1514. <https://doi.org/10.1111/1365-2664.13392>.
- Jenssen, B.M., Aarnes, J.B., Murvoll, K.M., Herzke, D., Nygård, T., 2010. Fluctuating wing asymmetry and hepatic concentrations of persistent organic pollutants are associated in European shag (*Phalacrocorax aristotelis*) chicks. *Sci. Total Environ.* 408, 578–585.
- Katagi, T., Fujisawa, T., 2021. Acute toxicity and metabolism of pesticides in birds. *J. Pest. Sci.* 46, 305–321. <https://doi.org/10.1584/jpestics.D21-028>.
- Kitowski, I., 2003. Trends on parental care in Montagu's harrier during nestling period in south east Poland. *Berkut* 12, 112–118.
- Lemmettyinen, R., Rantamäki, P., 1980. DDT and PCB residues in the arctic tern (*Sterna paradisaea*) nesting in the archipelago of southwest Finland. *Ann. Zool. Fenn.* 17, 141–146. <http://www.jstor.org/stable/23734044>.
- Lemmettyinen, R., Rantamäki, P., Karlin, A., 1982. Levels of DDT and PCB's in different stages of life cycle of the Arctic tern *Sterna paradisaea* and the herring gull *Larus*

- argentatus*. Chemosphere 11, 1059–1068. [https://doi.org/10.1016/0045-6535\(82\)90080-7](https://doi.org/10.1016/0045-6535(82)90080-7).
- Leroux, A., Bretagnolle, V., 1996. Sex ratio variations in broods of Montagu's harriers *Circus pygargus*. J. Avian Biol. 27, 63–69. <https://doi.org/10.2307/3676962>.
- Lewis, K.A., Tziliavakis, J., Warner, D., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess. 22, 1050–1064. <https://doi.org/10.1080/10807039.2015.1133242>.
- Liu, T., Liang, X., Lei, C., Huang, Q., Song, W., Fang, R., et al., 2020. High-fat diet affects heavy metal accumulation and toxicity to mice liver and kidney probably via gut microbiota. Front. Microbiol. 11, 1604. <https://doi.org/10.3389/fmicb.2020.01604>.
- Liu, X., Liu, C., Wang, P., Liang, Y., Zhan, J., Zhou, Z., Liu, D., 2019. Distribution, metabolism and metabolic disturbances of alpha-cypermethrin in embryo development, chick growth and adult hens. Environ. Pollut. 249, 390–397. <https://doi.org/10.1016/j.envpol.2019.03.017>.
- Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. Science 277, 504–509. <https://doi.org/10.1126/science.277.5325.504>.
- Mauck, R.A., Ricklefs, R.E., 2005. Control of fledging age in Leach's Storm-Petrel, *Oceanodroma leucorhoa*: chick development and pre-fledging mass loss. Funct. Ecol. 19, 73–80. <https://doi.org/10.1111/j.0269-8463.2005.00933.x>.
- Merleau, L.A., Lourdaux, O., Olivier, A., Vittecoq, M., Blouin-Demers, G., Alliot, F., et al., 2024. Pesticide concentrations in a threatened freshwater turtle (*Emys orbicularis*): seasonal and annual variation in the Camargue wetland, France. Environ. Pollut. 341, 122903. <https://doi.org/10.1016/j.envpol.2023.122903>.
- Mineau, P., 1982. Levels of major organochlorine contaminants in sequentially-laid herring gull eggs. Chemosphere 11, 679–685. [https://doi.org/10.1016/0045-6535\(82\)90179-5](https://doi.org/10.1016/0045-6535(82)90179-5).
- Mitra, A., Chatterjee, C., Mandal, F.B., 2011. Synthetic chemical pesticides and their effects on birds. Res. J. Environ. Toxicol. 5, 81–96. <https://doi.org/10.3923/rjet.2011.81.96>.
- Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N., Surai, P.F., 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Avian Poultry Biol. Rev. 11, 137–160.
- Moreau, J., Rabdeau, J., Badenhausser, I., Giraudeau, M., Sepp, T., Crépin, M., et al., 2022. Pesticide impacts on avian species with special reference to farmland birds: a review. Environ. Monit. Assess. 194, 1–48. <https://doi.org/10.1007/s10661-022-10394-0>.
- Monteiro, L.R., Furness, R.W., 2001. Kinetics, dose-response, excretion, and toxicity of methylmercury in free-living Cory's shearwater chicks. Environ. Toxicol. Chem. 20, 1816–1823. <https://doi.org/10.1002/etc.5620200827>.
- Narváez, C., Ríos, J.M., Píriz, G., Sanchez-Hernandez, J.C., Sabat, P., 2016. Subchronic exposure to chlorpyrifos affects energy expenditure and detoxification capacity in juvenile Japanese quails. Chemosphere 144, 775–784. <https://doi.org/10.1016/j.chemosphere.2015.09.060>.
- Nordstad, T., Moe, B., Bustnes, J.O., Bech, C., Chastel, O., Goutte, A., et al., 2012. Relationships between POPs and baseline corticosterone levels in black-legged kittiwakes (*Rissa tridactyla*) across their breeding cycle. Environ. Pollut. 164, 219–226. <https://doi.org/10.1016/j.envpol.2012.01.044>.
- Pan, Y., Chang, J., Xu, P., Xie, Y., Yang, L., Hao, W., Li, J., Wan, B., 2022. Twenty-four hours of Thiamethoxam: *in vivo* and molecular dynamics simulation study on the toxicokinetic and underlying mechanisms in quails (*Coturnix japonica*). J. Hazard Mater. 427, 128159. <https://doi.org/10.1016/j.jhazmat.2021.128159>.
- Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118, 1883–1891. <https://doi.org/10.1111/j.1600-0706.2009.17643.x>.
- Pelosi, C., Bertrand, C., Daniele, G., Coeurdassier, M., Benoit, P., Nélieu, S., et al., 2021. Residues of currently used pesticides in soils and earthworms: a silent threat? Agric. Ecosyst. Environ. 305, 107167. <https://doi.org/10.1016/j.agee.2020.107167>.
- R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rigal, S., Dakos, V., Alonso, H., Aunins, A., Benkő, Z., Brotons, L., et al., 2023. Farmland practices are driving bird population decline across Europe. Proc. Natl. Acad. Sci. USA 120, e2216573120. <https://doi.org/10.1073/pnas.2216573120>.
- Rodrigues, A., Gaffard, A., Moreau, J., Monceau, K., Delhomme, O., Millet, M., 2023. Analytical development for the assessment of pesticide contaminations in blood and plasma of wild birds: the case of grey partridges (*Perdix Perdix*). J. Chromatogr. A 1687, 463681. <https://doi.org/10.1016/j.chroma.2022.463681>.
- Salamolard, M., Butet, A., Leroux, A., Bretagnolle, V., 2000. Responses of an avian predator to variations in prey density at a temperate latitude. Ecology 81, 2428–2441. [https://doi.org/10.1890/0012-9658\(2000\)081\[2428:ROAAPT\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[2428:ROAAPT]2.0.CO;2).
- Salamolard, M., 1997. Utilisation de l'espace par le Busard Cendré *Circus pygargus*, superficie et distribution des zones de chasse. Alauda 65, 307–320.
- Stanton, R.L., Morrissey, C.A., Clark, R.G., 2018. Analysis of trends and agricultural drivers of farmland bird declines in North America: a review. Agric. Ecosyst. Environ. 254, 244–254. <https://doi.org/10.1016/j.agee.2017.11.028>.
- Sternalski, A., Mougéot, F., Bretagnolle, V., 2012. Carotenoid limitation and allocation priorities in asynchronous raptor nestlings. Biol. J. Linn. Soc. 105, 13–24. <https://doi.org/10.1111/j.1095-8312.2011.01773.x>.
- Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2014. The stress of being contaminated? Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Sci. Total Environ. 476, 553–560. <https://doi.org/10.1016/j.scitotenv.2014.01.060>.
- Teysier, A., Lens, L., Matthyssen, E., White, J., 2018. Dynamics of gut microbiota diversity during the early development of an avian host: evidence from a cross-foster experiment. Front. Microbiol. 9, 1524. <https://doi.org/10.3389/fmicb.2018.01524>.
- Veini, M., Tsitsiloni, O.E., Martini, S.M., Haritos, A.A., 1986. Multiple molecular forms of soluble esterase in the digestive system of the developing chicken. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 83, 775–781. [https://doi.org/10.1016/0305-0491\(86\)90145-8](https://doi.org/10.1016/0305-0491(86)90145-8).
- Warton, D.I., Duursma, R.A., Falster, D.S., Taskinen, S., 2012. Smatr 3—an R package for estimation and inference about allometric lines. Methods Ecol. Evol. 3, 257–259. <https://doi.org/10.1111/j.2041-210X.2011.00153.x>.
- Wieringa, A., Klaassen, R.H., Schlaich, A.E., Koks, B.J., 2019. Increased food provisioning by female Montagu's Harriers in years with food shortage weakens sex-specific roles in parental care. Ardea 107, 149–158.
- Wintermantel, D., Odoux, J.F., Decourtye, A., Henry, M., Allier, F., Bretagnolle, V., 2020. Neonicotinoid-induced mortality risk for bees foraging on oilseed rape nectar persists despite EU moratorium. Sci. Total Environ. 704, 135400. <https://doi.org/10.1016/j.scitotenv.2019.135400>.
- Wright, J., Markman, S., Denney, S.M., 2006. Facultative adjustment of pre-fledging mass loss by nestling swifts preparing for flight. Proc. R. Soc. A B 273, 1895–1900. <https://doi.org/10.1098/rspb.2006.3533>.
- Zhu, Y., Li, Y., Yang, H., He, K., Tang, K., 2021. Establishment of gut microbiome during early life and its relationship with growth in endangered crested ibis (*Nipponia nippon*). Front. Microbiol. 12, 723682. <https://doi.org/10.3389/fmicb.2021.723682>.