Feeding partridges with organic or conventional grain triggers cascading effects in life-history traits

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1. Introduction

In just five decades, farmland bird abundance has declined by 56% in the UK and similar patterns were observed in other Western European countries (Chamberlain et al., 2000; Robinson and Sutherland, 2002; Inger et al., 2015) and North America (Rosenberg et al., 2019). Although air pollution (specifically ozone) has been recently shown to be responsible for part of bird decline in United-States (Liang et al., 2020), there is overwhelming support that agricultural intensification, in particular habitat transformation and insect food resource loss, have been causing such trends (Robinson and Sutherland, 2002; Newton, 2017). Until recently, however, there was very little evidence that pesticides could also have contributed to such declines, but continental and country scale analyses have suggested it might be so (Goulson, 2014; Hallmann et al., 2014). Environmental stressors such as pollutants and pesticides may increase allergies and immunopathologies (Rowley and Monestier, 2005; Carey et al., 2006) leading to immune system disorders and reduce immunity and body condition (Carey et al., 2006; Franco et al., 2009; Lopez-Antia et al., 2018; Zhao et al., 2020). Birds’ immune systems rely on compounds that not only...
contaminants may have a positive effect on life-history traits at low dose (Majer et al., 2019). Indeed, the hormetic dose-response to environmental stressors predicts a biphasic dose response with positive effects at low dose becoming negative at a higher dose (Costantini et al., 2010; Mattson and Calabrese, 2010; Hashmi et al., 2014). This effect has been described in a wide range of organisms in response to different environmental stressors, and hormesis in birds has gained recent attention (Hashmi et al., 2014). Interestingly, hormetic responses may represent an evolutionary adaptation of metabolic systems that promotes survival for an organism living in a stressful environment, allowing bird populations higher flexibility to cope and survive with environmental perturbations (Costantini, 2009; Costantini and Borremans, 2019). Deciphering whether the effects of low doses of pesticide residues can be positive or negative is critical to determine the role of chronic exposure in the fate of farmland bird populations.

We report here on an experiment that aimed at filling this gap by quantifying the sublethal effects resulting from pesticide residues exposition on a range of life-history traits under realistic field conditions in the grey partridge, Perdix perdix, a charismatic farmland bird. This species has declined by about 90% over the last 50 years in Europe (Kuijper and Oosterveld, 2008; Aebischer and Ewald, 2010). Partridges are exposed to a wide range of pesticides through the food they forage in farmland fields and sub-lethal effects may contribute to the current decline of their populations though neither direct nor acute effects were described so far (Bro et al., 2015; Millot et al., 2015). In the present study, we tested the sub-lethal effects of being fed with food from organic or conventional farming, differing therefore in pesticide content, and controlling for its quality. We thus developed a novel experimental procedure, in which our 6-month-old hand-reared grey partridges were provided with natural grains being grown either from organic or conventional agriculture, i.e., seeds were not treated on their own but resulted from different cropping practices, thus food varied in pesticide content. Over 26 weeks for two consecutive years, we surveyed partridges' behaviour, breeding performance and bodily and physiological conditions, allowing to measure lagged and sublethal effects of naturally ingested low doses of pesticides in grains from conventional farms.

2. Materials and methods

2.1. Experimental design

For this experiment, we used 40 (2017) and 38 (2018) 6-month-old hand-reared grey partridges obtained from a farming centre. One male and one female were randomly housed in pairs (i.e., 20 and 19 pairs) in outdoor cages (580 × 109 × 170 cm). Pairs were acclimatized to the new boxes for three weeks before starting the experiments, with ad libitum tap water and food. At this stage, all were fed with organic wheat seeds (week –3 to week 0; see Fig. S1 for details and below for details about food). Pairs were then randomly assigned to either the “organic” group or the “conventional” group (10 pairs each in 2017 and 9 pairs in 2018). All life-history traits assessed in this study (see below) showed no difference between the two groups in 2017 and 2018 at start of the season, (week 0; see electronic supplementary material, Table S1).

The organic group was fed ad libitum with grains obtained from the harvest of certified organic crops (and purchased from organic producers). Diet was composed of two-thirds wheat and one-third maize in 2017 and wheat only in 2018. We changed the composition of food to get a replicate of our experiments (organic versus conventional). The ratio of two-thirds of wheat and one-third of maize in 2017 had been recommended by partridge breeders. As wheat and maize were provided ad libitum, birds had the
opportunity to choose their foods. In 2017 and 2018, organic wheat was from variety LG ABSALON and ATTLASS respectively (Table 1a). The conventional group was also fed with two-thirds wheat and one-third maize in 2017 and wheat only in 2018, but seeds were purchased from producers that grew the crops using conventional agriculture with various pesticide applications during the cropping season (Table 2). Wheat varieties involved in conventional treatment; NEMO was the same for both years (Table 1a). Unfortunately, we could not get this information for maize from producers. Moreover, birds of both groups in both years received organic food pellets, 2 days a week in 2017 (always Monday and Thursday) and 3 days a week in 2018 (always Friday to Monday), also ad libitum. The precise composition of these granules is given in electronic supplementary material, Table S2. The addition of granules was mandatory to maintain enough proteins to the diets since a strict grain diet would not allow birds to lay eggs.

Given grains differed between the two treatments regarding presumed pesticide loads, but also possibly regarding quality (since varieties differed), we quantified pesticide residues (Table 2), energy content (Table 1b) and searched for quality content of the different varieties (Table 1a). Pesticide residues were quantified by GIRPA (Beaucoûx, Loire Atlantique, France) using a multi-residual method with a gas chromatograph coupled to a mass spectrometer (GC-MS, ISO 17025, the limit of detection was 0.003 mg/kg). Pesticide residues were found at various concentrations in the conventionally grown grains (up to 0.048 mg/kg in wheat in 2018 and 2.90 mg/kg in maize in 2017) whereas no residues were detected in the organic grains (Table 2). These values are consistent with those found in other studies quantifying pesticide residues in cereal grains (Lozowicka et al., 2014; Mebdoua and Ounane, 2019). The energy content for both food types was also quantified as the heat released by combustion using a calorimeter (Table 1b). This was carried out twice at the Centre Européen de Recherches et d’Analyses (Dijon, France). There was no significant difference between the food ingested by the organic group and the conventional group (Table 1b). Protein class, specific weight, Zeleny index and grains hardness of the different wheat indicated that organic and conventional varieties had highly similar quality traits (Table 1a).

Water and food were replaced each week. During the 26-week season, from the end of January (week 0) to the end of July (week 26), birds were kept on a natural light cycle. After 3 weeks of acclimatization (week – 3 to week 0) when both groups were fed organic grains, the birds were assessed at least three different times (Fig. S1) to assess: (1) immunocompetence using a PHA test and physiological stress through measurement of different indices, (2) the intensity of carotenoid-based colouration and plasma carotenoid concentration, (3) the behaviour and body condition and (4) breeding traits. In 2017 only, we used the natural infection by intestinal parasites (coccidia) to assess the effect of food on intestinal parasite load. No anticoccidial drugs were provided during the experiment.

2.2. Intestinal parasites (coccidia)

To avoid unnecessary stress of repeated captures to collect individual faeces, we chose to analyse the intestinal parasitic load on pairs of birds. The day before the collection of faeces, the cages were cleaned carefully to remove all faeces. Each pair was left without disturbance for exactly 24 h, then fresh faeces were collected from the floor and the faecal samples were weighed to the nearest ± 0.01 g with an electronic balance (ScoutPro) and placed in a 50 mL Falcon tube. Samples were then gently homogenized with 14 mL of Sheather’s solution (45% sugar). Parasites were counted in a McMaster chamber with 600 µL of the sample solution which had been left for 10 min to allow the oocysts and eggs to float (Filippi et al., 2009). There were two counts for each faecal sample and the mean number of oocysts was used. Coccidia concentrations were expressed as the mean number of parasites per gram of faecal sample. Counts were performed at weeks 1, 4, 7, 10 and 13.

2.3. Haematocrit, white blood cell count and heterophil/lymphocyte ratio

A blood sample was taken from the brachial vein using a sterile needle and heparinised micro-capillary tubes. These tubes were then centrifuged at 5000 r.p.m. for 5 min immediately afterwards to determine haematocrit (the proportion of the tube filled with red blood cells divided by the total sample volume in the capillary tube,

Table 1
(a) Information regarding different wheat varieties, purchased from organic and conventional farmers in 2017 and 2018 (this information was not available for maize varieties). Data were obtained from ARVALIS company, the French arable crops Research and Development Institute (https://www.arvalis-infos.fr/index.html). (b) Energy content (in J per gram) for sample of 200 g wheat or maize crushed in 2017 and wheat only in 2018, analysed twice by calorimeter (Centre Européen de Recherches et d’Analyses, Dijon, France). There was no significant differences between the conventional and organic food in either year (the 95% confidence intervals overlap).

<table>
<thead>
<tr>
<th>Year</th>
<th>Wheat</th>
<th>Maize</th>
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<tbody>
<tr>
<td>2017</td>
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<td>Conventional</td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>Maize</td>
</tr>
<tr>
<td></td>
<td>Energy1</td>
<td>Energy2</td>
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<tr>
<td>1600</td>
<td>1600</td>
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<td>± 30</td>
<td>± 33</td>
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<td>± 33</td>
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<tr>
<td>38.6 ± 9.4</td>
<td>35.8 ± 11.1</td>
<td>25.4 ± 4.7</td>
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<tr>
<td>Week 6</td>
<td>Week 15</td>
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* The protein content is expressed in class on a scale from 1 (very low) to 9 (very high).
* Specific weight represents the density of a batch of wheat and is expressed in class from 1 (very low) to 9 (very high).
* Zeleny index (or dry gluten content) represents an indicator of the quantity and quality of proteins through their ability to swell in an acid medium.
as an index of anaemia) (Biard et al., 2015). Lengths were measured with an electronic calliper (accuracy of 0.1 mm). A thin blood smear was made from a droplet of the blood sample from each bird. The slides were air-dried and immediately fixed in absolute methanol for 5 min and stained with Giemsa solution for 40 min. The blood smears were examined under 1000 magnification with an oil immersion microscope to estimate the total numbers and proportions of different types of leucocytes. The total proportion of white blood cells (WBC) was estimated by counting the number of leucocytes found for 10000 erythrocytes. The ratio of the relative numbers of heterophils to lymphocytes (H/L ratio) for 100 leucocytes was used as an index of physiological stress (Ots et al., 1998). Blood sampling and all related counts were performed in weeks 0, 5 and 10.

2.4. Plasma carotenoid concentration

We centrifuged blood from the same samples immediately after collection (4000 r.p.m. at 4°C for 5 min) to recover the plasma which was stored in Eppendorf tubes at −80°C until analysis. The plasma carotenoid concentration was assessed using a standardised colorimetric technique (Biard et al., 2010). Twenty microliters of plasma were diluted in 180 μL of absolute ethanol; gently vortexed and flocculent proteins were precipitated by centrifugation at 1500g for 10 min. The optical density of the supernatant was determined with a spectrophotometer at 450 nm. This was performed in weeks 0, 5 and 10.

2.5. PHA assay

The ability to trigger a cell-mediated immune response was measured using a simplified phytohaemagglutinin (PHA) skin test protocol (Biard et al., 2009). Grey partridges were injected with 100 μL of 10 mg/mL of PHA (PHA-P, Sigma-Aldrich, Lyon, France) dissolved in phosphate-buffered saline solution (PBS) in the centre of the right-wing web (patagium). All individuals were injected in the morning between 9:00 and 12:00 a.m. The patagium thickness at the injection site was measured just before and exactly 24h after the injection using a pressure-sensitive thickness gauge with an accuracy of 0.01 mm (Teclock SM-112, Alpa SpA, Milano, Italy). Each individual was measured twice. The PHA test was performed only once per bird, at week 5.

2.6. Eye ring colouration

In the grey partridge, the red patch just behind the eye acts as a secondary carotenoid sexual trait, involved in mate choice (Svobodová et al., 2013). High-resolution digital images were taken...
without flash or zoom twice on each side of the head using the same digital camera (Olympus, Stylus TG-2) under standardized conditions (same light, same spatial position between camera and birds) to measure the intensity of the red colour of the eye ring in weeks 0, 5 and 10 (Fig. S1). A standard grey strip was placed close to the head to control for any subtle variation in illumination between images. For each image, we calculated the red (R), green (G), and blue (B) components of the eye ring using Adobe Photoshop (v 7.0) by selecting a square of 1 cm² of only the pigmented area (see the procedure in Lopez Antia et al., 2013). The same components were calculated for the grey reference to compensate for possible differences in colour between images. The average of R, G, and B components from the four pictures was then calculated. The intensity of red colouration (redness) was calculated as R divided by the average of R, G, and B. This was performed in weeks 0, 5 and 10.

2.7. General behaviour

The behaviour of individual birds in the aviaries was scored to derive a time budget, using cameras (Reconyx, UltraFireTM). After acclimatization for 30 min, 10 s-videos were recorded every minute for 2 h. The videos were then watched individually to score “active behaviour” (eating, moving, vigilant, grooming) and “passive behaviour” (sleeping, immobile) for each 10-s video. Only the first behaviour observed in video was recorded when several activities occurred within the 10 s. Video recordings were made in weeks 0, 5 and 10 at the same hour.

2.8. Body mass index

To monitor the body condition of grey partridges during the course of the experiment, right and left tarsus lengths were measured twice with a digital calliper with an accuracy of 0.1 mm at the beginning of the experiment (week 0). Body mass was recorded to the nearest 0.1 g using an electronic balance (Scout Pro) for weeks 0, 3, 6, 7, 8, 9 and 10 (Fig. S1). Using body mass and mean tarsus length (right and left), we evaluated the body condition by selecting a square of 1 cm² of only the pigmented area (see the procedure in Lopez Antia et al., 2013). The same components were calculated for the grey reference to compensate for possible differences in colour between images. The average of R, G, and B components from the four pictures was then calculated. The intensity of red colouration (redness) was calculated as R divided by the average of R, G, and B. This was performed in weeks 0, 5 and 10.

2.9. Clutch and egg sizes

The first eggs were laid in week 16 in 2017 and week 17 in 2018. Once the first egg was laid, all boxes were checked three times a week (on Monday, Wednesday and Friday) to collect eggs over the whole season (8 weeks). All eggs were measured (maximum length and diameter) using the same digital calliper and weighed to the nearest 0.1 g using an electronic balance (Scout Pro). In 2017 only, the eggs laid 4th and 5th in the laying sequence (laying ranks 4 and 5) were sacrificed to assess the thickness of eggshell (see Lopez-Antia et al., 2013 for the details of the method).

2.10. Statistical analyses

Prior to analyses, the normality and homoscedasticity of the variable distributions were checked and transformed appropriately if required (see below). The difference in reaction to PHA between the organic and conventional groups, sex and year was tested with a linear regression model (LM) with F-ratio statistics. The haematocrit, H/L ratio (log-transformed), carotenoid concentration (log-transformed), colour (log-transformed), body condition, inactivity (square root transformed) and egg volume were compared using Linear Mixed-Effects Models (LMM) including the treatment (organic versus conventional), sex, and year as fixed effects, week of measure as a repeated effect to account for repeated measurements (time effect in the statistical model). Bird identity was treated as a random effect except for egg volume and intestinal parasite analyses, in which the random effect was the pair identity, and the models did not include the sex. Eggshell thickness was analysed similarly but the model did not include the year as data were available only for 2017. Generalized Linear Mixed-Effects Models with negative binomial error (NB-GLMM) were used instead of LMMs to analyse the variation in the white blood cell counts and intestinal parasites, but the statistical procedure was identical to that for the LMMs. Egg-laying chronology was analysed using Cox mixed-effects model, using the pair identity as a random effect. The LMMs, NB-GLMMs and Cox model were associated with an analysis of deviance based on χ²-statistics for unbalanced design. For all tests, P-values are given at 5% significance level threshold. All statistical tests were performed using R software (v. 3.5.0, R Core Team 2018) implemented with nime for LMM, glmmADMB for NB-GLMM, car for deviance analyses, smatr for the scale mass index computation and coxme for Cox mixed-effects model.

3. Results

There were no significant differences between years and treatments in the survivorship of adult partridges. In the conventional group, there were five deaths (two in 2017 and three in 2018) while in the organic group there were three deaths (two in 2017 and one in 2018) (Fisher exact test: \( P = 0.67 \)). On the other hand, all behavioural, morphological and physiological traits either differed significantly or had significantly different trends between the two groups throughout the season.

Firstly, for the physiological traits, we found that the intestinal parasite load in the conventional group increased significantly during the course of the experiment while that of organic pairs remained stable (treatment × time interaction: \( P = 0.01 \)) (Fig. 1a). Moreover, the haematocrit of the conventional group
(both sexes, but particularly males) decreased significantly through the season, while it remained stable in the organic group (time x treatment x sex interaction: \( P < 0.01 \)) for both years (Fig. 2a, Table S3). For both sexes, the WBC count (treatment x time interaction: \( P < 0.0001 \)) and H/L ratio (treatment x time interaction: \( P = 0.02 \)) significantly increased in the conventional group while there was a decrease in the organic group, for both years (Fig. 2b and c, Table S3). Finally, the inflammatory response to PHA was significantly higher in the conventional group with a more marked effect in 2018 than 2017 (Fig. 2d). Linear regression model, treatment: \( F_{1,69} = 73.60, P < 0.0001 \); year: \( F_{1,69} = 123.69, P < 0.0001 \) for both sexes (sex: \( F_{1,69} = 1.52, P = 0.22 \); treatment x sex interaction: \( F_{1,69} = 0.41, P = 0.52 \)).

Secondly, for the behavioural traits, we found that eye-ring redness (carotenoid-based colouration) increased slightly for both males and females over the season in both years, but it increased and reached higher values in the organic males compared to the conventional males, especially in 2017 (time x treatment x year interaction: \( P = 0.03 \); Fig. 3a, Table S3). There was an even more marked difference for the concentration of plasma carotenoids for both years, which was similar for the two groups at the beginning of the season, but then decreased steadily in both sexes of the conventional group relative while it increased sharply in the organic group (time x treatment interaction: \( P < 0.01 \); Fig. 3b, Table S3). Finally, the inflammatory response to PHA was significantly higher in the conventional group with a more marked effect in 2018 than 2017 (Fig. 2d, Linear regression model, treatment x sex interaction: \( P < 0.01 \) and H/L ratio (treatment x time interaction: \( P = 0.52 \)) for the conventional group in 2017 and \( 12.43 \pm 2.26 \) versus \( 12.25 \pm 2.86 \) in 2018, \( N = 8 \) females for both years; Wilcoxon rank sum test: for 2017, \( W = 26, P = 0.95 \), for 2018: \( W = 28.5, P = 1.00 \). This absence of an effect of pesticide exposition on laying was confirmed by a Cox mixed effects regression model for the egg laying dynamics (treatment: \( \chi^2 = 1.17, df = 1, P = 0.28 \); year: \( \chi^2 = 0.0004, df = 1, P = 0.98 \); treatment x year: \( \chi^2 = 2.06, df = 1, P = 0.15 \)). However, pesticide exposure affected the egg volume which was lower in the conventional group than in the organic group, although this was only significant in 2017 (treatment x year interaction: \( P < 0.01 \); Fig. 1b, Table S3). On the other hand, eggshell thickness for the conventional group (mean ± s.e.m. in mm: \( 1.12 \pm 0.02 \) and \( 1.15 \pm 0.02 \) for the eggs with laying ranks 4 and 8 respectively) was greater than for the organic group (mean ± s.e.m. in mm: \( 0.99 \pm 0.03 \) and \( 1.02 \pm 0.04 \), Table S3).

4. Discussion

Although our experimental approach consisted in providing untreated grains differing only in the source (grains from organic farms and grains from conventional farms), we were able to detect a suite of cascading consequences on life-history traits after only 10 weeks, which led to significant divergences between the two groups for every trait we monitored in both years despite modest
sample sizes. However, we acknowledge that organic and conventional systems differ in a set of parameters, which may affect bird in various pathways. Organic farming may provision more continuous and more abundant resources to birds across the landscape and throughout the season. Indeed, the ban of synthetic herbicides and mineral fertilizers increases floral, but also insect diversity or density (Bengtsson et al., 2005; Gabriel and Tscharntke, 2007; Ponce et al., 2011). Moreover, organic farmland is often sown with a greater variety of crops than conventionally farmed land (Hole et al., 2005; Barbieri et al., 2017) and comprises larger areas of semi-natural elements (Gibson et al., 2007). These two processes however cannot affect our results since birds are hand-reared. Only grains origins differed in the two treatment groups since food pellets were identical, and the proportions and quantity of grains were also identical. Despite varieties differed between organic and the conventional diets, we detected no statistical difference in quality between the organic and the conventional diets, either for energetical content (Table 1a) or from producer’s information regarding protein contents (Table 1a). Finally, feeding rates were similar between the two groups of partridges (Table 1c). Therefore, the only remaining difference in the grain’s food provided to the two groups was the pesticide content: no detectable pesticide was found in organic food, but 1 to 4 pesticides were detected in the conventional food depending on the year. We are therefore confident that the differences detected in the behaviour and physiology between our two groups of partridges are the result of pesticide load, though we cannot exclude that another factor, unmeasured in this study, may further drive additional differences. The rapid development of different analytical techniques to assess simultaneously the detection and the quantification of different molecules of pesticides in avian blood samples (Rivera-Rodriguez et al., 2007; Taliansky-Chamudis et al., 2017; Byholm et al., 2018) will make it possible to quantify the prevalence of different molecules in wild grey-partridge in the near future.

Since the pesticide exposure did not increase mortality and the divergence appeared gradually with the time between groups, this indicates sub-lethal effects. The main effects of pesticide exposure included: (i) less-developed carotenoid-based traits and lower concentration of plasma carotenoids, (ii) more active immune systems (stronger response to PHA, increased concentration of white blood cells), (iii) physiological stress (lower haematocrit, higher H/L ratio), (iv) higher intestinal parasitic load, (v) higher behavioural activity (males) and higher body condition (females) and (vi) lower breeding investment (smaller eggs, though with thicker eggshell). Despite a change in the source of the conventionally grown grains between the two years, hence, a priori, different pesticide loads, the differences between groups were consistent between the years. The effects were stronger in 2017 than in 2018, as the pesticide load was also higher in 2017 than in 2018 (Table 2). Twenty-three active substances were used during the cropping season in 2017 with 4 detected as residues versus 11 active substances in 2018, with one residue (Table 2). The sexes also reacted differently: pesticide exposure in males resulted in more active birds than in the control group, with no apparent cost for body condition, while females traded body condition and breeding investment since pesticide-exposed females improved their body condition at the expense of egg size. This sex difference could be due to pesticides since they can act as endocrine disruptors through different pathways associated with the hypothalamic–pituitary–gonadal axis (Grue et al., 1997). This includes endocrine processes, hence the secretion of steroid hormones, potentially affecting the production of sexual ornaments and/or the motivation to breed. For example, some pesticides may mimic the action of oestrogens (steroid hormones), resulting in an increase in the oestradiol concentration (associated with a decrease in testosterone) and leading to a decrease in adult fertility (Khali et al., 2017). In some cases, pesticides may modulate the enzyme Cytochrome P450 aromatase responsible for the conversion of androgens to oestrogens (Saxena et al., 2015) and consequently the regulation of male sexual behaviour (Ubuka and Tsutsui, 2014).
Prolactin and oestradiol are also impacted and increased to different extents depending on the molecules involved (Mohanty et al., 2017; Pandey et al., 2017). The effects of pesticides on hormones have already been explored in the red-legged partridge, showing contrasting results (Lopez-Antia et al., 2013; 2015a). Such effects on sex-specific hormones in the grey partridge should be better assessed in future studies.

Partridges exposed to pesticides usually show physiological stress such as lower haematocrit, being indicative of anaemia, similarly to birds ingesting neonicotinoids (Mitra et al., 2011; Lopez-Antia et al., 2013). However, stress increased the activity of the immune system with a stronger response to PHA as well as an increase in the white blood cell count, an effect rarely reported so far (e.g., Lopez-Antia et al., 2013). Such an effect is well known for the human immune system structure and functions (Mokarizadeh et al., 2015) and an increase of the expression of the immune system was found with other contaminants such as mercury (Carey et al., 2006). More indices of quality of immune system such as the capacity of partridges to produce immunoglobulin should be designed in future studies to evaluate the delayed sublethal effects of pesticides on bird immunocompetence completely. Whatever the mechanisms involved, both a high level of expression of the immune system and the physiological stress may impact individual fitness like more damages by parasites and diseases (Van der Sluijs et al., 2015), a consequence that seems to be confirmed here by the increased intestinal parasitic load in the treated group compared to controls.

Plasma carotenoid concentrations and carotenoid-based colouration of partridges was also lower in the conventional group and given that red-eye colouration intensity as also found in red-legged partridges (Lopez-Antia et al., 2013, 2014, 2015), a likely result of functional trade-offs. Pesticides ingestion may trigger a detoxification process, the latter producing reactive oxygen species that must be neutralized by the constitutive antioxidant system including carotenoid (Moller et al., 2000). This neutralization thus depletes the carotenoids stock for eye-ring pigmentation, especially in the conventional group (Fairev et al., 2003; Baeta et al., 2008). Since red-eye colouration intensity is involved in mate choice (Svobodova et al., 2013, 2016), the increased behavioural activity of males in the conventional group may perhaps be interpreted as a response to decreased attractiveness to females. If this mechanism operates in the wild, it may have potential deleterious consequences in regard to mating success and/or predator detection since predation is a major cause of mortality in grey partridges in France (Bro and Millot, 2013).

Pesticides known in birds to perturb key reproductive hormones (Lopez-Antia et al., 2015a; Lv et al., 2016; Pandey et al., 2017), which may decrease egg production, delay egg-laying, reduce egg size and eggshell thickness (Hernández et al., 2008; Lopez-Antia et al., 2013, 2018, Liu et al., 2019). Here, we found mixed results: while females from the conventional group laid smaller eggs, which may affect chick survival (Blomqvist et al., 1997), their clutch size was similar to females from the organic group (see Lopez-Antia et al., 2015a for a similar result on red-legged partridges), and most surprisingly, they had actually thicker eggs. Pesticides such as organochlorides inhibit calcium pumps in the membrane of eggs and thus, reduce the transport of calcium carbonate from blood to the shell (Mitra et al., 2011), leading to the thinning of eggshell (Lopez-Antia et al., 2013; Gibbons et al., 2015). This result may suggest a trade-off between egg size and eggshell thickness: a reduced egg size may allow a higher investment in eggshell. We also found that pesticide-exposed birds had a higher body condition, an unexpected result since the reverse has generally been found (Subapiya et al., 2007; Lopez-Antia et al., 2013). We suggest that the consumption of pesticides may have induced dysfunctions of metabolic pathways associated with fat storage, as shown in rats exposed to persistent organic pollutants (Ruzzin et al., 2010). There are potential costs associated with bird overweight, such as reducing their ability to escape predators (Binazzi et al., 2011).

Finally, some of our results are consistent with a hormetic dose-effect, since we detected increased performance of exposed individuals to chronic doses of pesticide residues, at least in some traits, such as higher activated immune system and body condition. Immunological hormetic response (Calabrese, 2005; Costantini et al., 2010) and stimulatory effect of the chemical on the growth of birds have already been reported in chickens (Costantini et al., 2010; Hasmi et al., 2014 and references therein) although the several physiological and molecular mechanisms involved have not yet been fully elucidated (Costantini et al., 2010). However, whether these hormetic effects have positive effects for exposed birds is still questioned, as revealed by exponentially growing numbers of papers showing positive effects (even transgenerationally) on one hand, but some showing negative effects on the other hand (see Agathokleous and Calabrese, 2015; Calabrese and Agathokleous, 2020 for recent examples). Possible hormesis dose-response in partridges should be therefore further investigated as well as exploring a possible preconditioning effect (i.e., bird being better able to survive with exposure to higher levels of pesticide residue if encountered on subsequent occasions) as has been suggested in some birds (Costantini et al., 2010; Costantini, 2019).

5. Conclusion

Our innovative experimental design highlights that realistic pesticide exposure levels may have lagged sub-lethal consequences resulting in major life-history traits shifts. Although this has yet to be proven, these changes in life-history traits may impair individuals’ fitness in the wild. These shifts include, for instance, increased predation risk due to increased activity, lower mating success due to reduced secondary sexual traits, or lower survival due to higher intestinal parasite load, immune system disorders and fat storage. Our results thus shed new lights on farmland bird decline and their causes, by showing for the first time that ingesting low doses of pesticides may have lagged sub-lethal consequences on several major physiological pathways. There is, therefore, an urgent need to assess the long-term sub-lethal effects of pesticides on farmland birds, for pesticide risk assessment where only short-term effects are currently considered on model species under laboratory conditions, which may not reliably predict consequences of pesticide exposure on wild birds (EFSA, 2009).

Author statement

Moreau J: Funding acquisition, conception and design the study, acquisition of data, Formal analysis, drafting the article, final approval of the version, Monceau K: conception and design the study, Formal analysis, revising the article, final approval of the version, Crépin M: acquisition of data, Formal analysis, final approval of the version, Derouin Tochon F: acquisition of data, Formal analysis, final approval of the version, Mondet C: acquisition of data, Formal analysis, final approval of the version, Fraikin M: acquisition of data, Formal analysis, final approval of the version, Teixeira M: acquisition of data, Formal analysis, final approval of the version, Bretagnolle V: Funding acquisition, conception and design the study, drafting the article, final approval of the version.
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.envpol.2021.116851.

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Data accessibility

Once the paper has been accepted, data will be deposited in the Dryad.

Research ethics

All experiments complied with French laws on animal experimentation and all experimental protocols were approved by the Deux-Sèvres Committee of Animal Experimentation (APA FIS#9465-201703101551625). Experiments were conducted in the research facilities of the Zoodyssée authorised animal park Villiers-en-Bois, France) on behalf of Bernard Ragot (79/D87/2015).

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